STUDIES ON THE DOSE BANDING OF PACLITAXEL

by

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Dedication

This thesis is dedicated to one of the most important people to me, my late father, Yun Chang Xu, who has taught me a lot about the meaning of life. It was my great sorrow to lose him just before starting my Ph.D. study. However without his encouragement, I would not have been brave enough to move forwards to conduct this project. He has been and will always be a source of encouragement and inspiration throughout my life. He would forever be with me in my heart……

Abstract

Paclitaxel is an important chemotherapeutic agent and is used in the treatment of many solid tumours including ovarian cancer, NSCLC, and breast cancer, with further applications under evaluation in clinical trials. In current practice, the dosage of paclitaxel is calculated according to body-surface area (BSA). However the scientific validity of individualised BSA-based dosing has been doubted for many years. In the case of paclitaxel, alternative dosing strategies such as flat-fixed dosing and dose-banding have been considered. However, prior to the studies reported in this thesis, no evidence was available to support the pharmaceutical and clinical implications of such strategies.

This thesis includes a literature review, outlining the role of chemotherapy in the treatment of cancer and a detailed appraisal of paclitaxel from both clinical and pharmaceutical prospective. Robust stability data on ready-to-use preparations are required to support the inclusion of medicines in dose-banding (D-B) schemes. Studies on the physical and chemical stability of paclitaxel infusions are described of drug concentrations relevant to D-B.

A clinical and pharmacokinetic study is described which was designed to assess the clinical effect of paclitaxel D-B, and to compare D-B with individualised BSA-based dosing and flat-fixed dosing schemes. This study used area under the plasma concentration-time curve (AUC) to assess effect of dose schedule on exposure of tissues to the drug. This was considered an appropriate surrogate for therapeutic effect and toxicity. Validated methods for the processing and analysis of plasma samples were a pre-requisite for this study and the development and validation of these methods are described in Chapter 3 of this thesis.

External factors precluded implementation of the clinical-pharmacokinetic study and therefore a novel *ex vivo* pharmacokinetic model was designed to simulate the clinical PK study. This laboratory simulation was developed and scaled-down from *in vivo* data. This *ex vivo* study suggested there was no significant difference between the D-B dosing and the individualised BSA-based dosing as well as between the flat-fixed dosing and the individualised dosing of paclitaxel on the basis of likely exposure of the tissues to the drug.

Acknowledgement

I would like to express my deepest thanks to my supervisor, Professor Graham Sewell. Without him, this project would not be possible. My most heartfelt appreciations to him for his supervision, constant encouragement and unlimited patience during my whole Ph.D. study. I would like to express my sincere gratitudes to Professor John Brown who has kindly offered me invaluable advice and support whenever I was in need.

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Finally I would like to thank my family for their support throughout my Ph.D. and indeed my entire life. I am very lucky to be able to share my life with my beloved family. I would particularly like to thank my Mother 'Ji Mei', my father 'Yun Chang' and my grandparents. Without them I would not be where I am now. Lastly, I would like to thank most of all, my husband Alfred for being the greatest support during my Ph.D. and also my life.

Glossary

ABI-007	Abraxane, Albumin- bound nanoparticle paclitaxel
AC	Anthracycline and cyclophosphamide therapy
ACN	Acetonitrile
AE	Adverse event
AIC	The Akaike Information Criterion
ANC	Absolute neutrophil count
AUC	Area under the drug concentration-time curve
BP	British Pharmacopoeia
BSA	Body surface area
C.I.	Confidence interval
CE	Capillary electrophoresis
CIV	Continuous intravenous infusion
CL_{total}	Total (systemic) clearance
CL_{obs}	Total observed clearance
C_{max} (C_{MAX})	Peak plasma concentration
CMC	Critical micelle concentration
CN-E	Cyano end-capped
COREC	Central Office for Research Ethics Committees

CrEL	Cremophor EL
CRF	The case record form
CV	Coefficient of variation
D-B (DB)	Dose banding strategy
D_{DB}	Dose-banded dose
D_{flat}	Flat-fixed dose
D_{Ind}	Individualised dose
DLT	Dose-limiting toxicity
DOC	Docetaxel
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme-linked immunosorbent assays
FDA	Food and Drug Administration
FIA	Fluoroimmunoassay
FLAT	Flat-fixed dose (dosing)
GCP	Good clinical practice
G-CSFs	Granulocyte colony stimulating factors
GOG	Gynaecologic Oncology Group
HPLC	High performance liquid chromatography

HSA	Human serum albumin
HSRs	Hypersensitivity reaction
IND	Individualised dose (dosing) according to body surface area
IP	Intraperitoneal administration
IS	Internal standard
K	Elimination rate constant from the central compartment
LC-MS	Liquid chromatography and mass spectroscopy
LD_{50}	The doses at which 50% of the animals die in animal studies
LDPE	Low density polyethylene
LLE	Liquid-liquid extraction
LLOQ	Lower limit of quantification
LOD	Limits of detection
LOQ	Limits of quantification
LSS	Limited sampling strategy
MDR	Multidrug resistance
MRT	Mean residence time
MSKCC	Memorial Sloan-Kettering Cancer Center
MS-MS	Tandem mass spectrometry

MTD	Maximum tolerated dose
NCA	Non-compartmental pharmacokinetic analysis
NCI	National Cancer Institute
NICE	National Institute for Health and Clinical Excellence (UK)
NSCLC	Non-small cell lung cancer
ODS	Octadecylsilane
PAC	Paclitaxel
PD	Pharmacodynamics
PK	Pharmacokinetics
PO	Polyolefin
PP	Polypropylene
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidenefluoride
QC	Quality control
QUALY	Quality adjusted life years
REC	Research Ethics Committee
RIA	Radio-immunoassay
RP	Reverse phase

SAE	Serious adverse event
SAR	Serious adverse reaction
SCF	Stem cell factor
SPC	Summary of product characteristics
SPE	Solid phase extraction
$T_{1/2}$	Half-life of a drug
THF	Tetrahydrofuran
T_{max}	Time at which C_{max} is observed following administration of drug
UV	Ultraviolet detection
V_C	Volume of distribution for the central compartment
Vol_{inf}	Total volume of infusion
V_{ss}	Volume of distribution at steady state
WSSR	Weighed sum of squared residuals

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1. CHAPTER 1: LITERATURE REVIEW AND INTRODUCTION

1.1. Literature review

1.1.1 Cancer and cancer treatment

Cancer is a class of malignant diseases in different tissues, characterised by uncontrolled cell division and the ability to spread by local invasion and by metastasis through vessels of the blood or lymphatic system. It is one of the commonest diseases in the world that cause death. It is said that almost one third of the population in the West will develop cancer sometime in their lives. So far the cause of cancer is not clearly known yet. It arises from the mutations of DNA genes. These mutations always happen to the genes that regulate cell proliferation, thus causing uncontrolled cell division[1].

Current cancer treatment includes surgery, chemotherapy, radio-therapy, immunotherapy, endocrine therapy and other supportive care. The treatment may be curative or palliative depending on many factors such as the detection stage, the cancer type and size, and chemosensitivity[1].

Chemotherapy has been introduced to cancer patients since the 1970's. Many clinical studies have demonstrated chemotherapy contributes to the improvement in response rate, time to progression, prolongation of overall survival and relief of symptoms. However in recent years, the previous opinion has been challenged by supporting the role of chemotherapy mainly for palliation as it does not result in a significant improvement of the 5-year survival for patients. In a recent literature survey, chemotherapy (curative and adjuvant) was considered to have a minor contribution to the 5-year survival for adult patients: 2.3% (Australia) and 2.1% (USA), even if the

relative 5-year survival rate (1992-97) for 22 adult malignancies in Australia was 63.4%[2]. However, it is not easy to evaluate the role of chemotherapy when combined with surgery, radiotherapy or endocrine therapy for patients. There are also many other possible reasons that may answer for the failure of chemotherapy. It could be due to treatment delay, bad medical care (nursing/doctor), inappropriate regimen, combined drugs or other combined therapies, multi-drug resistance (MDR), poor health condition of patient, or poor bioavailability. It is therefore unjustifiable to claim that chemotherapy has an insignificant impact on patient survival unless any of the other above factors could be excluded.

On the other hand, chemotherapy was reported to significantly improve cancer survival and relieve symptoms and therefore is still supported by many practitioners[3,4,5,6].

1.1.2 The cell life cycle

Knowledge of the cell cycle is required to understand the following classification of chemotherapeutic agents and also the mechanism of action of paclitaxel. The cell life cycle of cancer cells is similar to that of normal cells. A cell life cycle normally starts from the ending of one cell division and finishes at the end of the next cell division. There are five phases contained in one cell life cycle (see Figure 1-1):

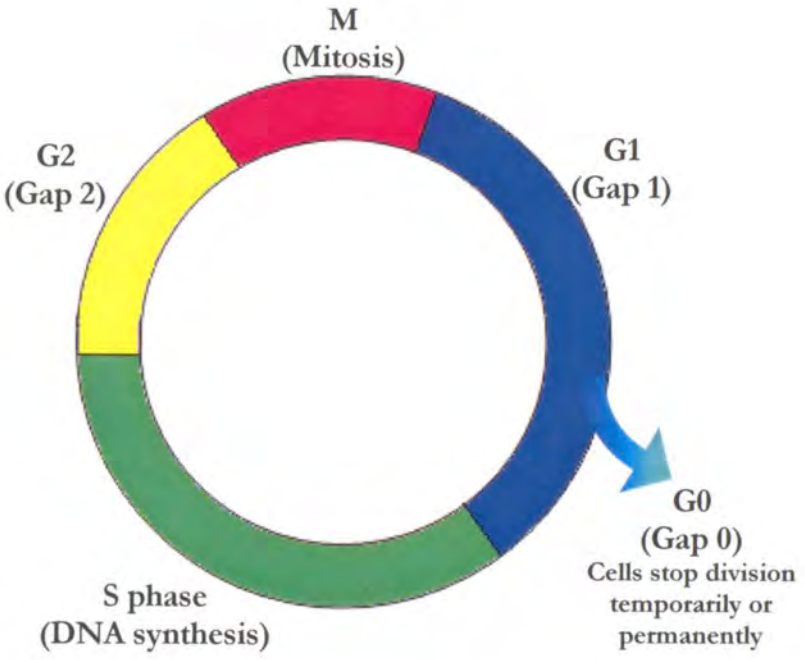

Figure 1-1 The cell life cycle

G0 (Gap0 phase), G1 (Gap 1 phase), S (synthesis phase), G2 (Gap 2 phase) and M (Mitosis phase)[7]. After mitosis, some cells can remain in the G0 phase without division, whereas others can enter the G1 phase with starting a new cell cycle. There is a dynamic balance in the number of the cells between the G0 phase and the G1 phase according to the growth-factor of the tissue or tumour. The G1 phase is associated with synthesis of protein, RNA and enzymes that are necessary for the synthesis of DNA. The G1 phase is followed by the S phase, where DNA is replicated. After the synthesis phase, cells enter the G2 phase where more RNA and proteins are synthesised in preparation for mitosis. Following the G2 phase, mitosis is initiated (where cell division takes place), which includes four subphases: prophase, metaphase, anaphase and telophase. At the end of mitosis, one cell is divided into two daughter cells. Each of these can then enter the cell cycle as mentioned above[7].

Chemotherapeutic agents can be divided into three main groups[8]:

I. Phase -specific drugs

This group of drugs exert a cytotoxic effect on one specific phase of the cell cycle. Drugs in this group only kill a certain number of cells that exist in specific phases of the cycle and they are usually used in intermittent dose schedules or in combination with other drugs. Most antimetabolites and plant alkaloids are phase-specific drugs. Examples include: methotrexate, cytosine arabinoside, paclitaxel and vincristine.

II. Cycle -specific drugs

The second group are cycle-specific drugs that have a broader cytotoxic band than the first group, and can kill active cells in any phase of the cell life cycle except for the G0 phase. However for some drugs, the cytotoxic effect varies between the different phases of the cell cycle. Many cytotoxic agents are defined in this group, for example, cyclophosphamide, busulfan, actinomycin D and carboplatin[8].

III. Cycle – non-specific drugs

The chemotherapeutic agents in this group exert a cytotoxic effect not only on the active cells that attend the cell division in the cell life cycle but also on the static cells that rest in the G0 phase, for example, carmustine[8].

1.1.3 Classification of chemotherapeutic agents

Based on the mechanism of cytotoxic activity or drug source, chemotherapeutic agents are generally grouped into the following 6 classes:

- The alkylating agents

The alkylating agents are a group of chemical compounds that can interact with DNA molecules by alkylation, thus changing or destroying the structure of DNA and its template which can finally stop the replication of DNA or lead to cell death. Alkylation

typically occurs at the guanine N-7 position and can result in inter- or intra- strand cross links[9]. The common alkylating agents include: busulfan, chlorambucil, carboplatin and cisplatin[8,9].

- The antimetabolites

Antimetabolites are a group of synthetic agents with similar structure to metabolites that are required in the metabolic synthesis of nucleic acids and DNA. These agents can replace the required metabolites such as purine, pyrimidine and folate during S phase, thus damaging the metabolic synthesis of nucleic acid and DNA. Therefore, this group is usually subdivided into purine, pyrimidine and folate antagonists. For example, 5-fluorouracil (5-FU), a representative of pyrimidine antagonists with similar structure to pyrimidine bases uracil and thymine, can exert a cytotoxic effect by incorporation into RNA and also by direct inhibition of thymidylate synthase[9]. Methotrexate (MTX), cytosine arabinoside (Ara-C), floxuridine and Deoxycoformycin are also included in this group[8,9].

- The plant alkaloids

This group of agents are all obtained from plants. There are three main different types included in this group: vinca alkaloids, taxanes and epipodophyllotoxins. Among them, the first two types of agents are antitubulin agents that can interfere with microtubules, stopping dynamics of microtubules and then arresting cells in certain cell-cycle phases. However, these drugs function in different ways: vinca alkaloids inhibit the polymerisation of microtubules and stop the formation of mitotic spindles during the M phase; taxanes induce the polymerisation of microtubules and stop cells in the G2 and M phases. As mentioned before, paclitaxel is a taxane (details about its mechanism will be mentioned in the following part)[7,8]. Epipodophyllotoxins include etoposide and

teniposide, which can break double strands of DNA by inhibiting the topoisomerase II enzyme that is required for the important activities of DNA such as transcription and replication[10].

- Anti-tumour antibiotics

These drugs inhibit cell division by interfering with the function or synthesis of DNA and RNA in many different ways including intercalation, DNA strand breakage and inhibition of the topoisomerase II enzyme. Doxorubicin is an example of this type, which exhibits its cytotoxic effect by the 3 above mechanisms. Other examples in this type of drug are dactinomycin, mitomycin C, and mitoxantrone[7,9].

- The hormones

The hormones or hormone-like agents exhibit their therapeutic effect by antagonising or blocking the naturally endogenous substances that stimulate tumour growth, thus inhibiting the tumour proliferation. For example, tamoxifen is used as an anti-oestrogen for the treatment of metastatic breast cancer because it is a competitive inhibitor of endogenous oestradiol with the oestrogen receptor[9]. Also, these hormones include dexamethasone, flutamide and aminoglutethimide[7,8].

- Miscellaneous agents

Miscellaneous agents are those that do not belong to any above group but have a cytotoxic effect. Crisantaspase is included in this group. Crisantaspase can convert asparagine to aspartic acid and ammonia, thus inhibiting the synthesis of asparagine[9]. Other miscellaneous agents include procarbazine and hydroxyurea[7].

1.1.4 Paclitaxel: discovery and development

1.1.4.1 Origins of paclitaxel

Paclitaxel is a taxane, one of the most important classes of chemotherapeutical agents, which has been incorporated into many protocols for the treatment of cancer chemotherapy such as breast cancer, ovarian cancer and non-small cell lung cancer. It was firstly documented that a chieftain of the Eburones, Catuvolcus at the time of "Gallic Wars" committed suicide by eating an extract from the yew tree, which was mentioned by Julius Caesar[11]. In 1962, a crude extract containing paclitaxel was obtained from the bark of the pacific yew, *Taxus brevifolia*, by the National Cancer Institute (NCI) in order to screen plant materials for anticancer activity[11]. In the next few years, Wani and his co-workers were assigned to test the extract using different materials and models. The extract exhibited the cytotoxic activity against human KB epidermoid carcinoma cells (derived from a nasopharyngeal tumour) firstly in 1964. Later, its cytotoxic activity was also observed in many *in vivo* models, e.g. the Walker 256 carcinosarcoma, P1534 leukaemia, and L1210 leukaemia models[12]. However, among these models, strong activity against P1534 leukaemia was determined[12]. In 1971, the active component, paclitaxel, was identified and the novel structure (Figure 1-2) was determined by both X-ray crystallography analysis and ^{1}H-NMR techniques[11]. However, it was not easy to develop this drug from the natural plant, which is one of the most slowly growing trees in the world, taking more than 100 years to mature. Also, paclitaxel has poor aqueous solubility. Both of these disadvantages brought into doubt the development of paclitaxel at that moment. It was not until 1979, when the unique mechanism of action for paclitaxel was revealed that paclitaxel began to attract world-wide attention[13,14]. Phase I clinical trials commenced in 1983 by the NCI, however during these trials paclitaxel was found to be associated with a variety of

toxicities, especially acute hypersensitivity. Phase II studies began in 1985 and Phase III studies started from 1990. In 1992, paclitaxel was initially approved to be used for the treatment of refractory ovarian cancer by the FDA[15,16].

1.1.4.2 Chemical and physical characteristics

Paclitaxel is a small molecule with a molecular weight of 853.91 (Figure 1-2). The chemical name of paclitaxel is 5ß,20-Epoxy-1,2a,4,7ß,10ß,13a-hexahydroxytax-11-en-9-one4,10-diacetate2-benzoate13-ester with (2R, 3S)-N-benzoyl-3-phenylisoserine with the formula, $C_{47}H_{51}NO_{14}$[17]. The molecule consists of a primary taxane ring and side chains at the C13 and C2 positions. The side chain in C13 has proved essential for the cytotoxicity of paclitaxel[15,18]. Paclitaxel and its analogue (docetaxel) that is synthesised from a precursor extracted from the needles of yew trees, share a very similar structure except for different functional groups in C10 and C5' (as shown in the coloured areas in Figure 1-3). Also, paclitaxel has poor water solubility due to its hydrophobic nature. Pure paclitaxel is a slightly white crystalline powder with a melting point of between 216°C and 217°C[17].

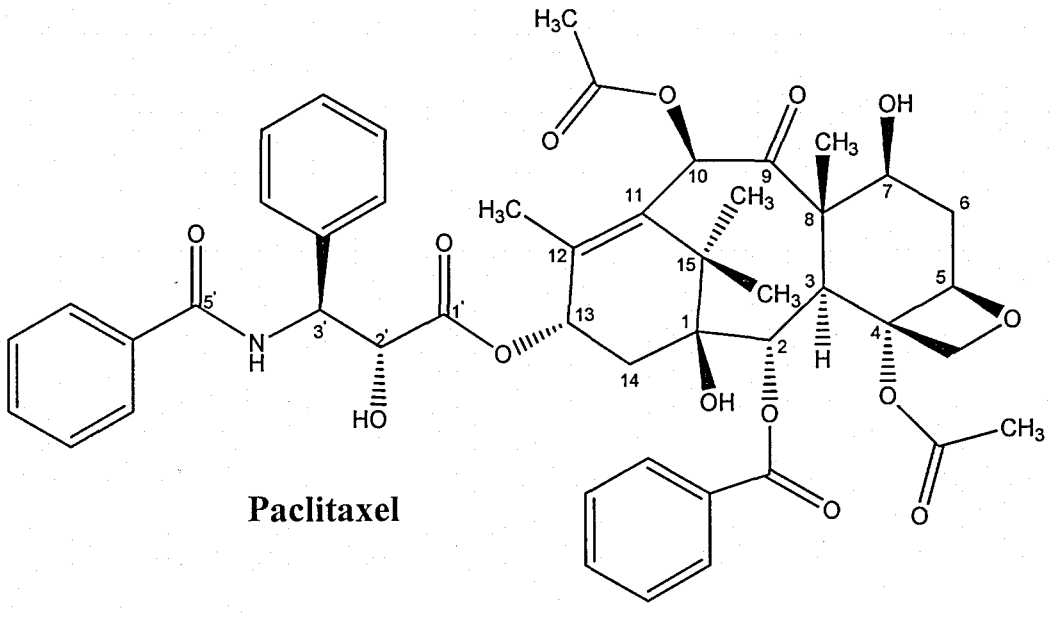

Figure 1-2 Chemical structure of paclitaxel

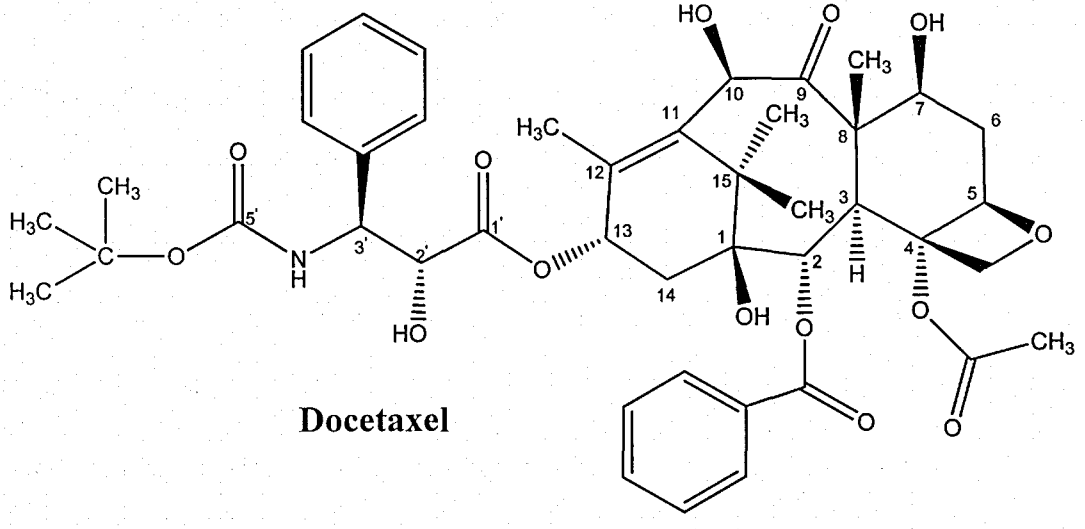

Figure 1-3 Chemical structure of docetaxel

1.1.4.3 Pharmacology

1.1.4.3.1 Microtubules and the detailed mechanism of action of paclitaxel

Microtubules play an important role in cell function. They help maintain the cellular skeleton and also take part in many cellular activities, e.g. cell division, intracellular

transport and cellular effluxion[19]. The basic structural unit of microtubules is a heterodimer which consists of both alpha- and beta- tubulin subunits[20]. Microtubule dynamics involve the polymerisation and disassembly of tubulins, which is required for cell cycle functions including mitosis and interphase phases. For example, during mitosis, the formation and separation of the mitotic spindle, the key process for cell division, is dependent on the polymerisation and disassembly of tubulins.

Taxanes are an example of an antitubulin agent that can block cell division by interrupting microtubule dynamics. As mentioned above, paclitaxel can stabilise the polymerisation of microtubules, unlike other earlier antitubulin agents such as vinca alkaloids that induce the disassembly of tubulins[19,21]. It produces a cytotoxic effect by binding to the beta-tubulin subunit during G2 and M phases in the cell cycle (see Figure 1-4[22]). This inhibits the disassembly of tubulins and stabilises microtubules, thus damaging normal microtubule function and stopping cell division in G2/M phases of the cell cycle, which would induce apoptosis (programmed cell death)[12,13,23]. As the mechanism of action for paclitaxel was better understood, the position of the active site for paclitaxel on the microtubule tubulins became more apparent[13,19]. Horwitz's group used a photoaffinity labelling method and identified that one taxol analogue, [^{3}H]3'-(p-azidoenzamido) taxol can bind to the N-terminal 31 amino acids of the beta-tubulin subunit in microtubules[24,25,26]. They also found another analogue [^{3}H]2-(m-azidobenzoyl) taxol which can bind to the peptide containing amino acid residues 217-233 on beta-tubulin[26,27]. Nogales and his co-workers explained the binding conformation of taxol in beta-tubulin by proposing a unique solution, a T-shaped structure[28]. Although the interaction of taxol and microtubules has been identified, the mechanism of inducing apoptosis after the binding of taxol to microtubules is still not clearly known[19].

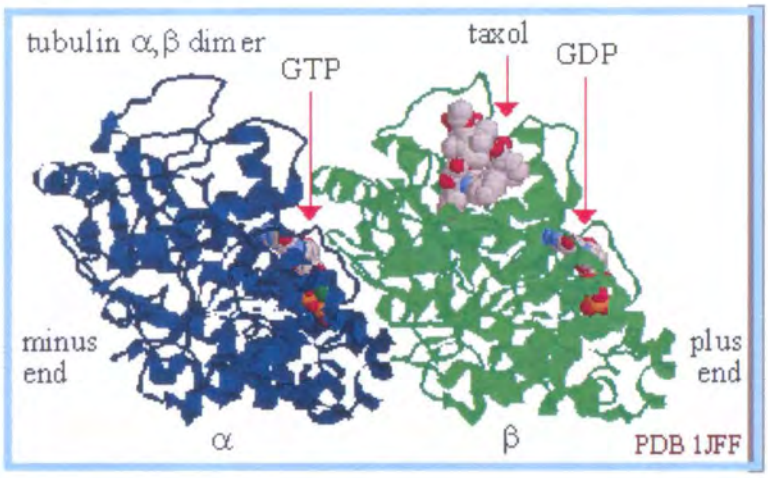

Figure 1-4 The binding of paclitaxel to beta-tubulin in microtubule

Interestingly, paclitaxel can only reversibly bind to beta-tubulin in a dimer form rather than a free tubulin. This binding is substoichiometric and approximately 1 mol of taxol binds to per mol of tubulin dimer[29,30]. This might suggest that the interaction of paclitaxel with microtubules is concentration dependent[13,26,31]. With low paclitaxel concentrations (< 10nM), the normal inhibition of mitosis was induced, followed by the induction of apoptosis. It may be because only a few taxol-binding sites on microtubules are occupied without increased polymer mass and changed conformation[21,26,31,32]. When taxol was used in a higher concentration (>1μM), the microtubules reorganised themselves into bundles (Figure 1-5) or formed asters of mitotic spindles (Figure 1-5) which resulted in the disruption of normal microtubule dynamics and arresting of cells in the G2/M phases of the cell cycle[13,20,26,33].This might be due to the disturbance of the equilibrium between soluble tubulin dimers and microtubules, leading to an increase in polymer mass[31].

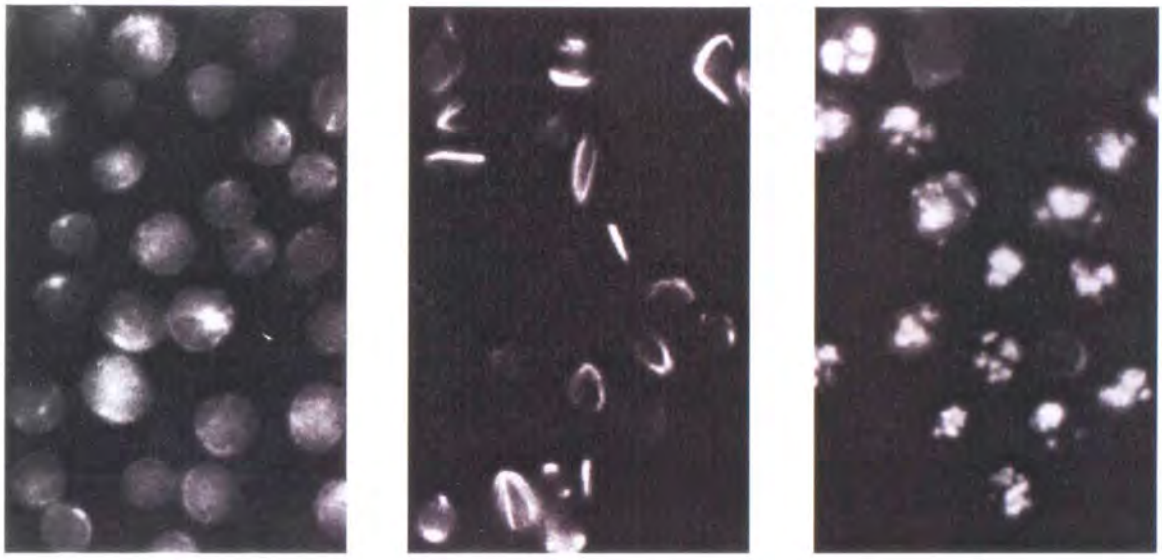

Figure 1-5 Microtubule Effects of Paclitaxel (Human Leukemia Cells Stained with Antitubulin Antibody, Indirect Immunofluorescence Microscopy)

A = normal cells, B = microtubule bundles, C = multiple asters of mitotic spindles [13]

In addition, paclitaxel may induce cytotoxic effects by increasing the expression of tumour necrosis factor α, which can also decrease tumour cell proliferation. This is considered a different route from the normal interaction of paclitaxel and microtubules[34].

Also, paclitaxel is known to enhance the radiation sensitivity of tumour cells. It is because the G2 and M phases, where cells are arrested by paclitaxel, are the most sensitive stages of the cell cycle to radiation[35]. Thus, paclitaxel has good potential to be combined with radiation therapy in clinical practice[36].

1.1.4.4 Adverse effects of paclitaxel

I. Hypersensitivity Reactions (HSRs)

At a very early stage, phase I trials demonstrated there were a variety of toxicities associated with the paclitaxel formulation. Among these, hypersensitivity reactions

(HSRs) were the most serious with a high incidence of major reactions, 25% – 30% in some studies[13]. These reactions were typically type I hypersensitivity reactions with dyspnea, flushing, rash, chest pain, tachycardia, hypotension, angioedema and generalised urticaria[13,15,37,38,39]. They usually occurred with the first or second dose and in the very beginning of infusion, or at least during the first hour of infusion. Even though later H_1, H_2 blockers and corticosteroids were used as premedication, HSRs were still observed in about 41% of all patients and the incidence of serious reactions approached 1.5% – 3%[39,40,41].

The formulation vehicle, Cremophor EL (CrEL) (see 1.1.4.5) was considered to be responsible for these hypersensitivity reactions because they had also been observed with other drugs formulated with CrEL[42,43]. Although the mechanism of hypersensitivity reactions have not been clarified completely, complement C3 activation can be caused by CrEL, thereby resulting in many reactions experienced by patients[41,43]. Sparreboom's group reported that the complement activation induced by CrEL is concentration dependent, which demonstrated the reason why a lower infusion rate can reduce the hypersensitivity reactions[44]. In addition, CrEL was found to induce the release of histamine in dogs, which may contribute to the hypersensitivity reactions[38,45].

II. Neutropenia

Neutrophils, part of the white blood cells, are derived in the bone marrow. There, pluripotent stem cells can proliferate and differentiate into different specific cell lines including the cell line to produce neutrophils. After many stages of differentiation, such as myeloblasts, promyelocytes and myelocytes, various blood cells including neutrophils will be formed with a high differentiation level and then distributed into

peripheral blood. The formation of blood cells (haematopoiesis) is regulated by a number of haematopoietic growth factors e.g. interleukin-3 (IL-3), IL-6, IL-11 and stem cell factor (SCF) and granulocyte colony stimulating factors (G-CSFs)[1]. These growth factors help to stimulate the primary cells and to increase proliferation by binding to receptors expressed on these cells.

Neutropenia is a major toxicity caused by paclitaxel itself[13]. It typically occurs on day 8 to 10 after starting treatment. Normally, there is a negative feedback mechanism following the appearance of neutropenia, which activates a series of factors (as mentioned above), thus stimulating resumption of haematopoietic activity to maintain the balance of blood cells. Therefore usually on day 15 to 21 after starting treatment neutropenia will recover. Sometimes severe neutropenia can occur (< 500 cells/mm^3). Currently, paclitaxel therapy is limited to patients with neutrophil counts of more than 1,500 cells/mm^3 [39]. Also, paclitaxel is commonly administered together with granulocyte colony-stimulating factors (G-CSFs), which can minimise the occurrence of neutropenia.

It has been reported that the occurrence of neutropenia is dose-limited and time dependent. The incidence of severe neutropenia increases with dose and time. Comparing both, it seems that time is more important[46,47,48]. Smith and his co-workers concluded 24-hour infusion of paclitaxel (250 mg/m^2) was associated with a higher incidence of neutropenia compared with 3-hour infusion of paclitaxel at the same dose[46].

III. Peripheral neuropathy

Peripheral neurotoxicity is another major dose-dependent toxicity induced by paclitaxel. It usually occurs when patients receive a high dose treatment (> 250 mg/m^2) or after

several courses at conventional doses (135 to 250 mg/m^2). It is normally associated with a series of symptoms such as numbness, tingling and paresthesia (e.g. burning pain) in a glove-and-stocking distribution. Usually, the incidence of common peripheral neuropathy is about 60% – 64%, of which 3% can be severe[39].

Many facts suggested that CrEL formulated in Taxol was responsible for peripheral neuropathy. Rowinsky's group found that compared with Taxol treated rats, less toxicity can be detected in the group of rats treated with non-CrEL paclitaxel[49]. This was in agreement with another study which showed docetaxel, which has a similar structure to paclitaxel but without CrEL in its formulation, was associated with less than one tenth of the neurotoxicity compared with paclitaxel formulated with CrEL[50]. Also, an obvious neurotoxicity can be observed with cyclosporin A, another anticancer drug formulated with CrEL as well[51]. Some peroxidation products in CrEL may lead to axonal degeneration and demyelination in peripheral neurological system, which presents a variety of neurotoxic symptoms as mentioned above[37,38,52]. It is noteworthy that central neuropathy is rarely caused by paclitaxel.

IV. Cardiac effects

Paclitaxel also has effects on the cardiovascular system. Obvious effects are hypotension, cardiac rhythm abnormalities, oedema and syncope. Among these toxicities, the first three occur in around 10% – 20% of all patients. The others are rare. Usually they are not serious without need for additional treatment[39].

V. Other toxic effects

In addition to the above adverse effects, other common toxicities of paclitaxel also include myalgia, arthralgia, infection, nausea, vomiting, diarrhoea etc. These occur less frequently than the above main toxicities. Also, with the exception of peripheral

neuropathy there is no obvious relationship between the severity of some toxicities and the dose and schedule of paclitaxel. They are not major concerns with paclitaxel administration[39].

1.1.4.5 Formulations

1.1.4.5.1 Cremophor EL (CrEL)

Cremophor EL is a non-ionic surfactant. The components included in CrEL are quite variable, consisting of polyoxyethylene glycerol triricinoleate 35[37]. It is widely used as a formulation vehicle to increase the aqueous solubility for drugs. As mentioned above, paclitaxel has very poor water-solubility, but it can be dissolved in CrEL and ethanol. The common commercial formulation (Taxol) contains paclitaxel in a mixture of CrEL and dehydrated ethanol (1:1, v:v)[38]. However, CrEL is not inert but is a pharmacologically active component. It has been widely known that a high amount of CrEL in Taxol formulation is associated with toxicity, including very severe reaction such as acute hypersensitivity[37,38,53]. Premedication is required in practice to prevent hypersensitivity, as described in other paragraphs (see 1.1.4.4). Also, it is clearly proven that CrEL affects the behaviour of paclitaxel in the body and can result in the non-linear pharmacokinetics of paclitaxel after administration[47,54], which makes it difficult for clinicians and pharmacists to monitor and evaluate the effect and side-effects of paclitaxel for the treatment of specific diseases. In addition, it was found that CrEL can leach the plasticiser diethylhexyl phthalate (DEHP) from PVC bags and the intravenous infusion administration system, which leads to the drug being prepared in glass or non-PVC infusion systems[38,55].

1.1.4.5.2 Abraxane (ABI-007)

To avoid the problems induced by the CrEL formulation, the exploration of new formulations for paclitaxel became an important topic in pharmaceutical research. An optimal formulation should be based on two main factors: it should have no serious side effects like acute hypersensitivity and also it must increase the aqueous solubility of paclitaxel.

The most successful recent formulation is abraxane, albumin-bound nanoparticle paclitaxel (ABI-007, nab paclitaxel)[37]. There are many advantages claimed for ABI-007. One is the basis that paclitaxel has a high affinity to human serum albumin (HSA), thus HSA was considered to be a good carrier for paclitaxel[56]. Also, to form ABI-007, the nanoparticle technology has been employed, which makes the unit size small enough to pass through the blood vessel wall to the tumour tissue without any need for a solvent. Therefore, Abraxane successfully decreases the serious acute hypersensitivity due to CrEL and avoids the need for premedication before administration. Furthermore, HSA can bind to both the albumin receptor (gp60) and secreted protein, acidic and rich in cysteine (SPARC) expressed in tumour endothelial cells, which helps the drug enter the tumour cells and accumulate in the tumour, thus increasing the intra-tumour concentration and the antitumour activity of the drug[37,57]. In addition to the increased antitumour activity, the new formulation exhibits a wider capability of infusion presentation. Abraxane can be reconstituted in normal saline at a concentration of 1 – 20 mg/mL compared with 0.3 – 1.2 mg/mL for Taxol formulation, which obviously reduces the infusion time and required volume[37,58]. At the same time it was demonstrated in a phase I study that abraxane had a higher MTD based on the fact that it can be administered safely at 300 mg/m^2 as a short infusion on a 21-day cycle in comparison to 175 mg/m^2 for paclitaxel[58]. Additionally, the choices of

infusion systems for the new formulation are more flexible without need to consider plasticisers leaching, compared with Taxol[37,58].

1.1.4.5.3 Other alternative formulations on study

In addition to the above ABI-007, many other potential alternative formulations for paclitaxel have been evaluated to overcome the disadvantages of CrEL. These include: co-solvents, emulsion systems, micro-encapsulation systems, cyclodextrines and paclitaxel prodrugs etc[59]. For example, some prodrugs e.g. Paclitaxel poliglumex (XYOTAX; CT-2103), PNU 166945 and DHA (docosahexaenoic acid) conjugated paclitaxel are being evaluated in phase I, II and III clinical trials[60,61,62]. Also, another micelle forming surfactant (Pacliex) and liposome formulation have been studied[63,64]. Some of them have shown clinical potential, although, unlike ABI-007, so far there is no obvious success on the exploration of these potential formulations to take the place of CrEL.

1.1.4.6 Indications

Paclitaxel has been used as a single agent treatment or combination treatment in a wide range of cancers in clinics[39,65]. Firstly, it has been used as adjuvant chemotherapy or as subsequent chemotherapy after failure of the initial treatment for breast cancer. Secondly, it has been applied successfully in the first-line treatment of ovarian cancer in combination with platinum-based compounds and it is indicated to be used for second-line treatments of ovarian cancer. Also, it is frequently combined with other drugs as first-line treatment for non-small cell lung cancer. In addition, it can be used as second-line treatment for aids related Kaposi's sarcoma (refractory to liposomal anthracycline). Furthermore, paclitaxel can also be used in other cancers such as head

and neck cancer, prostate cancer, bladder cancer and oesophageal cancer, some of which are still on study and need more safety and efficacy data[13].

1.1.4.7 Animal studies (preclinical studies)

In pre-clinical studies, the mechanism of action of paclitaxel was clarified by the Horwitz group[23] in 1979 as mentioned previously (details see 1.1.4.3). Also, the antitumour profile of paclitaxel was becoming broader and its schedule dependency and potential application in combination therapy were explored in this stage. Many more preclinical models were developed such as Madison 109 lung carcinoma, M5076 sarcoma and different human tumour xenografts including A431 vulva, A2780 ovarian, and L2987 lung etc. Paclitaxel was found to be active in most of these distal site tumour models[66]. In the study of comparison of paclitaxel activity against murine tumours and human tumours carried as xenografts in athymic mice, paclitaxel exhibited better activity against human tumour xenografts than murine tumours[67]. Paclitaxel also showed activity against human hepatic metastases of breast cancer, cutaneous metastases of bronchial cancer, a tumour of the base of the tongue and an ovarian tumour in other xenograft studies[67,68]. It was also reported that paclitaxel has better activity against solid tumours than leukaemia[69]. A schedule dependency and dose-response study in the M109 lung carcinoma model demonstrated a daily injection treatment was preferred rather than an intermittent injection schedule regardless of dose levels[66]. It was found in a study of combination therapy, combining paclitaxel with many anticancer drugs including VP-16 methotrexate and pentamethylmelamine did not, with the exception of cisplatin, contribute to a better effect compared with paclitaxel alone[66].

It was reported that early toxicology studies on paclitaxel were based on three different species. Both single doses and daily doses for 5 successive days by intraperitoneal administration were evaluated in Sprague-Dawley rats. The LD_{50} values (the dose at which 50% of the animals die) on both the single doses and on the 5 day schedule were 206 mg/m^2 and 51 mg/m^2 respectively. A similar 5-day schedule study was used in $CD2F_1$ mice, which showed an LD_{50} of 82 mg/m^2. Also, toxicity was also evaluated in beagle dogs by an intravenous route. Some typical toxic effects were found in the haematopoietic, gastrointestinal, lymphatic and reproductive systems. Myelosuppression was estimated, based on these data, as a dose-limiting toxicity (DLT) in humans. It was firstly noted that the CrEL vehicle resulted in hypotension in dogs due to vasodilation caused by histamine release, which can cause death. However, there was no obvious evidence of cumulative effect and repeated small doses contributed to less toxic effect[20].

Hamel et al[70] first showed that paclitaxel had a high affinity to plasma protein (about 92%) in rabbits by using a biochemical assay. In the study, paclitaxel was administered as a rapid intravenous bolus to rabbits. The disposition of paclitaxel was explained by a biexponential model of drug elimination and it was easily cleared from the central compartment[70]. In addition, it was indicated that the passage of paclitaxel into cells can be limited by the overexpression of the phosphorylated trans-membrane glycoproteins in cells, which function as efflux pumps for paclitaxel and many other chemotherapeutic agents including colchicines, vincristine and doxorubicin[20,71]. This is considered to be the main mechanism of multidrug-resistance (MDR)[20]. Also, in this stage, Schiff et al reported paclitaxel can increase the radiation sensitivity of human astrocytoma cells[72,73].

1.1.4.8 Early clinical studies

1.1.4.8.1 Phase I clinical trials

Early phase I clinical trials had been concluded by Susan's group in 1993[15]. Table 1-1 shows the details of paclitaxel studies with different schedules.

Table 1-1 Early Phase I clinical trials of paclitaxel (up to 1993)

Institution	*Schedule**	*Recommended phase II dose (mg/m²)*	*Dose-limiting toxicity*	*Premedication*
M.D. Anderson[74]	1h daily x 5	150 (30 x 5)	Leucopenia	No
Wisconsin[75]	6h daily x 5	150 (30 x 5)	Leucopenia	No/Yes
Dana-Farber[76]	24h daily x 5 (120 h)	150 (30 x 5)	Leucopenia, mucositis	No/Yes
Memorial[77]	3h	None	Hypersensitivity	No
Hopkins[78]	6h	Minimal prior treatment: 212; extensive prior treatment: 170	Leucopenia	No/Yes
Einstein[79]	6h	250	Neuropathy, leucopenia	No
San Antonio[80]	6h	225	Neuropathy, leucopenia	Yes
Einstein[81]	24h IV	250	Neuropathy, leucopenia	Yes
Hopkins[82]	24h CIV every 14-21 day	315	Mucositis	Yes
NCI Medicine Branch[83]	24h(with G-CSF)	250	Neuropathy	Yes
Gynaecologic Oncology Group[84]	Every 3 week IP	≤ 125(not recommended)	Abdominal pain	Yes

** treatment was planned every 3 weeks unless noted.*
CIV, continuous intravenous infusion; IP, intraperitoneal administration

All these trials made a contribution to the feasible dose range (135 – 250 mg/m^2) on the single-agent infusion schedule for phase II clinical trials and a 5-day schedule (30 mg/m^2 /day) was also suggested[12]. At the beginning of phase I trials, hypersensitivity reactions (HSRs) nearly stopped the development. These occurred very frequently with an incidence rate of 18% around all the patients[77]. The symptoms exhibited were variable as mentioned previously (see part 1.1.4.4). In some severe cases, these could lead to death. Later, premedication was successfully introduced to reduce the frequency and severity of HSRs. Without this, clinical trials could not have continued. Cremophor EL in Taxol formulation was identified as a possible cause of hypersensitivity at this stage. It was found that a shorter infusion schedule (3h) induced HSRs more frequently than a longer one (24h); therefore, the 24-hour infusion schedule repeated every 3 weeks was recommended for phase II clinical trials with premedication including diphenhydramine, dexamethasone, and a H2 receptor blocker[85]. In phase I clinical studies, the dose-limiting toxicity, neutropenia, was identified in humans. The times of its occurrence and recovery (details see 1.1.4.4) were agreed in many studies[74,77,79]. It was suggested that G-CSF was added in order to increase the dose of paclitaxel[83]. Also, in a combination study of paclitaxel and cisplatin, the sequence of administration was found to be crucial because paclitaxel used after cisplatin exhibited a 25 % less systemic clearance associated with potentially increased neutropenia. Based on the above, paclitaxel was preferred to be administered prior to cisplatin to maximise positive activity and minimise toxicities[86].

1.1.4.8.2 Phase II clinical trials

A serious problem at this stage limited the clinical development due to the limited supply of paclitaxel as a natural product (see Section 1.1.4.1, p.7). Due to a NCI

initiation, effort was put into the development of an alternative source. Semisynthetic paclitaxel was produced from a precursor, 10-deacetylbaccatin III, which was isolated from yew trees in Europe and India and from needles and twigs from ornamental yew shrubs. The source of the new semisynthetic taxane was renewable. To develop paclitaxel from renewable sources and to study another new analogues, Taxotere (Docetaxel), attracted more attention in chemistry and pharmaceutical areas (structure of docetaxel is presented in Figure 1-3)[12,15].

Table 1-2 Early Phase II clinical trials of paclitaxel (up to 1994)

Institution	*Number of patients*	*Tumour types*	*Dose (mg/m^2)*	*Infusion duration (h)*	*G-CSF*
GOG[87]	41	Ovarian	170 & 135*	24	No
Johns Hopkins[88]	40	Ovarian	250 & 200 (amended to 170&135)	24	No
Albert Einstein[89]	30	Ovarian	250 & 200*	24	No
MD Anderson[90]	25	Breast	250 & 200*	24	No
Multinational[91†]	159‡	Ovarian	175 vs.135 (randomization)	24 vs. 3	No
NCI Medicine Branch[92]	38	Ovarian	250	24	Yes
MSKCC[93]	26	Breast	250	24	Yes
M.D. Anderson[94]	25	Non-small-cell lung	200	24	No
ECOG[95]	24	Non-small-cell lung	250	24	No
ECOG[96]	28	Melanoma	250	24	No
M.D. Anderson[97]	25	Melanoma	250 & 200	24	No

** Lower dose used for high-risk patients.*
† Interim analysis.
‡ Two patients never received Taxol and were not evaluated for safety.
GOG means Gynecologic Oncology Group.
MSKCC means Memorial Sloan-Kettering Cancer Center
ECOG means Eastern Cooperative Oncology Group.

Due to the poor supply of the drug, the early phase II studies only covered some common tumours and suggested dosages by phase I trials. Table 1-2 listed the details of some phase II studies up to 1993. McGuire et al[88] firstly reported the clinical activity of paclitaxel for recurrent and refractory ovarian cancer in phase II trials with an overall response rate, 30%, which was agreed in several subsequent studies[87,89]. In 1991, the objective responses to breast cancer were about 56% and 62% based on two studies including patients without prior therapy or with only adjuvant therapy[90,93]. Also of significance were reports in later studies on breast cancer, where paclitaxel was found to give response rates of around 20% – 40% in the heavily pre-treated patients. Also, similar response rates were obtained in anthracyclines-resistant patients and those sensitive to anthracyclines, which was supposed to be the best treatment for breast cancer at that period[13,98]. Both randomised phase II studies conducted by ECOG and M.D.Anderson Cancer centre demonstrated good overall response rates of paclitaxel in non-small-cell lung cancer, 21% and 24%, respectively[94,95]. Also, paclitaxel demonstrated different activity against head and neck cancers[99]. In addition, at that stage, paclitaxel showed weak activity in melanoma and cervix cancer and was virtually inactive in renal, colon, and prostate cancer[96,97,100,101,102,103]. However, some of these sites still need to be re-evaluated.

Many phase II clinical trials were devoted to the optimisation of schedules. As suggested in phase I clinical trials, the 24-hour infusion schedule was most utilised in phase II clinical trials (as shown in Table 1-2) because it was associated with less hypersensitivity reactions. However, Eisenhauer et al reported the result of a randomised trial in ovarian cancer to evaluate the 3-hour and 24-hour schedules, each of which was conducted with two different doses, 135 and 175 mg/m^2. They found the 24-hour infusion schedule, compared with the 3-hour schedule, contributed to

more severe neutropenia regardless of doses. This study first demonstrated that the 3-hour infusion of paclitaxel was acceptable and safe at the beginning of the treatment (the rate of severe hypersensitivity was 4%) when used with proper premedication[91]. Thus, so far there was no proof to show a longer infusion schedule would be more effective than a shorter one and the 3-hour infusion schedule was more convenient in practice. Therefore, the 3-hour infusion schedule may be a better choice depending on whether a longer infusion schedule would result in better efficacy, which was proposed for investigation in future phase III studies. In addition to the 3-hour and 24-hour infusion schedules, a 96-hour infusion schedule was proposed for study because of some encouraging data obtained in preclinical trials that showed a high possibility of efficacy increased with longer exposure to the drug[104].

Although most phase II trials at the beginning employed the doses suggested by phase I trials (refer to Table 1-2), in the middle of this series of clinical trials, the high starting dose used had to be decreased due to severe neutropenia. For example, in McGuire's study[88], the starting dose of 250 mg/m^2 was eventually reduced to 135 mg/m^2 in order to minimise the adverse effects, especially neutropenia. In addition, another phase I trial on advanced ovarian cancer indicated that paclitaxel 135 mg/m^2 could be safely administered with cisplatin 75 mg/m^2 [86]. Since the 135 mg/m^2 dose had shown acceptable response with tolerable toxicities, it was proposed for phase III clinical studies. In 1992, the 135 mg/m^2 dose was first approved by the FDA. In addition, there was no clear evidence that G-CSF can improve the maximal tolerated dose (MTD) but it had been shown to shorten the stage of neutropenia. Patients without G-CSF pre-treatment could endure the 180 and 200 mg/m^2 doses rather than 250 mg/m^2 because the latter dose would cause many intolerable problems such as neutropenia, neurotoxicity, myalgias, arthralgias and fatigue[88,90]. Besides, with the exploration of dose

intensity, it was proposed for phase III studies to determine if a higher dose contributed to better response.

Many *in vitro* experiments were carried out during this period to understand the mechanism of radiation sensitivity induced by paclitaxel[35]. *In vivo* experiments were still on-going. The combination of radiation therapy and paclitaxel was promising in the treatment of several kinds of tumours such as breast, head and neck and non-small-cell lung cancer[15]. Phase I clinical trials were designed to study the effect of paclitaxel on radiation therapy using different schedules[105,106].

1.1.4.8.3 Phase III clinical trials

In this stage, much effort focused on the study of doses and schedules in different cancer therapies, which made the role of this drug more understandable and formed dosage regimens for some cancers that would be optimised in the future.

- **Ovarian cancer**

A phase III study was sponsored by NCI to compare the effect of paclitaxel (135 mg/m^2) and cisplatin (75 mg/m^2) against a standard regimen of cyclophosphamide (750 mg/m^2) and cisplatin (75 mg/m^2)[107]. Although neutropenia occurred more often due to paclitaxel and there was no significant improvement in overall response rates with paclitaxel group, the cisplatin/paclitaxel regimen could reduce the risk of recurrence by 32% over that obtained with cisplatin/cyclophosphamide. The cisplatin/paclitaxel regimen obviously improves the duration of progression-free survival[107,108]. All these data showed the combination of paclitaxel and cisplatin had a high potential to become the new standard therapy for advanced ovarian cancer.

Another important phase III trial for ovarian cancer at this stage was designed to study the dose and schedule. In this trial, 407 patients were randomised to get one of two doses of paclitaxel (135 mg/m^2 or 175 mg/m^2) and also one of two schedules (3-hour or 24-hour infusion) [109]. Although there was no statistical difference in overall survival between different groups, a delay in time to progression (about 5 weeks) was observed with the 175 mg/m^2 treated group. This non-significant relationship of dose and response may be due to the small difference between the doses. The preliminary data showed the 3-hour schedule was associated with significantly less neutropenia (only 18%) than the 24-hour schedule (around 71%) and was also proven to be safe at the beginning of infusion providing premedication was used[109]. However, until the response and survival were reported, the 3-hour infusion schedule was not believed to be as a safe and effective schedule as the 24-hour schedule[109]. In addition, the 3-hour schedule was more convenient for outpatient treatment. Other toxicities could be tolerated and seemed more related to the higher dose without obvious schedule dependence[13,108]. Based on these results, the 175 mg/m^2 by a 3-hour infusion schedule would be approved by FDA for the treatment of refractory and recurrent ovarian cancer.

- **Breast cancer**

Some important phase III clinical trials on breast cancer were concluded as shown below (see below Table 1-3).

Table 1-3 Some phase III clinical studies of paclitaxel doses and schedules on breast cancer (up to 1998)[110]

Study	*Paclitaxel dose (mg/m^2)/schedule*	*Evaluable patients*	*Overall response (%)*
Nabholtz[111]	135/3h vs. 175/3h	227 vs. 223	22 vs. 29
Peretz[112]	175 to MTD/3h vs. 175 to MTD/24h	521 (total)	29 vs. 32
CALGB 9342[113]	175/3h vs. 210/3h vs.250/3h	325 (total)	21 vs. 28 vs.22
NSABP B-26[114]	250/3h vs. 250/24h	516 (total)	40 vs. 50
MDACC[115]	250/3h vs. 140/96h	88 vs. 91	23 vs. 30

I. Infusion schedule

Following phase II trials, the issue of the infusion schedule attracted much attention to determine if a longer infusion time would contribute to higher efficacy. In single-agent studies, the efficacies of the 3-hour schedule and 24-hour schedule were compared in the treatment of metastatic breast cancer. The Peretz group evaluated a randomised study where the 3-hour and 24-hour infusion schedules were given to patients at a same dose, 175 mg/m^2 [112]. The results showed no significant difference in response rate and survival between these two groups, i.e. there was no obvious advantage for the 24-hour schedule compared with the other schedule. More grade 3 or 4 neutropenia was associated with the 24-hour schedule, whereas more peripheral neuropathy was seen in the 3-hour schedule. Another similar trial, NSABP B-26[114], was reported, where the same two different schedules (3-hour & 24-hour) were compared but with a higher dose, 250 mg/m^2. Although a significantly higher response rate was observed with the 24-hour schedule (50%), compared with the 3-hour schedule (40%), there was still no obvious difference in overall survival between both the 24-hour schedule (21 months) and the 3-hour schedule (20.7 months)[114]. Since there was no evidence that a longer

infusion schedule was associated with improved efficacy and also the shorter infusion (3-hour) seemed safer than the longer one (24-hour), the 3-hour infusion schedule became a preferred single-agent infusion schedule in the treatment of breast cancer.

Further studies examined weekly administration of paclitaxel rather than the three-week schedule. It seemed that weekly administration could lead to a cumulative density of drug in plasma. Moderate doses by this schedule had been studied with good response rates and less toxicity[116,117]. However, further investigations are still ongoing in this area.

II. Dose-response

The issue of dose-response addressed at the phase II stage was also studied extensively in phase III studies. Among all these trials, two of them contributed to a relatively clear dose schedule in breast cancer. The first one was developed by Nabholtz et al[111], in which two doses, 135 mg/m^2 and 175 mg/m^2 were compared by 3-hr infusion every 3 weeks. There was no statistical difference in overall response rates between these two schedules, 22% (the lower dose) and 29% (the higher dose). However, a significant difference in time to progression (i.e. the median time to disease progression), was identified as 4.2 months (the higher dose) and 3.0 months (the lower dose). These results were confirmed by another clinical trial, CALGB 9342[113]. In this trial, doses of paclitaxel as a single agent for metastatic breast cancer, 175 mg/m^2, 210 mg/m^2 and 250 mg/m^2, were studied by a 3-hour infusion schedule every 3 weeks. No significant difference was found in response rate and overall survival, although, time to treatment failure was shown longer with the higher dose (250 mg/m^2) than the lower ones. More frequent toxicities, especially neutropenia and peripheral neuropathy, were associated with the highest dose, 250 mg/m^2. So far there was no obvious evidence to show that

a higher dose (250 mg/m^2) was worth development, although it had a longer time to treatment failure. Also, a 175 mg/m^2 dose as a single-agent treatment for metastatic breast cancer had been proven to be effective and safe with well tolerated toxicities. Therefore, paclitaxel 175 mg/m^2 by 3-hour infusion every 3 weeks was considered a reasonable choice as a single-agent treatment of breast cancer.

III. Combination therapy

Another focus in the phase III stage was combination therapy. With good single-agent activity, paclitaxel was studied with many other chemotherapy agents, which helped maximise the use of this drug in breast cancer therapy. The combination of paclitaxel and doxorubicin was found promising in the treatment of breast cancer with a significantly higher overall response (47%) than those obtained separately in their single-agent studies[118]. In addition, in the studies of paclitaxel with platinum-compound combination, paclitaxel and cisplatin could result in high response rates for advanced breast cancer, even for those with anthracycline resistance. However severe overlapping toxicity, especially neurotoxicity, became a barrier to this combination[119,120]. However, paclitaxel combined with carboplatin was found feasible in practice due to good response and well-tolerated toxicity[121,122]. Other combination studies involved 5-FU, vinorelbine, cyclophosphamide and anti-HER2 antibody. Many of these are still ongoing.

- **Non-small-cell lung cancer (NSCLC)**

Both phase II trials on patients with NSCLC run by the Eastern Cooperative Oncology Group (ECOG) and M.D. Anderson Cancer Centre showed paclitaxel had good overall response rates of 21% and 24% respectively[94,95], which encouraged ECOG to design a Phase III clinical trial to observe the effect of paclitaxel on survival

between 1993 and 1994[119]. This trial was designed to study the survival effect of the combination of paclitaxel (two levels: 135 mg/m^2 and 250 mg/m^2) with cisplatin rather than a single agent, which was based on the fact found by ECOG that the addition of cisplatin may contribute to a relatively better survival rate[119]. Etoposide-cisplatin was included as the reference because this combination had previously received a high 1-year survival rate (25%)[123]. Eventually, better responses and longer survival were obtained with the two groups of paclitaxel and cisplatin (paclitaxel 135 and 250 mg/m^2), compared with the results from the reference group (see below Table 1-4).

Table 1-4 Responses and survival rates in a phase III study by ECOG[124]

Evaluation	*Etoposide-cisplatin (reference group)*	*Paclitaxel-cisplatin (135 mg/m^2)*	*Paclitaxel-cisplatin (250 mg/m^2) G-CSF*
Response (%)	12.3	26.3	31.0
Survival (% 1-year)	31.3	37.3	40.4

The above results were reflected in another randomised Phase III study developed by the EORTC lung cancer study group where the treatment of paclitaxel with cisplatin was compared with the cisplatin/teniposide treatment. A significantly improved response rate (47%) was obtained with paclitaxel containing group, compared with 29% obtained by another group[125].

1.1.4.8.4 Pharmacokinetic studies with early clinical trials

Data on the pharmacokinetics characteristics of paclitaxel had been collected since early clinical trials. The classic pharmacokinetic study involves 4 parts: absorption, distribution, metabolism and elimination (ADME). However, as mentioned above,

paclitaxel is poorly water soluble indicating poor absorption with oral administration. There was not much data reported about absorption of paclitaxel in the early stages of its development and all recent studies focussed on the intravenous route. This review therefore concentrates on the distribution, metabolism and elimination of paclitaxel by intravenous route.

- Plasma protein binding and distribution

Paclitaxel exhibited a high affinity for plasma proteins (95% – 98%) by dialysis or ultracentrifugation methods[79,126]. The actual protein binding may be a little less than the above values due to the inevitable binding of unbound paclitaxel to these dialysis or ultracentrifugation devices. Also, paclitaxel showed a large volume of distribution at steady-state (Vss =50 to 400 L) indicating extensive tissue distribution in humans[18,75,79,81,85,127,128]. Also of note is that paclitaxel was found only in trace amounts in human cerebrospinal fluid (CSF) in a single-patient study on leukaemia by a 24-hour infusion[82].

- Metabolism and elimination

Although paclitaxel has extensive tissue binding, it can be eliminated easily from the body. Both *in vitro* and *in vivo* studies identified 2 NADPH-dependent paclitaxel metabolites, 6α-hydroxypaclitaxel and 3'-p-hydroxypaclitaxel. 6α-hydroxypaclitaxel was reported as the major metabolite[129,130]. The *in vitro* study also demonstrated that cytochrome P450 isozymes may be responsible for the metabolism of paclitaxel[129]. The main two CYP450 enzymes were identified. CYP2C8 is mainly responsible for the formation of the major metabolite, 6α-hydroxypaclitaxel, whereas the other metabolite, 3'-p-hydroxypaclitaxel is formed by CYP3A4[129,131,132]. Therefore, individual differences in the expression of CYP450s may partly answer for the interpatient variability in systemic clearance. Also, the induction or inhibition of these CYP450s may lead to

some potential drug interactions between paclitaxel and other co-administered drugs that are metabolised by the same enzymes to paclitaxel[18]. Renal clearance of paclitaxel was very low (approximate 5%)[15,75,80,81]. Although the complete excretion of paclitaxel in the human body was not clearly known, the main excretion was considered due to biliary clearance, and hepatic metabolism with biliary excretion and tissue binding may be accounting for the bulk disposition of paclitaxel in the body[15,20,130].

- Pharmacokinetic models and related parameters

Paclitaxel exhibited a nonlinear disposition after intravenous administration in early clinical trials. Its disposition was demonstrated by a biexponential model (two-compartment model) of drug elimination in many early phase I studies[75,79,80,81,82,86]. Later, the data collected using more sensitive HPLC methods were also described by triphasic models (three-compartment models)[127,133,134]. The specific information on pharmacokinetic models will be clarified later (see part 1.1.5.3). In addition, a two-compartment model involving a saturable distribution process was considered the best model to describe the data obtained in a study on children with 24-hour infusion administration[128].

Among the clinical trials, variable mean peak plasma paclitaxel concentrations (C_{max} values) and the area under the plasma concentration and time curve (AUC) were obtained. C_{max} and AUC were found to be increased disproportionately with dose[127,133,134]. Shorter schedules contributed to a higher mean peak plasma concentration (C_{max}) than a longer schedule[18]; for example, the 24-h infusion was found with a one-tenth of C_{max} that was obtained with the 3-h infusion[127].

Sonnichsen et al[18] summarised the data from many published studies and plotted the relationship between AUC and administered dosages as shown below (Figure 1-6):

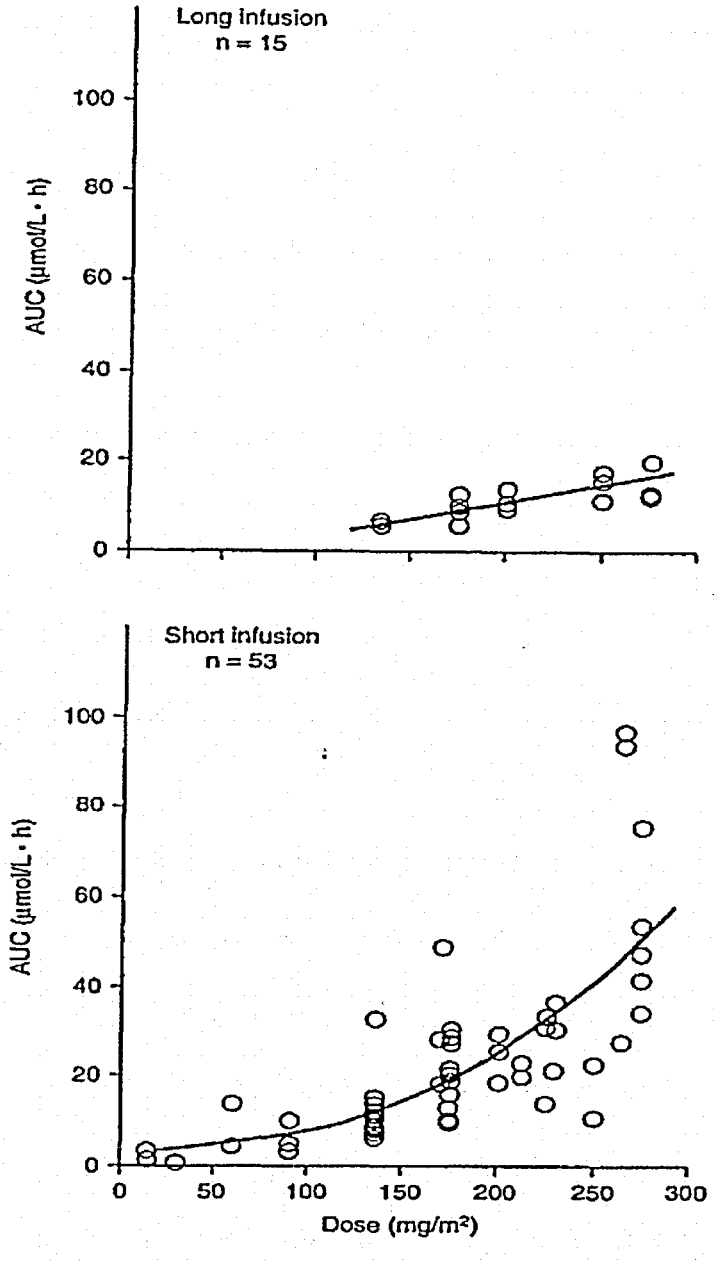

Figure 1-6 Paclitaxel area under the plasma concentration versus time curve (AUC) versus administered paclitaxel dosage from published adult studies

This above figure shows clearly that systemic clearance of paclitaxel decreased with the increasing dosage or shorter infusion (≤ 6 hours) as also showed in other studies[127,135]. An obvious dose-dependent elimination was observed with shorter infusion, but at

lower doses especially with prolonged infusion (≥ 24-h), this dose dependent clearance was not obvious. The reason was considered that plasma concentrations rarely exceeded the affinity constant for elimination (K_m)[18]. However, saturable elimination was an exception at a relatively high dose or with a short infusion, where only small increases in dosage may increase the AUC (as a surrogate of systemic exposure) greatly, resulting in a high risk of serious paclitaxel toxicity[18].

1.1.5 Pharmacokinetic studies on paclitaxel

1.1.5.1 Analytical techniques for determination of paclitaxel in plasma

1.1.5.1.1 Liquid chromatography assays

- High performance liquid chromatography (HPLC) and ultraviolet (UV) detection.

With the exploration of the anticancer activity of paclitaxel since 1970s, a variety of analytical methods were developed to detect paclitaxel in biological fluids. HPLC plus UV detection was applied first during an early phase I study at Albert Einstein Cancer Centre[79]. Since then, HPLC became the main tool to study paclitaxel in biological fluids in the later clinical trials because of its many advantages, for example, more sensitive, simpler, easy to use (less sample processing), compared with other analytical assays at the time[75,80,81]. Nearly all the assays during that period employed ultraviolet detection (UV) for paclitaxel determination. Paclitaxel exhibited a distinct absorption maximum at the wavelengths of 227 to 230 nm[136,137,138,139]. In 1993, the Huizing's group had described their HPLC assay with lower limits of detection (LOD), 0.007 μmol/L and 0.009 μmol/L, respectively in plasma and urine[127].

During the last decade, HPLC combined with a UV detector continues to be the most common technique for paclitaxel determination in biological fluids. Many pharmaceutical studies have concentrated on optimising the conditions of this system in order to obtain better sensitivity and convenience (Table 1-5). Two main LC columns were included in these studies: reverse phase octylsilica (C8) and octadecylsilica (C18, ODS)[136,137,138,139]. Some studies preferred the octylsilica analytical column rather than normal ODS columns because the former was believed to give a good resolution of paclitaxel and its metabolites[136,137]. Comparatively, a C18 RP column used by Martin's group could only resolve and identify paclitaxel itself rather than its metabolites[138]. This was also confirmed in Supko group's study[140]. However, both columns produced good lower limits of detection (LOD) and limits of quantification (LOQ) for paclitaxel in plasma, 5 – 15 ng/mL and 10 – 30 ng/mL respectively[136,138,141,142]. The limits of quantification for paclitaxel can be improved to 4 ng/mL by employing a guard column[47].

The selection of a mobile phase seems flexible based on Table 1-5. A combination of acetonitrile – methanol – ammonium acetate buffer (0.02M, PH 5) was considered to result in optimal separation[138,140]. It was usually run under isocratic conditions with a flow rate of 1.0 – 2.0 mL/min[136,138,139]. In addition, it was found that the addition of tetrahydrofuran (5%) sharpened peaks and enhanced stability of paclitaxel[138].

The internal standards used for the determination of paclitaxel (Table 1-5) included docetaxel, cephalomanine N-octylbenzemide, n-hexyl p-hydroxybenzoic acid, and 2'-Methylpaclitaxel[47,136,138,139,140,141]. Docetaxel was believed a good internal standard, not only because of its similar structure and physical properties, but also due to its wide availability[138].

- High performance liquid chromatography and mass spectrometry (LC-MS)

With the further study of pharmacokinetics of paclitaxel, the need to identify its metabolites became important for studying the behaviour of paclitaxel in the body. Mass spectrometry (MS) as a detection method was used increasingly to study the metabolites of paclitaxel[143,144] Also, tandem mass spectrometry (MS-MS) was induced to analyse paclitaxel and its metabolites due to the higher specificity possible[145,146]. Comparatively, MS-MS not only provides the information about molecular weight but also gives more information on molecular structure and help identify the metabolites. In this system of LC-MS/MS-MS, reverse phase ODS (C18) micro-bore columns were commonly used because these operate at a low flow rate (0.1 – 0.2 mL/min) required for mass spectrometry[143,144,145,146]. Usually, atmospheric pressure ionisation and electrospray ionisation were employed to ionise the sample by using positive ion control[143,144,145,146]. By using mass spectrometry as a detector, the LOD has been reported to decrease to 1 ng/mL in some cases[143,145].

Table 1-5 Summary of HPLC analytical methods for determination of paclitaxel since 1995

Reference	Mobile Phase	Column	Detector	Internal Standard	LOD or LOQ	Precision or Accuracy
Sparreboom et al. 1995[136]	Acetonitrile Methanol Ammonium acetate buffer	Reverse phase C8 analytical column 5µm	UV 227nm	2'-Methylpaclitaxel	LOD 15ng/ml LOQ 25ng/ml	Between-day and within-day precision ≤ 7.3%
Huizing et al. 1995[137]	Acetonitrile Methanol Ammonium acetate buffer	Reverse phase C8 analytical column 5µm	UV 227nm	No	LOQ 10ng/ml	Accuracy: 95% - 97% Precision: 1.2% - 8.5%
Gianni et al. 1995[47]	Acetonitrile water	Superspher C18 4 µm 125x4mm protected with a lichrosphere C18, 5µm, 4x4mmm	UV 230nm	Cephalomanine	LOQ 4ng/ml	Intra-assay: 10.6% and 2.8%(0.0625 and 8 µmol/l) Inter-assay: 10.2% and 9.7% (0.0625 and 8 µmol/L)
Martin et al. 1998[138]	Acetonitrile Ammonium acetate buffer Tetrahydrofuran	Nurcleosil Reverse phase C18 5µm	UV 227nm	Docetaxel	LOD 11.5nM (10ng/ml) LOQ 29nM (25ng/ml)	Variabilities Intra-assay: 1.54% - 6.34% Inter-assay : 2.97% - 11.18%
Sparreboom et al. 1998[139]	Methanol Tetrahydrofuran Ammonium hydroxide Water	Reverse phase C18 5µm	UV 230nm	Docetaxel	LOQ 10ng/ml	Intra-run and inter-run variabilities both ≤ 3%
Supko et al. 1999[140]	Acetonitrile Methanol Ammonium acetate buffer	A cyanopropyl contained column and an octylsilica analytical column by an automated column switching method	UV 227nm	N-octylbenzemide	LOQ 6nM (5ng/ml)	Day to day accuracy (100.2%) and precision (RSD 11.7%)

Lee et al. 1999[141]	Acetonitrile Phosphoric acid Water	a Capcell-pak C18 UG120 column and a Capcell-pak C18 UG120 guard column	UV 227nm	n-hexyl p-hydroxy benzoic acid	LOD 5ng/ml LOQ 10ng/ml	Variabilities Intra-day : 0.4 - 2.2% Inter-day : 0.6 - 7.8%
Panchagnula et al. 1999[147]	Ammonium acetate buffer Methanol Isopropanol	Reverse phase C18 column 5µm, 250x4.6mm	UV 230nM	Docetaxel	LOD 30ng/ml LOQ 100ng/ml	Accuracy (recovery): 82%-119%
Mader et al. 2002[142]	Sol A (for SPE): methanol-water Sol B: Ammonium acetate buffer Acetonitrile	Reverse phase C8 5µm	UV 229nM	No	LOD11ng/ml LOQ < 50nM	Inter-assay relative standard deviation :1.3-3.2%: Accuracy : 0.9-2.7%
Badea et al. 2004[148]	Acetonitrile Water	Two chromatographic system (SP: pentafluorophenyl, 5µm)	UV 225-229nm	No	LOD 0.072µg/ml LOQ 0.240µg/ml	Repeatability (R.S.D) : 0.67%

LOD, limit of detection, defined as the concentration of compound giving a signal-to-noise ratio greater than 3:1.

LOQ, limit of quantification, defined as the lowest concentration that can be measured with accuracy and precision ≤ 20%.

- Others: capillary electrophoresis (CE)

In addition to HPLC, capillary electrophoresis (CE) with UV detection was used for determination of paclitaxel in biological fluids[149]. In this case the LOD value was 20 ng/mL based on the concentration of compound giving a signal-to-noise ratio greater than 5:1. The precision and accuracy were both acceptable (< 15%).

1.1.5.1.2 Immunoassays and bioassays

Since HPLC methods have limitations e.g. strict sample preparation and high financial and time cost, many immunoassays and bioassays appeared in literature. These include radio-immunoassay (RIA), indirect competitive inhibition enzyme immunoassays and competitive enzyme-linked immunosorbent assays (ELISA), fluoroimmunoassay (FIA) and receptor protein-based assay[150,151,152,153,154,155]. Compared with HPLC, most of these methods are sensitive and do not require sample pretreatment. ELISA, as the most common immunoassay, was proved highly sensitive, and can detect paclitaxel in biological fluids at sub-ng/mL levels[155]. Also, a FIA method with a fluorescent-labelled antigen involved was shown to be effective with a lower limit of detection of 5.86 ng/mL[154]. In addition, bioassays were able to detect paclitaxel in human plasma through a competitive format where paclitaxel and fluorescent-labelled or peroxidase-labelled paclitaxel competed for tubulin binding[153,156]. However, generally these immunoassays and bioassays are labourious and some of them are more expensive. More importantly, they cannot resolve paclitaxel from metabolites and degradation products with a similar main structure to paclitaxel, and therefore lack specificity.

1.1.5.1.3 Sample preparation

Plasma samples for analysis are a complex matrix containing electrolytes, cells and proteins. Since the optimal ultraviolet wavelength for paclitaxel UV detection is

relatively low and the assay is susceptible to interference by other molecules, there is a need for sample preparation to extract paclitaxel from the biological matrix prior to assay. Many different approaches have been tried to isolate, clean-up and extract paclitaxel, including liquid-liquid extraction (LLE), solid phase extraction (SPE), protein precipitation and also column switching methods[79,136,137,138,143,157]. Among these methods, SPE and LLE are most commonly used in practice. They both produce good recovery of paclitaxel of around 80 – 97%[47,136,137,138,146]. Different solutions can be used for LLE such as diethyl ether and tert-butyl methyl ether[138,141]. Also, diethyl ether showed good volatility (easy to evaporate from extract) with a good recovery (about 90%)[138]. However, compared with LLE, SPE was believed simpler and more reliable. There were a variety of SPE cartridges that have been used. Cyano SPE cartridges were used in many studies with a good recovery, 80 – 90%[137,143,146]. C18 and C2 SPE cartridges have also been used[144,157,158].

Combining LLE and SPE did not improve recovery. The Sparreboom group employed both techniques in their study without improving on the sensitivity and recovery obtained by Huizing group where only SPE was used as sample pretreatment[136,137].

1.1.5.2 Recent pharmacokinetic studies

- Absorption and oral paclitaxel study

The systemic bioavailability of paclitaxel after oral administration in humans is less than 6%[159]. This poor bioavailability precludes the oral route. The low water solubility of paclitaxel, which would otherwise explain the low oral absorption, has been resolved by the addition of CrEL in the formulation (Taxol) as mentioned in Section 1.1.4.5 (p.16). Other factors responsible for low bioavailability include the poor absorption from the intestinal tissue due to the overexpression of the ATP-dependent

glycoproteins (Pgp) in columnar cells, which behave as efflux pumps for paclitaxel[160,161]. A second important factor to contribute to the poor bioavailability is the first-pass extraction and metabolism by cytochrome P450 enzymes, especially CYP2C8 and CYP3A4, which stop paclitaxel from entering the systemic circulation[162]. However, in view of the convenience of oral paclitaxel, some studies were devoted to oral administration of the drug[159,162]. A possible strategy was promoted, based on the above, to increase the oral bioavailability of paclitaxel by inhibiting the function of Pgp and CYP3A4[159,162]. Cyclosporin A was chosen to combine with paclitaxel because cyclosporin A has been proven as an inhibitor to both Pgp and CYP3A4 and also *in vivo* experiments showed it can increase the bioavailability of paclitaxel[163,164,165]. The study demonstrated the feasibility of oral paclitaxel-cyclosporin A as a dosing schedule, by which a high systemic drug exposure was successfully achieved[162]. However, much research is required to achieve the application of oral administration of paclitaxel in practice.

- Metabolism and elimination

In recent years, the disposition of paclitaxel in the body is becoming clearer. The primary route of paclitaxel clearance is by hepatic metabolism and biliary excretion[166]. The main metabolites, especially 6α-hydroxypaclitaxel and 3'-p-hydroxypaclitaxel, were detected in many studies with human plasma and faeces[47,167,167]. In addition, dihydroxypaclitaxel, an inactive metabolite, was formed by successive hydroxylation of CYP2C8 and CYP3A4 on different positions of the molecule[167]. All metabolites were less toxic than paclitaxel itself; therefore metabolism is confirmed as an important detoxification pathway for paclitaxel[167,168]. The variability of paclitaxel metabolism was attributed to the interindividual differences of CYP450 enzymes or /and drug-induced interactions[166,169,170].

- Pharmacokinetics and related studies

The disposition of paclitaxel was clearly shown to be nonlinear and complex. In two studies[47,54], both 3-hour and 24-hour schedules were studied. A representative plasma paclitaxel concentration-time curve is shown below (Figure 1-7), where the mean peak plasma paclitaxel concentrations (C_{max}) and the AUC values showed a clear nonlinear relationship and also dose dependence was observed with 3-hour infusion schedule, e.g. a 30% increase in dose resulted in an 80% increase in C_{max} and a 75% increase in AUC during 3-hour infusion. However, both the nonlinear relationship and dose dependence were not obvious with 24-hour infusion schedule[47,54]. In addition, as mentioned before (see Section 1.1.4.8.4, p.32), the systemic clearance (CL) and the volume of distribution at steady state (Vss) decreased with increased doses and shorter infusion time.

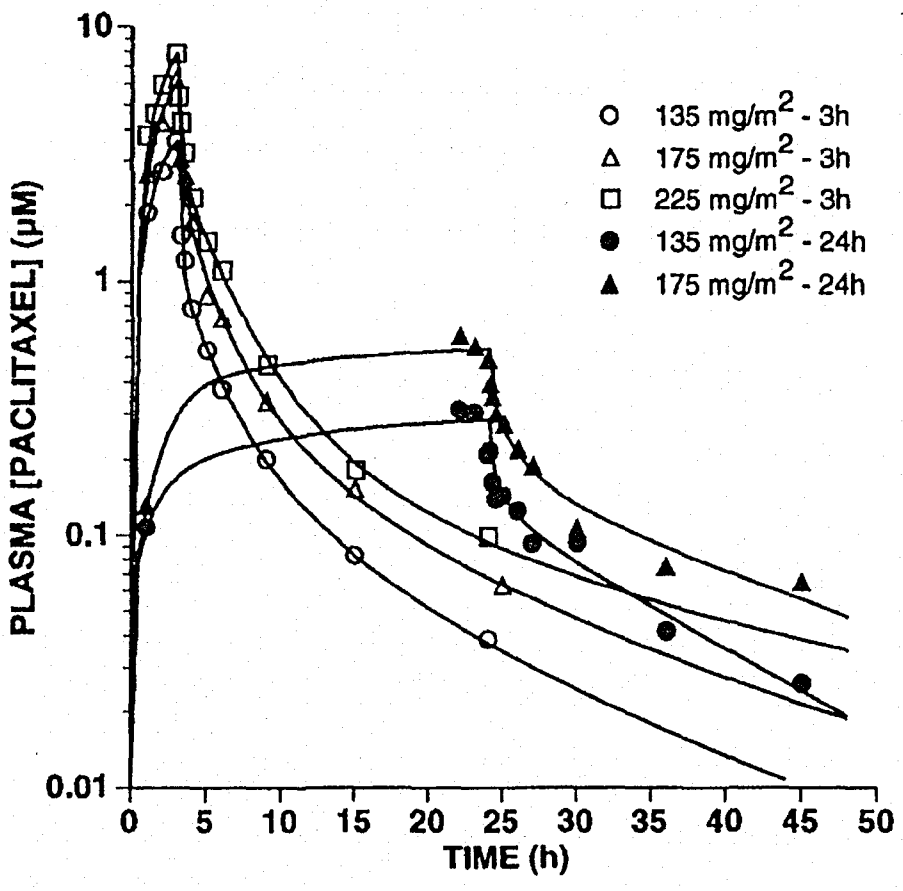

Figure 1-7 Paclitaxel plasma concentration-time profiles of representative patients who recived the dfferent infusion doses (135, 175 or 225 mg/m^2) and schedules (3 or 24 hour)[47]

This pharmacokinetic behaviour of paclitaxel was previously believed to be due to saturable metabolism at relative high doses, given a very short infusion. However, later studies demonstrated that CrEL, the vehicle in the current formulation (Taxol), contributed to the nonlinear pharmacokinetics of paclitaxel after administration[171,172]. Sparreboom's group demonstrated by *in vivo* and *in vitro* studies that CrEL has a high affinity for paclitaxel by trapping paclitaxel in micelles mainly composed of polyoxyethylene glycerol triricinoleate, thus decreasing the free drug fraction in the plasma[172]. Also, in this report the affinity of CrEL to paclitaxel was proved higher than that of red blood cells (RBC) and human serum albumin (HSA), which suggests the

influence of CrEL on the pharmacokinetic behaviour of paclitaxel in the body is significant. More studies were developed to understand the influence of CrEL on paclitaxel disposition[44,173,174]. CrEL was shown to change the metabolism and distribution of paclitaxel and also limit the activity of paclitaxel by trapping paclitaxel in micelles and decreasing unbound drug in plasma because only the free fraction of paclitaxel is active. In a study of the pharmacokinetics of unbound paclitaxel during 1-hour and 3-hour infusion[174], the previous non-linear relationship described above disappeared. Instead, with 3-hour infusion, an obvious increase in paclitaxel AUC was seen and the systemic clearance (CL) of paclitaxel was decreased compared with the results obtained during a 1-hour infusion. Comparatively, with a longer infusion, the AUC of CrEL was decreased but with increased CL. That may explain why a shorter infusion schedule is associated with higher CrEL-related toxicities like hypersensitivity reactions (HSR). Although CrEL influenced paclitaxel disposition, other factors that may lead to the non-linear relationship in the pharmacokinetic study of total paclitaxel cannot be excluded, for example a saturable mechanism involved in metabolism and distribution and also the alteration of P-glycoproteins mediated biliary secretion by CrEL[175]. Further studies in this area are ongoing.

1.1.5.3 Pharmacokinetic models

So far many pharmacokinetic models have been used to describe paclitaxel pharmacokinetics in the body, for example, non-compartment, one-compartment, two-compartment and three-compartment pharmacokinetic models. Kearns has demonstrated that models like the one-compartment model which only include a linear relationship, cannot describe the data of paclitaxel pharmacokinetics well as shown in the following figure (Figure 1-8). Comparatively, two-compartment and three-

compartment models can be fitted for the data because they both contain non-linear processes. Both models involve two processes after drug administration, distribution and elimination, which are both first -order saturable processes described by Michaelis-Menten Kinetics.

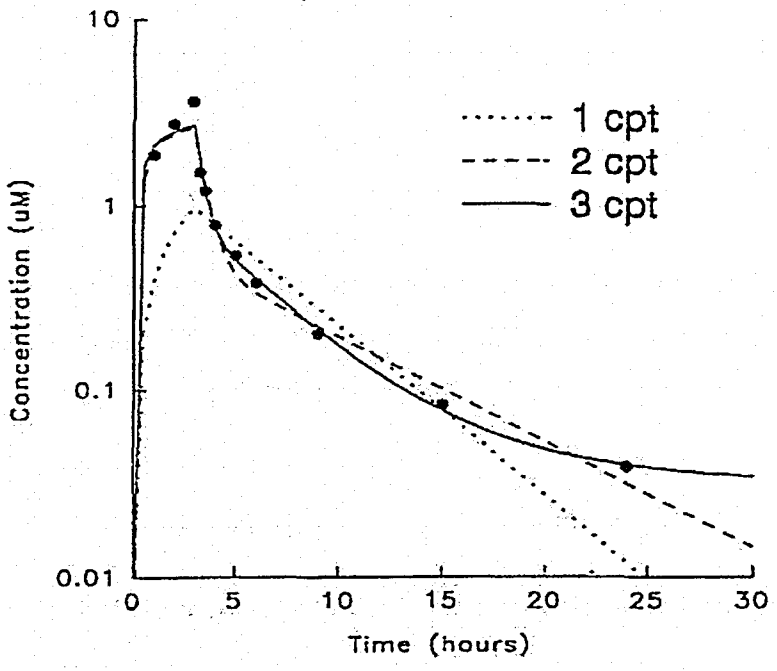

Figure 1-8 Comparison of linear one-, two-, and three-compartment (cpt) models fits for the data from one patient treated with paclitaxel 135 mg/m^2 by 3-hour infusion[48]

The following figure (Figure 1-9) demonstrates the basic structures of two- and three-compartment elimination models. A classic two-compartment model (Model B) describes the kinetics of drug disposition between central part (Compartment 1, systemic circulation) and peripheral part (Compartment 2, tissues and organs where the drug equilibrates slowly). A three-compartment model (Model A) contains a third compartment (compartment 3) which differentiates the tissues with much lower equilibration rates, such as fat and bone, from other tissues with higher equilibration rates (liver and kidney). Unlike the distribution and elimination processes, the

pharmacokinetic behaviour of drug between the central compartment and the third compartment is described by zero-order kinetics as shown in Figure 1-8[48].

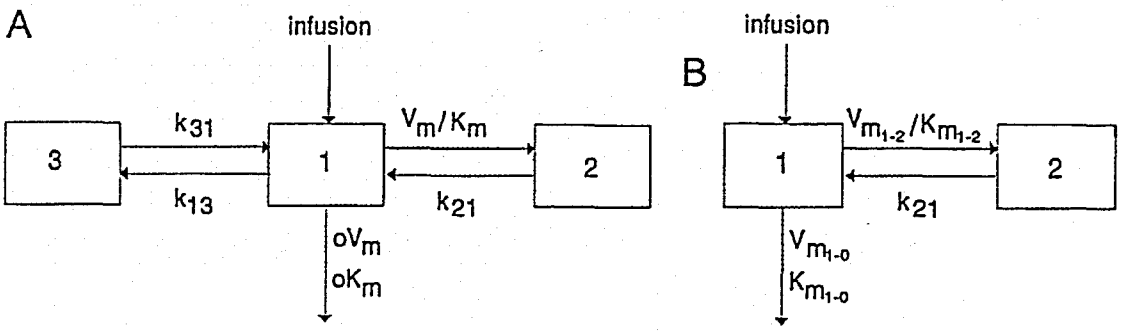

Figure 1-9 Schematic of paclitaxel disposition by both three-compartment and two-compartment models: A = three-compartment model and B = two-compartment model[48]

1.1.5.4 Pharmacokinetic and pharmacodynamics (PK-PD) relationships

It is difficult to define the real anti-tumour response of paclitaxel, which makes it difficult to evaluate the pharmacokinetic and pharmacodynamic relationship. Currently, the most common way to observe the pharmacodynamics of paclitaxel after administration is to evaluate the major toxicity, neutropenia, due to paclitaxel itself as a surrogate of activity[20,47,48]. Usually, Grade 3 and Grade 4 toxicities (neutrophil count < 1.0×10^9 /L) would be noted in the studies[176]. In the early clinical trials, neutropenia had been reported to be related to several pharmacokinetic parameters, for example, area under the plasma concentration curve (AUC), peak plasma concentration (C_{max}), steady-state plasma concentration (Css) and drug exposure time above a certain threshold concentration[54,127,128,177]. Later, neutropenia was found to be more related to the time at which paclitaxel plasma concentration was above a threshold value rather than other PK parameters. Most recent studies applied the duration of paclitaxel plasma concentration above 0.05 μmol/L ($T_{\geq 0.05\ \mu mol/L}$)[47,54,178]. Different threshold

concentrations, including 0.03 μmol/L, 0.05 μmol/L and 0.1 μmol/L, were evaluated in one study and 0.05 μmol/L was confirmed as the optimal threshold[47]. In the same study, a sigmoid E_{max} model was introduced to describe the relationship of $T_{\geq 0.05\ \mu mol/L}$ and neutropenia. The typical curve obtained is shown below (Figure 1-10). The sigmoid E_{max} model was a good fit for the data collected with different doses and schedules. It was thus applied in a later study[54].

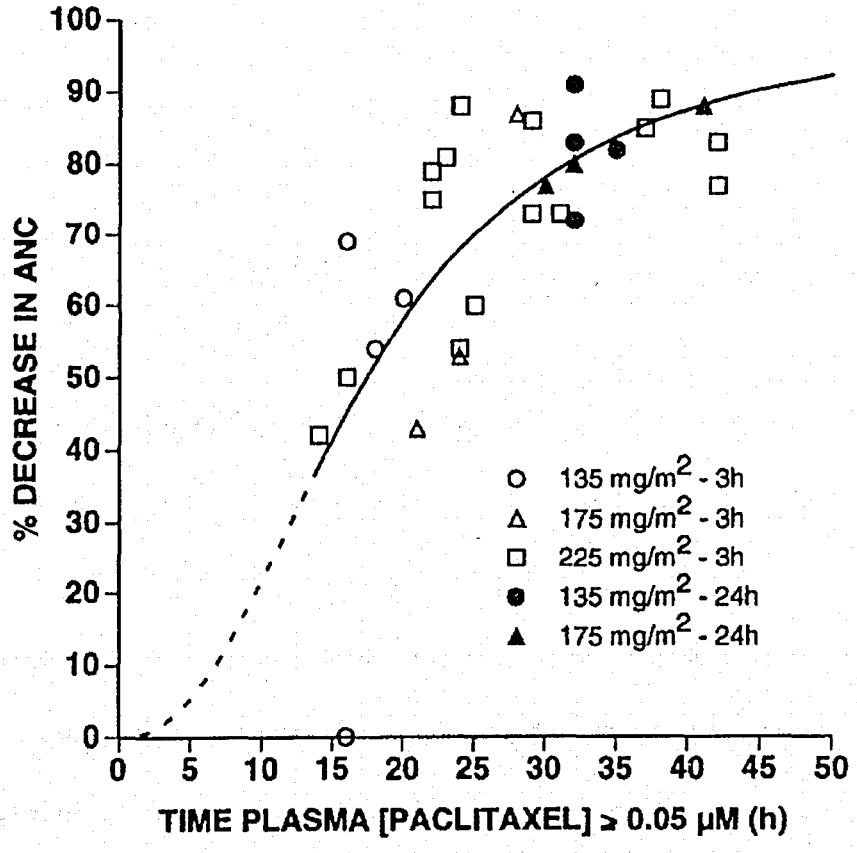

Figure 1-10 The sigmoid E_{max} model to describe the pharmacokinetic/ pharmacodynamic relationship between $T_{\geq 0.05\ \mu mol/L}$ and reduction in the absolute neutrophil count (ANC)[47,48]

1.1.6 Clinical use and clinical experience with paclitaxel

Paclitaxel has been used clinically for over 15 years. The experience on paclitaxel in practice has been greatly enhanced over the last 10 years but is still compromised by problems associated with its formulation and pharmacology. In this section, the related concerns and experience on paclitaxel are reviewed.

1.1.6.1 Clinical experience and effectiveness of paclitaxel in major diseases

1.1.6.1.1 Non-small cell lung cancer (NSCLC)

As mentioned in the above section on early clinical trials, paclitaxel combined with one of the platinum compounds has become the standard treatment for NSCLC. The combination of paclitaxel with cisplatin produced response rates of 35 to 47%[179,180,181]. In early studies, carboplatin demonstrated comparable activity with less toxicity when combined with paclitaxel[182,183,184,185]. Recent studies reported that the combination of paclitaxel and carboplatin produced a range of response rates from 7% to 25% and median survival times of approximately 8 months, although this regimen was associated with a 10 – 17% incidence of neuropathy[186,187,188]. Currently, paclitaxel and carboplatin (CP) has become one of the standard regimens used globally[188]; for example, the combination of paclitaxel (225 mg/m^2) and carboplatin (target AUC = 6 mg/ml×min) administrated every 3 weeks is most commonly used in USA[189].

Weekly paclitaxel schedules are active, well tolerated and provide adequate dose intensity. Response rates of 32% and 39% have been reported with weekly paclitaxel in patients with NSCLC[190,191]. Recently, Belani's group concluded paclitaxel 100 mg/m^2 weekly for 3 of 4 weeks with carboplatin (AUC = 6 mg/mL/min) administered on day 1 can result in the most favourable therapeutic index with better response rate (32%) and median overall survival (49 weeks) compared with two slightly different

regimens[189]. Also, in another study[192], paclitaxel (112.5 mg/m^2) on day 1 and 8 followed by carboplatin (AUC = 5/6 mg/mL/min) on day 1 every three weeks was related to a good response rate (45%) and median overall survival (11 ± 2 months). Both studies prove that weekly paclitaxel is more practical and relatively well tolerated. However, further studies are needed to compare weekly paclitaxel schedule and the standard "every-3-week" regimen.

1.1.6.1.2 Breast cancer

Paclitaxel has emerged as an important agent in the treatment of advanced breast cancer due to its efficacy, tolerability and also its lack of cross-resistance with anthracyclines. In the UK, paclitaxel is usually used for the treatment of advanced breast cancer in combination with one of the anthracyclines when initial chemotherapy (including one anthracycline) has failed or is inappropriate[65]. Paclitaxel has been recently indicated as adjuvant therapy for node-positive breast cancer after anthracycline and cyclophosphamide treatment. Paclitaxel has been studied in many randomised clinical trials as first-line treatment for advanced breast cancer, although, it has not been licensed[65]. Recently, many clinical trials were conducted to explore the efficacy of paclitaxel combined with an anthracycline. In a phase III trial[193], doxorubicin, paclitaxel and also the combination of both as first-line treatment were studied. As single agents both drugs demonstrated comparable activity without significant difference but their combination contributed to superior overall response rate and median time to treatment failure (TTF). However, no survival or quality of life scores were improved in this trial in the combination study compared with the single-agent study[193]. In addition, it was reported that the combination of paclitaxel and doxorubicin resulted in a high incidence of cardiac toxicity (20%)[194], which may be explained by the interaction of paclitaxel and doxorubicin. The vehicle, Cremophor EL

(CrEL), was reported to alter the pharmacokinetics of doxorubicin, by resulting in decreased systemic clearance and increased exposure of doxorubicin in the tissue and plasma[195]. It was also demonstrated that paclitaxel/CrEL can also affect the disposition of epirubicin, another anthracycline, by interfering with the metabolism of epirubicin[196]. Therefore, there will be more focus on the interaction between these two classes of drugs, taxane and anthracyclines, which will influence the viability of combination therapy with these drugs. Apart from anthracyclines, paclitaxel has been combined with vinorelbine, another kind of anti-tubulin agent with an opposite function to paclitaxel[197]. It was suggested that this combination may be one of the most effective therapeutic options for metastatic breast cancer due to its excellent antitumour activity, less toxicity and also smaller dose requirements for each one[197].

1.1.6.1.3 Ovarian cancer

Although surgical intervention is still the first treatment for ovarian cancer, chemotherapy has played an important role in treatment. Many clinical trials were conducted to study the role of the combination of paclitaxel with cisplatin in the treatment of ovarian cancer[198,199,200]. Two randomised trials strongly support that paclitaxel/cisplatin can produce better efficacy than the "standard" treatment of cyclophosphamide/cisplatin[198,200]. Also, in another study run by the Gynecologic Oncology Group (GOG), the combination of paclitaxel with cisplatin was preferred as the initial treatment option rather than cisplatin or paclitaxel alone because of a better toxicity profile with this combination[199]. Currently, the combination of paclitaxel and a platinum drug has become the standard treatment for primary ovarian cancer. Although platinum alone, for example, cisplatin or carboplatin, can be used in the first-line treatment, currently nearly 75% of women with ovarian cancer receive paclitaxel/platinum combination as first-line treatment because of potential increased

efficacy due to the addition of paclitaxel[201]. For metastatic ovarian cancer, paclitaxel has also become the alternative after first-line treatment of cisplatin or carboplatin has failed[201]. In addition, the paclitaxel/carboplatin combination has been reported to give a better toxicity profile than paclitaxel/cisplatin and also gives comparative activity[202]. Therefore, in the UK, paclitaxel is usually combined with carboplatin in clinical practice. Also, in a recent study[203], 12 cycles of single-agent paclitaxel were used for maintenance after patients with advanced ovarian cancer had a clinically defined complete response to initial paclitaxel/platinum treatment, which contributed to a significantly prolonged duration of progression-free survival (PFS).

1.1.6.2 Summary of paclitaxel indications

1.1.6.2.1 The United Kingdom (UK)

- First-line therapy

The National Institute for Health and Clinical Excellence (NICE) recommended that paclitaxel in combination with one platinum-based compound (cisplatin or carboplatin) or platinum monotherapy is considered as the standard first-line therapy and subsequent therapy for ovarian cancer (usually following surgery) and additional courses of the first-line treatment should be considered if the previous response was adequate[201]. Currently, paclitaxel is licensed in the UK as the first-line therapy in combination with cisplatin for patients with advanced NSCLC (stage III or IV) who are not candidates for potential curative surgery and/or radiation therapy[65]. Although some randomised clinical trials showed paclitaxel combination therapy to be effective as the first-line treatment for advanced breast cancer, it has not been licensed in this indication so far[65].

- Second-line therapy

Paclitaxel can be chosen with other licensed drugs as the second-line therapy for patients with ovarian cancer who have not been treated with this drug as their first-line therapy[201]. Compared with docetaxel, paclitaxel is not recommended as the second-line treatment for NSCLC[65]. In the UK, paclitaxel is currently licensed as monotherapy for the treatment of metastatic breast cancer where the standard initial anthracycline-containing treatment has failed or is inappropriate[65].

- Adjuvant therapy

The National Institute for Health and Clinical Excellence (NICE) recommended that paclitaxel is indicated as the adjuvant treatment of node-positive breast cancer following anthracycline and cyclophosphamide (AC) therapy[204]. Adjuvant treatment with paclitaxel should be regarded as an alternative to extended AC therapy[204].

1.1.6.2.2 Other countries

- The United States (USA)

Paclitaxel is indicated as first-line and subsequent therapy for the treatment of advanced ovarian cancer. As first-line therapy, paclitaxel is indicated in combination with cisplatin[205]. Also, it is indicated for the adjuvant treatment of node-positive breast cancer [205]. For NSCLC, paclitaxel is licensed with cisplatin as the first-line treatment for patients who are not candidates for potentially curative surgery and/or radiation therapy but it is not used for the second-line treatment[205]. In the USA, paclitaxel is licensed as a single-agent or combination treatment option for the treatment of metastatic breast cancer after failure of first combination chemotherapy (containing anthracycline) and also the relapse within 6 months of adjuvant chemotherapy[205].

Paclitaxel is indicated for the second-line treatment of AIDS-related Kaposi's sarcoma[205].

- Canada

Similarly, paclitaxel has been indicated as the first-line treatment and the second-line treatment for ovarian cancer and also it is considered as the first-line treatment for NSCLC[206]. Paclitaxel is indicated as adjuvant therapy for node-positive breast cancer and also as the second-line therapy for breast cancer in Canada[206]. In addition, paclitaxel is licensed in Canada as the treatment of AIDS-related Kaposi's sarcoma (refractory to liposomal anthracycline)[206].

- China

In China, paclitaxel is mainly used for the treatment of women with ovarian cancer and breast cancer[207]. Also, it is indicated as the first-line treatment of NSCLC in combination with one platinum-based compound[207]. In addition, paclitaxel can be used to treat other cancers like head-neck cancer, AIDS-related Kaposi's sarcoma and oesophageal cancer[207].

1.1.6.3 Pharmacoeconomics and cost

Pharmacoeconomics, as one sub-discipline of health economics, applies the principles and methodologies of health economics to the pharmaceutical field[208]. Health economics analyses the supply and demand for healthcare and helps the government and health decision-makers make decisions and understand consequences[208]. Pharmacoeconomics focuses on the study of cost and effects of a pharmaceutical product, which has attracted a lot of attention due to rapid growth in healthcare expenditure and increased stringent resources. The pharmacoeconomic evaluation

includes: cost minimisation analysis (CMA), cost effectiveness analysis (CEA), cost utility analysis (CUA), and cost benefit analysis (CBA)[208].

The high cost of paclitaxel made it difficult for decision-makers to consider it as the standard first-line treatment of advanced ovarian cancer in the early stage of drug development. Many pharmacoeconomic studies were conducted to help make a decision. An early cost-utility analysis was performed in Canada to evaluate the incremental cost-effectiveness of the paclitaxel and cisplatin combination (TP)[209]. As first-line treatment, the cost of TP treatment was approximately four-fold greater on a per-cycle base than that of the cyclophosphamide and cisplatin (CP) treatment. With progression-free survival benefit and patient treatment preferences included, an incremental cost of between Can$ 12,000 and Can$ 24,000 per quality-adjusted progression-free year was obtained with TP treatment[209]. However, this study confirmed that the TP regimen provided a substantial quality-adjusted progression-free survival at a reasonable cost to the Canadian health care system[209]. Similarly, another cost-effectiveness analysis was conducted to determine the cost structure of advanced ovarian cancer and evaluate the cost-effectiveness of TP treatment compared with previous CP treatment in six European countries, the UK, the Netherlands, Germany, Spain, France and Italy[210]. The TP regimen was associated with improved life expectancy compared with the CP treatment and the incremental cost-effectiveness of TP ranged from US$6,403 to US$11420 per year saved. These results suggested paclitaxel/cisplatin (TP) as the first-line therapy of advanced ovarian cancer[210]. NICE concluded eleven cost-effectiveness analyses and three cost-utility analyses, all of which showed that paclitaxel/platinum combination was more costly and more effective than control treatments. Two published UK cost-effectiveness analyses showed the incremental cost per life-year gained for paclitaxel/platinum ranged between £7173

and £12417. One published UK cost-utility analysis was mentioned in the NICE guidelines. The incremental cost per quality-adjusted life year (QALY) for the paclitaxel and platinum combination was £5273 compared with carboplatin alone.

In a recent study of advanced NSCLC[211], the efficacies of three regimens including docetaxel/cisplatin, paclitaxel/cisplatin and paclitaxel/carboplatin were compared using a pharmacoeconomic method. In this trial, no significant difference in efficacy was obtained among all three regimens. But a cost-minimisation analysis showed that, with equal efficacy, docetaxel/cisplatin resulted in a cost saving of US$2951 per patient in terms of the doses and schedules. This was mainly due to the lower cost of docetaxel[211]. A rapid review supported by the NHS (UK) reported, for the first-line treatment of NSCLC, a baseline incremental cost per life-year gained for paclitaxel/cisplatin was £8537, compared to best supportive care (BSC) based on a range of trial data and protocols[212]. NICE guidelines concluded that vinorelbine/cisplatin was less costly and more cost-effective compared to paclitaxel/carboplatin for the treatment of NSCLC in a US-based cost-minimisation analysis[213].

There are limited data about the economic evaluations of paclitaxel for the treatment of breast cancer reported in the NICE guidelines. Thus, future studies are expected in this area.

1.1.7 Methods of chemotherapy dose calculation

1.1.7.1 Rationale of individualised dosing

Most cytotoxic drugs have very narrow therapeutic windows, for example, a very small change in dose may result in very severe toxicity or less efficacy. Also, there is high inter-individual variability in drug clearance, which makes over- or under-dosages of

anti-cancer drugs relatively common. Both of these problems existing in oncology practice emphasise the need for correct dosing of anti-cancer drugs in order to maintain the effect of drugs, reduce the inter-individual variability in pharmacokinetics and decrease the potential occurrence of toxicity[214,215]. Therefore, individualised dosing is commonly used in oncological practice. Many body measures were tried to calculate the individualised dose, for example, body surface area, lean body mass, ideal body weight, adjusted ideal body weight and body mass index[216,217,218]. However among all the measures, body-surface area became the most commonly used parameter in practice because all the others lack scientific proof to support their applications.

1.1.7.1.1 Body surface area (BSA)

Individualised dosing according to body surface area (BSA) was established due to the relationship of BSA with some specific characteristics of patients, such as blood volume, glomerular filtration rate (GFR) and basal metabolic rate, and also due to the extrapolation of starting dosage used in animal studies which was usually based on body surface area (BSA) and body weight[214,215].

There are many methods that have been reported to calculate BSA such as the DuBois formula, Boyd formula, GG formula, Heycock formula and Takahira formula[219]. Among all these formula, the DuBois formula is most widely used especially in western countries due to its perceived accuracy, although this formula is quite old and was produced by DuBois and Du Bois in 1916. They studied 9 patients of various age, size and shape and derived this formula (as shown below) to calculate BSA only using body weight and body height[216]. However, this formula was challenged in many later studies[220,221,222,223]. Gehan and George found this formula showed good accuracy only when applied to a small population size[220]. Also, it was suggested that the Du Bois

formula would underestimate BSA for small individuals especially children[221,223], although, this formula is still the most commonly used in current practice. In a recent Japanese investigation where several different formulas were compared to calculate a typical Japanese patient, the Du Bois formula exhibited excellent accuracy to estimate BSA compared with others[219].

The DuBois formula[216]: BSA (m^2) = weight (kg) $^{0.425}$ × height (cm) $^{0.725}$ × 0.007184

As mentioned above, individualised BSA based dosing is the most commonly used practice in dosing cytotoxic drugs. However, the role of this individualised dosing has been doubted and re-evaluated for many years[178,214,215,224,225,226]. The two main reasons for this are concluded as follows: firstly, the scientific rationale of BSA is lacking[214,226]. The use of BSA in humans was derived from animal studies where doses were calculated using BSA. However, the fact that the difference in size between animals and humans was far greater than that between humans was neglected[178,214]. Secondly, for most anti-cancer drugs, the BSA was found to have a poor relationship with PK and PD parameters and could not decrease the inter-patient variability in drug clearance (CL)[214,215,224,225,226].

The role of BSA for paclitaxel dosing is questionable. One study suggested that the individual variability in drug exposure to unbound and total paclitaxel was reduced 50% by individualised BSA based dosing, compared to flat-fixed dosing[227]. Also in this study, body surface area and other body measures like weight and lean-body mass were all significantly related to unbound and total paclitaxel clearance. The author attributed this observation to the Cremophor EL (CrEL) in Taxol, which can highly bind to paclitaxel in plasma and has a small distribution volume in the body close to the total blood volume that links to body surface area (BSA)[227]. The influence of BSA on

variability in paclitaxel pharmacokinetics was mainly related to the behaviour of CrEL, which also agreed with other studies[172,173,174]. Paclitaxel dosing by BSA was also recommended by Felici's group[215]. However, the above conclusion was debated by Egorin and he pointed out that in the above study the correlation of pharmacokinetics and pharmacodynamics was lacking, i.e. no relationship between BSA and toxicity was determined, which made it difficult to evaluate the role of BSA[226]. At the same time, another study was conducted to evaluate the relationship between BSA and pharmacokinetic parameters and also between BSA and toxicity[178]. In this study, a different conclusion was obtained suggesting that individualised BSA based dosing was not necessary. Although BSA was inversely correlated with the area under the curve of the plasma concentration-time profile (AUC), there was no significant relationship found between body surface area (BSA) and neutropenia. Thus, the author supported that fixed dosing of paclitaxel would simplify the administration of this drug[178].

1.1.7.1.2 Body weight

In addition to body surface area, body weight is also used to calculate doses of many pharmacological agents in practice. However, it is not recommended for dosing chemotherapeutic agents. Firstly, it is not correct to use only body weight to calculate doses because for the same body weight, there can be different body sizes for individuals, either larger or smaller. Also, there is evidence that the rates of many physiological processes are lower in larger individuals than in smaller individuals[224]. Rather than BSA based dosing, dosing by body weight cannot reduce the relative dose to body weight as body size increases[224]. Furthermore, as mentioned above, the requirement for a correct dose in the chemotherapeutic area is higher than in other therapeutic areas. A small deviation from the correct dose can be life-threatening.

When it comes to individualised dosing, a BSA-based dose is better than a dose based on body weight.

1.1.7.1.3 Pharmacokinetic guided dosing

Another individualised dosing method called pharmacokinetic guided dosing was developed for chemotherapy. This is a method whereby the dose for drugs would be decided using PK information which is normally obtained from data of populations rather than an individual[228]. The aim of this method is to optimise dosage for anti-cancer drugs and thus decrease the inter-patient pharmacokinetic variability. Carboplatin is a successful candidate for PK guided dosing. In some early studies, the clearance of carboplatin was defined to be linearly correlated with the glomerular filtration rate (GFR) and AUC could be predicted by GFR[229,230]. Thus, the current formula was produced based on GFR as shown in the equation below[229]:

$$\text{Dose} = \text{AUC} (\text{GFR} + 25)$$

However, for most anti-cancer drugs, the complexity of drug pharmacokinetics such as metabolism to active species and multiple elimination mechanisms makes it difficult to simplify dose calculation using pharmacokinetic guided dosing. Paclitaxel is an example for this case because its pharmacokinetic behaviour has been found to be complicated as mentioned earlier (Section 1.1.5.2, p.42). To develop this method further for anti-cancer drugs, there is a need to have a clear understanding of their pharmacokinetics and the relationship between pharmacokinetics and pharmacodynamics.

1.1.7.1.4 Therapeutic drug monitoring

Many other dosing methods are currently being developed for individualised dosing. Among them, therapeutic drug monitoring (TDM) is a potential method that is being studied. Using TDM, the dose of drugs can be adjusted according to blood–level monitoring. Thus, the variability of PK can be controlled using TDM. It has been tested with many drugs and Methotrexate is a good example of the application of TDM[231]. The variability of PK can be decreased with TDM. Another example, Etoposide[232], was associated with reduced variability in leukocyte nadir using PK monitoring. However, this technique failed to determine the initial dose of the drug for patients[219]. Also, it makes it difficult to study the relationship between drug doses and the outcome of treatment[228]. Moreover, its high cost and inconvenience make it difficult to introduce TDM into common clinical practice[219,228].

1.1.7.2 Flat dosing

Flat-fixed dosing means a fixed dose level for all patients regardless of any specific subject-related factors, such as body size or BSA[233]. Many medicines are administered at a flat-fixed dose for adults except for those with narrow therapeutic windows, especially for cytotoxic drugs. As mentioned previously, nearly all cytotoxic drugs are dosed based on body-surface area (BSA). However, in recent years the accuracy of individualised BSA-based dosing has been questioned. Flat-fixed dosing was recommended as a simple way in many reports[214,215,226]. It has been demonstrated to be successfully applied to several agents, for example, phenylbutyrate, PKI166, R115777 and SCH66336[234,235,236,237]. Therefore, it was suggested to use flat-fixed doses as the starting doses in early clinical trials, which would be calculated based on an average body surface area of 1.86 m^2 [214]. Also, dose adjustment on the following cycles of

treatment would be performed based on the toxicity of each patient [215]. In the early stage, it may help study the relationship between patient variables (including BSA) and PK parameters of the drug of interest.

An important study to support flat-fixed dosing of paclitaxel has been mentioned previously (Section 1.1.7.1.1, p.58). In this study[178], a fixed dose, 360mg of paclitaxel was given to all women patients. Finally, although BSA was found related to some PK parameters like AUC and clearance rather than $T_{>0.05\ \mu mol/L}$, there was no obvious correlation between BSA and toxicity (neutropenia) and also only $T_{>0.05\ \mu mol/L}$ was significantly correlated with the nadir absolute neutrophil count. Due to all the above results, it was believed that fixed dosing of paclitaxel would be feasible because it would simplify the administration of paclitaxel.

Compared with individualised dosing according to BSA, flat-fixed dosing can facilitate the following advantages[178,214,215,226]:- (a) for pharmaceutical companies, it is more convenient and economical for manufacture, storage and shipping; (b) for hospitals, it can reduce time and cost to prepare a same dose for all patients rather than individualised doses; (c) it may reduce dosing error involved in the calculation of BSA. However, any change in dose may cause unacceptable toxicity or lower drug efficacy. Therefore, there should be sufficient data to support a decision as to administer cytotoxic drugs at a flat-fixed dose.

1.1.7.3 Dose banding (D-B) strategy based on BSA

In view of the disadvantages of conventional individualised dosing (BSA based), a dose banding (D-B) strategy was developed based on the former method by Plumridge and Sewell in the UK[238]. Doses of cytotoxics calculated on an individual basis (for example, BSA) are defined into different *bands*. A pre-determined standard dose (usually mid-

point of one band), instead of individual doses within the band, will be given by combination of several pre-filled syringes or infusions (hospital or commercial). Maximum variation between the individual doses & the standard dose is 5%. The following figure (see Figure 1-11) shows how D-B scheme works:

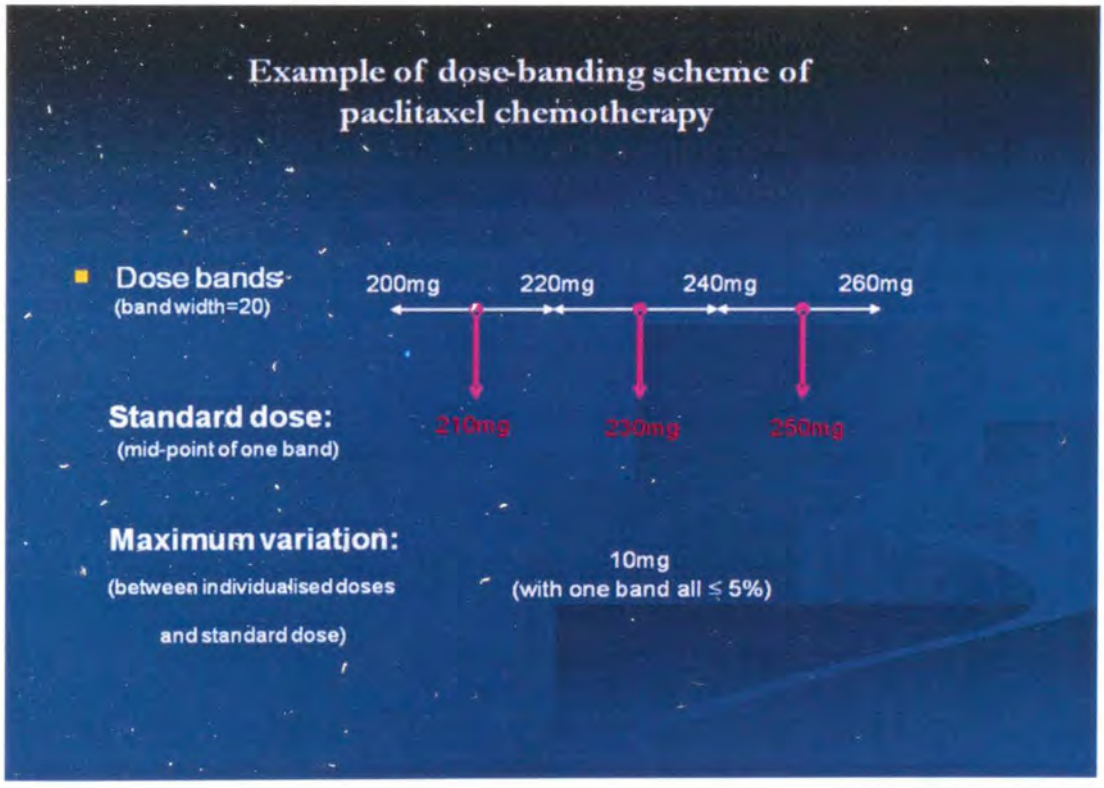

Figure 1-11 D-B scheme of paclitaxel with a predetermined band width of 20 mg

Although banded doses are still calculated based on BSA, this D-B strategy facilitates the following advantages:- (a) standard pre-filled packages (syringes or infusions) are immediately available, which can reduce waiting time for patients and provide convenience for out-patients; (b) it can conduct a well-planned cytotoxic reconstitution workload that may reduce dosing error; (c) it may reduce cost for preparation; (d) more importantly, it facilitates batch preparation of standard pre-filled packages (syringes or infusions) under GMP, which facilitates quality-control testing.

A validated shelf-life of 14 days up to 3 months is required for drugs to be considered for D-B dosing. So far, the D-B method has been successfully applied to many cytotoxic drugs in hospital practice, for example cyclophosphamide, methotrexate, doxorubicin, epirubicin, and 5-fluorouracil (5-FU)[239,240,241].

1.1.8 Summary of paclitaxel dosing

As mentioned previously, there has been much debate on the role of BSA for paclitaxel dosing. Although previous results showed that individualised BSA-based dosing can reduce the individual variability of drug exposure, it has been agreed in many studies that the influence of BSA on the variability of paclitaxel pharmacokinetics was mainly related to the behaviour of Cremophor EL (CrEL) in Taxol® formulation[172,173,174]. CrEL, which can bind tightly to paclitaxel in plasma, has a small distribution volume in the body, close to the total blood volume, and it is this that links plasma levels to BSA[227]. In another study, BSA was inversely correlated with the area under the curve (AUC) of the plasma concentration-time profile but no significant relationship between BSA and the major toxicity, neutropenia, was confirmed. As a result, the author supported flat-fixed dosing of paclitaxel to simplify the administration of this drug[178]. Clearly, no obvious evidence so far can be provided to support individualised BSA-based dosing of paclitaxel, although it is the licensed dose-strategy. Furthermore, in a recently published study, a fixed dose of 175 mg weekly administration was studied and recommended as a simple and effective option for paclitaxel treatment by comparison with a BSA-normalised dose of 100 mg/m^2 previously studied[242]. However, there was insufficient evidence to support flat-fixed dosing of paclitaxel in this study because it did not include a comparison group (individualised BSA dose).

1.2. Introduction to this study

1.2.1 Rationale for this study

In recent years, paclitaxel has become one of the most important chemotherapeutic agents widely used in the treatment of many cancers such as ovarian cancer, NSCLC, and breast cancer. Many investigations of its potential application are ongoing, for example, Kaposi's sarcoma, leukaemia and gastric cancer.

Conventional individualised dosing according to BSA lacks the rigorous credibility and its value has been doubted in many ways. Also, it brings inconvenience to the hospital and patients, and is a potential source of error in dose calculation. Some alternative methods or improvements based on the current chemotherapeutic dosing method are expected. Flat-fixed dosing has been recommended but it is clear that a fixed dose can cause the over- or under- dosage for patients with lower- or higher- body sizes. Further investigation should be performed for specific drugs and with certain patient types (e.g. emaciated or obese patients) to support this simple flat-fixed dosing.

D-B strategy may be a promising way to dose paclitaxel. However, to be considered for this strategy, a robust stability period (> 14 days) is required for drug infusions. Although some previous data demonstrated paclitaxel infusions (0.3 mg/mL) had a sufficient stability period of up to 28 days at 2 – 8 °C, this study involved the investigation of the physical and chemical stability of paclitaxel infusions of relevance to dose-banding. This study aimed to confirm the feasibility of D-B application to paclitaxel and identify a range of pre-filled infusions to finally develop a D-B scheme for paclitaxel chemotherapy.

To date there have been very few clinical studies to compare different dosing methods for paclitaxel. Further clinical investigation is required to evaluate the D-B approach on

paclitaxel chemotherapy when compared with individualised BSA dosing and the flat-fixed dosing. A protocol for a clinical pharmacokinetic (PK) study was developed, however due to the unavailability of patients, an *ex vivo* PK study was developed and conducted to simulate this designed clinical PK study.

1.2.2 Aims and objectives

The aim of this study was to investigate the evidence to support the feasibility of a D-B strategy on paclitaxel chemotherapy in comparison with other dosing strategies. The main objectives were:

a. To investigate the physical and chemical stability of paclitaxel infusions
b. To develop the D-B scheme for paclitaxel chemotherapy
c. To optimise and develop a sensitive analytical method for quantification of paclitaxel in human plasma by SPE and HPLC-UV
d. To evaluate the stability of paclitaxel in human plasma and during the assay procedure
e. To design a bio-equivalence clinical PK study and to devise a clinical protocol
f. To simulate the above clinical PK study by developing an *ex vivo* PK model based on literature data and to conduct data analysis by WinNonlin
g. To conclude the evidence of D-B application on paclitaxel chemotherapy and to identify future work based on the above studies

1.2.3 Thesis overview

This thesis includes 6 chapters.

Chapter 1 gives broad background information about paclitaxel and outlines the objectives of this study.

The background information described the discovery of paclitaxel and its subsequent development including its origin, physical and chemical characteristics, mechanism of action, and formulations. Early and recent pharmacokinetic studies were summarised and the updated information about paclitaxel PK and PK/PD were included. The analytical techniques used for paclitaxel quantification, especially in biological fluids, were reviewed. Recent clinical experience with paclitaxel was reported. The dosing strategies in chemotherapy were reviewed, and the dose-banding strategy was introduced. Finally the objectives of this study were outlined.

Chapters 2 to 5 represent the main body of this thesis.

In Chapter 2, the physical and chemical stability of paclitaxel infusions in a pharmaceutical environment (at 5°C) was investigated to establish the feasibility of the D-B application. A validated HPLC-UV method was used to determine the chemical stability. Physical stability was assessed by visual appearance and by using a liquid particle counter. Sufficient stability data were obtained in this study which facilitated the D-B application on paclitaxel. A D-B scheme for paclitaxel was proposed, which could be used in the later studies.

In Chapter 3, a sensitive paclitaxel assay in human plasma was developed and validated. The sample preparation including protein precipitation and SPE, and chromatographic parameters were optimised to achieve a high sensitivity and selectivity. Also in this chapter, paclitaxel stability in human plasma after long- and short-term storage and under different storage conditions was studied which provided the robustness of this analytical method and guided the design of the later clinical PK studies.

Chapter 4 includes a protocol for a clinical PK study designed to investigate the clinical validity of a D-B strategy compared with individualised BSA dosing and flat-fixed

dosing for paclitaxel chemotherapy. However, this clinical study was not conducted because of external factors and may be performed in the future.

Chapter 5 presents an *ex vivo* pharmacokinetic study to replace the above clinical PK study. This model was designed on literature data for the *in vivo* handling and elimination of paclitaxel. The details of model development, scale-down of the system and details of conducting the study were described. Results of pharmacokinetic and bio-equivalence analyses by WinNonlin software were reported.

Chapter 6 is a concluding discussion of all of this work and also makes proposals for future studies.

2. CHAPTER 2: PHYSICAL AND CHEMICAL STABILITY OF PACLITAXEL INFUSIONS TO FACILITATE DOSE-BANDING

2.1. Introduction and aim of study

In recent years, the scientific validity of individualised dosing according to BSA has been questioned in many reports[214,215,224,225]. To date, no strong evidence has been found to support this individualised dosing for paclitaxel chemotherapy[178,226]. The need to simplify conventional individualised dosing (BSA based) for paclitaxel has increased due to increased demand and the need to reduce delays in treatment of patients.

As mentioned before, the dose-banding (D-B) method may be a promising and more convenient way to dose paclitaxel. It has been successfully applied to many chemotherapeutic drugs, for example, methotrexate, doxorubicin, and epirubicin[239,240,241]. Compared with conventional individualised dosing, D-B offers the following advantages: reduced waiting time for patients, reduced workload for pharmacy and nursing staff, and facilitating batch preparation and quality control testing[238].

However, dose-banding requires a reasonable stability period (> 14 days) for the drug infusions to enable these to be pre-made. Previous reports have demonstrated that paclitaxel infusions (0.1 – 1.2 mg/mL) have a variable stability period of 2 to 28 days at different storage conditions, refrigerated (4 or 5°C), ambient temperature (22 or 25°C) and 32°C, with or without light protection[243,244,245,246]. Physical stability was the limiting factor in shelf-life of paclitaxel infusions[244,247]. This study was undertaken to confirm the physical and chemical stability of paclitaxel infusion (0.3 mg/mL and 1.0 mg/mL) to enable batch-scale preparation and the inclusion of paclitaxel in the dose-banding

schemes. Through this study, the feasibility of D-B of paclitaxel would be confirmed and the optimal concentration range of pre-filled infusions could be determined to enable a D-B scheme for paclitaxel chemotherapy to be developed.

2.2. Methods

2.2.1 Chemical and reagents

Paclitaxel concentrate solution (100 mg/16.7mL): Batch 06003 and expiry 03/2008, Batch no. BJ07015C and expiry 08/2009, supplied by Hameln Pharmaceuticals (Gloucester, UK).

Ecoflac plus 0.9% w/v sodium chloride infusions: 500 mL Batch no. 4191A141 and expiry 04/2007, low density polyethylene (LDPE) bottles, provided by B/Braun Company (Melsungen, Germany).

Freeflex polyolefin (PO) infusion bags 0.9% w/v, sodium chloride: 500 mL Batch no. UB7312 and expiry 11/02/2009, obtained from Fresenius Kabi (Cheshire, UK).

0.2 μm Minisart filters: Lot no. 16534 and expiry 06/2006, from Sartorius (Epsom, UK). Ammonium Acetate powder (≥ 99.99%): from Sigma-Aldrich (Dorset, UK).

All analytical or HPLC grade solvents: from Fisher Scientific (Leicestershire, UK).

2.2.2 High performance liquid chromatography (HPLC)

The system comprised a Prostar 210 solvent delivery module (Varian, UK), a Spectro Monitor 3000 variable wavelength UV detector (LDC/Milton Roy, Florida, USA) and a SP4400 ChromJet Integrator (Thermo Separation Products, Florida, USA) and loop-valve injector. The sample (20 μL) was injected into a Waters ODS2 column (150 × 4.6 mm, 5 μm; Waters, Hertfordshire, UK). The mobile phase consisted of 50%

Acetonitrile, 45% 20 mM Ammonium Acetate buffer (PH 5) and 5% tetrahydrofuran (THF) run at a flow rate of 1.0 mL/min. The detection was by UV at 227 nm and sensitivity was set to 0.005 AUFS. An external standard method, with bracketing of samples, was used.

2.2.3 Validation of HPLC method

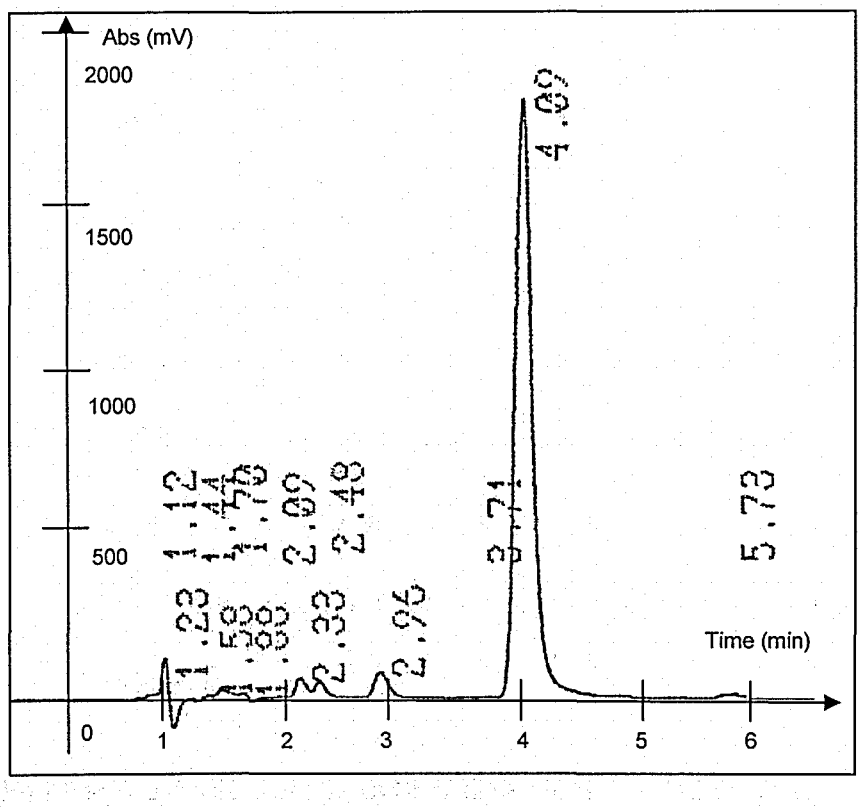

Figure 2-1 Typical chromatogram of paclitaxel by HPLC method described in Section 2.2.2

Retention time = 4.09 min, Concentration = 20 μg/mL

A typical chromatogram is shown in Figure 2-1. The retention time of paclitaxel peak was 4.1 minutes.

2.2.3.1 Calibration plot

Three sets of calibration plots have been produced in different weeks based on freshly made standard solutions (diluted in deionised water): 10 μg/mL, 20 μg/mL, 40 μg/mL, 80 μg/mL and 100 μg/mL. A good linear correlation between peak height and paclitaxel concentration over the range of 10 μg/mL – 100 μg/mL was demonstrated with an average correlation coefficient R^2 of 1. The slope of the calibration plots ranged from 10390 to 10592 and the y-intercept ranged from 783.9 to 8433.2 as shown in Table 2-1. The mean calibration plot (n = 3) is shown in Figure 2-2.

2.2.3.2 Intra- and inter-day precision

The intra-day precision and inter-day precision were calculated by injecting 10 μg/mL and 80 μg/mL paclitaxel solutions (n = 6) on 3 different days. The intra-day precision (CV) was 3.05% (10 μg/mL) and 0.68% (80 μg/mL) and the inter-day precision (CV) was 4.06% (10 μg/mL) and 1.62% (80 μg/mL). These were considered acceptable (≤ 15%) according to FDA guidance[248].

Table 2-1 The characteristics of the calibration plots for the chemical stability of paclitaxel infusion

Study	*Slope*	*Y-intercept*	R^2
1st	10390	1166.4	1.00
2nd	10681	783.9	1.00
3rd	10592	8433.2	1.00
Mean	10554	3461.2	1.00

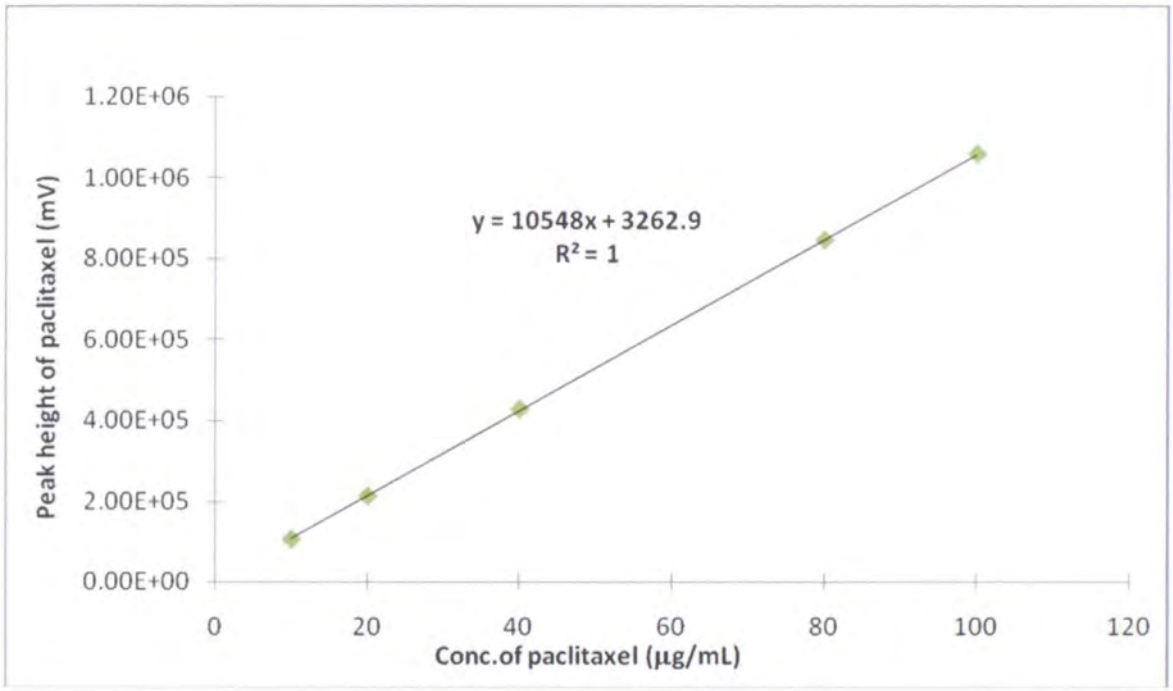

Figure 2-2 The mean calibration plot of paclitaxel assay based on three studies on different days: describing the relationship of the peak height to the concentration of paclitaxel

2.2.3.3 Stability indicating ability of assay

This experiment observed the effect of stress inflicted by variation of temperature, pH and the inclusion of an oxidative agent on paclitaxel stability. This was to ensure that the main paclitaxel analyte peak was resolved from any degradation product peaks. The experimental was according to the guidance (CPMP/ICH/281/95[249]) by the European Agency for the Evaluation of Medicinal Products. Any change of retention time and signs of degradation would be studied. 0.5 mL of paclitaxel (15 µg/mL in deionised water) was separately mixed with 1 mL deionised water, 1 mL of 6% hydrogen peroxide, 1 mL of 1 M hydrochloric acid, 1 mL of 1 M sodium hydroxide. The first sample was exposed to heating (at 55°C water bath for 1 hour) and the rest three samples were kept at room temperature for 1 hour. 0.5 mL paclitaxel (15 µg/mL) with

1 mL deionised water as a control sample was stored in the refrigerator at 5°C for 1 hour. The acidic and alkaline samples were neutralised and all samples were diluted approximately prior to assay. The paclitaxel concentration in each sample was estimated based on the calibration plot (Figure 2-2). The drug remaining (%) was obtained by comparing the test samples with the control sample. Peak homogeneity of the paclitaxel peak was determined by comparing the ratio of peak heights measured at different wavelengths (227 nm and 245 nm) for the test samples to that of the control sample.

The study results are shown in Table 2-2 and some typical chromatograms are demonstrated in Appendix 1 (p.279). On exposure to heating, oxidative degradation, acid hydrolysis, paclitaxel concentration (remaining %) was determined at 15.24 μg/mL (99.6%) and 15.52 μg/mL (101.4%), and 2.56 μg/mL (16.7%) at the retention time of 4.10, 4.09 and 4.09 min respectively. No paclitaxel peak was observed in the solution subjected to alkaline degradation, indicating the complete degradation of the drug. All the degradation products appeared at a retention time of around 1.0 – 3.0 min and none of them interfered with the paclitaxel peak. Peak purity of the paclitaxel peak was above 97% in all cases (control, heating and oxidative condition) except for the acid hydrolysis group (79%). Based on the evidence (peak height and peak purity) obtained here and the purpose of the study, this HPLC method was likely to be stability-indicating for paclitaxel.

Table 2-2 Stability indicating ability of the HPLC assay for paclitaxel

Treatment	*Retention time (min)*	*Paclitaxel concentration (μg/mL)*	*Paclitaxel remaining (%)*	*Peak purity (%)*
Control	**4.09**	**15.30**	**100**	**100.00**
Heating (55°C)	**4.10**	**15.24**	**99.6**	**99.50**
Oxidative degradation	**4.09**	**15.52**	**101.4**	**97.80**
Acid hydrolysis	**4.09**	**2.56**	**16.7**	**79.18**
Alkaline hydrolysis	—	—	—	—

Aim to observe any effect of elevated temperature (55°C), oxidative degradation, acid hydrolysis and alkaline hydrolysis on paclitaxel LC assay
n = 2

2.3. Experimental

To enable sufficient sample volumes to be taken for analysis and to avoid compromising the physical stability of paclitaxel infusions, separate sets of samples, cohort 1 and cohort 2, were used to determine chemical stability, visual appearance, weight and pH change (cohort 1) and sub-visual particulate counts (cohort 2).

2.3.1 Preparation of paclitaxel infusions

All infusions were prepared in a Class II safety cabinet under EU Class A conditions, and in accordance with the principles of Good Pharmaceutical Manufacturing Practice.

0.3 mg/mL paclitaxel infusion: 25 mL of 0.9% sodium chloride solution was removed from a 500 mL infusion bag or bottle (n = 3 each) and an identical volume of paclitaxel stock solution (6 mg/mL) was added, resulting in about 0.3 mg/mL of paclitaxel infusion.

1.0 mg/mL paclitaxel infusion: 83.3 mL of 0.9% sodium chloride solution from a 500 mL infusion bag or bottle (n = 3 each) was removed and an identical volume of paclitaxel stock solution (6 mg/mL) was added, resulting in about 1.0 mg/mL of paclitaxel infusion.

Two sets of 3 infusions for each concentration/container combination were prepared. All containers were labelled and stored in a pharmaceutical refrigerator (at 2 – 8°C) in light protected overwraps.

2.3.2 Preparation of the external standard paclitaxel solution

Paclitaxel stock solution (6 mg/mL) was diluted volumetrically in 0.9% sodium chloride solution on each test day by a factor of 100 to produce the external standard solution (60 μg/mL).

2.3.3 Sampling, testing schedule and discontinuation of the study

Infusion set 1 (Cohort 1): All sample solutions were analysed immediately after preparation (Day = 0) for chemical stability (HPLC assay) and physical stability (visual appearance, pH and weight change). These infusions were then subjected to all the above tests on the following testing days (t = 2, 8, 10, 13, 15, 17, 20, 22, 24, 27, and 29 days). During this study, if precipitate (number of particles ≥ 1) appeared in any sample container, testing of this sample was terminated; otherwise the study was continued. For all testing details refer to the relevant parts (Section 2.3.4 and 2.3.5, p.78 and 79).

Infusion set 2 (Cohort 2): Samples were taken from each infusion container for sub-visual particle counting on Day 0 and the following days (t = 3, 6, 9, 12, 16, 19, 22, 25, 30 days). Samples were analysed for up to 30 days. However, if any precipitate

appeared in a container, this container should be excluded from sub-visual particulate analysis.

2.3.4 Determination of physical stability

Visual inspection

On each testing day, all samples before sampling would be checked by eye for any change of colour, clarity and precipitation under the fluorescent light against (a) white and (b) black backgrounds and was compared with one reference infusion bag or bottle (containing no paclitaxel). Any change of clarity and presence of crystals or particles was recorded.

Sub-visual appearance inspection

Sub-visual particles at 10 μm and 25 μm levels were quantified by using an APSS-200 parenteral particle counting system (Particle Measuring Technique Ltd, Worcestershire, UK). This method was compliant with the method of the British Pharmacopoeia[250]. This system consisted of an LS-200 syringe sampler, a LiQuilaz particle counter and an integrated computer. Test samples were put into particle-free containers and a 10 mL volume would be taken into the system for analysis by the "light-blockage" method. Each sample was measured for over 7 times and the average cumulative counts were recorded at both 10 and 25 μm levels.

Weight control

All infusion containers from Cohort 1 were weighed before and after sampling using a calibrated Sartorius balance (Epsom, UK). Results after each storage period were compared with those obtained last time. Any change in weight was recorded as percentage (± %) change.

pH study

The pH measurement of infusions was taken using a Hanna HI98230 pH meter (Bedfordshire, UK) fitted with a glass electrode. The pH meter was calibrated using standard buffers (pH 4 and 7) before each session. Each sample was measured 3 times and the average value was recorded.

2.3.5 Determination of chemical stability

Chemical stability of paclitaxel infusions was assessed using the stability-indicating HPLC assay described previously (see Section 2.2.2, p.71). Individual test samples were diluted by a factor of 10 with deionised water and then injected in duplicate. The external standard was injected using the bracket injection technique (in the sequence of standard-test-test-standard). The ratio of the average peak heights between test sample and external standard was used to estimate the remaining concentration (refer to equation 1). The percentage (%) of paclitaxel remaining was calculated against the initial concentration.

$$Ct = \frac{PHt}{PHs} \times Cs \qquad \text{Equation 1.}$$

Ct = the concentration of the test sample; Cs = the concentration of the external standard; PHt = the average peak height of the test sample; PHs = the average peak height of the external standard

2.3.6 Acceptance criteria of chemical and physical stability

<u>Chemical stability</u>

The drug was considered to be chemically stable if the variance of paclitaxel assay was ≤ 5% of the initial concentration (assay range from 95% to 105%)[251].

Physical stability

The drug was considered physically stable under all following conditions if :- (a) there was no presence of any particle, colour and clarity change in the solution; (b) there was no obvious difference in pH of the solution and no significant change in weight due to moisture loss; (c) the particle count at 25 μm level was ≤ 3/mL in the solution.[244,246,250]

2.4. Results

Data for the physical and chemical stability of paclitaxel infusions in this study are summarised in Table 2-3, below:

Table 2-3 Summary of physical and chemical stability of paclitaxel infusions under refrigerated condition (2-8°C)

Concentration	*Container*	*[a]Stability period (days)*	*Weight change (±) (≤%)*	*pH range*	*Assay (%)*	*Sub-visual appearance*
0.3 mg/mL	**[b]PO**	20	0.018	3.64 - 3.73	98.5 – 103.6	Pass
	[c]LDPE	29	0.183	3.58 - 3.71	97.7 – 104.8	Pass
1.0 mg/mL	**PO**	15	0.014	3.42 - 3.53	98.8 – 102.8	Pass
	LDPE	20	0.196	3.42 - 3.49	97.1 – 103.3	Pass

[a] stability period is defined as time (days) that infusion was compliant with acceptance criteria.
[b] PO: Freeflex polyolefin infusion bags
[c] LDPE: Ecoflac low density polyethylene infusion bottles
n = 3 (each combination)

2.4.1 Determination of physical stability

- Visual Inspection

All infusions were evaluated for visual appearance on each working day. The 0.3 mg/mL infusions were visually clear for up to 20 days in PO bags and over 29 days in LDPE bottles without any sign of precipitation, crystallisation and colour change

observed in all containers. Conversely, the 1.0 mg/mL infusions remained clear for 15 days in PO bags and over 20 days in LDPE containers.

After the above determined periods, a few crystal-like particles were present in some containers. Although no longer considered stable, these infusions, on longer storage (approximately 3 – 5 days) became more turbid and colour changed from slight white, white to heavily white. This happened to all containers. The example of clarity change has been shown in Figure 2-3. The dense cloudiness was observed in PO bags (after 20 days storage) and in the LDPE bottles (after 40 days storage). This occurred in samples of higher concentration (1.0 mg/mL) earlier than samples of lower concentration (0.3 mg/mL).

Figure 2-3 Example of turbidity change of paclitaxel infusion (0.3 mg/mL) in PO bags

(a) reference bag; (b) light turbidity after 24 days storage; (c) moderate turbidity after 27 days storage; (d) heavy turbidity after 29 days storage

- Weight change (%) of infusion samples during the study periods

This measurement was to study any weight change of samples due to loss or gain of moisture during storage between each test time. The results are shown in Table 2-3. No significant weight loss (all ≤ 0.2%) was found in any of the study infusions within the shelf-life (refer to Table 2-3). The weight loss for 0.3 mg/mL infusions was less than 0.018% (PO bags) and 0.183% (LDPE bottles). And the weight loss for 1.0 mg/mL infusions was less than 0.014% (PO bags) and 0.196% (LDPE bottles). The weight loss for LDPE bottles was slightly larger than for PO bags, regardless of concentration.

- pH measurement

Physical stability was also considered in terms of pH change over the storage periods (Table 2-3). No obvious change in pH was found for all infusions over storage. Additionally, there was no significant difference in the pH values observed between PO bags and LDPE bottles at the same concentration (refer to Table 2-3). For 0.3 mg/mL infusions, pH values ranged from 3.58 to 3.73 and for 1.0 mg/mL infusions pH ranged from 3.42 to 3.53.

- Sub-visual appearance

The sub-visual particle counting study was performed on a separate cohort of containers. Table 2-4 shows the results of average cumulative counts of particles of ≥ 10 μm and ≥ 25 μm with storage periods of up to 30 days. The results were variable. Results on Day 0 seemed generally higher than Day 3. Figure 2-4 and Figure 2-5 show the plots of particle counts at both size thresholds in terms of storage periods. At 10 μm (Figure 2-4), the particle counts were higher for 1.0 mg/mL samples compared to

0.3 mg/mL samples. No significant increase in the counts was seen for all these samples during storage (30 days). At 25 μm (Figure 2-5), nearly all results were below 3 particles /mL (British Pharmacopeia limit[250]). But a marked increase in the counts at 25 μm was observed in 0.3 mg/mL infusions and 1.0 mg/mL infusions after 22 and 16 days, respectively, both in PO bags.

Table 2-4 Results of sub-visual particle counts for paclitaxel infusions stored at refrigerated conditions (2 – 8°C)

Day	0.3mg/ml				1.0mg/ml			
	PO		LDPE		PO		LDPE	
	≤10μm	≤25μm	≤10μm	≤25μm	≤10μm	≤25μm	≤10μm	≤25μm
0	46.27	0.73	56.13	0.33	127.40	3.20	209.67	2.00
3	33.50	0.30	32.93	0.07	63.07	1.03	167.33	1.07
6	32.50	0.17	30.93	0.37	101.40	1.17	140.30	0.50
9	37.90	0.43	31.67	0.10	71.13	1.03	120.40	0.53
12	34.77	0.17	26.13	0.13	69.60	0.73	107.73	0.50
16	40.80	0.47	28.83	0.17	87.97	0.93	93.93	0.23
19	50.10	0.60	39.80	0.27	86.00	1.73	89.13	0.70
22	57.50	0.57	47.57	0.47	112.03	1.63	110.77	0.70
25	65.00	1.65	53.63	0.43	92.13	1.57	98.50	0.83
30	68.85	1.05	64.00	0.77	103.30	2.20	91.17	0.63
Range	32.50 – 68.85	0.17 – 1.65	26.13 – 64.00	0.07 – 0.77	63.07 – 127.40	0.73 – 3.20	89.13 – 209.67	0.23 – 2.00

Unit: (number of particle)/mL
According to the British pharmacopoeia, the average number of particles present per unit should be ≤25 particles/mL (≥10μm) and ≤3 particles/mL (≥25 μm)[250].

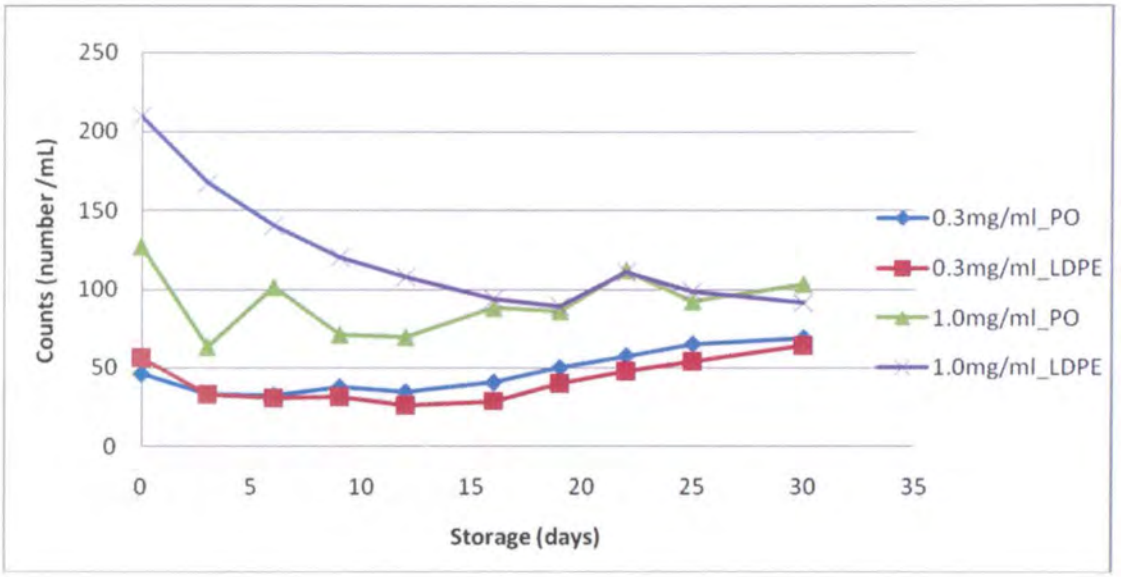

Figure 2-4 Plot of particle counts versus storage time (at ≥ 10 μm level) in the sub-visual particle counting study of paclitaxel infusions

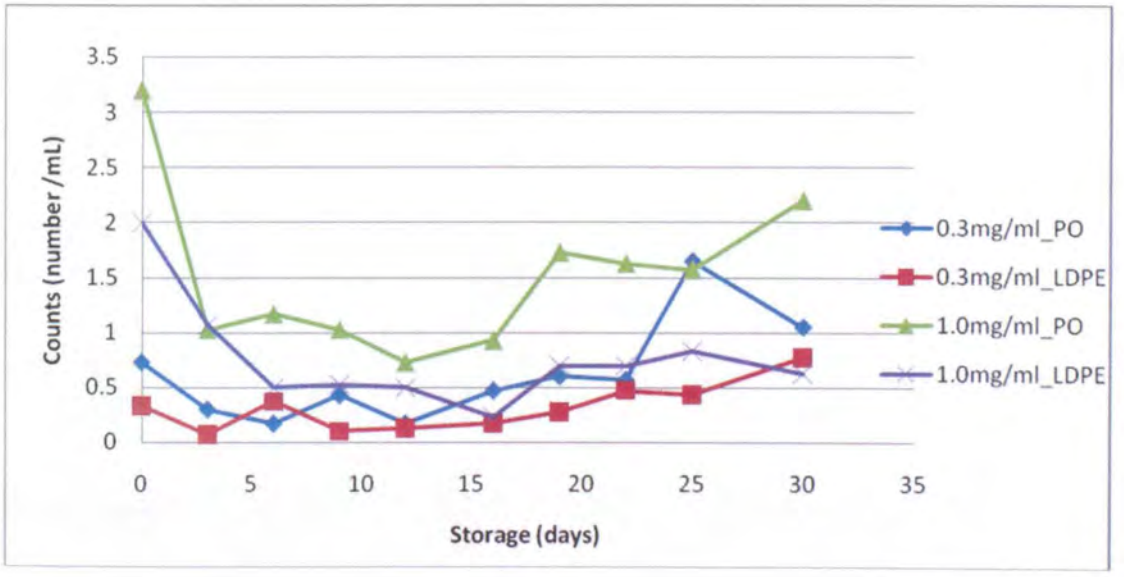

Figure 2-5 Plot of particle counts versus storage time (at ≥ 25 μm level) in the sub-visual particle counting study of paclitaxel infusions

2.4.2 Determination of chemical stability by HPLC

The chemical stability of paclitaxel infusions (0.3 mg/mL and 1.0 mg/mL) in 0.9% w/v sodium chloride solution is shown in Table 2-5.

Table 2-5 Chemical stability of paclitaxel infusions with storage by HPLC — Remaining drug as % of initial concentration (RSD%)

Day	*0.3 mg/mL (PO)*	*0.3 mg/mL (LDPE)*	*1.0 mg/mL (PO)*	*1.0 mg/mL (LDPE)*
0	100	100	100	100
2	98.5 (1.3)	97.7 (1.4)	101.4 (2.5)	99.5 (1.7)
8	102.5 (2.3)	102.3 (1.2)	98.8 (2.2)	97.1 (1.7)
10	99.7 (2.1)	100.9 (0.6)	102.8 (0.8)	103.3 (3.6)
13	103.4 (0.8)	103.7 (0.7)	102.3 (1.3)	101.1 (2.0)
15	103.6 (1.3)	103.9 (0.9)	102.4 (2.3)	101.9 (1.7)
17	102.3 (2.6)	104.8 (2.0)	—	99.8 (1.4)
20	100.1 (0.4)	100.6 (2.3)	—	99.9 (3.5)
22	—	99.1 (1.2)	—	—
24	—	100.7 (0.9)	—	—
27	—	103.2 (2.2)	—	—
29	—	101.1 (0.7)	—	—
Assay range	98.5-103.6	97.7-104.8	98.8-102.8	97.1-103.3

— Study terminated due to precipitate observed in one or more of tested containers under study.
Data shown are mean assay values for 3 replicate containers.

The HPLC result was expressed as an average remaining percentage (%) of the initial concentration. A variability within ± 5% of the initial concentration is normally considered acceptable[251]. During study periods, the assay range for 0.3 mg/mL infusions was at 98.5 – 103.6% (PO bags) and 97.7 – 104.8% (LDPE bottles); for 1.0

mg/mL paclitaxel remained at 98.8 – 102.8% (PO bags) and 97.1 – 103.3% (LDPE bottles). There was no sign of drug degradation, identified as additional LC peaks during the study periods in this study.

2.5. Discussion

2.5.1 Stability of paclitaxel in 0.9% w/v sodium chloride infusion

The stability of paclitaxel in 0.9% w/v sodium chloride solution was mainly decided by physical stability of the infusions. There was no significant change in pH and loss in weight during storage. The physical stability was limited by the formation of whitish precipitation. The maximum shelf-lives of paclitaxel infusions at different concentrations and in different containers have been compiled in Table 2-3. At the concentration of 0.3 mg/mL, paclitaxel was physically and chemically stable in sodium chloride solution for 20 days in PO bags and 29 days in LDPE bottles; at 1.0 mg/mL, paclitaxel was stable for 15 days in PO bags and 20 days in LDPE bottles. These stability data were in agreement with the study of Kattige[244], where paclitaxel at concentrations of 0.3 mg/mL, 0.75 mg/mL and 1.2 mg/mL in 0.9% w/v sodium chloride remained stable at 5°C for 28, 20 and 12 days, respectively.

It is clear that the physical stability was a function of drug concentration since in this study the maximum stability was produced at a low concentration (0.3 mg/mL) at the same storage condition. Also, it has been previously reported that a low storage temperature (refrigerated at 2-8°C) and light protection can contribute to better stability of paclitaxel infusions[244,245].

It was interesting that Ecoflac LDPE bottles provided better physical stability of paclitaxel in sodium chloride solution compared with Freeflex PO bags. A possible

explanation may be that the LDPE containers exhibited a slightly lower sub-visible particle count of the larger (≥ 25 μm) particle size. This could decrease the possibility of "seeding" of paclitaxel precipitation.

The physical stability of paclitaxel was limited by the formation of paclitaxel precipitation[247,252]. In this study, a few pieces of crystallised needle-like particles began to appear in the infusions after the shelf-life. With more storage time, visible gross precipitation was observed and infusions turned cloudy. This phenomenon was observed in previous studies[244,245,246,247,252]. It was believed that dilution of paclitaxel concentrate (formulated in CrEL and ethanol) in aqueous solutions can dramatically reduce the solubility of paclitaxel, which can cause precipitation[247,252]. CrEL molecules can form micelles at concentrations above critical micelle concentration (CMC). The micelles can trap paclitaxel (see Figure 2-6) in the core to increase its solubility. At concentrations above CMC, the solubility of drug increases linearly with the concentration of CrEL[253]. During dilution of paclitaxel concentrate, the concentration of CrEL reduces dramatically causing micelle fragmentation, therefore the solubility of paclitaxel decreases sharply. In addition, the precipitation may be caused by "seeding" of some particles in solution. Agitation or shaking during preparation of infusions may also induce the precipitation. Further investigation should be performed to clarify the mechanism of precipitation (see suggestions for future work, Section 6.2, p.240).

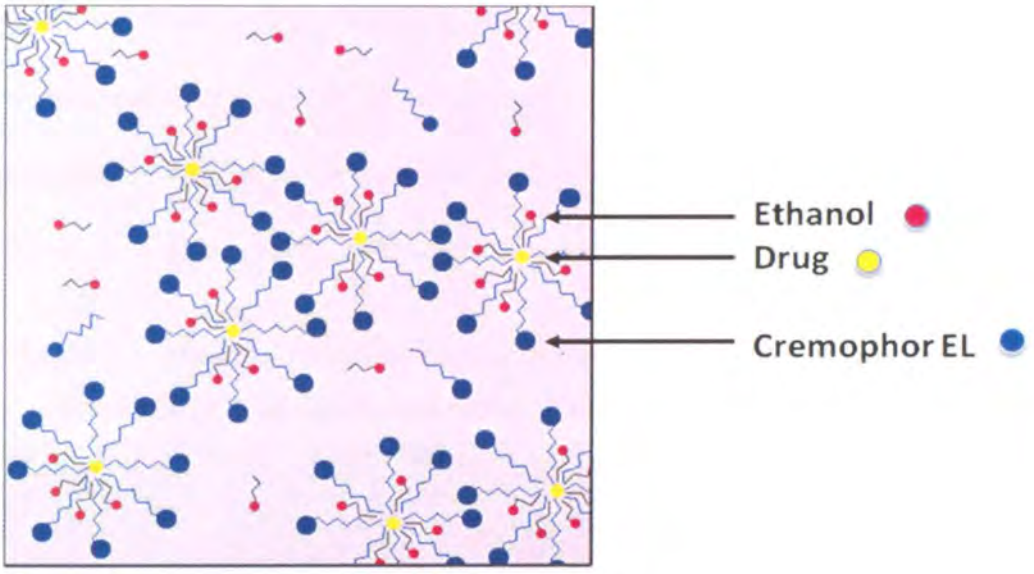

Figure 2-6 Schematic diagram of micelles structure (formed by Cremophor EL) exhibiting the relationship between paclitaxel, ethanol and Cremophor EL

Determination of chemical stability was terminated after precipitates appeared in samples. Prior to the appearance of precipitation, there was no significant loss of drug (all loss < 5%). However, after 35 storage days when visible precipitates were obvious, the paclitaxel concentration remaining was at 83.9% (0.3 mg/mL in PO bags), 95.3% (0.3 mg/mL in LDPE bottles), 81.5% (1.0 mg/mL in PO bags) and 94.5% (1.0 mg/mL in LDPE bottles). Infusions in PO bags in particular, at both concentrations, had become heavily cloudy (gross precipitation). Substantial drug loss (15 – 20%) accompanied this gross precipitation, which has been mentioned in previous studies[246,252]. These observations suggested that the precipitate was composed of paclitaxel and not only formulation excipients.

To confirm whether the cloudy precipitation was caused by paclitaxel itself, samples were injected after filtration via 0.2 μm membrane filters. A significant decrease of paclitaxel was seen in the cloudy samples (Table 2-6). Before filtration, 83.9% (0.3

mg/mL) and 81.5% (1.0 mg/mL) of paclitaxel was detected in PO bags; after filtration there was only 42.2% and 39.2% remaining for 0.3 mg/mL and 1.0 mg/mL samples, respectively. Therefore, it was clear that the formation of cloudy precipitation involved paclitaxel itself, thus reducing the paclitaxel concentration significantly.

Table 2-6 Effect of filtration on paclitaxel loss in infusions with heavy precipitate (PO bags) compared with clear infusions (LDPE bottles)

	0.3 mg/mL		*1.0 mg/mL*	
	***PO bags**	**LDPE bottles**	***PO bags**	**LDPE bottles**
After [a]F (μg/mL)	109.21	315.14	320.46	967.93
Before F (μg/mL)	258.67	309.93	816.84	963.03
[b]Recovery (%)	42.17	101.70	39.23	100.53

**: Cloudy samples*
[a] : filtration through a 0.2 μm membrane
[b]: Recovery % = Drug after F/Drug before F × 100
n = 3 (each)

2.5.2 Sub-visual particle counting

To provide sufficient sample volume for testing, the sub-visual particle counting study was performed on a second cohort of infusions. The particle measurement was based on the light obscuration which can determine the size of particles and the number of particles according to size. All cleaning and measurement accorded to the British Pharmacopoeia (BP) guidance[250]. Samples after being fully mixed were drawn by an LS-200 syringe sampler and tested by a LiQuilaz particle counter.

In this study, the particle counts on Day 0 were higher than Day 3. This may have been because bubbles in the infusions resulting from the preparation of infusions caused

interference. However for the subsequent sampling days, the particle counts seemed to settle and be reduced especially at 25 μm at the earlier sampling points (Table 2-4).

According to the BP guidance for drug preparation in containers within a nominal content of > 100 mL, the average number of particles present per unit should be ≤ 25/mL at 10 μm level (≥ 10 μm) and ≤ 3/mL at 25 μm level (≥25 μm). In this study, the sub-visual particle counts at 10 μm level were all beyond the reference value during the study period. This had also occurred in a previous study[244]. In practice, not all drug infusions can be judged by the British Pharmacopoeia standard, which are intended for licensed injectable medicines. Paclitaxel is very hydrophobic and nearly insoluble in aqueous solution. This may cause the formation of sub-visual particles when paclitaxel is dispersed in an aqueous solution. Therefore, it is necessary to pass paclitaxel infusions through an inline filter (≤ 0.22 μm) before administration[65]. This measure is to protect a patient from the risk of pulmonary embolism, but could also result in sub-therapeutic levels of infusions reaching the patient due to retention of drug on the filter.

In this study, the sub-visual particle counts at 25 μm level were nearly all within the acceptable range at both concentrations (0.3 mg/mL and 1.0 mg/mL) for up to 30 days. A visible increase of particle counts at 25 μm at both concentrations (PO bags) occurred after 22 and 16 days. This matched quite well with the stability shelf-lives obtained (20 and 15 days respectively) in the first cohort of infusions. It may be useful to interpret obvious changes in sub-visual particle counts as an indication of impending physical instability of paclitaxel infusions.

2.5.3 Perspectives of the current evaluation method for physical stability of paclitaxel infusions

Physical stability, as opposed to chemical stability, plays a more important role in the stability of paclitaxel infusions. However, most paclitaxel stability studies focus on chemical stability[243,245]. In these studies for evaluation of physical stability, it was mainly dependent on visual appearance, which was subjective and may over-estimate the stability of the drug. Therefore there is a need for more sophisticated methods for assessment of physical stability than visual appearance. Liquid sub-visual particle counting may offer a partial answer to this as obvious changes in sub-visual particle counts in this experiment has indicated the impending physical instability of paclitaxel infusions. However, many laboratories are reluctant to use this method with cytotoxic drugs because of risks of occupational exposure to laboratory staff.

2.5.4 Development of dose-banding (D-B) scheme for paclitaxel chemotherapy

Through this study, a range of stability periods from 15 to 29 days was obtained for paclitaxel (0.3 – 1.0 mg/mL in 0.9% w/v sodium chloride solution) stored at 2 – 8 °C with light protection. A maximum stability period of 29 days was defined for paclitaxel 0.3 mg/mL infusions in Ecoflac LDPE bottles. These stability periods were considered sufficient to facilitate the application of D-B to paclitaxel chemotherapy.

In addition to the requirement of sufficient stability, there are some other criteria to produce a D-B scheme for a drug:- (a) all the dosage regimens applied in current clinical practice should be considered; (b) a broad range of BSA must be considered to ensure that all patients would be included; (c) the variation of the banded doses must

be controlled within limits of a maximum deviation (±) of 5% from the provided (individualised) dose[238].

Based on the above criteria, a feasible D-B scheme for paclitaxel was devised as shown in Table 2-7. A broad range of dosage regimens including 80 – 225 mg/m^2 covered all currently used protocols for paclitaxel in clinical practice. Also, the range of BSA covered in the scheme was from 1.60 m^2 to 2.0 m^2, well including the average reference BSA values suggested by Mosteller[254]. All doses were grouped into 20 bands, where the band widths were not completely equal. Among these bands, the band width (BW) of 15 mg was only for 185 – 200 mg band. Below this band, all band widths were 10 mg. Above this band, all band widths were 20 mg. In each case the maximum variances between all banded doses (DB) and individualised doses calculated based on BSA were ≤ 5%. The standard dose (normally mid-point of each band) could be provided by 6 types of pre-made infusions in total, as shown in Table 2-8. The infusion concentrations were less than or equal to 0.5 mg/mL and all banded doses could be provided by combination of 2 to 4 pre-made infusions (Table 2-7).

In general, the total infusion volumes of banded doses administered to patients (as shown in Table 2-7) would not present any clinical problems. Any patients with fluid restrictions (e.g. because of renal failure) would need individual infusions of higher concentration prepared in lower volumes.

Table 2-7 Dose-banding scheme for paclitaxel chemotherapy over doses ranging from 125–460mg

Dose bands (mg)	*std dose (mg)*	*Max. Dev (%)*	*Pre-made infusions (mg)*	*No. Pre-infusion*	*Total volume of infusions (mL)*
125-135	*130*	4.00	100mg + 20mg +10mg	3	350
135-145	*140*	3.70	100mg + 20mg +20mg	3	350
145-155	*150*	3.45	100mg + 50mg	2	350
155-165	*160*	3.23	100mg + 50mg + 10mg	3	400
165-175	*170*	3.03	100mg + 50mg + 20mg	3	400
175-185	*180*	2.86	100mg + 50mg + 20mg + 10mg	4	450
185-200	*190*	5.00	100mg + 50mg + 20mg + 20mg	4	450
200-220	*210*	5.00	200mg + 10mg	2	550
220-240	*230*	4.55	200mg + 20mg + 10mg	3	600
240-260	*250*	4.17	200mg + 50mg	2	600
260-280	*270*	3.85	200mg + 50mg + 20mg	3	650
280-300	*290*	3.57	200mg + 50mg + 20mg + 20mg	4	700
300-320	*310*	3.33	300mg + 10mg	2	1050
320-340	*330*	3.13	300mg + 20mg + 10mg	3	1100
340-360	*350*	2.94	300mg + 50mg	2	1100
360-380	*370*	2.78	300mg + 50mg + 20mg	3	1150
380-400	*390*	2.63	300mg + 50mg + 20mg + 20mg	4	1200
400-420	*410*	2.50	200mg + 200mg + 10mg	3	1050
420-440	*430*	2.38	200mg + 200mg + 20mg + 10mg	4	1100
440-460	*450*	2.27	200mg + 200mg + 50mg	3	1100

Std dose, standard dose; Max. Dev, maximum deviation between standard dose and individualised doses within a band

Table 2-8 Paclitaxel pre-made "standard" infusions for the D-B scheme shown in Table 2-7

Pre-made infusions (mg)	*Infusion bag size (mL)*	*Volume replaced by paclitaxel 6 mg/mL (mL)*	*Final volume (mL)*	*Final concentration (mg/mL)*	*Infusion shelf-life (days)*	
					PO	*LDPE*
10	50	1.7	50.0	*0.2*	*> 20*	*> 29*
20	50	3.3	50.0	*0.4*	*15-20*	*20-29*
50	100	8.3	100.0	*0.5*	*15-20*	*20-29*
100	250	16.7	250.0	*0.4*	*15-20*	*20-29*
200	500	33.3	500.0	*0.4*	*15-20*	*20-29*
300	1000	50.0	1000.0	*0.3*	*20*	*29*

3. CHAPTER 3: OPTIMIZATION AND DEVELOPMENT OF PACLITAXEL ASSAY IN HUMAN PLASMA

3.1. Introduction and Aim of Study

3.1.1 Analytical methods for paclitaxel assay in human plasma

Paclitaxel exerts cytotoxic activity at concentrations as low as 50 nM[47], which requires highly sensitive analytical methods to quantify paclitaxel in clinical studies. The paclitaxel molecule lacks a specific chromophore and detection at a lower UV wavelength of 227 – 230 nm is therefore used for HPLC assay of paclitaxel. However, many endogenous compounds in plasma and some co-administered drugs also strongly absorb at these wavelengths. A high selectivity is thus required for the paclitaxel assay. Table 3-1 shows some examples of co-administered drugs with paclitaxel chemotherapy such as analgesics, anti-emetics and other anti-cancer drugs, which could be encountered in clinical studies.

As described previously (Section 1.1.5.1, p.36), a number of analytical methods have been developed for paclitaxel assay, including HPLC[139,140,142,148], LC-MS[143,145], electrophoresis[149], immunoassays and other bioassays[154,155,156]. These are normally combined with sample preparation methods such as solid-phase extraction (SPE)[143,255], protein precipitation and liquid-liquid extraction (LLE)[145,149]. The HPLC-UV method is still the main tool for paclitaxel analysis in biological fluids used in combination with sample preparation methods due to its wide availability. This combination has achieved acceptable levels of LOD (5 – 15 ng/mL) and LOQ (10 – 30 ng/mL) (refer to Section 1.1.5.1).

However, few previous HPLC assays have demonstrated good selectivity and specificity in the presence of co-administered drugs in human plasma[137,140,255]. In addition, some of these previous methods have not been sufficiently documented the details of method development for sample preparation and HPLC analysis. This has made it difficult to reproduce similar assay sensitivity and selectivity using previously published methods[139,142].

Table 3-1 Potential co-administered drugs with paclitaxel chemotherapy

Co-administered drugs	Dose	Peak plasma or steady state concentration
Dexamethasone	20 mg	252 ng/mL*[256]
Ranitidine	150 mg	440-545 ng/mL[257]
Cyclizine	50 mg tid PO	70 ng/mL[258]
Metoclopramide	20 mg (tablets)	44 ng/mL[259]
Disodium pamidronate (Aredia)	90 mg per day, i.v.	1.38 μg/mL[260]
Carboplatin	300-500 mg/m^2	42.5 μg/mL*[261]
Doxorubicin	20 mg/ m^2	8.34 μg/mL[262]
Tamoxifen	20 mg daily PO	134.4 ng/mL[263]
Clonazepam	0.5 mg bid PO	7.1-23.6 ng/mL[264]
Granisetron (Kytril Ampoules)	2 mg per day, i.v.	—
Ondansetron	32 mg i.v.	—

** Labelled peak plasma concentrations were calculated based on literature.*
The chemical structures of all these co-administered drugs refer to Appendix 2 (p.285).

3.1.2 Paclitaxel stability in human plasma

In studies on pharmaceutical formulations, paclitaxel exhibited limited stability[244,246], mainly because of physical instability and drug precipitation. Paclitaxel stability in human plasma is a crucial aspect to consider in the development of bioanalytical methods and in turn, in the subsequent design of clinical studies. It is also important in the evaluation of bioavailability of new formulations of paclitaxel, such as nano-particulate albumin-bound paclitaxel[265,266]. Previous studies on this subject have been reported, but these have either used assay methods that are not fully validated, or have not considered the complete isolation of the drug from the biological matrices and the analytical procedure. According to previous reports, paclitaxel was stable in human plasma at −20°C for over 2 months and for up to 2 years[138,255]. Also, it can tolerate up to 3 cycles of freezing and thawing[255,267,268]. It has also been suggested that paclitaxel plasma samples can be kept at room temperature for up to 4 hours prior to analysis without any loss[267,269]. Extracted paclitaxel samples were shown to be stable in LC autosamplers for up to 24 hours[268,269,270]. However, to date there are no detailed published data about paclitaxel stability over the complete sample preparation and analytical process. Also many previous studies lack rigour and clarity of conditions used.

3.1.3 Aim of this study

This first part of this study aimed to optimise and validate a sensitive and selective HPLC assay for the quantification of paclitaxel in human plasma in the presence of co-administered drugs. This method was required for a clinical pharmacokinetic study to assess the likely clinical effect of dose-banding.

The second part of this study evaluated paclitaxel stability in plasma samples after long and short term storage, after freeze/thaw cycles, and under different conditions to establish stability at each stage of the clean-up and assay process. This work was undertaken as part of the method development and validation of a paclitaxel assay for clinical and pharmacokinetic studies on different paclitaxel dosing strategies such as dose banding[238]. Such methods would also find use in evaluation of the bio-equivalence of different paclitaxel formulations.

3.2. Materials and Methods

3.2.1 Chemicals and reagents

Paclitaxel drug concentrate (6 mg/mL): 100 mL from Teva Pharmaceuticals (Leeds, UK), stored at 5°C with light protection.

The internal standard (docetaxel): 5 mg powder (HPLC grade) from Sigma-Aldrich Ltd (Dorset, UK), kept at −20°C with light protection.

Co-administered drugs (listed below)

Ondansetron (2 mg/mL): CP Pharmaceutical Ltd (Wrexham, UK);

Dexamethasone sodium phosphate (8 mg/mL for injection): Faulding Pharmaceuticals Plc (Warwickshire, UK);

Kytril Ampoules (1 mg/mL): Roche Products Ltd (Hertfordshire, UK);

Carboplatin (10 mg/mL): Mayne Pharma Plc (Warwickshire, UK);

Doxorubicin hydrochloride (2 mg/mL): Dabur Pharma Ltd (Solan, India);

Clonazepam, Metoclopramide hydrochloride, Ranitidine, Pamidronate disodium, Tamoxifen and Cyclizine: obtained from Sigma-Aldrich Ltd (Dorset, UK).

Raw human plasma (citrated mixed pool): 500 mL per bottle, supplied by First Link (Birmingham, UK).

Other reagents:

Deionised water was produced by an Elga water purification system (Marlow International, Buckinghamshire, UK);

Acetonitrile (ACN), Methanol (MeOH), Ethanol, Tetrahydrofuran (THF) were all HPLC grade supplied by Fisher Scientific (Loughborough, UK);

Ammonium acetate (≥ 99.99% purity) was from Sigma-Aldrich Ltd (Dorset, UK);

All other reagents were analytical grade from Fisher Scientific (Loughborough, UK).

3.2.2 Materials

Bond Elut cyano end-capped (CN-E) SPE cartridges, Bond Elut C8 and C18 cartridges (all 500 mg, 3 mL) were supplied by Varian (Oxford, UK);

15 mL polypropylene centrifuge tubes were from Fisher Scientific (Loughborough, UK);

Millipore polyvinylidenefluoride (PVDF), Chromacol polytetrafluoroethylene (PTFE), Nylon, and polypropylene (PP) syringe filters (all 0.22 μm, 13 mm id.) were all from Fisher Scientific (Loughborough, UK);

Auto-sampler vials (2 mL, Chromacol 2-SV) and vial caps were all from Fisher Scientific (Loughborough, UK);

Regenerated cellulose filter membranes (50 mm, 0.45 μm) for degassing the mobile phase were from Sartorius (Epsom, UK);

U300 ultrasonic bath (sonicator) was from Ultrawave Ltd (Cardiff, UK);

Vortex mixer (FB15013) was from Fisher Scientific (Loughborough, UK);

5702R centrifuge was from Eppendorf Ltd (Cambridge, UK);

Chromaband 12-position vacuum SPE manifold was from Fisher Scientific (Loughborough, UK);

All glassware (Grade A), pipettes and pipette tips, and other general laboratory materials were all from Fisher Scientific (Loughborough, UK).

3.2.3 High-performance liquid chromatography (HPLC)

The HPLC system consisted of a model PU-2080 pump, a model AS-2055 auto-sampler and a model MD-2010 diode array detector (all from Jasco, Essex, UK). Data were collected and processed by the EZChrom software (Agilent, West Lothian, UK). A stainless steel narrow-bore column packed with Spherisorb ODS2 (5 μm, 2.1 × 150 mm) (Waters, Herts, UK) was used in conjunction with a 4 × 2.0 mm C18 guard cartridge (Phenomenex, Macclesfield Cheshire, UK).

3.3. Experimental

3.3.1 Part I: Optimisation and validation of paclitaxel assay in human plasma

The first part of this study describes the method development of paclitaxel assay in human plasma. Most experimental conditions involved in the chromatographic analysis and the sample preparation (SPE, protein precipitation) were optimised. All conditions of paclitaxel analytical assay in human plasma were finalised and validated. The following flow chart (Figure 3-1) shows the experimental procedures of optimisation and validation of this paclitaxel assay.

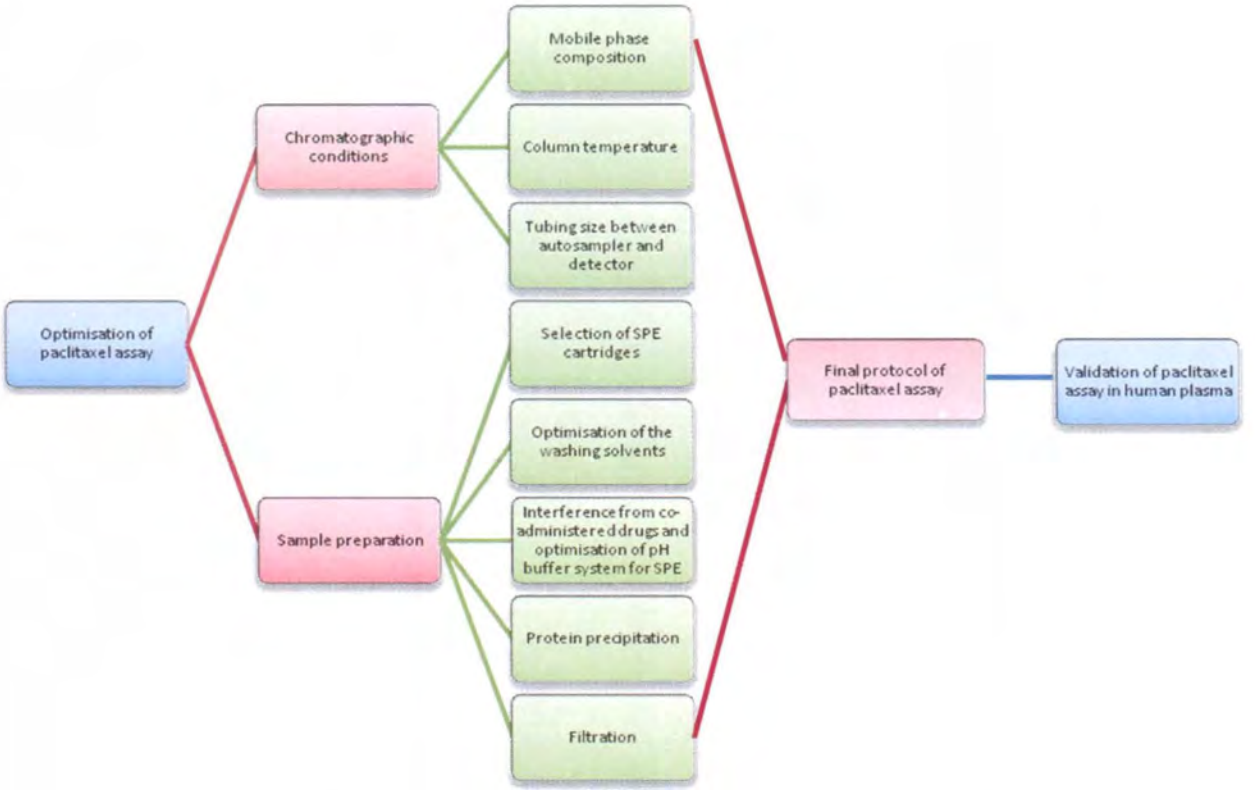

Figure 3-1 Flow chart of optimisation and validation of paclitaxel assay in human plasma including sample preparation and HPLC assay in Part I of this study

During method development, different mobile phases (see Table 3-2) were used but all other basic chromatographic conditions were generally the same (refer to Section 3.2.3, p.100). Any change in conditions would be specified in later sections.

Table 3-2 Different mobile phases used in the optimisation of HPLC assay of paclitaxel

HPLC method	*Mobile phase conditions*
Method A	ACN/THF/0.02M Ammonium acetate buffer (pH 5.0), 50/5/45 (v/v)
Method B	ACN/0.02M Ammonium acetate buffer (pH 5.0), 50/50 (v/v)
Method C	ACN/THF/0.02M Ammonium acetate buffer (pH 5.0), 50/2/48 (v/v)

ACN, acetonitrile; THF, tetrahydrofuran
Mobile phase was run at a flow rate of 0.2 mL/min.
All other chromatographic conditions were same for these three methods (A, B and C).

Table 3-3 demonstrates the original procedure of SPE method used in this study, which was based on a previous study[137].

Table 3-3 Solid phase extraction of paclitaxel in human plasma adapted from literature

	Method from literature (*Huizing*[137])	Method used in this study
SPE cartridge	Cyano Bond Elut, 1 mL	Cyano Bond Elut, 500 mg
Condition 1	2 mL MeOH[a]	5 mL MeOH
Condition 2	2 mL 0.01M AA[b]	5 mL 0.01M AA
Loading	1 mL sample matrix[c]	5 mL sample matrix
Wash 1	2 mL 0.01M AA	5 mL 0.01M AA
Wash 2	2 mL MeOH-0.01M AA[d]	5 mL MeOH-0.01M AA
Wash 3	1 mL hexane	2 mL hexane
Drying	Under full vacuum	Under full vacuum
Elution	2 mL 0.1% TEA in ACN[e]	1.2 mL 0.1% TEA in ACN
Evaporation	Under nitrogen stream at 30°C	Under nitrogen stream at 30°C
Reconstitution	In 200 μL mobile phase	In 500 μL mobile phase

[a]: MeOH = Methanol
[b]: 0.01M AA = 0.01M ammonium acetate buffer (pH 5.0)
[c]: sample matrix contained 50% plasma sample with 50% 0.2 M ammonium acetate buffer (pH 5.0).
[d]: MeOH- 0.01M AA = Methanol- 0.01 M pH 5 ammonium acetate buffer (2:8, v:v)
[e]: TEA = triethylamine; ACN = acetonitrile

3.3.1.1 Optimisation of chromatographic conditions

During method development, the mobile phase and some other chromatographic conditions were adjusted and optimised to achieve good sensitivity and selectivity of the assay.

3.3.1.1.1 Optimisation of mobile phase composition

During method development, the mobile phase was adjusted twice to achieve both selectivity and sensitivity of the HPLC assay. Table 3-2 shows different mobile phases used during method development. The original mobile phase (Method A) used was 50% ACN, 5% THF and 45% 0.02 M Ammonium acetate buffer (pH 5.0), which had been applied in previous studies reported in this thesis (Section 2.2.2, p.71). The first adjustment was made when the internal standard (docetaxel) was introduced because the separation between paclitaxel and docetaxel peaks was found poor. The resolution (Rs) of both peaks was only 1.29 (usually require > 1.5[271]) with the original mobile phase as shown in Figure 3-2. A variety of mobile phases with different compositions were tested to obtain a good separation for these two peaks. The summary of results is shown in Appendix 3 (p.290). Good separation (Rs = 2.93, shown in Figure 3-2) was achieved with the mobile phase containing 50% ACN and 50% 0.02 M ammonium acetate buffer (pH 5.0). Thus, the mobile phase (Method B) consisting of 50% ACN/50% 0.02 M Ammonium acetate buffer (pH 5.0) was applied in Section 3.3.1.2.3 and 3.3.1.2.4 (p.114 and 122).

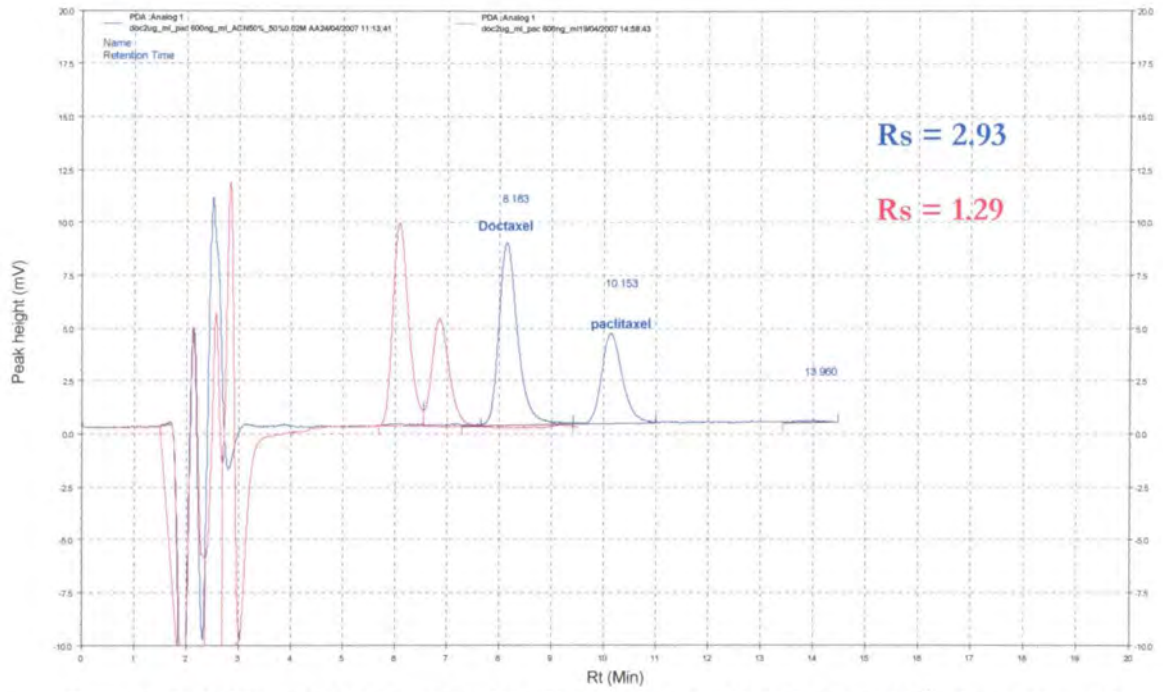

Figure 3-2 Chromatograms with different mobile phases (1st changing of mobile phase)

ACN/THF/0.02 M ammonium acetate (pH 5.0), 50/5/45 (v/v) (—)
ACN/0.02 M ammonium acetate (pH 5.0), 50/50 (v/v) (—)
Concentration of paclitaxel = 600 ng/mL; Concentration of docetaxel = 2 μg/mL

Although good separation was achieved for paclitaxel and docetaxel with the mobile phase (Method B) of 50% ACN and 50% ammonium acetate buffer, the target limit of quantification for paclitaxel (10 ng/mL in plasma) could not be detected with the above mobile phase. The absence of THF in the mobile phase obviously reduced the sensitivity of the HPLC assay since paclitaxel at 10 ng/mL had been clearly identified with the previous mobile phase including 5% THF. Therefore, a lower percentage of THF (< 5%) was decided to be added to increase the sensitivity.

Mobile phases with different percentages of THF (1%, 2% and 3%) were tested. The results are shown below (Table 3-4).

Table 3-4 Optimisation of the percentage of THF in the mobile phase for paclitaxel HPLC assay (2nd changing of mobile phase)

% THF in mobile phase	Resolution	% increase in Peak height
0	2.93	0
1	2.31	49
2	*1.99*	*63*
3	1.64	71

THF, tetrahydrofuran
Test solution: paclitaxel 50 ng/mL in ACN/water (50/50, v/v) (equivalent to paclitaxel 10 ng/mL in plasma)
0% THF: 50%ACN/50% 0.02M ammonium acetate buffer (pH5.0)
1% THF: 50%ACN/1%THF/49% 0.02M ammonium acetate buffer (pH5.0)
2% THF: 50%ACN/2%THF/48% 0.02M ammonium acetate buffer (pH5.0)
3% THF: 50%ACN/3%THF/47% 0.02M ammonium acetate buffer (pH5.0)

With increased percentage of THF in the mobile phase, the assay sensitivity (% increase in peak height) increased with a concurrent decrease in resolution. Among these compositions, 2% THF was considered optimal because it provided adequate sensitivity as well as good separation for the peaks. The mobile phase (Method C) with 2% THF was selected for further use in optimisation and validation of the method.

3.3.1.1.2 Control of column temperature

To obtain good reproducibility and avoid the influence of environmental temperature, a column oven (serial no.9227, John Chromatography, UK) was incorporated into the HPLC system. A simple study was conducted to define the influence of temperature on the paclitaxel assay. A standard sample was re-injected at different column temperatures (26 – 35°C). The chromatograms are shown in Figure 3-3. The retention times and peak heights of both peaks had not changed greatly at different temperatures. As a result, temperature seemed not to have obvious influence on both

paclitaxel and docetaxel peaks. However, for consistency, the column was maintained at 25°C in all future studies.

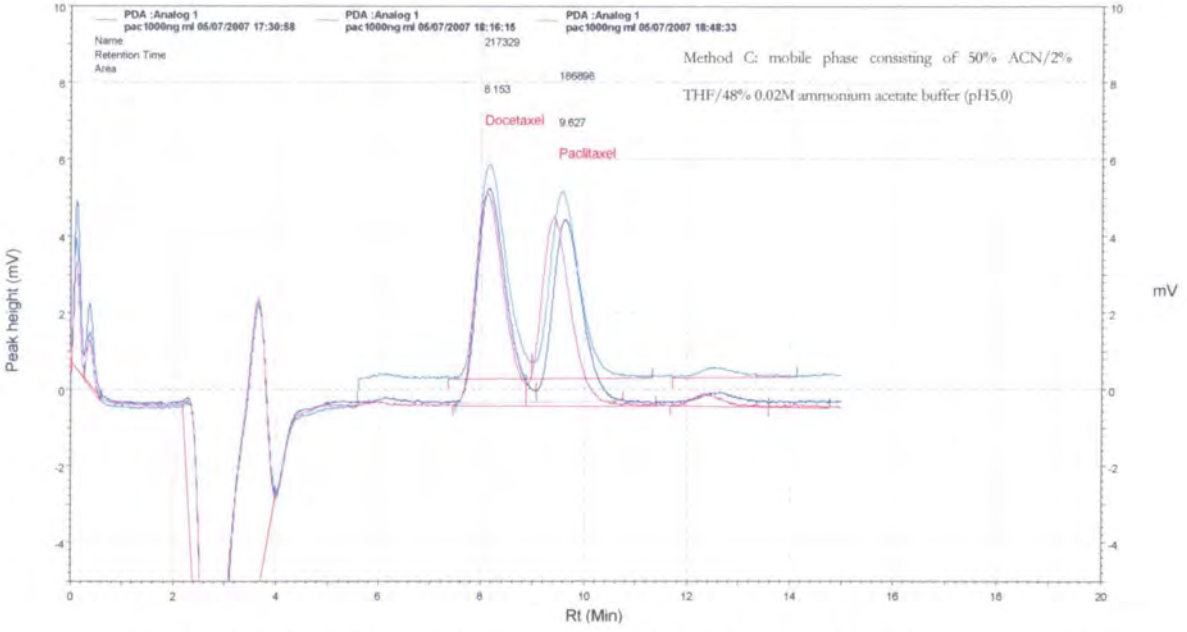

Figure 3-3 Influence of temperature on paclitaxel and the internal standard (docetaxel)

Column temperature: 26°C (—); 30°C (—); 35°C (—)

3.3.1.1.3 Optimisation of tubing size between auto-sampler and detector

To increase the sensitivity of the HPLC assay, the tubing size between auto-sampler and detector was also optimised. Three different tubing sizes were tested. As shown in Figure 3-4, a smaller tubing size contributed to higher peak efficiency. Among these three sizes (7/1000 inch, 10/1000 inch, and 15/1000 inch), 7/1000 inch tubing offered the best peak efficiency, producing the best sensitivity of the assay. Thus the 7/1000 inch tubing was used in all subsequent studies.

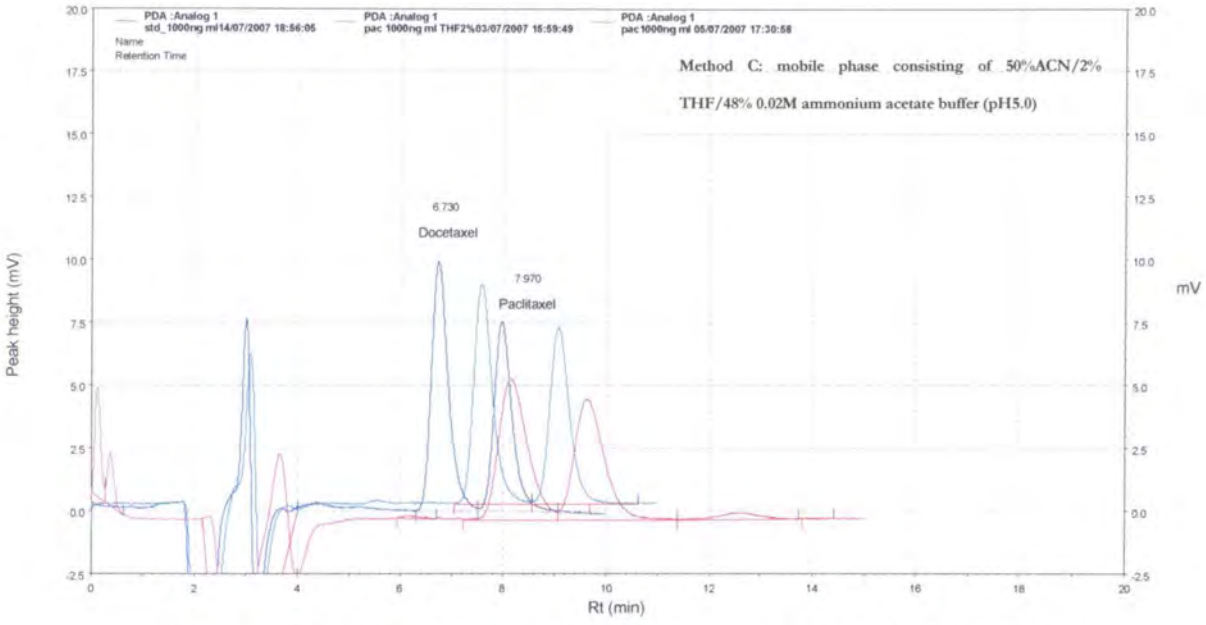

Figure 3-4 Effect of different tubing sizes on LC chromatograms

Tubing between auto-sampler and detector; 7/1000 inch (—); 10/1000 inch (—); 15/1000 inch (—)

3.3.1.2 Optimisation of sample preparation

3.3.1.2.1 Selection of SPE cartridges

This experiment tested the efficiencies of different SPE cartridges for paclitaxel extraction from plasma. Since paclitaxel is very insoluble (non-polar) in aqueous matrices such as plasma, some potential SPE cartridges were selected on their ability to retain lipophilic compounds or to retain lipophilic compounds with polar functional groups. The SPE cartridges tested were cyano-bond Elut (CN-E), C8 and C18. The cartridges were evaluated according to recovery and reproducibility. The experimental procedure was shown as below:

a) An appropriate volume of raw human plasma (mixed pool citrated, First link Ltd, UK) was defrosted from −20°C in a water bath (30°C).

The plasma was subjected to centrifugation at 3000 g (4°C) for 10 minutes and then the supernatant was aspirated for use.

b) The paclitaxel solution (6 μg/mL) was freshly diluted from the stock solution (6 mg/mL) using ACN/water (50/50, v/v).

c) The test sample (paclitaxel 200 ng/mL in plasma) was produced by mixing 200 μL of paclitaxel solution (6 μg/mL) with 5.8 mL of plasma in a centrifuge tube (15 mL), followed by vortexing for 1 minute.

d) 6 mL of 0.2 M ammonium acetate buffer (pH 5) was added to the plasma solution in (c), above, with mixing.

e) 5 mL of this solution (d) was transferred onto one conditioned SPE cartridge as described in Table 3-3.

f) Following washing, elution and evaporation steps, the sample was finally reconstituted in 500 μL of ACN/water (50/50, v/v) and a 20 μL sample was injected onto the HPLC system in duplicate.

g) Each type of cartridge was tested in duplicate (n = 2).

Table 3-5 Optimisation of SPE cartridges for paclitaxel assay in human plasma

SPE cartridge	CN-E	C8	C18
R%	105.7%	100.6%	77.9%
CV%	5.20%	4.40%	1.60%

R% : recovery %
CV%: reproducibility (coefficient of variance)
N= 4 injections (each cartridge)
Method A (refer to Table 3-2) was used.

Table 3-5 showed recoveries (R%) of paclitaxel were 105.7% (CN-E), 100.6% (C8), and 77.9% (C18) with CV%s of 5.2%, 4.4% and 1.6%, respectively. The

chromatograms obtained for this experiment are shown in Appendix 4 (p.292). CN-E contributed to a better recovery with an acceptable reproducibility. Also, it was found that the CN-E cartridge resulted in a slightly faster flow during sample loading compared to C18 and C8 cartridges; the latter two cartridges were readily clogged by the plasma samples. With maximal recovery of paclitaxel and the faster flow (reduced preparation time), CN-E was selected as the optimal SPE cartridge for further use.

3.3.1.2.2 Optimisation of the strength and volume of washing solvents

During method development, the wash step plays a very important role because it can sometimes dramatically improve the sensitivity and selectivity of the assay by removing impurities which have a lower affinity for the sorbent compared to the analyte. In this study, washes were conducted in three steps of increasing lipophilicity which involved the following components: buffer solution → MeOH-buffer → hexane.

i. Optimisation of Wash 2 (MeOH/buffer): washing strength

Among the above wash steps, Wash Step 2 was the most important part. This step normally consisted of an aqueous mixture with an organic composition of 5 – 50%. Although in the literature 20% MeOH in the combination was generally used for paclitaxel, the strength of Wash 2 still required further investigation to optimise the percentage of MeOH.

a) Preparation of the sample matrix

Paclitaxel samples (60 ng/mL) were diluted freshly in ACN/water (50/50, v/v) from the stock solution (6 mg/mL). Sample matrix was made by mixing 2 mL of paclitaxel sample (60 ng/mL) with an equal volume of 0.2 M ammonium acetate buffer (pH5), followed by vortexing for 1 minute.

b) Preparation of a series of wash solvents with increasing solvent strength

The 0.01 M ammonium acetate buffer (pH5) was mixed with different amounts of MeOH to make up 20%, 40%, 50%, 60%, 80% and 100% MeOH in 0.01 M ammonium acetate buffer.

c) SPE procedure

3 mL of sample matrix was applied onto each SPE cartridge (CN-E) and washed separately by the above range of different Wash 2 solvents. Finally each extract was reconstituted in 300 µL of ACN/water (50/50, v/v). All other procedures were identical as those described previously (Table 3-3). The experiment was performed in duplicate for each wash solvent and extracted samples were analysed using Method A (Table 3-2).

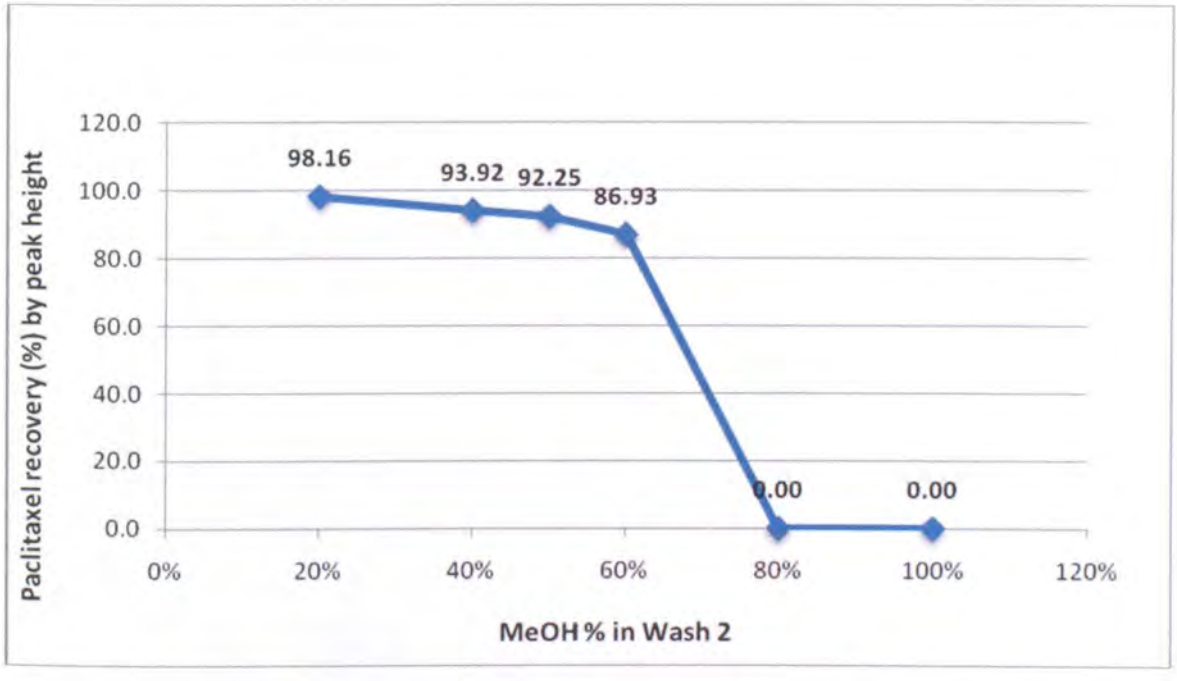

Figure 3-5 Effect of altering the MeOH% in Wash 2 solvent (MeOH-buffer) during SPE clean-up of paclitaxel

With reference to Figure 3-5, it was shown that paclitaxel recovery decreased with increasing MeOH% in Wash 2 solvent. 20% MeOH in Wash 2 contributed to the best recovery of paclitaxel (98%). A higher percentage of MeOH (over 20%) compromised the recovery of paclitaxel and the sensitivity of the assay. Since no obvious drug loss was observed with 20% MeOH in Wash 2, a lower percentage of MeOH (< 20%) was not necessary to test in this experiment as a lower percentage (< 20%) may compromise the clean-up of plasma samples. Considering these results, a 20% composition of MeOH was optimal and would be applied in the later study.

ii. Optimisation of Wash 1: volume of buffer

This was to test if 5 mL of Wash 1 buffer was optimal for plasma samples and whether there was a need to increase the volume of Wash 1.

Different volumes of Wash1 (0.01 M ammonium acetate buffer), 5 mL (standard), 10 mL and 15 mL, were applied to wash different cartridges (CN-E) after plasma samples (paclitaxel 400 ng/mL with potential interference drugs) were loaded. Peak height results with 10 and 15 mL of Wash 1 were compared with standard wash volume (5 mL) and the remaining recovery % was calculated by comparison with the standard group.

Table 3-6 Influence of different volumes of Wash 1 on drug recovery (R%) by peak height

Vol. [a]Wash 1	[b]R% of Paclitaxel	R% of [c]Docetaxel	R% of [d]Ratio (paclitaxel/IS)
5 mL (standard)	100	100	100
10 mL	101	103	99
15 mL	102	106	97

[a]Wash 1: 0.01M ammonium acetate buffer (pH 5.0)
[b] R%: recovery % compared with the standard group (5 mL)
[c]docetaxel as internal standard (IS)
[d]Ratio: peak height ratio of paclitaxel versus docetaxel
Samples were analysed using Method B (Table 3-2)
n=2 (each volume)

Based on Table 3-6, an increased volume (10 or 15 mL) seemed to slightly increase the recovery of paclitaxel and docetaxel. It may be because an increased volume of Wash 1 contributed to less impurity and thus better recovery for both drugs. However, an increased volume (10 or 15 mL) of Wash 1 had no significant influence on peak height ratio of these two drugs and dramatically increased the experimental time. Since in the calibration study the peak height ratio of both drugs was of interest and a shorter assay time was preferred in any clinical study, a 5 mL Wash 1 was still used for 500 mg CN-E cartridges.

iii. Wash 3: hexane

Wash 3 step was tested to determine if this step could be simply removed or a higher % of hexane would have influence on paclitaxel recovery. Results are showed in Table 3-7. There was no significant difference in drug recovery and sample purity between groups with or without the Wash 3 step. However considering that a real blood sample collected in the clinical study was a very complicated matrix with a lot of interference

from co-administered drugs and endogenous products, Wash 3 (hexane) was still kept because it may further remove organic non-polar impurities in clinical blood samples.

Table 3-7 The influence of different volumes of Wash 3 (hexane) on paclitaxel recovery during SPE clean-up

Volume hexane (mL)	0 mL	*2 mL (standard)*	4 mL
R% by peak height	97	*100*	100

Test sample: paclitaxel 60 ng/mL in acetonitrile/water (50/50, v/v)
R%: paclitaxel recovery % compared with the standard group (2 mL hexane) by peak height
Samples were analysed using Method A (Table 3-2).

3.3.1.2.3 Determination of interference from co-administered drugs and optimisation of pH buffer system during SPE

This experiment aimed to define any potential interference caused by the co-administered drugs to paclitaxel and docetaxel (the internal standard, IS) in aqueous solutions, and to determine if there was any interference on the peaks of interest (paclitaxel and docetaxel) in plasma after SPE clean-up. The final target was to remove all interference by optimising the pH of buffers used during SPE.

i. Determination of any potential interference from individual co-administered drugs

All stock solutions of drugs including paclitaxel, docetaxel (IS) and the individual solutions of the other 11 co-administered drugs were diluted in ACN/water (50/50, v/v) to make up the appropriate concentrations based on peak plasma concentrations (or steady state concentrations) obtained from literature (refer to Table 3-1). The concentration details of all aqueous test solutions are shown in the third column of Table 3-8. All co-administered drugs were tested individually as well as together with paclitaxel to determine any potential interference.

Table 3-8 Preparation of test solutions (including paclitaxel, docetaxel and co-administered drugs) for interference determination

Drugs	Stock concentration	Concentration in [a]aqueous sample	Concentration in [b]plasma samples for SPE
Paclitaxel	*6 µg/mL	1200 ng/mL	**10 & 400 ng/mL**
Docetaxel	*100 µg/mL	2 µg/mL	**400 ng/mL**
Dexamethasone	4 mg/mL	2 µg/mL	**400 ng/mL**
Ranitidine	5 mg/mL	2 µg/mL	**400 ng/mL**
Cyclizine	*1 mg/mL	0.5 µg/mL	**100 ng/mL**
Metoclopramide	1 mg/mL	0.2 µg/mL	**40 ng/mL**
Disodium pamidronate (Aredia)	1 mg/mL	5 µg/mL	**1 µg/mL**
Carboplatin	10 mg/mL	25 µg/mL	**5 µg/mL**
Doxorubicin	2 mg/mL	10 µg/mL	**2 µg/mL**
Tamoxifen	*2 mg/mL	2 µg/mL	**400 ng/mL**
Clonazepam	*1 mg/mL	100 ng/mL	**20 ng/mL**
Granisetron	1 mg/mL	5 µg/mL	**1 µg/mL**
Ondansetron	2 mg/mL	5 µg/mL	**1 µg/mL**

*Most stocks were prepared in deionised water except * which were prepared in ethanol.*
[a]: aqueous samples (individual drug mixture with paclitaxel) were used for determination of interference from individual co-administered drugs with paclitaxel and docetaxel;
[b]: plasma samples including paclitaxel, docetaxel and all co-administered drugs were used for the determination of interference from plasma samples using SPE.

The individual retention times of all the test drugs obtained with the LC assay are listed in Table 3-9. Among these co-administered drugs, only the Granisetron and Ondansetron peaks could potentially interfere with the paclitaxel peak. Metoclopramide may also cause interference with the docetaxel peak.

Table 3-9 HPLC retention times of paclitaxel, the internal standard (docetaxel) and other co-administered drugs

Drugs	Retention time (min)
Paclitaxel	10.20
Docetaxel	8.17
Dexamethasone	2.22
Ranitidine	3.51
Metoclopramide	8.48
Cyclizine	*ND*
Disodium pamidronate	*ND*
Clonazepam	4.93
Doxorubicin	4.76
Carboplatin	2.09
Tamoxifen	*ND*
Granisetron	9.06
Ondansetron	9.40

All assays were run with the mobile phase (Method B) of ACN/0.02 M ammonium acetate (50/50, v/v).
ND: no visible peak was detected within the running time of 20 minutes.

ii. Determination of interference in plasma samples after SPE

This procedure was to check if the interference as mentioned above could be removed through SPE. Two drug mixtures including paclitaxel, docetaxel and co-administered drugs in human plasma were prepared based on the drug concentrations shown in Table 3-8 (paclitaxel at both 10 ng/mL and 400 ng/mL). Both drug mixtures were subjected to SPE (refer to Table 3-3), followed by the HPLC assay (Method B, refer to Table 3-2). The chromatograms after SPE of plasma containing paclitaxel 10 and 400 ng/mL are shown below in Figure 3-6 and 3-7, respectively.

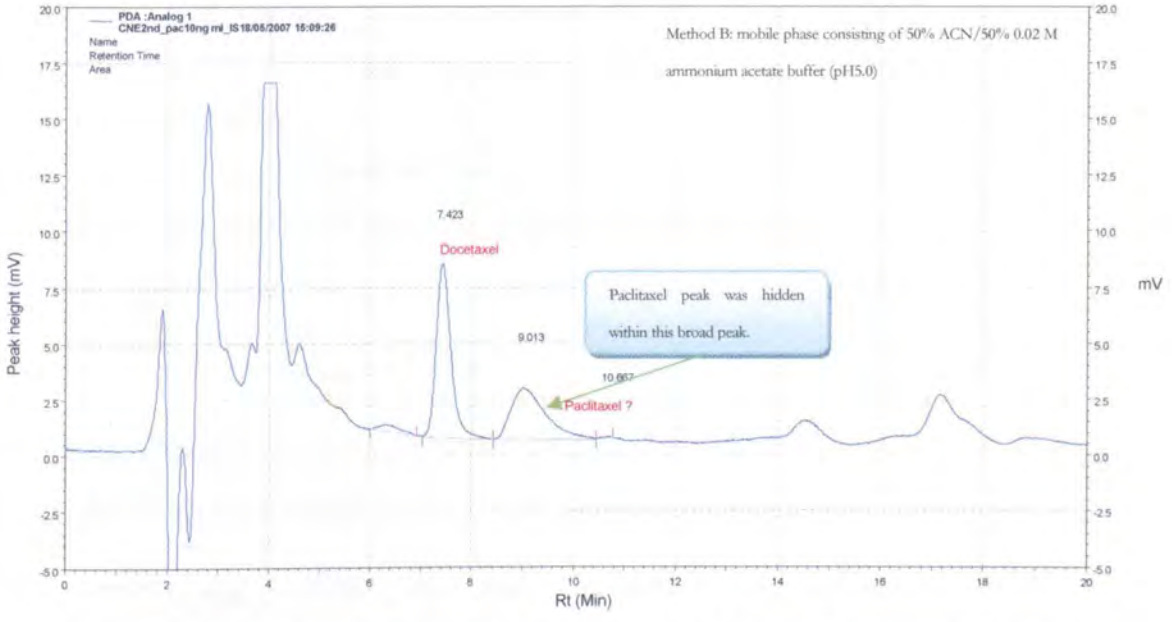

Figure 3-6 A chromatogram of paclitaxel (10 ng/mL) extracted from a spiked plasma sample containing the internal standard (docetaxel, 400 ng/mL) and other co-administered drugs

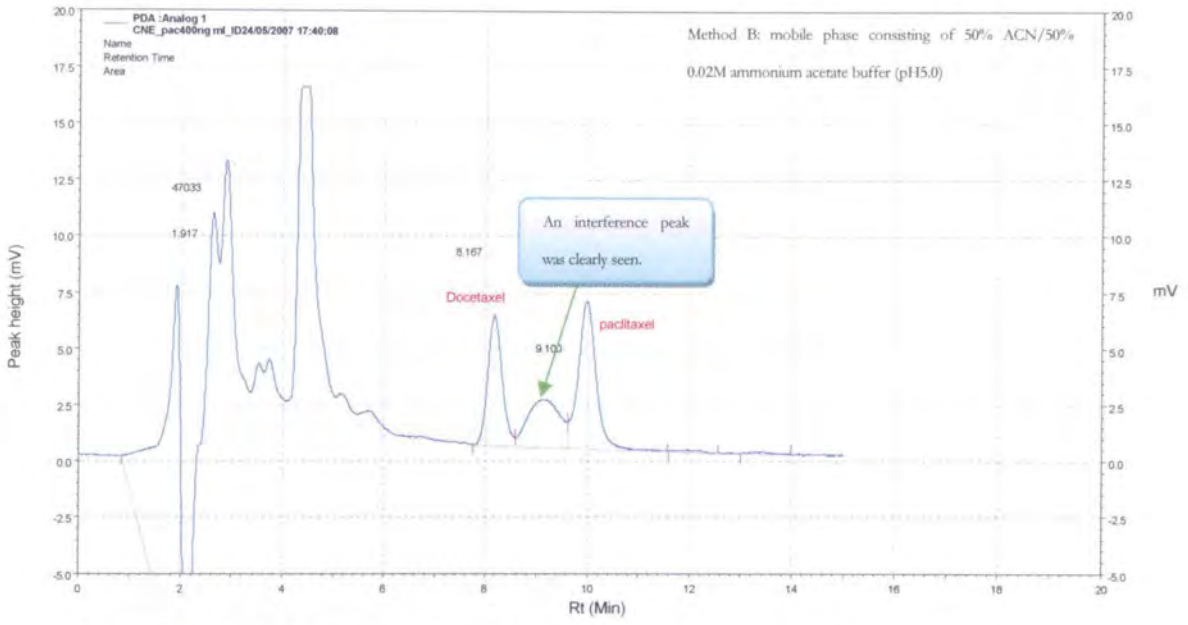

Figure 3-7 A chromatogram of paclitaxel (400 ng/mL) extracted from a spiked plasma sample containing the internal standard (docetaxel, 400 ng/mL) and other co-administered drugs

At 10 ng/mL, the paclitaxel peak could not be recognised at around the retention time of 10 min but the peak tailing of the broad peak hinted that the paclitaxel peak may be obscured by this interfering peak (Figure 3-6). At a paclitaxel concentration of 400 ng/mL, the paclitaxel and docetaxel peaks experienced interference from a small broad peak with a retention time of 9.1 min, which may have been Granisetron or Ondansetron. Using the SPE system defined in Table 3-3, interference from certain co-administered drug could not be removed successfully.

iii. Optimisation of the pH buffer system for SPE to remove the potential interference from some co-administered drug

Since the interference due to co-administered drugs was not removed by SPE (pH of buffers = 5), this experiment was designed to optimise the pH buffer system so as to remove the interference for both paclitaxel and docetaxel.

Buffers at different pHs (1-11) were prepared as shown in Table 3-10.

Table 3-10 Preparation of different pH buffers used for solid-phase extraction (SPE) during paclitaxel assay in human plasma

pH	Buffer system
1.0	0.1 M HCL adjusted using 0.1 M KCL
2.0	0.1 M HCL adjusted using 0.1 M KCL
3.0	0.1 M formic acid adjusted by triethylamine
4.0	0.1 M Sodium acetate adjusted by glacial acetic acid
5.0	0.1 M Sodium acetate adjusted by glacial acetic acid
6.0	0.1 M Sodium acetate adjusted by glacial acetic acid
7.0	0.1 M Na_2HPO_4 buffer adjusted by 0.1 M HCL
8.0	0.1 M Na_2HPO_4 buffer adjusted by 0.1 M HCL
9.0	0.1 M Na_2HPO_4 buffer adjusted by 0.1 M HCL
10.0	0.1 M Na_2HPO_4 buffer adjusted by 0.1 M NaOH
11.0	0.1 M Na_2HPO_4 buffer adjusted by 0.1 M NaOH

To make certain buffer system (e.g. pH 1), all ammonium acetate buffers (pH 5) in the original protocol (Table 3-3) were replaced by this pH buffer (e.g. pH 1.0 buffer).

Drug mixtures were prepared by spiking paclitaxel, docetaxel and other co-administered drugs into plasma (the concentration of paclitaxel = 120 ng/mL and all other drug concentrations refer to Table 3-8). Plasma samples were subjected to SPE at different pH buffer systems (pH 1 – 11), followed by the HPLC assay (Method B). The SPE procedure was generally the same as the previous one (shown in Table 3-3) but all ammonium acetate buffers in the previous protocol were replaced, in turn, by each of the above buffers. For example, to test the pH 1.0 system, the conditioning buffer, Wash 1 and buffer in Wash 2 were all replaced by the pH 1.0 buffer.

Table 3-11 Results of optimisation of buffer pH used during SPE for paclitaxel assay in human plasma

pH	paclitaxel peak	Resolution
1.0	ND	ND
2.0	ND	ND
3.0	Fully separated	3.0
4.0	—	ND
5.0	Partly separated	1.0
6.0	Partly separated	1.0
7.0	Partly separated	1.1
8.0	Partly separated	0.6
9.0	Not separated	ND
10.0	Not separated	ND
11.0	Not separated	ND

ND: no paclitaxel peak observed
—: Experiment failed because of instability of the pH buffer
Fully separated: paclitaxel peak was completely separated with other peak.
Partly separated: paclitaxel peak was not completely separated with other peak.
Not separated: paclitaxel peak was not seen and not separated at all.

Of the buffer systems tested (Table 3-11), only the pH 3.0 buffer system contributed to good selectivity and less interference. At pH 3.0, the interference peak caused by some co-administered drugs was completely removed at the retention times of paclitaxel and docetaxel. At pH < 3 and pH > 8, there was no peak of interest detected on the chromatograms, which indicated paclitaxel may not be stable under very acidic or basic conditions, or the SPE bonded phase may be destroyed due to hydrolysis cleavage (pH < 3) and dissolution (pH > 8). Between pH 5 – 8, interference due to some co-administered drugs (Granisetron or Ondansetron) still existed and paclitaxel could not be fully separated.

Typical chromatograms of extracted samples (with and without paclitaxel and docetaxel) from plasma using the pH 3.0 buffer system are shown in Figure 3-8.

Compared with the previous pH 5.0 buffer system (Figure 3-7), the pH 3.0 buffer system could remove the interference peak from the peaks of interest. This could be seen in both the control sample (only including co-administered drugs) and the test sample (containing all co-administered drugs, paclitaxel and docetaxel) as shown in Figure 3-8.

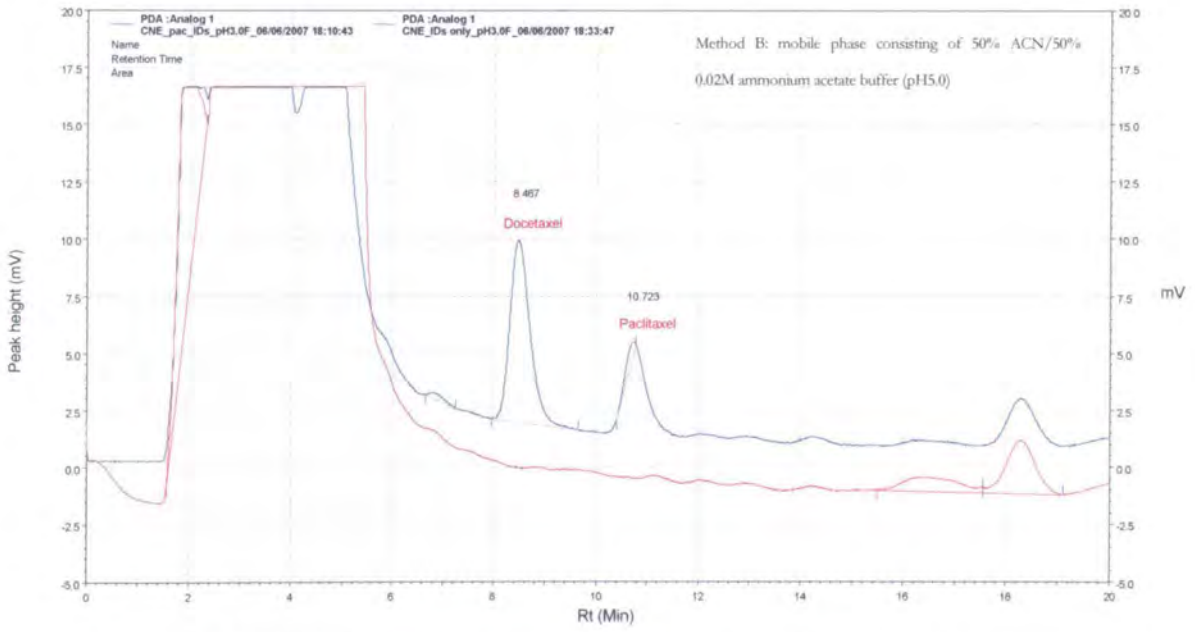

Figure 3-8 Typical chromatograms of plasma samples after SPE extraction (pH 3.0 system)

— : (Test sample) plasma spiked with paclitaxel (120 ng/mL), internal standard (docetaxel, 400 ng/mL) and all co-administered drugs

— : (Control sample) plasma spiked with all co-administered drugs but without paclitaxel and docetaxel

Recoveries (%) of drugs at pH 3.0 were compared with those at pH 5.0 (previous system). With respect to the pH 3.0 system during SPE, the recoveries of paclitaxel and docetaxel were 96.7% and 95.7%, respectively. With the pH 5.0 buffer system, paclitaxel and docetaxel had recoveries of 109% and 110%, respectively.

From this experiment, pH 3.0 buffer system was proven to be the optimal buffer system for SPE, as the previous interference peak due to other co-administered drug

was successfully removed and the paclitaxel peak clearly separated from other peaks. Recoveries of paclitaxel and docetaxel were fairly good at pH 3.0 compared with those obtained at pH 5.0. And the precisions (CV%) of the recoveries at pH 3.0 were 4.7% and 4.4% for paclitaxel and docetaxel, respectively (n = 4).

However, the application of the pH 3.0 buffer system during SPE was also associated with some disadvantages. Firstly, during sample loading the SPE cartridge was easily blocked at pH 3.0 due to protein precipitation compared with previous pH 5.0 system; secondly, peak sensitivity (peak height) seemed reduced compared with the previous pH 5.0 system. However, these problems were resolved by the addition of protein precipitation (Section 3.3.1.2.4, see below) and the adjustment of mobile phase compositions (Section 3.3.1.1.1, p.104), respectively.

3.3.1.2.4 Protein precipitation

After optimisation of the pH buffer system for SPE (Section 3.3.1.2.3, see above), a major problem of cartridge blockage during loading occurred more frequently. This may be due to pH 3.0 being closer to the isoelectronic point (pI) of proteins in human plasma, which can result in protein precipitation.

Figure 3-9 shows examples of blocked cartridges during loading with the pH 3.0 buffer system. At the bottom of the cartridge, a layer of precipitated protein is visible. This had seriously increased the experimental time for each SPE experiment and caused experimental failure.

To resolve the above problem, a pre-SPE step of protein precipitation was introduced. Sample matrix was made by mixing an equal volume of pH 3.0 buffer with the plasma sample in a 15 mL centrifuge tube. This sample was left to stand for 1 hour at 2 – 8°C

with light protection before it was centrifuged at 3000 g for 10 min (at 4°C). 5 mL of the supernatant would be subjected to SPE, followed by HPLC assay (Method B).

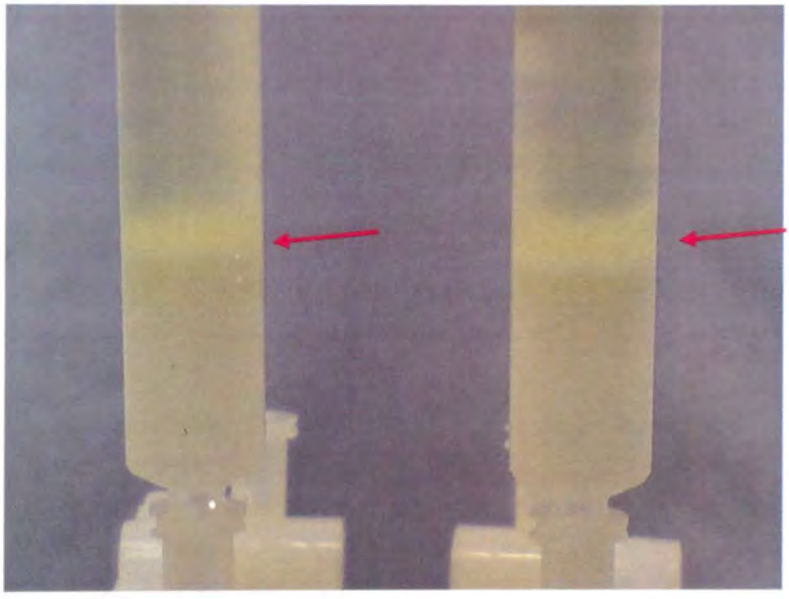

Figure 3-9 Photograph of blocked SPE cartridges during sample loading

→ *The layers of precipitated protein that blocked the cartridges*

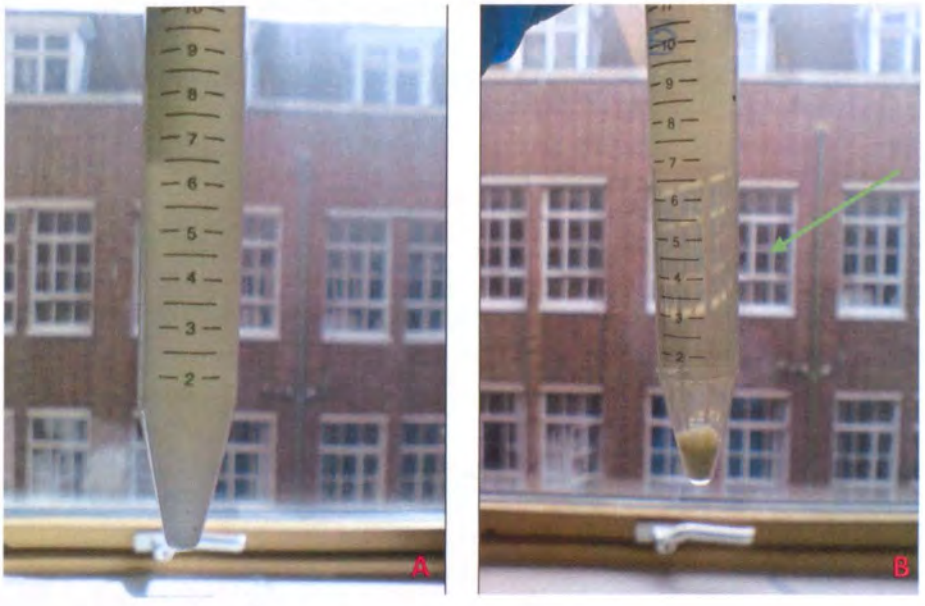

Figure 3-10 Photograph of a sample matrix before (A) and after the protein precipitation step (B)

→ *The supernatant part that would be subjected to SPE*

It was observed that the addition of the protein precipitation step could clarify the plasma sample (Figure 3-10). After the introduction of this step, the loading time was reduced by nearly 70% (from about 40 min to 12 min). This pre-SPE step successfully prevented SPE cartridges from being blocked. This step did not affect the recoveries of paclitaxel and docetaxel (101% and 97%, respectively).

3.3.1.2.5 Filtration

After SPE a few visible particles from plasma were observed in some extracted samples. To prevent these particles from damaging the HPLC column, the reconstituted samples were passed through a 0.2 μm filter membrane before injection.

Polyvinylidenefluoride (PVDF) membranes are commonly used to filter aqueous samples, and were considered to be suitable for this study where drugs were reconstituted in ACN/water (50/50, v/v). However, an experiment was still conducted to further assure that PVDF was the optimal membrane with less leaching and no interference with peaks of interest. Many filter membranes made of different materials including PVDF, polytetrafluoroethylene (PTFE), Nylon, and polypropylene (PP), were tested for comparison.

The blank control (ACN/water, 50/50) was drawn using a syringe and needle, and then passed through each filter in turn prior to HPLC assay (Method A, refer to Table 3-2). All the chromatograms for each filter membrane are shown in Appendix 5 (p.297). It was interesting that a large peak at a retention time of 28 – 31 min was observed with all types of filters. This large peak was identified as a leaching component from the membrane themselves as no peak was found with a blank sample before filtration and a blank sample exposed only to the needle and syringe. However, this large peak did not interfere with either paclitaxel (Rt = 6.8 – 7.0 min) or docetaxel

(Rt = 6.0 – 6.2 min). Among all these types of filters, PVDF filter resulted in a much cleaner baseline; others produced noise peaks (especially PP membrane), some of which appeared at retention time of around 4 – 10 min and definitely could cause some interference to paclitaxel and docetaxel. On this basis, PVDF filters were selected for use in later studies. In addition, to avoid any unnecessary interference due to membrane leaching, the filters were pre-washed with ACN/water (50/50, v/v) and dried in air before use.

Additionally, recoveries of paclitaxel and docetaxel after filtration through PVDF filters were checked as shown in Table 3-12. The average recovery (%) was 99.0% for paclitaxel and 98.6% for docetaxel (n = 2), which showed filtration through a PVDF membrane had no significant influence on the recovery of either drug.

Table 3-12 Recovery (%) of paclitaxel and docetaxel after filtration through PVDF membranes

	Paclitaxel	*Docetaxel*
Recovery %	99.0	98.6

n=2
Concentration of paclitaxel = 600 ng/mL; Concentration of docetaxel = 2 μg/mL
Samples were analysed using Method A (refer to Table 3-2).

3.3.1.3 Final statement of experimental conditions for the paclitaxel assay in human plasma

3.3.1.3.1 Chromatographic conditions

The finalised chromatographic conditions after optimisation were described in Table 3-13. These were used in all subsequent studies.

Table 3-13 Chromatographic conditions after optimisation for use in paclitaxel assay in human plasma

Instruments	a model PU-2080 pump, a model AS-2055 auto-sampler and a model MD-2010 diode array detector (all from Jasco)
Tubing size between autosampler and detector	7/1000 inch
Column	Waters Spherisorb ODS2 column (5 µm, 150 × 2 mm) in combination with a 4 × 2 mm C18 guard cartridge
Column temperature	25°C
Mobile phase	50% ACN / 2% THF / 48% 0.02 M Ammonium acetate buffer (pH 5.0), degassed by filtration and sonication prior to use
Flow rate	0.2 mL/min
Detection wavelength	227 nm

3.3.1.3.2 Protein precipitation and solid-phase extraction (SPE)

120 µL of internal standard solution (10 µg/mL) was added to 3 mL of the plasma sample, followed by addition of 2.88 mL of pH 3 buffer (0.1 M formic acid) followed by vortexing and kept at 2 – 8°C with light protection (for protein precipitation by pH change). After one hour, the sample matrix was subjected to centrifugation (at 4°C) at 3000 g for 10 minutes. 5 mL of the supernatant (equivalent to 2.5 mL of the paclitaxel plasma sample) was introduced onto the 500 mg cyano Bond Elut (CN-E) SPE cartridge which was pre-conditioned using 6 mL MeOH and 6 mL pH3 buffer. The cartridge was then washed with 5 mL of pH3 buffer, 5 mL of MeOH/pH3 buffer (2/8, v/v) and 2 mL of hexane. Next, the cartridge was dried under full vacuum and eluted using 1.2 mL of ACN with 0.1% triethylamine in 3 aliquots of 0.4 mL each,

followed by sonication for 2 minutes. The sample was collected in a 2 mL amber screw auto-sampler vial (Chromacol 2-SV, Fisher Scientific, Loughborough, UK). The elution sample was evaporated dry under a nitrogen stream at 30°C and finally the residue was reconstituted in 500 µL ACN/water (50/50, v/v). After passing this sample through a 0.22 µm PVDF filter, 20 µL of sample was injected onto the HPLC column in duplicate. The bracket injection method by injecting unextracted external standard solutions between extracted samples was used to calculate the individual recovery % for each extracted sample.

3.3.1.4 Validation of the paclitaxel analytical assay in human plasma

3.3.1.4.1 Preparation of docetaxel stock and working stock solutions of paclitaxel and docetaxel

Docetaxel stock solution (2 mg/mL): Prepared by dissolving an appropriate amount of docetaxel powder into pure ethanol and stored in the freezer (–20°C) with light protection.

Paclitaxel working stock solution (6 µg/mL): Freshly diluted from paclitaxel stock (6 mg/mL) in ACN/water (50/50, v/v) and stored at 2 – 8°C with light protection.

Docetaxel working stock solutions (100 and 10 µg/mL): Both freshly diluted from the stock (2 mg/mL) in pure ethanol and stored in the freezer (–20°C) with light protection.

3.3.1.4.2 Preparation of unextracted standard solutions in ACN/water

Paclitaxel working stock solution (6 µg/mL) was freshly diluted volumetrically to make up 50, 100, 200, 500, 1000 and 1500 ng/mL standard solutions in ACN/water (50/50, v/v), with the addition of the appropriate volumes of docetaxel working stock solution

(100 μg/mL) as internal standard to give a final docetaxel concentration of 2 μg/mL in each standard solution.

3.3.1.4.3 Preparation of calibration standard samples in human plasma

Six calibration standards were prepared in citrated human plasma by two steps.

Step 1: Preparation of the plasma solutions for Step 2 (Table 3-14)

Table 3-14 Preparation of the plasma solutions for use in calibration standards (Step 1)

Paclitaxel concentration (ng/mL)	Paclitaxel (6 μg/mL)	Docetaxel (100μg/mL)	ACN/water (50/50,v/v)	Plasma volume	Total volume
1500	1.5 mL	120 μL	0.5 mL	3.88 mL	6 mL
1000	1.0 mL	120 μL	1.0 mL	3.88ml	6 mL
500	0.5 mL	120 μL	1.5 mL	3.88ml	6 mL
200	0.2 mL	120 μL	1.8 mL	3.88ml	6 mL
100	0.1 mL	120 μL	1.9 mL	3.88ml	6 mL
50	50 μL	120 μL	1.95 mL	3.88 mL	6 mL

Concentration of docetaxel in each sample =2 μg/mL
Each solution was prepared in a 15 mL-centrifuge tube.

Step 2: Prepare the calibration standards and quality control samples using the above plasma solutions in Step 1 (Table 3-15)

Table 3-15 Preparation of calibration standards and quality control samples (Step 2)

Concentration (ng/mL)	Plasma solutions made in Step 1	Plasma volume	Total volume
*300	0.6 mL of 1500 ng/mL paclitaxel	2.4 mL	3 mL (n = 7)
200	0.6 mL of 1000 ng/mL paclitaxel	2.4 mL	3 mL (n =4)
*100	0.6 mL of 500 ng/mL paclitaxel	2.4 mL	3 mL (n = 7)
40	0.6 mL of 200 ng/mL paclitaxel	2.4 mL	3 mL (n =4)
20	0.6 mL of 100 ng/mL paclitaxel	2.4 mL	3 mL (n =4)
*10	0.6 mL of 50 ng/mL paclitaxel	2.4 mL	3 mL (n = 7)

Final concentration of docetaxel in each sample = 400 ng/mL
Each preparation was made in a 15 mL centrifuge tube.
** indicates quality control (QC) samples.*

3.3.1.4.4 Calibration plot and linearity

In this study, all results were based on peak height of drugs, as peak height results contributed to a higher precision, compared with peak area results. Three calibration plots were separately produced by plotting the average peak height ratio of paclitaxel and internal standard (docetaxel) against the known paclitaxel concentration on three different weeks. For each plot, the above 6 standard plasma samples were analysed in triplicate (three determinations per concentration). The linearity of the regression lines was calculated by the method of least squares. The correlation co-efficient (R^2), y-intercept and slope of the regression line were calculated. A R^2 value of ≥ 0.99 was acceptable for bioanalytical assays[271].

Concentrations were back-calculated from the average calibration curve. The maximum deviation of these concentrations from the nominal (known) concentrations should be within ±20% at the LLOQ (lower limit of quantification) level and with

±15% at other concentrations. The coefficient of variation (CV) should be less than 20% at the LLOQ level and 15% at other concentrations[248].

3.3.1.4.5 Intra- and inter-day accuracy

The accuracy describes the closeness of the mean measured concentration to the nominal concentration of paclitaxel, and was reported as the percentage of the measured concentration with respect to the nominal concentration. In this study, the intra- or inter-day accuracy was determined by at least 6 determinations of each QC (quality control) sample (10 ng/mL, 100 ng/mL and 300 ng/mL) within-day or between-days. The intra- and inter-day accuracy should be above 80% at the LLOQ level and above 85% at other concentrations[248].

3.3.1.4.6 Intra- and inter- day precision

The precision describes the reproducibility of the repeated individual measures of an analyte, which was represented by the coefficient of variation (CV). The intra- or inter-day precision (CV) was calculated based on at least 6 determinations at each QC sample within-day or between-days. The intra- and inter-day precision (CV) should be within ± 20% at the LLOQ level and ± 15% at other concentrations[248].

3.3.1.4.7 Recovery (%)

Recovery of an analyte was to describe the extraction efficiency of the analytical procedure to extract the analyte and internal standard from the plasma matrix, which was determined with respect to an unextracted standard that represented 100% recovery[248]. In this study, the recovery (%) of paclitaxel or the internal standard was calculated by the percentage of the average peak height (paclitaxel or the internal standard) in the extracted plasma sample with respect to the unextracted external

standard (representing 100% recovery) at three QC concentrations. Although the recovery (%) data may be less than 100%, it should be reproducible.

3.3.1.4.8 Limit of detection (LOD) and lower limit of quantification (LLOQ)

In this study, the limit of detection (LOD) was determined based on the standard deviation of the response and the slope of the calibration curve, expressed as[272]:

$$\text{LOD} = 3.3\,\sigma/S$$

σ is the standard deviation of the response, estimated by the standard deviation of y-intercepts of the regression lines. S is the mean slope of the calibration curves.

The lower limit of quantification (LLOQ) was defined as the lowest concentration measured with an accuracy of 80 – 120% and a precision of ≤ 20%[248].

3.3.2 Part II: Paclitaxel stability in human plasma

3.3.2.1 Stability indicating ability of the paclitaxel HPLC assay

This HPLC method was validated to be stability-indicating by accelerated degradation of paclitaxel under stressed conditions. Paclitaxel solutions (500 ng/mL) made in ACN/water (50/50, v/v) were subjected to either: control (at 5°C), heating (at 55°C), oxidative (6% H_2O_2), acidic (1 M hydrochloric acid) and alkaline (1 M sodium hydroxide) conditions for 1 hour before analysis (the procedure refers to Section 2.2.3.3, p.74). Typical chromatograms are shown in Figure 3-11. Table 3-16 shows there was no significant loss of paclitaxel on exposure to heating (55°C) and oxidative conditions. A 48.2% loss of paclitaxel was observed under acidic conditions and no paclitaxel peak was found after treatment under alkaline conditions. A decreased peak purity (56.5%) was observed after treatment under acidic conditions. No degradation

products interfered with the paclitaxel peak. Similar results have been reported previously [244,246]. It was conducted that this LC assay was stability-indicating for paclitaxel.

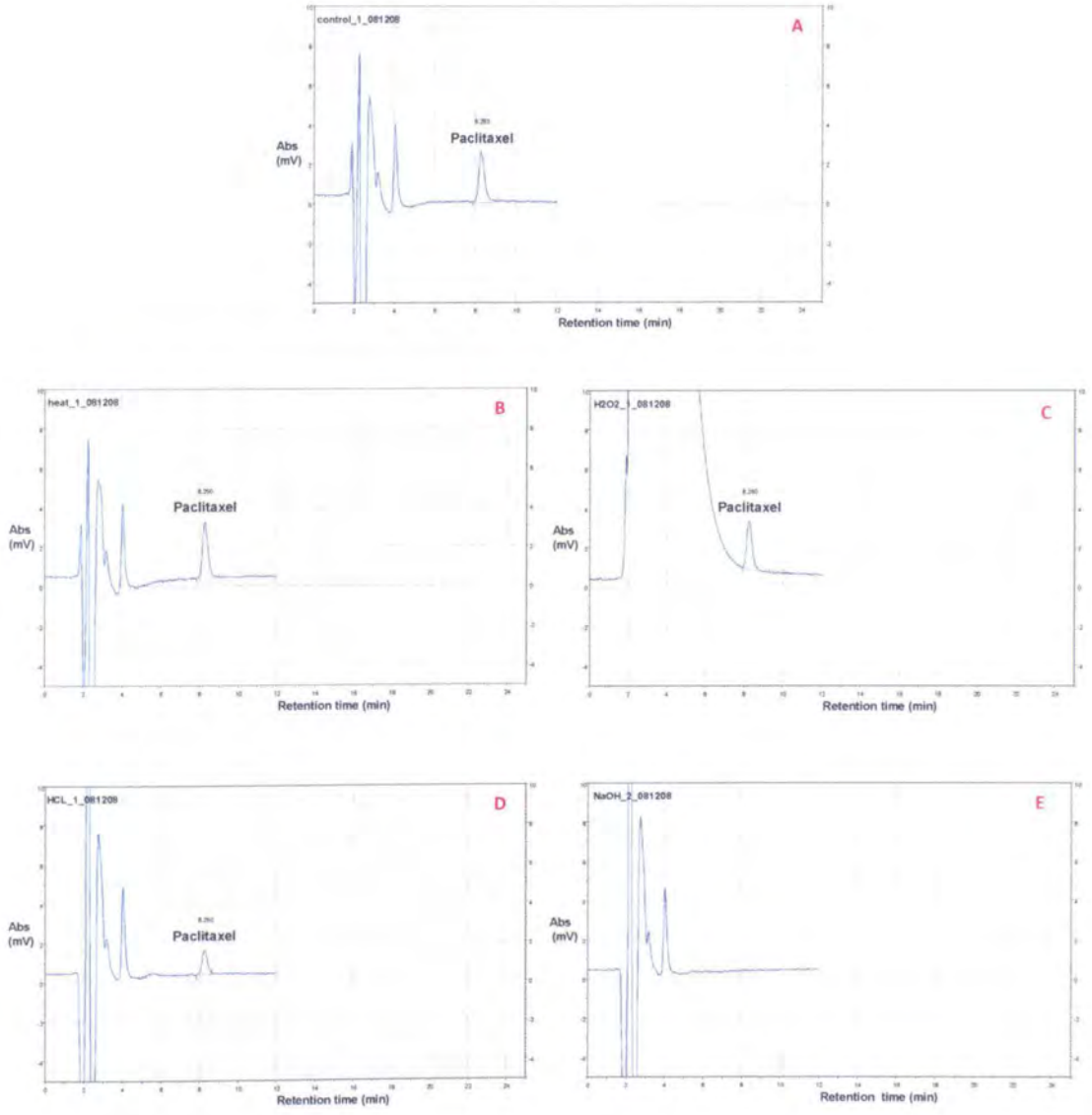

Figure 3-11 Typical chromatograms of paclitaxel solutions (500 ng/mL) in the stability indicating study

A. Control (kept at 5°C for 1 hour); B. Heating (in 55°C water bath for 1 hour); C. Oxidative condition (mixed with 6% H_2O_2 for 1 hour); D. Acid hydrolysis (mixed with 1 M hydrochloric acid for 1hour); E. Alkaline hydrolysis (mixed with 1 M sodium hydroxide for 1 hour);
Chromatographic conditions: an ODS2 column (5 μm, 150 × 2mm) in combination with a 4 × 2 mm C18 guard cartridge; a mobile phase consisting of 50% ACN/2% THF/48% 0.2M ammonium acetate buffer (pH 5.0)

Table 3-16 Stability indicating capability of the LC assay under different conditions (Heating, oxidative, acidic and alkaline)

Treatments	Retention time (min)	Quantity (ng/mL)	Quantity remaining (%)	Peak purity (%)
Control	8.28	500	100	100.00
heating (55°C)	8.27	509.02	101.8	99.36
Oxidative condition	8.27	487.76	97.6	99.94
Acid hydrolysis	8.25	259.18	51.8	56.46
Alkaline hydrolysis	—	—	—	—

n = 2 (each treatment)
Control: kept at 5°C
Oxidative condition: hydrogen peroxide (6%, v/v)
Acid hydrolysis: 1 M hydrochloric acid (pH =0)
Alkaline hydrolysis: 1 M sodium hydroxide (pH = 14)

3.3.2.2 Preparation of paclitaxel plasma samples

Paclitaxel plasma samples (study samples) were prepared in citrated human plasma at concentrations of 30 ng/mL and 300 ng/mL in volumetric flasks, followed by vortexing for over 1 minute and gently inverting the flasks 20 times. Samples of plasma (3 mL) were then transferred into separate 15 mL polypropylene tubes (Fisher, Loughborough, UK). All plasma samples were stored at −20°C in light protected overwraps.

3.3.2.3 Paclitaxel stability in human plasma

3.3.2.3.1 Long-term stability study

At least three replicates of each plasma sample (30 ng/mL and 300 ng/mL) were thawed at room temperature and analysed at different time intervals during the study period (up to 3 months). The mean peak height ratios of paclitaxel vs. internal standard were used to calculate the observed concentrations of paclitaxel in the stability samples in comparison with day zero results of the long-term stability study.

3.3.2.3.2 Short-term stability at room temperature

Paclitaxel plasma samples (30 ng/mL and 300 ng/mL, n = 4) were thawed from −20°C to room temperature (22 – 24°C) and kept at room temperature for over 5 hours before protein precipitation and SPE. The mean peak height ratios of paclitaxel vs. internal standard (docetaxel) were compared with those samples analysed immediately after being thawed without standing at room temperature.

3.3.2.3.3 Freeze and thaw stability

Three replicates at each of two paclitaxel concentrations of 30 ng/mL or 300 ng/mL were frozen at −20°C after preparation for 24 hours and then thawed at room temperature (22 – 24°C). The freeze/thaw (F/T) cycle was repeated three times. Samples were analysed after the third cycle. The mean peak height ratios were compared with samples not subjected to F/T cycles.

3.3.2.3.4 Stability of elution samples in the refrigerator

12 replicates of each plasma sample (30 and 300 ng/mL) were subjected to SPE on the same day. Elution samples were then kept in the refrigerator (2 – 8°C). Two replicates of elution samples at each concentration were evaporated, reconstituted and analysed on day 0, 3, 7, 10 and 14 after refrigeration.

3.3.2.3.5 Stability of the reconstituted samples in the autosampler

Reconstituted samples in ACN/water (50/50, v/v) at two paclitaxel concentrations (30 ng/mL and 300 ng/mL) were analysed on Day 0 and then kept in the autosampler at room temperature (20 – 24°C). Stability was checked after different autosampler residence times (up to 72 hours). The % paclitaxel remaining was calculated with respect to the Day 0 concentration.

3.4. Results

3.4.1 Part I: Validation of paclitaxel analytical assay in human plasma

Peak height ratios at 6 different paclitaxel concentrations are listed in Table 3-17. A typical chromatogram is shown in Figure 3-12. Paclitaxel had a retention time of 8.0 minutes and the internal standard (docetaxel) had a retention time of 6.8 minutes.

Table 3-17 Details of LC peak height ratios (paclitaxel / internal standard) obtained at different concentrations in the calibration studies

Paclitaxel concentration (ng/mL)	*10	20	40	*100	200	*300
Study 1	0.042	0.084	0.162	0.375	0.781	1.093
	0.041	0.085	0.158	0.367	0.778	1.060
	0.044	0.088	0.156	0.370	0.765	1.067
	0.043	0.082	—	0.380	0.758	1.072
	0.044	—	—	0.382	—	1.053
	0.041	—	—	0.378	—	1.080
	0.040	—	—	0.380	—	1.082
Study 2	0.043	0.084	0.153	0.343	0.772	1.101
	0.043	0.078	0.150	0.346	0.760	1.072
	0.043	0.078	0.147	0.341	0.753	1.068
	0.042	0.080	0.152	0.337	0.772	1.074
	0.044	—	—	0.336	—	1.067
	0.046	—	—	0.336	—	1.070
	0.043	—	—	0.389	—	1.114
Study 3	0.038	0.066	0.135	0.333	0.684	1.112
	0.038	0.072	0.131	0.344	0.680	1.114
	0.039	0.069	0.127	0.346	0.678	1.115
	0.041	0.064	0.142	0.339	0.659	1.114
	0.038	0.068	—	0.341	—	1.079
	0.037	—	—	0.342	—	1.077
	0.037	—	—	0.332	—	1.056
	—	—	—	—	—	1.064
Mean	***0.041***	***0.077***	***0.147***	***0.354***	***0.737***	***1.082***
Std	0.003	0.008	0.012	0.020	0.046	0.021
RSD%	*6.39*	*10.54*	*7.95*	*5.55*	*6.30*	*1.91*

** indicate quality control (QC) samples (used for calculation of intra- or inter-day precision/accuracy and recovery data)*
Ratio: mean peak height ratio of paclitaxel vs. docetaxel
Std : standard deviation
RSD%: relative standard deviation (%) = CV%

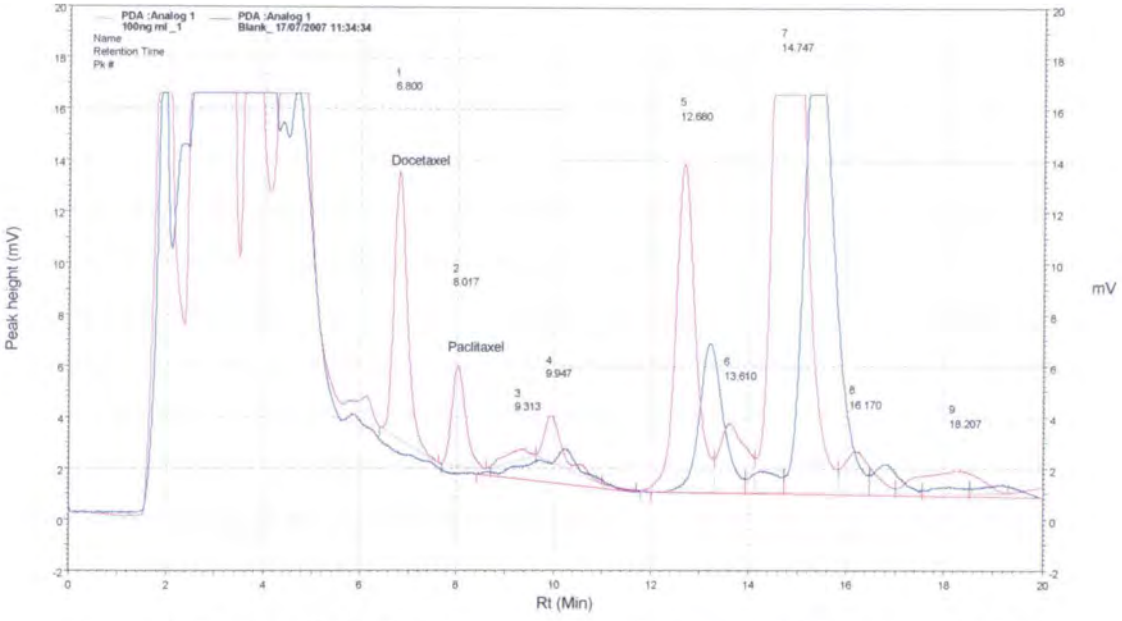

Figure 3-12 A typical chromatogram of paclitaxel (100 ng/mL) and the internal standard (docetaxel 400 ng/mL) extracted from a plasma sample (—) compared with a blank plasma sample (—)

3.4.1.1 Calibration plot and linearity

A linear range of 10 – 300 ng/mL was produced for paclitaxel in human plasma. The mean calibration plot (Figure 3-13) was produced based on the mean peak height ratios (paclitaxel/ internal standard) versus the known concentrations of paclitaxel: Ratio = 0.0036 × (Conc. Paclitaxel at ng/mL) + 0.0036, with an average correlation coefficient (R^2) of 0.9997. The least-squares regression data of three calibration plots are shown in Table 3-18. The correlation coefficients (R^2) were all better than 0.9978. The slopes of calibration plots were all 0.0036 and the y-intercept ranged from –0.01 to 0.016.

The relative standard deviations (R.S.D. %) of the peak height ratios were 6.39% at 10 ng/mL and were all below 15% at other concentrations. Also, the deviations of the

measured concentrations from the nominal concentrations were less than 20% at 10 ng/mL and below 15% at other concentrations.

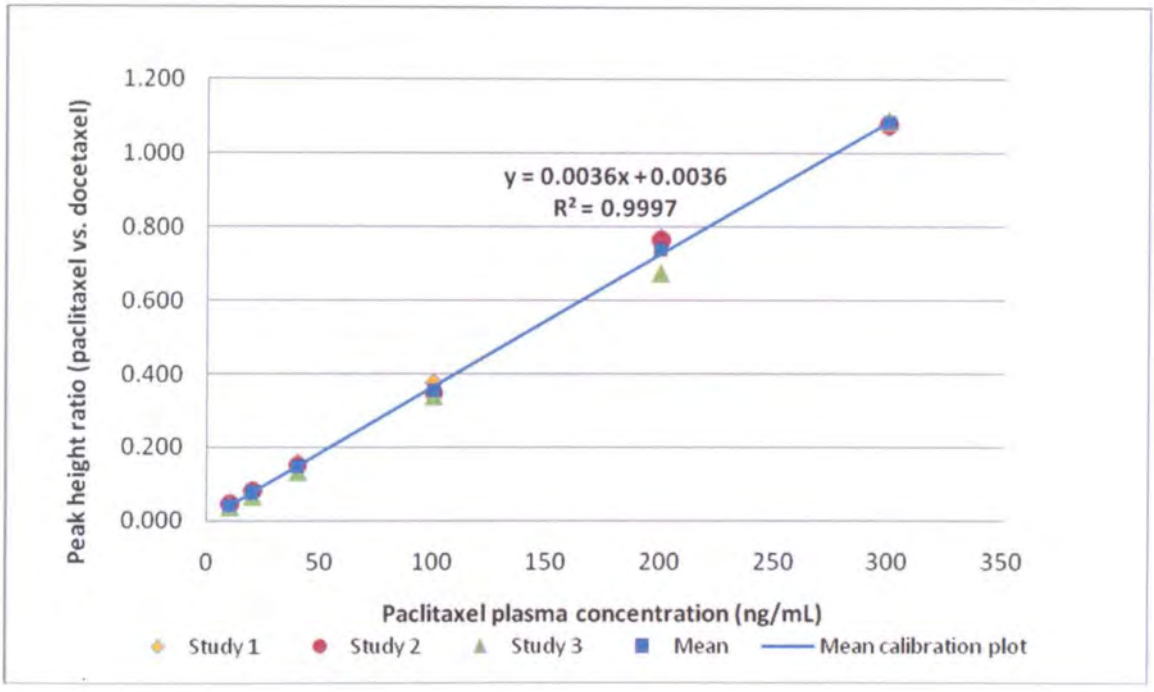

Figure 3-13 Mean calibration plot for LC assay of paclitaxel in human plasma based on three calibration studies carried on different weeks

Table 3-18 Mean R^2, slope and y-intercept of calibration plots in three calibration studies

Study	1st	2nd	3rd	Mean
R^2	0.9979	0.9981	0.9978	0.9979
Slope	0.0036	0.0036	0.0036	0.0036
y-intercept	0.0155	0.0052	-0.0100	0.0036

R^2: the correlation co-efficient of a calibration plot

3.4.1.2 Intra- and inter-day accuracy and precision

The assay performance data are shown in Table 3-19. The intra-day accuracy was 104.8%, 97.4% and 99.8% at paclitaxel concentrations of 10, 100 and 300 ng/mL, respectively, and the inter-day accuracy ranged from 97.4% to 104.8%. The deviations of intra- and inter- day accuracy were all within 5%.

The intra-day precision was 3.6, 2.8 and 1.8% and inter-day precision were 6.4, 5.6 and 1.9% at 10, 100 and 300 ng/mL levels, respectively. They were all below 10% at all concentrations.

3.4.1.3 Recovery

The average recovery (R%) of paclitaxel was 111.7% for 10 ng/mL, 97.5% for 100 ng/mL, and 93.3% for 300 ng/mL, with a CV% of 3.3%, 2.0% and 8.7%, respectively. For the internal standard (docetaxel), an average R% was 97.3% obtained with an overall CV% of 6.5% (Table 3-19).

Table 3-19 Validation characteristics of paclitaxel assay in human plasma

Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)		Precision (%)		Recovery %
		Intra-	Inter-	Intra-	Inter-	
10	10.48	104.77	104.77	3.61	6.38	111.7± 3.7
100	97.38	97.38	97.38	2.82	5.55	97.5 ± 1.9
300	299.56	99.81	99.85	1.78	1.91	93.3 ± 8.1

Intra-, intra-day; Inter-, inter-day
$n \geq 7$ for intra-day accuracy/precision
$n \geq 21$ for inter-day accuracy/precision and recovery %

3.4.1.4 Limit of detection (LOD) and lower limit of quantification (LLOQ)

Based on the standard deviation of the response and the slope of the calibration plot (criteria refer to Section 3.3.1.4.8, p.131), the LOD of this assay was defined as 6.6 ng/mL.

Since in this study the low concentration (10 ng/mL) was quantified with acceptable accuracy of 104.8% (within 80 – 120%) and precision of < 6.4% (below 20%), a paclitaxel concentration of 10 ng/mL was defined as the LLOQ according to the criteria (Section 3.3.1.4.8, p.131).

3.4.1.5 Specificity

Different batches of blank plasma samples (no drug contained) were tested using the optimised method for any interference due to endogenous products in the plasma. No endogenous peaks were observed interfering with paclitaxel and the internal standard (a typical chromatogram is shown in Figure 3-12).

Using the optimised mobile phase (Table 3-13), 11 co-administered drugs were tested again for the chromatographic characteristics. The details of retention times of these drugs are shown in Table 3-20, which showed Metoclopramide and Granisetron possibly causing interference to paclitaxel. However, the SPE procedure removed all interference peaks for both paclitaxel and docetaxel (Figure 3-14).

Table 3-20 Chromatographic characteristics of potential co-administered drugs with paclitaxel

Drugs	Rt (min)
Paclitaxel	7.9
Docetaxel	6.7
Dexamethasone	2.2
Ranitidine	3.6
Cyclizine	—
Metoclopramide	8.4
Pamidronate	—
Carboplatin	1.9
Doxorubicin	4.8
Tamoxifen	—
Clonazepam	4.3
Granisetron	8.9
Ondansetron	9.7

Rt: retention time

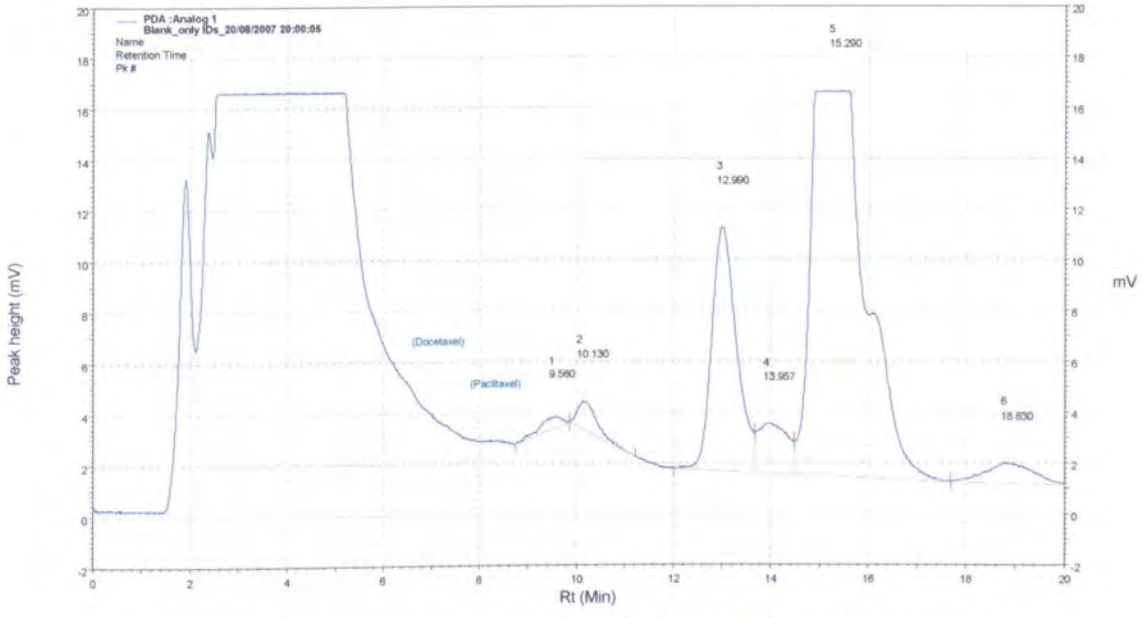

Figure 3-14 A typical chromatogram of a blank control sample containing all potential co-administered drugs only

3.4.2 Part II: Paclitaxel stability in human plasma

The aim of the stability study for paclitaxel in plasma was to support the experimental design of later clinical studies and to test the robustness of the methods used. In the FDA guidance of Bioanalytical Method Validation[248], the precision and accuracy determined at each calibration standard concentration should not exceed 15% except for at the LLOQ, where it should not exceed 20%. Therefore, for the stability of paclitaxel in plasma, the acceptance criteria of accuracy (the remaining ratio %) and precision were defined to be within ±15% at both test concentrations (30 ng/mL and 300 ng/mL).

3.4.2.1 Long-term stability

Results are shown in Table 3-21. Paclitaxel (300 ng/mL in plasma) was stable for up to 3 months with intra-day precision from 1.0% to 4.0% during the study period. Paclitaxel 30 ng/mL was found stable for up to 2 months with intra-day precision from 2.5% to 6.7%. Paclitaxel concentration remained at 107.2% (30 ng/mL) and 105.7% (300 ng/mL) after 2 months storage at −20°C and at 97.9% (300 ng/mL) after 3 months storage.

Table 3-21 Long-term stability of paclitaxel in plasma at –20°C

Nominal concentration (ng/mL)	Day	Ratio [a]	Observed concentration (ng/mL) [b]	Remaining % [c]	n [d]	CV % [e]
30	0	0.096	30.00	100.00	3	2.49
	20	0.094	29.52	98.38	3	2.70
	35	0.093	29.27	97.56	6	2.49
	54	0.092	28.73	95.76	3	6.65
	60	0.103	32.14	107.15	4	4.78
300	0	1.106	300.00	100.00	3	1.25
	9	1.119	303.71	101.24	3	1.72
	16	1.076	292.02	97.34	3	1.29
	23	1.140	309.29	103.10	3	2.05
	32	1.099	298.13	99.38	3	4.00
	60	1.168	316.98	105.66	3	1.76
	71	1.158	314.25	104.75	3	1.00
	92	1.083	293.72	97.91	3	1.55

Storage conditions: –20 °C in polypropylene tubes in light protected overwraps.
[a] Ratio = mean peak height ratio of paclitaxel vs. docetaxel
[b] Observed concentration = (ratio on Day n/ ratio on Day 0) × Conc. on Day 0
[c] Remaining % = (observed conc. / Day 0 conc.) × 100 %
[d] n = number of replicates
[e] CV% = intra-day reproducibility/precision

3.4.2.2 Short-term temperature stability

After 5 hours at room temperature, paclitaxel in plasma remained at 99.6% for 30 ng/mL (intra-day precision 6.0%), and at 100.3% for 300 ng/mL (intra-day precision 1.5%), compared with samples analysed immediately after thawing (Table 3-22).

Table 3-22 Short-term (5 hours) stability of paclitaxel at room temperature

Nominal concentration (ng/mL)	Ratio	Observed concentration (ng/mL)	[a]Paclitaxel remaining %	n	CV %
30	0.10	29.88	99.60	4	5.96
300	1.11	301.01	100.34	4	1.46

Storage conditions: room temperature (22 – 24℃) with light protection for over 5 hours before further treatment.
[a]paclitaxel remaining % with respect to samples analysed immediately after thawing

3.4.2.3 Freeze and thaw stability

After 3 x F/T cycles, paclitaxel remaining was at 105.1% and 102.6% with intra-day CV% of 3.9% and 1.1% for 30 and 300 ng/mL respectively. This means paclitaxel was stable for up to 3 × F/T cycles without loss compared with freshly made samples (Table 3-23).

Table 3-23 Freeze and thaw (F/T) stability of paclitaxel after 3 F/T cycles

Nominal concentration (ng/mL)	Ratio	Observed concentration (ng/mL)	Paclitaxel remaining %	n	CV%
30	0.10	31.52	105.07	3	3.91
300	1.13	307.80	102.60	3	1.11

3.4.2.4 Stability in the elution solvent

The stability of paclitaxel in the elution solvent (ACN plus 0.1% triethylamine) after extraction (kept at 2 – 8°C) is shown in Table 3-24. After 14 days of storage, the average peak height ratios (paclitaxel/IS) were 0.095 and 1.135 with inter-day precisions of 5.3% and 3.2%, at 30 ng/mL and 300 ng/mL, respectively.

Table 3-24 Paclitaxel stability (30ng/ml & 300ng/ml) in elution solvent at refrigerator (2 – 8°C)

Concentration 30ng/mL	Day	Ratio	Observed concentration (ng/mL)	Paclitaxel remaining %	CV%
	0	0.102	30.00	100.00	4.01
	3	0.098	28.67	95.58	4.41
	7	0.089	26.20	87.34	2.59
	10	0.092	27.06	90.19	2.42
	14	0.094	27.70	92.34	3.20
	Mean	0.095			
	SD	0.005			
	Inter-day CV%	5.262 (n = 10)			
Concentration 300ng/mL	Day	Ratio	Observed concentration (ng/mL)	Paclitaxel remaining %	CV%
	0	1.109	300.00	100.00	0.6
	3	1.094	296.00	98.67	0.60
	7	1.185	320.61	106.87	0.94
	10	1.157	313.03	104.34	0.99
	14	1.129	305.26	101.75	0.41
	Mean	1.135			
	SD	0.037			
	Inter-day CV%	3.235 (n = 10)			

Elution solvent: acetonitrile plus 0.1% triethylamine
n = 4 replicate injections (duplicate injections for each of 2 samples) at each concentration on each day
SD = standard deviation
Inter-day CV%: inter-day reproducibility (n = 10)

3.4.2.5 Stability of reconstituted paclitaxel extracts in the autosampler

The stability of paclitaxel in the autosampler was tested at room temperature to determine if samples were stable during autosampler runs or if they could be re-injected in case of instrument or sample run failure. After reconstitution in ACN/water (50/50, v/v), paclitaxel samples were kept in the autosampler and stability was determined after 24, 48 and 72 hours separately. Paclitaxel was found stable for up to 72 hours since it remained at 104.2% and 101.2% of initial concentrations, for 30

ng/mL and 300 ng/mL, respectively, with an average precision of 6.1% and 1.1% (Table 3-25).

Table 3-25 Summary of paclitaxel stability after different residence times in the autosampler

	Concentration (ng/mL)	Paclitaxel remaining %	n	CV%
Over 24hr	30	101.02	4	2.52
	300	98.52	12	1.04
Over 48hr	30	99.02	3	4.55
	300	101.80	6	2.75
Over 72hr	30	104.19	7	6.06
	300	101.15	6	1.12

Storage conditions: kept in the autosampler at room temperature (20 – 24 ℃)

3.5. Discussion

3.5.1 Sample pretreatment

Protein precipitation by pH adjustment was successfully introduced to resolve the problem of cartridge blockage. The addition of pH 3.0 buffer (0.1 M formic acid plus triethylamine) into the plasma sample followed by standing for 1 hour before SPE reduced the solubility of plasma proteins and thus induced protein precipitation. Through this protein precipitation step, the loading time was greatly reduced (around 70%) and the loading speed was about 0.3 – 0.5 mL/min. With this step, the recovery of paclitaxel and docetaxel was not compromised at 100.8% (paclitaxel) and 97.3% (docetaxel).

As for SPE, there are many types of cartridges available such as cyano (CN), C2, C4 and C18, which have been applied to extract paclitaxel from biological fluids[137,142,144,157]. Since plasma is an aqueous environment and paclitaxel is very non-polar, all these above SPE cartridges may be considered. To optimise recovery, three types of SPE

cartridges were evaluated (Section 3.3.1.2.1, p.108). They were end-capped cyano (CN-E), C8 and C18 with increased retention strength. They all provided extraction of paclitaxel with acceptable precision. However, both C8 and C18 had stronger retention for paclitaxel which could potentially reduce the extraction recovery. In a clinical study a high sensitivity is required as paclitaxel concentrations in plasma could vary. Thus CN-E was selected in this study because it contributed to a higher recovery for paclitaxel and less blockage of the cartridge. Moreover, in this study CN-E was compatible with docetaxel with reproducible recovery data, which agrees with Rosing et al[273].

The increase in volume of Wash 1 did not improve the clean-up effect of SPE. 20% solvent in Wash 2 was found to contribute to an acceptable recovery (98.1%) of paclitaxel. Although the hexane wash step (Wash 3) during SPE procedure did not seem to have significant difference on drug recovery and clean-up effect to the plasma sample, it was still kept in the procedure because it may remove some organic impurities with high hydrophobicity in the clinical studies where the sample matrix is more complex.

The pH buffer system had been optimised to remove all potential interference due to certain co-administered drugs (Section 3.3.1.2.3, p.114). At pH 1.0 and 2.0, the SPE bonded phase may suffer from hydrolysis cleavage or drugs were unstable under very acidic conditions so there was no peak of interest identified. At pH $\geq$ 5.0, the paclitaxel peak still experienced interference by certain co-administered drugs and could not be quantified. At pH 3.0, the interference due to certain co-administered drugs was removed and paclitaxel and the internal standard were separated clearly. No significant drug loss was found with paclitaxel and the internal standard and the peak purity was above 99% for paclitaxel and over 97% for docetaxel. Although the pH 3.0 buffer

system for SPE reduced sensitivity of the assay and caused blockage of the cartridge during sample loading, these problems were resolved by optimisation of the mobile phase and addition of the protein precipitation step.

By comparing 4 different types of filter membranes (all 0.22μm), PVDF was considered optimal because it is widely used for aqueous samples and it also contributed to less leaching and interference to peaks of interest (see Appendix 5, p.297). However PVDF filters were still pre-washed before use to avoid any unnecessary interference.

Also, it was interesting to find that syringe needles could leach some materials that may cause interference to both peaks of paclitaxel and docetaxel and different types or batches of needles behaved differently. Therefore, before using needles these were pre-washed with ACN/water (50/50, v/v). However, no leaching and interference was found to be associated with syringes in this study.

3.5.2 Chromatography

The column used was a Waters Spherisorb narrow-bore ODS2 column (5 μm, 150 ×2 mm). A narrow-bore column was used instead of a conventional 4.6 mm ID column because the former could increase the efficiency and sensitivity and was also compatible with LC-MS (planned in later studies). This type of column showed very good reproducibility in performance between different batches. It was also very lasting and reliable as no obvious loss in column performance was observed after frequent use over 1 year. The addition of a guard cartridge contributed to further cleaning the sample, reducing the pump back-pressure and increasing the column life.

The size of tubing used between the auto-sampler and detector was optimised. Smaller tubing size (7/1000 inch) gave a better sensitivity and efficiency, presumably by reducing band-broadening. Changing the column temperature did not affect peak performance in this study.

In this study, various mobile phases at different compositions were evaluated and the results are shown in Appendix 3 (p.290). During method development in this study, the mobile phase was optimised twice and finalised as 50% ACN/2% THF/48% 0.02 M Ammonium acetate buffer (pH5.0) for optimal selectivity and sensitivity. The addition of THF contributed to higher peak efficiency and a sharp peak, which agreed with Martin et al[138]. Both drugs of interest were found to be stable in pH 5.0 mobile phase.

Many different compounds have been previously used as the internal standard in LC assay of paclitaxel, such as cephalomanine, docetaxel, N-octylbenzemide and 2-methyl paclitaxel (Section 1.1.5.1, p.36). Although 2-methyl paclitaxel is a good internal standard to paclitaxel, it is difficult to obtain. Thus in this study, docetaxel was considered a good choice not only because it shared similar chemical and physical properties to paclitaxel but also it was widely available.

3.5.3 Validation of paclitaxel assay in human plasma

This sensitive and specific analytical method has been optimised and developed to quantify paclitaxel in human plasma for the designed clinical study (the study protocol is shown in Chapter 4).

3.5.3.1 Linearity and LLOQ

Results showed acceptable linearity between the peak height ratios versus concentrations with a mean correlation co-efficient (R^2) of 0.9997. The LLOQ has been defined as 10 ng/mL with acceptable accuracy and precision. The sensitivity of this method is comparable with most HPLC assays used for paclitaxel, where the LLOQ or LOQ was usually around 10-25 ng/mL[136,137,138,140]. The sensitivity is sufficient for the determination of paclitaxel in human plasma since the therapeutic window for paclitaxel is usually above plasma concentration 50 nM (≈ 43 ng/mL)[47,54]. In other words, a concentration below 50 nM is usually beyond the range of interest.

The coefficient of variation (CV%) of the peak height ratios were below 15% at all paclitaxel concentrations including LLOQ, and the deviation of the measured concentrations from nominal concentrations were all below 15% at each level. These are all acceptable according to the FDA criteria (Section 3.3.1.4.4, p.129).

3.5.3.2 Intra- and inter- day accuracy and precision

The intra- and inter-day accuracy was all within 100±5% at each QC concentration. And the intra- and inter- day precision at each QC concentration was below 6.4%. According to the acceptance criteria (Section 3.3.1.4.5 and 3.3.1.4.6, p.130 and 130), these results were adequate, indicating that this assay is accurate, reproducible and robust.

3.5.3.3 Recovery

The average recovery was 100.8% for paclitaxel and 97.3% for docetaxel, with an average CV% of less than 8.0% and 6.5%, respectively. The extraction recovery was considered to be acceptable and reproducible compared with previous reports, where

the average paclitaxel recovery was around 80 – 90% and docetaxel recovery was around 94 – 105%[136,137,138,139,140,255].

3.5.3.4 Selectivity

Blank plasma samples containing no drug showed endogenous products caused no interference for both paclitaxel and the internal standard. In addition, plasma samples with all drugs (including paclitaxel, the internal standard and other potential co-administered drugs) and control plasma samples containing only co-administered drugs demonstrated no other drug could cause interference to the peaks of interest after SPE. Therefore, the selectivity of this method was excellent in terms of many endogenous products and co-administered drugs, which is very important in a real clinical study. No previously reported LC assay method for paclitaxel has demonstrated this level of selectivity.

3.5.4 Paclitaxel stability in human plasma

This long-term stability study showed paclitaxel at 30 ng/mL and 300 ng/mL can be stored at –20°C in plasma for at least 2 months without obvious degradation. This agreed with previous studies where paclitaxel at 44 ng/mL and 750 ng/mL were studied in glass tubes[138]. Although, paclitaxel was reported stable in frozen plasma for over 2 years[255], no specific data and concentrations were shown to support this conclusion. In addition, paclitaxel demonstrated good stability for up to 3 months when kept at –70°C[269]. Overall, storage of 2 months for paclitaxel in frozen plasma was considered sufficient for conducting normal clinical and pharmacokinetic studies where samples would be batched for analysis within 2 months.

The results of short-term room-temperature stability also met the acceptance criteria. Paclitaxel (30 ng/mL and 300 ng/mL) were stable when kept at room temperature for up to 5 hours prior to any treatment. This agreed with previous studies[255,267,269] and gave confidence should any delay happened during the sample preparation process.

After 3 × F/T cycles, paclitaxel was stable without loss compared with freshly made samples. This confirms findings of other studies[255,267,268] and allows for the possibility that thawed samples can be refrozen and subsequently re-thawed for analysis at a later date.

Paclitaxel showed good stability in the elution solvent (ACN plus 0.1% triethylamine) after extraction, for at least 2 weeks when kept at 2 – 8°C. This offers the paclitaxel assay significant flexibility. Elution samples can be stored at 2 – 8° C for a short period (up to 2 weeks) and evaporated, reconstituted and analysed within 14 days without any degradation.

In this study, the single run of each plasma sample by HPLC took 23 min. During each calibration study, there were 33 plasma samples in total for analysis and each one was injected in duplicate. Therefore, the total HPLC analysis time required for each calibration study was over 30 hours including the analysis time of external standard solutions (freshly made in ACN/water). The 72-hour stability in the autosampler provided confidence that samples could be left in the autosampler for lengthy batch analysis without compromising assay validation. This is a longer stability period than reported in previous studies where a 24-hour stability period was reported[268,269,270]. This improvement in stability may have resulted from the use of different solvents for the reconstitution of drugs.

It was concluded that the optimisation and validation of the analytical methods for the determination of paclitaxel in plasma were suitable for clinical and pharmacokinetic studies to evaluate the effect of dose-banding of paclitaxel.

4. CHAPTER 4: A CLINICAL AND PHARMACOKINETIC STUDY FOR COMPARISON OF BSA-BASED INDIVIDUALISED DOSING, DOSE-BANDING AND FLAT-FIXED DOSING FOR PACLITAXEL CHEMOTHERAPY

4.1. Introduction

As described previously, individualised BSA based dosing for paclitaxel chemotherapy is associated with certain limitations. The dose-banding (D-B) strategy or flat-fixed dosing may be promising for paclitaxel chemotherapy. In Chapter 2, sufficient stability periods (15 – 29 days) have been demonstrated with paclitaxel infusions (0.3 – 1.0 mg/mL) at 2 – 8°C, which facilitates the application of the D-B strategy (normally, 14 days is required).

A clinical pharmacokinetic study was designed to evaluate the D-B strategy and flat-fixed dosing by comparison with conventional individualised BSA-based dosing on paclitaxel chemotherapy. Pharmacokinetic measures such as C_{max} and AUC will be used as surrogates of tissue exposure to drug resulting from these three dosing strategies. To avoid excessive blood sampling and patient's inconvenience during sampling, a validated previously published limited sampling strategy (LSS)[274] will be employed in this study.

4.2. Experimental

To initiate this clinical pharmacokinetic study, the following protocol was developed for discussion with potential clinical collaborators and, eventually, submission to Research Ethics and Trust Research and Development Committees.

4.2.1 General information

I. **Protocol Title:** Clinical and pharmacokinetic (PK) study on paclitaxel for comparison of BSA-based individualised dosing, D-B strategy and flat-fixed dosing using a limited sampling strategy (LSS) for PK assessment

Simplified Title on Patient Information Sheet and GP Information Sheet: Paclitaxel blood levels with "dose-banded" dose & individualised dose & flat dose

Protocol identifying number:

Research Ethics Committee (REC) reference number:

EudraCT number:

Date of last version:

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4.2.2 Background information

4.2.2.1 Literature review

Rationale of individualised dosing

Most cytotoxic drugs have a very narrow therapeutic window, which requires individualised dosing of these drugs in order to maintain the optimal effect of drugs and decrease the potential occurrence of toxicity[214,215]. Individualised dosing based on body surface area (BSA) has become the most commonly used practice in chemotherapeutic area. However, this conventional individualised dosing based on BSA lacks the scientific proof and its accuracy has been doubted in many ways (refer to Section 1.1.7.1.1, p.58). It also brings a lot of inconvenience to the hospital and patients, for example, serious delay in chemotherapy, drug wastage and heavy workload in preparation. Demand is increasing in the chemotherapeutic area to optimise this conventional individualised dosing or develop a more efficient alternative method.

Dose-banding (D-B) dosing strategy based on BSA

Dose banding (D-B) strategy was developed based on the above conventional individualised dosing by Plumridge and Sewell in the UK[238]. The concept of this D-B strategy has been introduced previously (Section 1.1.7.3, p.63). This D-B strategy was developed on the basis of individualised dosing. It offers many advantages including reduced waiting time for patients, well-planned cytotoxic reconstitution workload (may reduce dosing error), reduced cost for preparation, facilitated batch preparation and quality-control testing.

Flat-fixed dosing

As introduced previously (Section 1.1.7.2, p.62), flat-fixed dosing means a fixed dose given to all patients. This method is widely used on non-cytotoxic medicines without

narrow therapeutic windows. In terms of the disadvantages of individualised dosing based on BSA, flat-fixed dosing was recommended because it was more convenient and economical for manufactures, hospitals and patients[214,215,226]. However, any change of dose may cause unacceptable toxicity or reduced drug efficacy. Therefore, there should be sufficient data to support a decision to administer cytotoxic drugs at a flat-fixed dose.

Current clinical practice of paclitaxel chemotherapy

Paclitaxel (Taxol) is a type of antitubulin agent that can block cell division by interrupting microtubule dynamics. Paclitaxel has been widely used in the treatment of many cancers in the UK (NICE guidance):

- **Breast cancer:** as the adjuvant therapy following the standard anthracycline and cyclophosphamide (AC) therapy for node-positive breast cancer and the second-line treatment (monotherapy) for metastatic breast cancer
- **Ovarian cancer:** as the first-line treatment in combination with a platinum drug for primary ovarian cancer or as the second-line treatment (monotherapy) for metastatic ovarian cancer
- **Advanced non-small cell lung cancer:** as the first-line treatment in combination with a platinum drug for advanced non-small cell lung cancer (NSCLC)
- **AIDS-related Kaposi's sarcoma:** as the second-line treatment for AIDS-related Kaposi's sarcoma (refractory to lipomal anthracycline)

The common dosage schedules for the above cancers are shown in the following:

- **Breast cancer:** for the adjuvant therapy, a dose of 175 mg/m^2 over 3-hour intravenous infusion repeated every 3 weeks for 4 courses is recommended, following standard anthracycline and cyclophosphamide (AC) therapy. Paclitaxel 175 mg/m^2 over 3-hour infusion with a 3-week interval between courses is suggested for the second-line treatment of breast cancer.

- **Ovarian cancer:** paclitaxel 175 mg/m^2 over 3-hour infusion or 135 mg/m^2 over 24-hour infusion, followed by a platinum compound, at 3-weekly intervals, is recommended for the first-line treatment. For the second-line therapy, paclitaxel monotherapy at a dose of 175 mg/m^2 is given over 3-hour infusion with a 3-week interval between courses.
- **Advanced NSCLC:** paclitaxel 175 mg/m^2 over 3-hour infusion, followed by a platinum compound, at a 3-weekly interval, is recommended for the first-line treatment of NSCLC.
- **AIDS-related Kaposi's sarcoma:** the recommended dose is 100 mg/m^2 administered as a 3-hour intravenous infusion every two weeks.

Dosing strategies for paclitaxel chemotherapy

For the role of individualised dosing for paclitaxel chemotherapy, there are many arguments rising. The reduced inter-patient variability with respect to individualised dosing was mainly due to the behaviour of Cremophor EL (CrEL) in Taxol® formulation[172,173,174]. No significant relationship was identified between BSA and the pharmacodynamic measurement of neutropenia. Thus, no obvious evidence so far can be provided to support the individualised dosing for paclitaxel. Flat-fixed dosing has been suggested for paclitaxel chemotherapy in some studies[178,242]. However, to date, there is lack of enough evidence to support flat-fixed dosing for paclitaxel and more investigation should be conducted to address this issue.

As mentioned previously for the application of D-B strategy, a robust stability period (> 14 days) is required. The long-term physical and chemical stability study (see Chapter 2, p.70) showed that paclitaxel infusions (0.3 mg/mL in 0.9% sodium chloride) stored at 2 – 8°C have a long shelf-life of between 20 – 29 days. This enables paclitaxel to be a suitable drug for the application of D-B strategy.

Furthermore, there have been very few clinical studies to compare these three dosing methods for paclitaxel chemotherapy. Thus, the questions arise if conventional individualised dosing can be simplified using the D-B approach or flat-fixed dosing? Which method would be best among them? This coming study will address these issues. A 3-way cross-over study will be designed to compare conventional individualised dosing, D-B strategy and flat-fixed dosing for paclitaxel chemotherapy.

4.2.2.2 Statement

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

4.2.2.3 Study population

Patients aged from 18 to 70 with solid tumour history may be assessed for eligibility, subject to the inclusion and exclusion criteria (refer to Section 4.2.5.1 and 4.2.5.2, p.165).

4.2.3 Trial objective and purpose

The objectives of this study are to evaluate this dose-banding (D-B) strategy and flat-fixed dosing on paclitaxel chemotherapy by comparison with conventional BSA-based individualised dosing method. Pharmacokinetic measures will be used as surrogates of tissue exposure to drug resulting from these three dosing strategies. Patients will be randomly assigned to three groups. In each group, patients will be given paclitaxel by these three dosing methods in different sequences. The null-hypothesis is that there is no clinically significant difference in deviations of pharmacokinetic measures, e.g. AUC and C_{max}, between D-B method and individualised dosing and between flat-fixed dosing and individualised dosing.

4.2.4 Trial design

4.2.4.1 Aims

Primary objective:

To observe any clinical significant difference in deviation of AUC, CL and C_{max} between D-B strategy, individualised dosing (BSA-based) and flat-fixed dosing methods by using non-compartment and compartment based pharmacokinetic (PK) models.

Secondary objective:

To study the relationship between pharmacokinetic (PK) parameters, e.g. AUC, T_{max} and C_{max}, and pharmacodynamic (PD) measures, e.g. neutropenia, for paclitaxel and to determine if there is any difference in the PK-PD relationship between D-B dosing, individualised dosing (BSA-based) and flat-dosing methods.

4.2.4.2 Study Design (Study Flow Chart)

This is a prospective open label, 3-period cross-over study with a three week wash-out period between treatments, assuming that there are no period or carry-over effects. Standard pre-medications should be given to all patients before the study. At study entry, patients will be randomised to three different cohorts. In each cohort, patients will be given 3 cycles of paclitaxel treatment based on three dosing methods in certain sequence. The allocation of dosing sequences is shown in the following table:

Table 4-1 Cross over design of paclitaxel treatment using three different dosing methods

Treatment cohorts	*Cohort A*	*Cohort B*	*Cohort C*
Cycle 1	DB	IND	FLAT
Cycle 2	IND	FLAT	DB
Cycle 3	FLAT	DB	IND

DB = D-B dosing, IND = individualised dosing, FLAT = flat fixed dosing

The study flowchart is shown below (Figure 4-1):

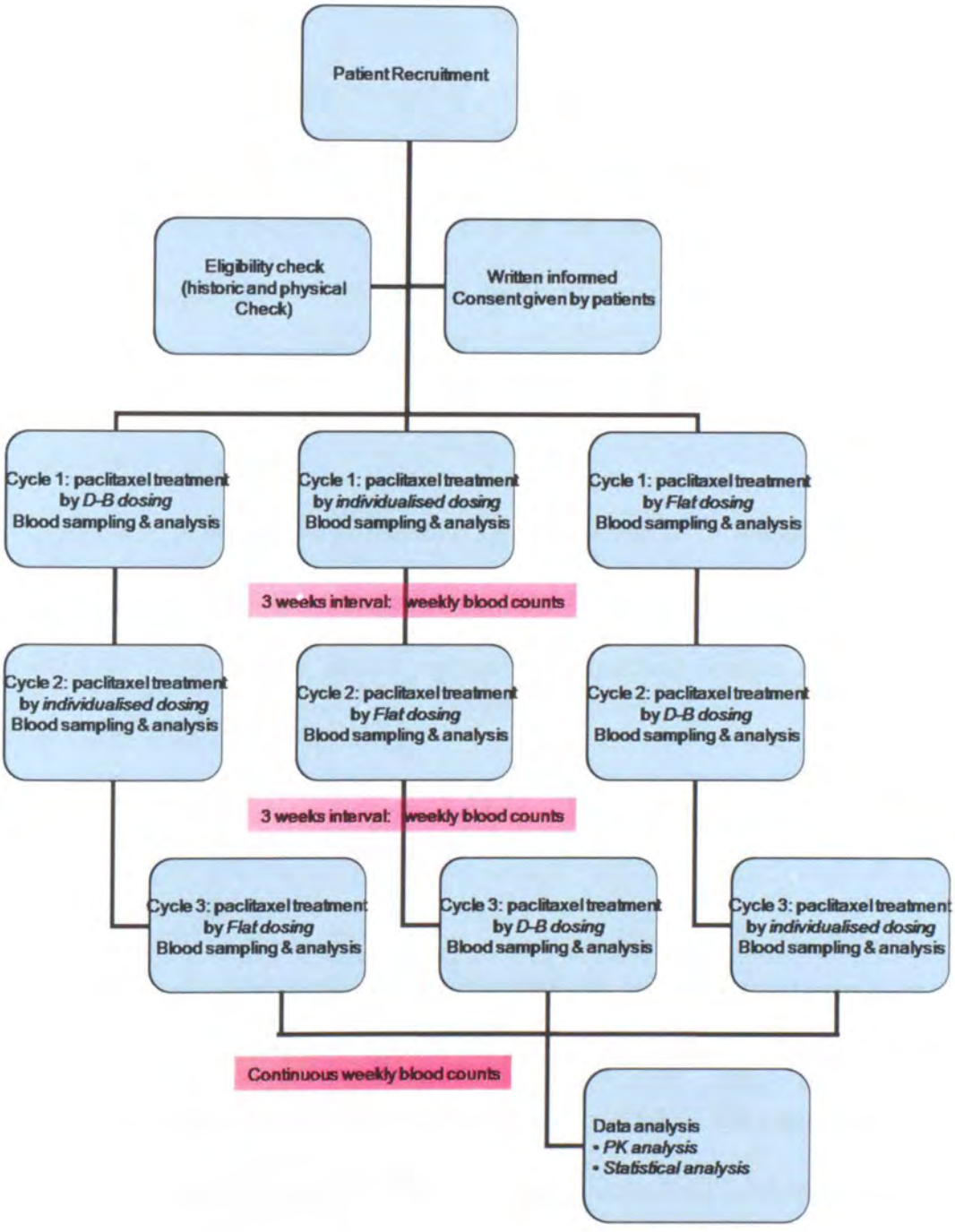

Figure 4-1 Study flowchart of clinical pharmacokinetic study in comparison of different dosing strategies for paclitaxel chemotherapy

4.2.4.3 Randomisation

This is an open label study. However, patients will be randomised into different cohorts to ensure there will be no bias caused by sequence of treatments. Block (restricted) randomisation will be used to keep the balance of the number and the similarity of patients in each group. The details of patient allocation will be kept in hospital pharmacy for later checking.

4.2.4.4 Study duration

The expected maximum study duration for one patient is 13 weeks. After initially inviting a patient, a maximum period of 4 weeks will be needed before the treatment starts. Study involves three formal paclitaxel chemotherapy courses, including three follow-up periods of three weeks.

4.2.4.5 Study treatment

Dosage and schedule

Subjects will receive any paclitaxel dose intravenously by a 3-hour infusion schedule repeated every 3 weeks. The dosage of 175 mg/m^2 will be used to conduct the BSA doses. Patients' BSA will be produced based on the Dubios formula: BSA (m^2) = weight (kg)$^{0.425}$ × height (cm)$^{0.725}$ × 0.007184. D-B doses will be determined according to the dose-banding scheme of paclitaxel chemotherapy (refer to Table 2-7, pg. 93). For flat dosing, a fixed dose of 300 mg will be given to patients based on an average BSA value, 1.75 m^2.

Preparation of paclitaxel infusions

Paclitaxel 6 mg/mL concentrate will be provided as a UK licensed product. All infusions used in this study will be prepared using the same batch of paclitaxel

concentrate. Paclitaxel infusions (0.3 mg/mL in 0.9% sodium chloride solution) for individualised dosing and flat dosing, and pre-filled infusion bags for D-B strategy (details of concentrations and volumes refer to Table 2-8, p.94) will be made under appropriate hospital pharmacy conditions.

Packaging and quality control

All paclitaxel infusions should be clearly labelled immediately after preparation with type of dosing, drug concentration and content, and date of production and expiry.

Quality control testing will be conducted for all drug infusion bags. 1 mL aliquot of solution will be removed from each infusion bag using a 1 mL syringe and contained in a labelled polypropylene tube. All quality control samples will be stored at 2 – 8°C in the hospital pharmacy and will be sent to the clinical pharmacy laboratory of Kingston University for analysis. All quality control samples will be subject to a validated HPLC assay (refer to Section 2.2.2, p.71).

4.2.4.6 Criteria for Discontinuation

Subjects would be withdrawn from the study if they reject to continue treatment, or thought to be drug intolerant, or any failure to fulfil criteria in Section 4.2.5.1 and 4.2.5.2 (p.165) during study, or if an adverse event reported to the Data Monitoring Committee was considered to be drug related and harmful to the subject.

4.2.5 Selection and withdrawal of subjects

This study will be proposed to patients by their oncologist (study co-investigator) prior to commencement of their chemotherapy. Patients will be assessed for eligibility and will be given Patient Information sheets. Patients will make decisions by referring to the information sheet and discussions with their family and GP. Patients who decide

to participate in this study after initial invitation need to return to the hospital within 2 days to give written informed consent. All these should be completed prior to commencement of treatment.

4.2.5.1 Inclusion criteria

- Patients aged over 18;
- Solid tumour history and single or combination paclitaxel chemotherapy considered as a suitable therapeutic option.
- Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 (refer to Appendix 6, p.302);
- No more than one prior chemotherapy or radiotherapy during 4 weeks before treatment;
- At least 4 weeks after surgery;
- Adequate bone marrow function;
- Required initial laboratory data as follows: absolute neutrophil count [ANC] ≥ 1.5×10^9 /L; platelet ≥ 100×10^9 /L; haemoglobin ≥ 6.0 mmol/L; bilirubin < 1.5 mg/dL; aspartate aminotransferase (AST) and alanine aminotransferase < 2.5 × upper limit of normal; creatinine clearance ≥ 60 mL/min;

4.2.5.2 Exclusion criteria:

- Age >70 years
- Patients who have had previous paclitaxel chemotherapy
- Serious, concurrent, or uncontrolled infections
- A history of severe acute hypersensitivity reaction
- Pregnancy and lactation
- Baseline neutrophils <1.5 x 10^9 /L or platelets <100 x 10^9 /L
- Serious hepatic or renal dysfunction

4.2.5.3 Subject withdrawal criteria

Subjects will be free to withdraw from the trial at any stage. These subjects will be replaced to maintain the sample size required for statistical significance. In the event of withdrawal, patients will get continuous standard treatment (paclitaxel treatment by conventional individualised dosing) and regular check-up under full care of the clinical team until the completion of this study.

4.2.6 Treatment of subjects and samples

4.2.6.1 Patient treatment

4.2.6.1.1 Prior to study (Baseline data)

Before study entry, the following information will be collected and recorded in the case record form (CRF).

- General information: initial, gender, age, date of birth, and ethnicity
- Patient study number and study cohort
- Height (m)
- Weight (kg)
- Indication for paclitaxel chemotherapy
- Medical history: especially including the information about prior chemotherapy and radiotherapy
- Physical examination
- Complete blood counts
- Routine chemistry and electrolyte
- Tumour measurement

4.2.6.1.2 On days of Paclitaxel treatment

On the day of paclitaxel treatment, patients will be premedicated with corticosteroids, antihistamines and H_2 antagonists according to trust policy. Paclitaxel doses will depend

on the cohorts that patients randomised into. Individualised doses will be produced using the common DuBois formula. (refer to Section 4.2.4.5, p.163). D-B based doses will be depending on the Dose-banding scheme for paclitaxel chemotherapy (see Table 2-7, p.93). A 300 mg dose of paclitaxel will be given to patients in the flat dosing cohort. All these doses will be administered intravenously for 3 hours under the supervision of an experienced clinician. During treatment, appropriate supportive care will be available in case of hypersensitivity. At defined time points, blood will be taken for analysis (details refer to Section 4.2.6.2, p.168). On the day of paclitaxel treatment, the following information will be taken and recorded in the case record form (CRF):

- Type of dose administered (exact dose of individualised or D-B or flat dosing)
- Treatment cycle number
- Date and clock-time of administration
- Time taken to administer the infusion
- Planned and actual blood-taking time
- All concomitant medications
- Information on radiotherapy, if applicable
- Concomitant illnesses
- Treatment toxicity (refer to NCI Common terminology Criteria for adverse event v3.0)

4.2.6.1.3 Follow up

Medical history, physical examination, complete blood counts, routine chemistry and tumour measurements should be performed before each study cycle. Complete blood count and differential WBC counts should be performed weekly.

4.2.6.2 Sampling and sample analysis

4.2.6.2.1 Blood sampling and limited sampling strategy (LSS)

For patient's convenience, a validated previously published limited sampling strategy (LSS) will be employed in this study[274]. Pharmacokinetic sampling will be only performed before infusion (t =0), at 1, 6 and 24 hour after start of the 3-hour infusion. Each time, 12 mL of blood will be taken from a vein in the opposite arm to the one used for drug infusion and contained in a heparinised tube. After each sampling, the cannula will be flushed with saline and each time before the next sampling, 4 mL of flush liquid should be discarded from the cannula. A total amount of 48 mL of blood will be needed for each patient during one cycle of treatment and 144 mL of blood will be taken over the three study cycles for each patient.

4.2.6.2.2 Sample analysis

Blood samples after collection will be immediately put in the ice and subject to centrifugation at 3,000 g for 30 minutes at 4°C. The plasma supernatant will be transferred into labelled screw-cap polypropylene tubes and stored frozen at –20°C. All plasma samples will be transported in dry ice to the clinical pharmacy laboratory (Kingston University) and kept at –20°C. The samples will be subject to a validated solid phase extraction (SPE) and high-performance liquid chromatography (HPLC) analysis (refer to Chapter 3, p.125) within a month from the collection date. During the process, data on original tubes (blood collecting tubes) will be carefully transferred and labelled accordingly including the following information:

- Patient initials and patient study number
- Study cohort
- Type of dose administered

- Cycle of treatment
- Date
- Sampling time points (pre-set) and actual sampling time points

4.2.6.2.3 Data analysis

The area under the paclitaxel concentration versus time curve (AUC) will be calculated from a limited sampling strategy based on the equation[274]: AUC = 4.7 (concentration at 1 hour) + 10 (concentration at 6 hours) + 0.63; Total body clearance (CL) can be estimated based on the following relation: CL = dose ÷ AUC; The time above the threshold concentration of 0.05 μmol/L ($T_{>0.05\mu mol/L}$) will be calculated based on the equation[274]:

$T_{>0.05\ \mu mol/L}$ = 282 (concentration at 24 hours) + 9.8

Concentration-time profiles of paclitaxel will be estimated using WinNonlin software. All the data will be fitted to non-compartment and two- or three- compartmental pharmacokinetic models.

4.2.7 Assessment of efficacy

In this study, drug exposure to tissues using different dosing ways, including individualised dosing, D-B dosing and flat-fixed dosing, will be evaluated using the pharmacokinetic (PK) parameter, AUC, as a surrogate measure. Difference between these methods is expected to be relatively small because there should be no large differences in paclitaxel doses using these three dosing methods. By using surrogate PK measures, this study will require less time and fewer patients than to evaluate clinical efficacy and long-term toxicity outcomes..

4.2.8 Assessment of safety

4.2.8.1 Definitions

4.2.8.1.1 Adverse event (AE)

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

4.2.8.1.2 Adverse reaction of an investigational medicinal product

All untoward and unintended responses to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship

4.2.8.1.3 Unexpected adverse reaction

An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unapproved investigational product or summary of product characteristics (SPC) for an authorised product).

When the outcome of the adverse reaction is not consistent with the applicable product information, this adverse reaction should be considered as unexpected.

The term "severe" is often used to describe the intensity (severity) of a specific event. This is not the same as "serious," which is based on patient/event outcome or action criteria.

4.2.8.1.4 Serious adverse event (SAE) or serious adverse reaction (SAR)

Any untoward medical occurrence or effect that:

- results in death,
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect.

Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.

4.2.8.2 Expected adverse drug reactions

- Acute hypersensitivity reactions (mainly flushing and rash)
- Bone marrow suppression
- Infection
- Neurotoxicity (mainly peripheral neuropathy)
- Bradycardia
- Hypotension
- Nausea
- Vomiting
- Diarrhoea
- Mucosal inflammation
- Alopecia
- Arthralgia

- Myalgia
- Injection site reactions (including localised oedema, pain, erythema, induration, on occasion extravasation can result in cellulitis)
- Severe elevation in aspartate aminotransferase (AST)
- Severe elevation in alkaline phosphatase

4.2.8.3 Expected serious adverse events

- Heart failure
- Pneumonitis
- Hepatic impairment
- Death

4.2.8.4 Recording, evaluation and reporting of adverse events

Treatment-related toxicity with the drug and dosages employed in the trial is not expected to deviate from that encountered in routine clinical practice. See part 4.2.8.2 for potential toxicities and refer to the summary of product characteristics (SPC) of Taxol by Bristol-Myers Squibb Pharmaceuticals Ltd for further details. To monitor the safety of this trial, all the adverse events (AEs) should be recorded in CRF. Serious adverse events (SAEs) should be reported to sponsor and sponsor will be responsible for evaluating causality (SAE→ SAR) and whether SARs are expected or unusual. Suspected unexpected serious adverse reactions (SUSARs) should be reported to MHRA. All SARs will be reviewed by trial office every two weeks.

4.2.9 Statistics

4.2.9.1 Study statistician

To be confirmed

4.2.9.2 Statistical methods to be employed

The hypothesis testing approach is applied in this study. $\Delta(AUC_{observed} - AUC_{predicted})$ will be determined for dose-banding (or flat-fixed) and individualised dosing, respectively. When comparing the two dosing strategies (e.g. D-B and IND), only the size of the deviations are important, not whether they are positive or negative. Therefore, $\sqrt{(\Delta(AUC_{obs} - AUC_{pred}))^2}$ will be calculated, to get the positive value of the deviations expressed in their original scale.

Differences (d) between deviations for dose-banding (or flat-fixed dosing) and individualised dosing will be calculated:

$$\sqrt{(\Delta(AUC_{observed} - AUC_{predicted})_{DB})^2} - \sqrt{(\Delta(AUC_{oberved} - AUC_{predicted})_{IND})^2} = \Delta_d$$

Δ_d will be analysed with a two-sided one-sample t-test (H_0: $\Delta_d = 0$, H_1: $\Delta_d \neq 0$).

In this study, the significance level is 0.05. Therefore, if the significance value obtained in the t-test is less than 0.05, H_0 is rejected and H_1 is accepted, which means there is significant difference between these two dosing strategies, and vice versa.

4.2.9.3 Sample Size determination

This is a two-sided equivalence test, with the significant level (α) of 5% and a power ($1-\beta$) of 80%. The minimal sample size should be determined by the sample size formula for a bioequivalence study as follows:

$$n = \sigma_d^2(t_{(1-\alpha)} + t_{(1-\beta)})^2/((\mu_A-\mu_B)-\delta)^2$$

σ_d is the standard deviation of δ; $t_{(1-\alpha)}$ and $t_{(1-\beta)}$ are the fractiles in the t-distribution for the levels of significance (α) and power ($1-\beta$); $\mu_A-\mu_B$ is the baseline difference between intra-patient AUC values in samples taken on 2 different cycles; δ is clinical significant difference between individualised dose and banded (or flat-fixed) dose and can be

defined as 0.20 × AUC_{IND} (IND is reference group) as a 20% of baseline difference is usually considered significant[275].

In this study, $t_{(1-\alpha)}$ and $t_{(1-\beta)}$ are 1.96 and 0.8416, respectively. μ_A-μ_B was assumed as 1.42 μmol·h/L as the intra-patient difference when the same dose is given a patient twice. A clinical acceptable difference (δ) is 4.33 μmol·h/L with the standard deviation (σ_d) of 3.02 μmol·h/L from previous studies[47,127,276]. Therefore, the minimal sample size in each cohort is 9 in this study.

4.2.9.4 Number of subjects to be enrolled

At least 9 patients are required in each cohort.

4.2.10 Direct access to source data/documents

All study investigators have direct access to all the trial data and documents at any time. The investigators will permit trial-related monitoring, audits, REC review, and regulatory inspections.

4.2.11 Quality control and quality assurance

- QC samples (blinded to operator) of paclitaxel infusions from all different drug preparations will be analysed.
- QC samples of spiked plasma with known paclitaxel concentrations (blinded to operator) will be analysed in parallel with patient plasma samples to provide QC for the assay system.
- Review meetings will be held monthly at the Hospital and will include all study investigators and collaborators.
- The study will also be regularly reviewed at the Trials Steering meeting in Cancer Research UK, and feedback from the results of the trial will be presented to the Cancer Research UK trials meeting

4.2.12 Ethics

Patient Consent

All patients will freely give their informed consent to participate in the study. Patients may decide to withdraw from the study at any time without prejudice to their future care.

Patient confidential information

In CRF, only the patient initial, date of birth and identification number will be included. Any publication would only include the patient identification number.

Ethical approval

Ethical approval will be obtained by submission to COREC.

Declaration of Helsinki and ICH Good Clinical Practice

The study is to be carried out in conformation with the spirit and the letter of the declaration of Helsinki, and in accord with the ICH Good Clinical Practice Guidelines

Limited sampling strategy

A limited sampling strategy will be applied in this study for blood taking, which can avoid excessive blood sampling and inconvenience for patients.

4.2.13 Data handling and record keeping

All electronic data and paper documents will be stored in secure and locked offices in the hospital pharmacy and the clinical pharmacy department at Kingston University. Data will be available only to the investigators involved in this study.

4.2.14 Financial and insurance matters

Indemnity will be in accordance with the policy of the sponsor trust.

4.2.15 Publication policy

The results of the study will be reported and published in a specialist medical oncology or oncology pharmacy journal, as appropriate.

Authorship will include all listed study investigators, with Mrs Jing Xu listed as the first author.

4.3. Discussion

After designing the study, it was found that breast cancer and non-small cell lung cancer patients eligible to receive paclitaxel chemotherapy were recruited into other national and international clinical trials. This meant it was unlikely that it would be possible to recruit sufficient patients into this study in the finite time-period available. An *ex vivo* pharmacokinetic model was decidedly developed for paclitaxel and a simulation study using this model was conducted to evaluate the D-B strategy and flat-fixed dosing by comparison with BSA-based individualised dosing.

5. CHAPTER 5: EX VIVO SIMULATION STUDY

5.1. Introduction and aim of study

The original intention of this project had been to conduct a clinical study to compare the effects of dose-banding, flat-fixed dosing and individualised-dosing of paclitaxel using a cross-over study with a pharmacokinetic measure (AUC) to determine the amount of drug available to the tissues with each approach. However, the designed clinical and PK study was not conducted in this project because of the difficulty in patient recruitment in the restricted time-period as mentioned in Chapter 4. As an alternative it was decided to explore the use of an *ex vivo* simulation to assess the relative effect of dose-banding compared to conventional chemotherapy with doses individualised according to BSA. Flat-fixed dosing is also under consideration for clinical use[178] and was also included in this study.

Although it is possible to estimate the likely effect dose-banding may exert from theoretical calculations based on variation from the prescribed dose, this would not take into account various other factors introduced by the dose-banding system. These include the accuracy of compounding infusions on a batch scale, the effect of administering more than one infusion in combination, to give the required dose, the rate of administration of multiple infusions compared to a single infusion, and the variation between the banded dose and prescribed dose for each individual patient. Expert practitioners of dose-banding[238,277] and oncologist prescribers[278] have all recommended that clinical or pharmacokinetic studies are conducted to provide evidence of the safety and efficacy of dose-banding.

The aim of this study was to pilot an *ex vivo* system to evaluate the effect of dose-banding. Although it was envisaged from the outset that such a simplified system would compromise the level of information available, it would have the advantage of lower costs and of sparing patients the inconvenience of a clinical trial. At this early stage, the main goal was to evaluate the *ex vivo* system for this purpose and to consider potential refinements that would enhance the system for future use.

The pharmacokinetics of paclitaxel in the human body is very complex (See Chapter 1). It is impossible to reproduce the complicated PK behaviour outside the body by using only simple equipment. However, given the purpose of this study, to compare different dosing strategies for paclitaxel, a simple simulated *ex vivo* model was considered reasonable since all treatment variations would be subjected to the same system.

To date, no other similar study has been reported in the literature to evaluate dosing strategies. This *ex-vivo* PK model was developed based on the PK information of paclitaxel available from literature and this model enabled key aspects of the PK behaviour of paclitaxel (e.g. clearance) to be reproduced. For example, the area under the curve of plasma concentration versus time (AUC) and peak plasma concentration (C_{max}) could be used as comparative measures. Simulation studies were run using the model developed to compare these main PK parameters among different dosing strategies. This could be used to identify any major differences between the different dosing strategies for paclitaxel. At the same time, the *ex vivo* PK model would be tested and validated through this study and the potential of this novel approach and its potential applications could be evaluated.

5.2. System design

5.2.1 Literature basis

Figure 5-1 summarises some available pharmacokinetic information about paclitaxel in literature. The main PK parameters, including the mean total clearance (CL) and the mean distribution volume of the central compartment (V_C), were applied in the *ex vivo* study in order to simulate the behaviour of paclitaxel in the central compartment (systemic circulation) of the body.

Table 5-1 Pharmacokinetic parameters of paclitaxel based on literature

Authors	Schedule ($mg/m^2 \cdot h$)	C_{max} (uM)	AUC (uM·h)	Cl total ($L/h/m^2$)	T ½ α (h)	T ½ β (h)	T ½ γ (h)	V_{ss} (L/m^2)	MRT (h)	^aV_C (L/m^2)	PK model
Gianni L[47]	—	—	—	—	—	—	—	—	—	[b]3.80	2-comp
Huizing MT[279]	135-3	2.54	9.37	17.66	0.20	1.44	14.43	98.32	7.37	5.10	3-comp
	135-24	0.23	7.31	21.81	0.09	2.24	49.76	656.76	43.74	2.83	
	175-3	4.27	16.81	12.69	0.27	2.34	18.75	99.25	9.22	4.94	
	175-24	0.43	9.30	23.55	0.14	1.96	19.63	269.20	23.33	4.76	
Fogli S[280]	100-1	9.10	13.86	7.61	0.24	3.23	*ND*	13.15	1.79	2.64	2-comp
		8.96	14.45	8.25	0.19	2.77	*ND*	14.02	1.66	2.26	
		8.94	12.73	8.57	0.23	4.17	*ND*	14.63	1.70	2.84	
Mross K[281]	150-1	8.85	16.88	11.64	0.21	3.35	*ND*	76.00	*ND*	3.53	2-comp
	175-1	10.12	17.01	12.18	0.30	3.27	*ND*	65.00	*ND*	5.27	
	200-1	15.01	27.86	9.00	0.27	3.07	*ND*	46.00	*ND*	3.51	
	225-1	17.05	31.04	8.98	0.33	0.33	*ND*	58.00	*ND*	4.28	
	250-1	22.45	41.01	8.05	0.33	3.10	*ND*	36.00	*ND*	3.83	
Mross K[242]	175mg (fixed) -3	3.70	7.25	19.70	0.39	10.02	*ND*	*ND*	*ND*	11.09	
Ohtsu T[54]	105-3	2.60	9.23	13.38	*ND*	9.90	*ND*	74.70	*ND*	*ND*	Non-comp
Gianni L[47]	135-3	3.30	10.90	14.80	*ND*	9.20	*ND*	*ND*	*ND*	*ND*	Non-comp
Ohtsu T[54]	135-3	3.94	13.14	12.84	*ND*	16.00	*ND*	113.10	*ND*	*ND*	Non-comp
Gianni L[47]	175-3	5.90	18.50	11.40	*ND*	6.50	*ND*	*ND*	*ND*	*ND*	Non-comp
Ohtsu T[54]	180-3	5.23	19.28	11.40	*ND*	13.70	*ND*	81.70	*ND*	*ND*	Non-comp
Grasselli G[282]	200-3	10.30	31.50	8.20	*ND*	22.40	*ND*	*ND*	*ND*	*ND*	Non-comp
		7.60	23.80	11.60	*ND*	12.60	*ND*	*ND*	*ND*	*ND*	Non-comp
Ohtsu T[54]	210-3	7.90	27.15	10.74	*ND*	13.30	*ND*	58.90	*ND*	*ND*	Non-comp
Gianni L[47]	225-3	7.60	24.30	11.60	*ND*	7.40	*ND*	*ND*	*ND*	*ND*	Non-comp
Ohtsu T[54]	240-3	9.02	31.19	9.06	*ND*	14.60	*ND*	55.60	*ND*	*ND*	Non-comp
Ohtsu T[54]	270-3	13.91	47.67	6.72	*ND*	11.60	*ND*	33.60	*ND*	*ND*	Non-comp
Mean				***12.14***						***4.33***	

Abbreviations: Schedule, dose (mg/m^2) and infusion time (hour); C_{max}, peak plasma concentration; AUC, area under the plasma drug concentration and time profile; CL_{total}, the total (systemic) clearance; $T_{1/2\ \alpha}$, alpha half-life; $T_{1/2\ \beta}$, beta half-life; $T_{1/2\ \gamma}$, gamma half-life; V_{ss}, volume of distribution at steady state; MRT, mean residence time; V_C, volume of distribution for the central compartment; Non-comp, non-compartmental analysis; 2-comp, two-compartment model; 3-comp, three-compartment model.

[a]V_C: most of V_C values were calculated by the following equation of $V_C = CL * T_{1/2\ \alpha} / 0.693$, except for the [b]labelled from the literature.

ND : no data available

5.2.2 Model design

The purpose of this *ex vivo* model was to reproduce the main pharmacokinetic behaviour of paclitaxel in the "central" compartment of the body. The design of the model is shown in Figure 5-1.

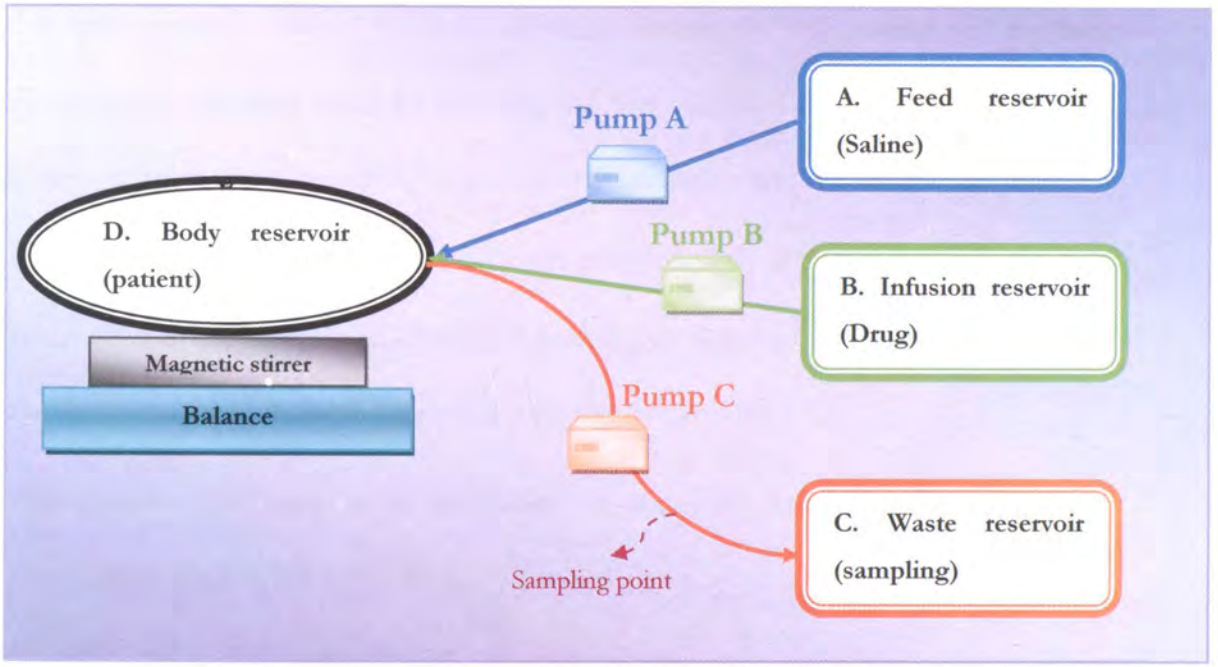

Figure 5-1 Experimental design of *ex-vivo* PK model for paclitaxel dosing

This system consisted of 4 reservoirs: A, B, C and D. Reservoir A (Feed reservoir) represents the feed-back of the body that can keep the volume of the central blood system in balance. Reservoir B (Infusion reservoir) contains drug infusion, which is infused to the "patient" (Reservoir D represents "the central blood system") during administration; Reservoir C (waste reservoir) is for collecting the waste and drug will be sampled from the outlet tube as shown in Figure 5-1. The rate at which flow is pumped from reservoir D to reservoir C would replicate the total clearance of paclitaxel from the central compartment. The magnetic stirrer was to make sure the solution in Reservoir D mixed rapidly. A balance was to monitor the weight of the body reservoir ("the central blood compartment") and ensure this was kept constant

with inlet and outlet flows during the study, and this ensured that the volume of the "central compartment" was kept constant.

The rates of inlet flow (A and B) and the outlet flow (C) were controlled by Pump A, B and C. The flow rate of B (F_B) was the drug infusion rate and the flow rate of C (F_C) was the elimination rate of the drug (the total clearance of drug); weight of the body reservoir (D) was kept stable by adjusting the flow rate of A (F_A). Over the first three hours (infusion stage), pump A, B and C worked together and the relation of flow rates (F) was: $F_A + F_B = F_C$; after the infusion stopped, only the pumps from the feed reservoir and waste reservoir continued working so that $F_A = F_C$. (NB: the weight of the body reservoir was kept constant during the whole study.)

The accuracy and precision of the pumps (A, B and C) were tested and details are presented in Section 5.4.3.1 (p.192).

5.2.3 Scale-down

For practical purposes it was necessary to scale down the *ex vivo* model, but at the same time it was essential to ensure that PK parameters were representative of the *in vivo* situation. Therefore, the system characteristics and the dose-banding (D-B) scheme were scaled-down, as shown below:

5.2.3.1 Scale-down of the system characteristics

Considering the steady state in the 1-compartment infusion model, where

$Rate_{in} = Rate_{out}$ ($Rate_{in}$, the rate into the system for a drug; $Rate_{out}$, the rate out of the system for a drug).

Therefore,

$$\frac{Dose \times BSA}{Infusion\ time} = CL \times BSA \times Css$$

Equation 5-1

Dose, drug dose; CL, total clearance; Css, steady state concentration (= C_{max}).

By rearranging Equation 5-1,

$$Css = \frac{Dose}{CL \times infusion\ time}$$

Equation 5-2

Also,

$$AUC = \frac{Dose}{CL}$$

Equation 5-3

AUC, area under the drug concentration and time profile

$$K = \frac{CL}{Vc}$$

Equation 5-4

K, elimination rate constant from the central compartment; Vc, volume of distribution of the central compartment.

Based on Equation 5-2, 5-3 and 5-4, to ensure both C_{max} (Css), AUC and K represent the *in vivo* situation (these parameters keep constant with scale-down), Dose, V_C and CL should be reduced by the same factor. The reducing factor was decided by practical conditions such as the amount of drug used (and cost), the volume of available containers, and the working limit of infusion pumps; for example, the reduced V_C should be small enough to be contained in a closed system to avoid occupational exposure to paclitaxel. Considering all the above factors in the study design, these three parameters were reduced by a factor of 20 for the *ex vivo* study. Therefore, for each

individual "patient", these three systematic parameters were calculated by the following equations:

$$Dose_{(BSA)} = 175\ mg/m^2 \times BSA / 20 \qquad \text{Equation 5-5}$$

$$V_{C\ (BSA)} = 4.33\ L/m^2 \times BSA / 20 \qquad \text{Equation 5-6}$$

$$CL_{(BSA)} = 12.14\ L/h/m^2 \times BSA / 20 \qquad \text{Equation 5-7}$$

Both Vc (4.33 L/m²) and CL (12.14 L/h/m²) were based on literature values (refer to Figure 5-1*).*

5.2.3.2 Scale-down of the D-B regimen for paclitaxel

To administer a scale-down dose, the dose-banding (D-B) scheme was adjusted and the standard doses for all bands were scaled down by a factor of 20 (refer to Table 5-2). The 3rd and 5th columns describe the standard doses and combination of "pre-made" infusions (after scale-down) that would be applied in the *ex vivo* study.

Table 5-2 Dose-banding (D-B) Scheme for paclitaxel chemotherapy after being scaled down by a factor of 20 for the *ex vivo* study

Dose bands (mg)	[a]Std dose (mg)	Std dose scaled down (mg)	[b]Max. Dev. (%)	Pre-made infusions (mg)	[c]No. infusions	Total volume of infusions (mL)
125-135	130	*6.5*	4.00	5mg+1mg+0.5mg	3	350
135-145	140	*7.0*	3.70	5mg+1mg+1mg	3	350
145-155	150	*7.5*	3.45	5mg+2.5mg	2	350
155-165	160	*8.0*	3.23	5mg+2.5mg+0.5mg	3	400
165-175	170	*8.5*	3.03	5mg+2.5mg+1mg	3	400
175-185	180	*9.0*	2.86	5mg+2.5mg+1mg+0.5mg	4	450
185-200	190	*9.5*	5.00	5mg+2.5mg+1mg + 1mg	4	450
200-220	210	*10.5*	5.00	10mg+0.5mg	2	550
220-240	230	*11.5*	4.55	10mg+1mg+0.5mg	3	600
240-260	250	*12.5*	4.17	10mg+2.5mg	2	600
260-280	270	*13.5*	3.85	10mg+2.5mg+1mg	3	650
280-300	290	*14.5*	3.57	10mg+2.5mg+1mg+1mg	4	700
300-320	310	*15.5*	3.33	15mg+0.5mg	2	1050
320-340	330	*16.5*	3.13	15mg+1mg+0.5mg	3	1100
340-360	350	*17.5*	2.94	15mg+2.5mg	2	1100
360-380	370	*18.5*	2.78	15mg+2.5mg+1mg	3	1150
380-400	390	*19.5*	2.63	15mg+2.5mg+1mg+1mg	4	1200
400-420	410	*20.5*	2.50	10mg+10mg+0.5mg	3	1050
420-440	430	*21.5*	2.38	10mg+10mg+1mg+0.5mg	4	1100
440-460	450	*22.5*	2.27	10mg+10mg+2.5mg	3	1100

[a]*Std dose: standard dose given by the pre-made infusions*
[b]*Max. Dev.: maximum deviation of standard dose from prescribed dose (BSA-based dose)*
[c]*No. infusions: number of pre-made infusions to conduct one standard (banded) dose*

To facilitate the scaled-down standard doses, the pre-made infusions had to be diluted further (Table 5-3). Compared with the original dose-banding scheme, the volume of each type of pre-made infusions was kept the same, but the drug amount in each type of pre-made infusions was scaled down by a factor of 20 and thus the final concentration of each was reduced by a factor of 20 as shown in the table.

Table 5-3 Preparation of paclitaxel pre-made infusions for dose-banding (D-B) scheme in the *ex vivo* study

Pre-made infusions (mg)	Infusion bag size (mL)	Volume replaced by paclitaxel 0.3mg/ml (mL)	Final volume (mL)	Final conc. (mg/mL)
0.5	50	1.7	50.0	*0.01*
1	50	3.3	50.0	*0.02*
2.5	100	8.3	100.0	*0.025*
5	250	16.7	250.0	*0.02*
10	500	33.3	500.0	*0.02*
15	1000	50.0	1000.0	*0.015*

5.3. Materials and Methods

5.3.1 Materials and reagents

Paclitaxel concentrate 6 mg/mL (Batch no.: 05C21NB, Expiry date: 03-2007) was supplied by Teva Hospitals (Leeds, UK);

The internal standard (Docetaxel stock 2 mg/mL): docetaxel powder from Sigma Aldrich (Dorset, UK) was dissolved in pure ethanol, giving a concentration of 2 mg/mL;

Sodium chloride (batch no. 106K0083, purity ≥99.5%) used for *ex vivo* studies was from Sigma Aldrich (Dorset, UK);

BondElut cyano end-capped (CN-E) SPE cartridges (Part no.: 12102007; 1mL, 100mg) were all supplied by Varian (Oxford, UK);

Different volumes of Freeflex® infusion bags containing 0.9% sodium chloride solution (50 mL: lot no. WKS141 and expiry in 10-2009; 100 mL: lot no. 13BAS231 and expiry in 01-2010; 250 mL: lot no. WI7308 and expiry in 09-2010; 500 mL: lot no.

WK7328 and expiry in 10-2010; 1000 mL: lot no. 14BA7102 and expiry in 01-2011) all from Fresenius Kabi Ltd (Runcom, UK);

3 Colleague ™ volumetric infusion pumps (serial no. 99BC2129AA; serial no. 9030134CK; serial no. 9030221CK) were supplied by Baxter Healthcare Corporation (Deerfield, USA);

The Kern KB10000-1 balance (Max 10100 g and d = 0.1 g) was purchased from Fisher Scientific Ltd (Loughborough, UK);

Normal infusion administration sets (non air vented with 15 μm filter) for Colleague volumetric pumps (lot no. 08A31V149 and expiry in 12-2012) and special vented paclitaxel sets (non-PVC with a 0.22 μm in-line filter) for Colleague pumps (lot no. 07F08V720 and expiry in 05-2012) were all supplied by Baxter Healthcare (Berkshire, UK);

Magnetic stirrer/hotplate (Jenway model 1000) was supplied by Bibby Scientific Ltd (Essex, UK)

5.3.2 High-performance liquid chromatography (HPLC)

The HPLC instrumental conditions were described previously in Chapter 3 (refer to Section 3.2.3 and 3.3.1.3.1, p.100 and 125).

5.4. Experimental

5.4.1 Validation of the analytical method

5.4.1.1 Preparation of validation standard samples in 0.9% sodium chloride

Fresh paclitaxel stock solution (6 mg/mL) was withdrawn from the manufacturer's vial (stored at 2 – 8°C in a light protected overwrap) using a venting needle, a needle and a

syringe. The extracted stock solution was volumetrically diluted using 0.9% sodium chloride solution (from Freeflex infusion) to the following concentrations: 20, 40, 100, 300, 600 and 1200 ng/mL.

5.4.1.2 Preparation of unextracted drug solutions of paclitaxel and internal standard in ACN/water (50/50, v/v)

Fresh paclitaxel stock solution (6 mg/mL) was diluted appropriately to make up the following concentrations: 100, 200, 500, 1500, 3000 and 6000 ng/mL, with 2 μg/mL of internal standard added to each. These samples were used for calculating of recovery of drugs from the standard samples.

5.4.1.3 SPE and sample analysis

The 100 mg cyano Bond Elut SPE cartridge (Varian, Oxford, UK) was pre-conditioned by 2 mL of Methanol (MeOH) and 2 mL of pH 3 buffer. Paclitaxel standard sample (2 mL) and 80 μL of internal standard (10 μg/mL) were added onto the pre-conditioned cartridge. The cartridge was then washed with 2 mL of pH 3 buffer and 2 mL of MeOH/pH 3 buffer (2:8, v:v). The cartridge was then dried under full vacuum and eluted using 500 μL of acetonitrile (ACN) with 0.1% triethylamine. The elution sample was evaporated under a nitrogen stream at 30°C and finally the residue was reconstituted in 400 μL ACN/water (1:1). 20 μL was injected on to the HPLC column in replicates. Freshly made unextracted samples containing paclitaxel and internal standard were injected between standard samples. The individual recovery (%) of paclitaxel and docetaxel was calculated by comparing the peak height of each drug in the standard sample to that in the unextracted sample.

5.4.1.4 Validation of HPLC method

3 replicates of each standard solution (20, 40, 100, 300, 600 or 1200 ng/mL) were analysed using the above HPLC method. The study was repeated on different days (n = 3). A typical chromatogram is shown in Appendix 7 (p.304). A calibration plot (mean of calibration plots, Figure 5-2) was produced to describe the relationship of the mean peak height ratios (paclitaxel versus docetaxel) and the known concentrations of paclitaxel: Ratio = 0.0041 × (Conc. paclitaxel) − 0.0256, with an average correlation coefficient (R^2) of 0.9999 obtained. The validation characteristics were obtained based on at least 6 determinations of each quality control standard (20, 300 and 1200 ng/mL) on each working day. Results are summarised in Table 5-4. The intra- and inter-day precision (CV%) ranged from 2.82% to 13.07% and the intra- and inter-day accuracy ranged from 2.77% to 9.27% at 20, 300 and 1200 ng/mL levels. The limit of quantification (LOQ) was 20 ng/mL in this study with acceptable inter- precision and inter-accuracy (both < 15%). The average recovery (%) was 89% for paclitaxel and 81% for the internal standard (docetaxel) with all CV < 15%. All of these characteristics were acceptable according to FDA guidance[248].

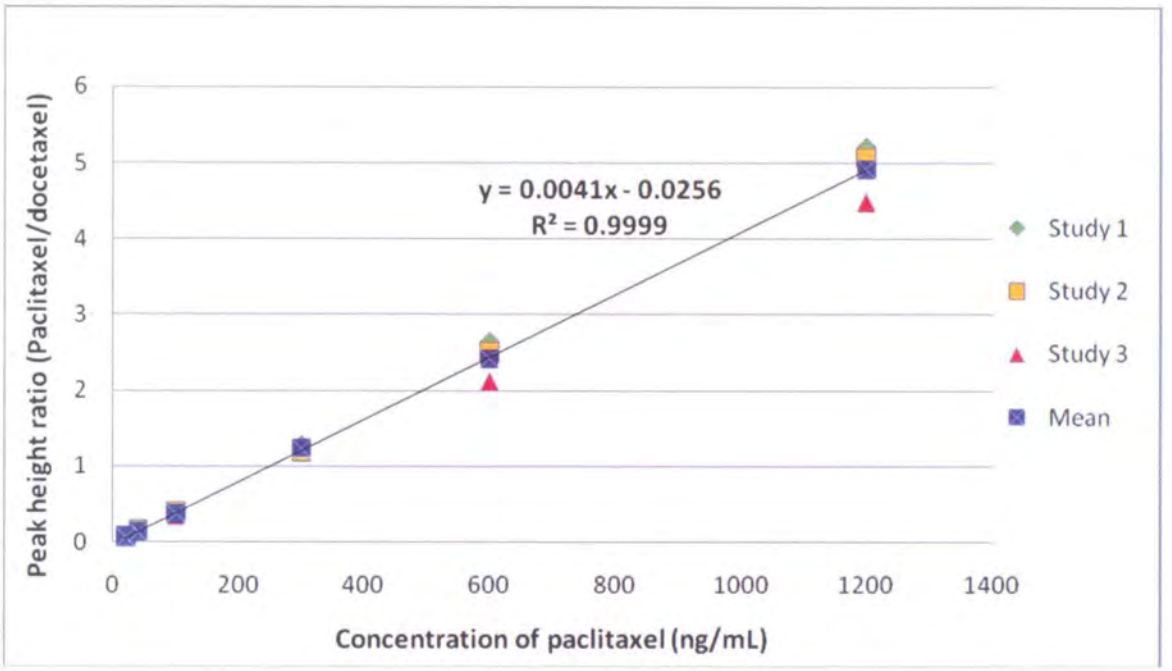

Figure 5-2 The mean calibration plot of paclitaxel assay for the *ex vivo* study

The relationship between peak height ratios (paclitaxel / docetaxel) and the concentrations of paclitaxel in standard solutions

Table 5-4 Summary of validation characteristics of paclitaxel assay for the *ex vivo* studies

Nominal concentration (ng/mL)	Measured concentration (ng/mL)	Accuracy (%)		Precision (%)	
		Intra-day	Inter-day	Intra-day	Inter-day
20	21.48	4.42	9.27	6.18	13.07
300	308.02	5.01	6.71	5.11	6.85
1200	1206.36	2.77	7.47	2.82	7.51

6 replicates (n=6) of each quality control sample were analysed on each of three different working days;
Precision (%) and accuracy (%) were calculated using peak height ratio of paclitaxel versus docetaxel.

5.4.2 Preparation of infusion solutions for *ex-vivo* studies

5.4.2.1 D-B infusion bags after scale down

0.3 mg/mL paclitaxel infusion (working stock): 5 mL of 0.9% sodium chloride solution in a 100 mL infusion bag was replaced by 5 mL of paclitaxel concentrate (6 mg/mL), giving a final concentration of 0.3 mg/mL. This was made freshly before preparation of the pre-infusion bags for one study and temporarily (< 1 hour) stored at 2 – 8° C with light protection before use.

Paclitaxel pre-made infusions for dose-banding after scale-down: Table 5-3 shows the preparation of the paclitaxel standard infusions for dose-banding after scale-down. For each *ex vivo* experiment, pre-made infusions were prepared according to specific need. Normally, preparation would be conducted one day before the *ex vivo* experiment. The prepared infusion bags were all kept in a light-protected overwrap and stored in the refrigerator (2 – 8°C) overnight before use.

5.4.2.2 Saline solution (0.9% w/v sodium chloride) for the body reservoir (20L)

a. 180 g sodium chloride salt was accurately weighed using the balance;
b. The above 180 g sodium chloride salt was dissolved in deionised water in a 2 L glass beaker and then transferred into a 25 L polypropylene container;
c. The beaker was washed with deionised water for more than 3 times and all rinsing solution was transferred into the 25 L container;
d. More deionised water was added until the 20 L level line was reached within the 25 L container. Finally the solution was well mixed in the container by agitation.

5.4.3 Testing of the system

Before starting the *ex vivo* study, the Colleague infusion pumps and the balance software were tested for the accuracy and validity.

5.4.3.1 Pump testing

To reduce error in flow rate, during this simulation study Baxter Colleague Volumetric Infusion pumps were used to connect the feed and infusion reservoirs to, and from the central compartment. These pumps are specially designed to deliver i.v. drugs to patients in clinics. They can reduce the error in dosing and flow rate to a minimal level.

However, different pumps can have different performance characteristics. The aim of this part of the study was to check pump accuracy and determine whether existing errors in flow rates would be acceptable.

A broad range of flow rates (166, 333, 500, 667, 833, 1000 and 1166 mL/hr) were tested on the Colleague pumps. Fresh deionised water was pumped at these varied flow rates. The water was collected and weighed every minute, thus providing a flow rate in mg/min. The measured flow rates were converted into mL/min using the above results. For each pump, each flow rate was tested in triplicate. The average results were used for analysis. The following figures (Figure 5-3, Figure 5-4 and Figure 5-5) show the relationship of measured flow rates and set flow rates with pumps.

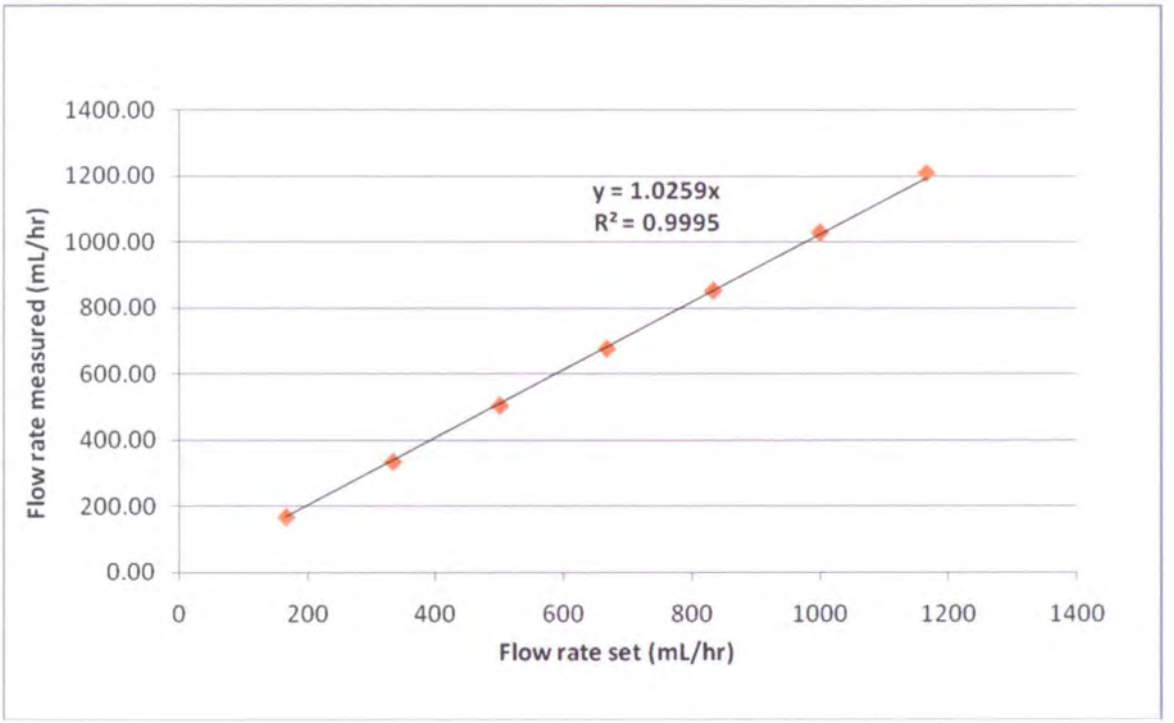

Figure 5-3 The relationship between measured flow rates and set flow rates on Pump A (Colleague™ volumetric infusion pump, serial no. 99BC2129AA)

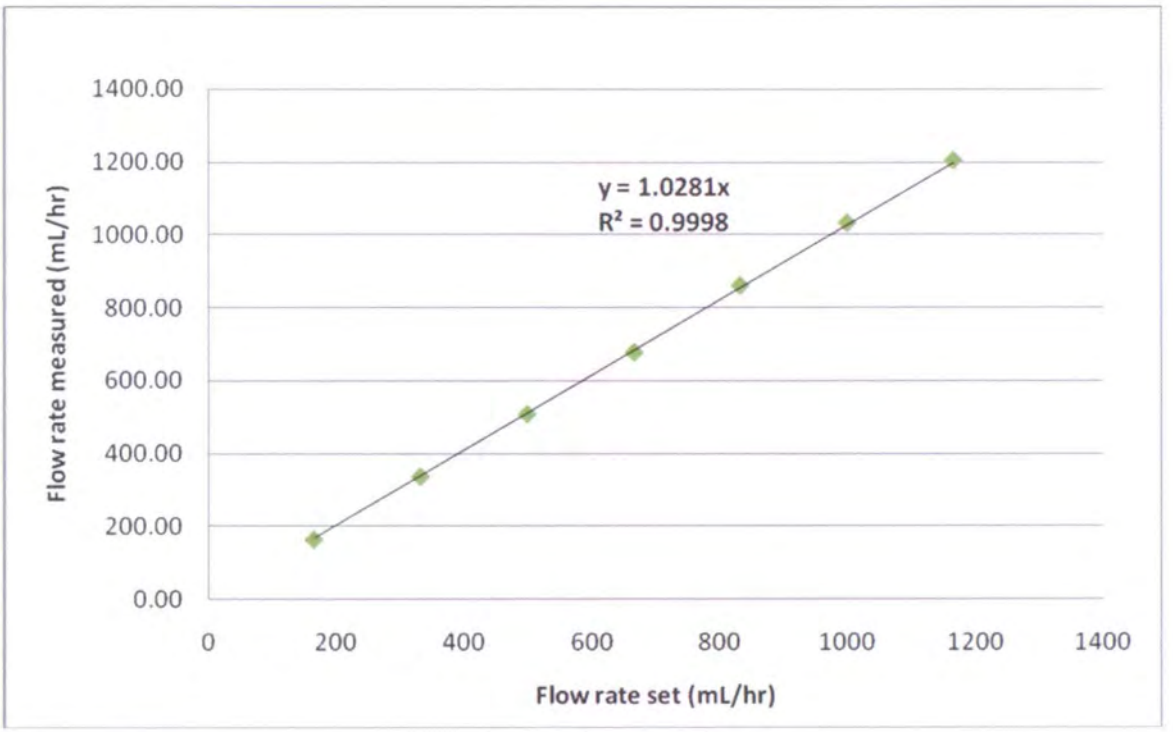

Figure 5-4 The relationship between measured flow rates and set flow rates on Pump B (Colleague™ volumetric infusion pump, serial no. 9030134CK)

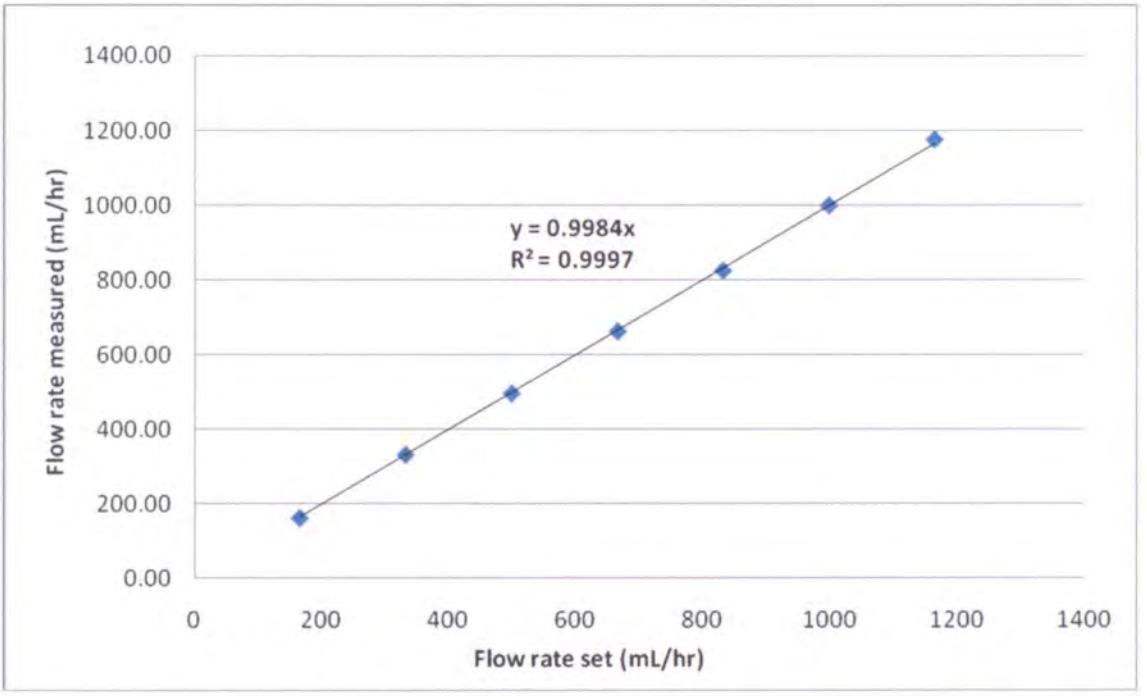

Figure 5-5 The relationship between measured flow rates and set flow rates on Pump C (Colleague™ volumetric infusion pump, serial no. 9030221CK)

Based on the above 3 figures, all the linear relationships are good (all $R^2 > 0.999$). All the slopes are close to 1 (ideal value). In other words, all 3 pumps have very good accuracy. The overall errors of the slopes were less than 2.81 %, which means the difference between the plots (error in flow rate) is very small.

5.4.3.2 Balance and software testing

In this study, the Kern balance was connected to a PC and data were recorded and analysed by the software of balance connection SCE-3.0 (version 3.1, Kern & Sohn, Balingen, Germany). This software directly imported the weighing data into a windows application. The data were then transferred to Windows Excel. Using the time-controlled program, weighing values of the body reservoir were logged with time. This information helped control the flow rates of the three pumps to achieve system balance.

The accuracy of the weighing system (the balance and software) was tested in two ways:

Test 1: Weighing of a beaker containing 0.9% sodium chloride solution in a static state for 1 hour (weight was logged per minute)

The variance of weight (the change of weight) was 0.1 g over 1 hour.

Test 2: Weighing of a beaker containing 0.9% sodium chloride solution with a magnetic stirrer working inside for over 6 hours (weight was logged every 2 minutes)

The weight was increased by a percentage of 0.2 % after 6 hours, compared with the original weight (time zero).

These above changes in weight were all considered insignificant.

5.4.4 *Ex-vivo* study

5.4.4.1 Flow-chart of the study

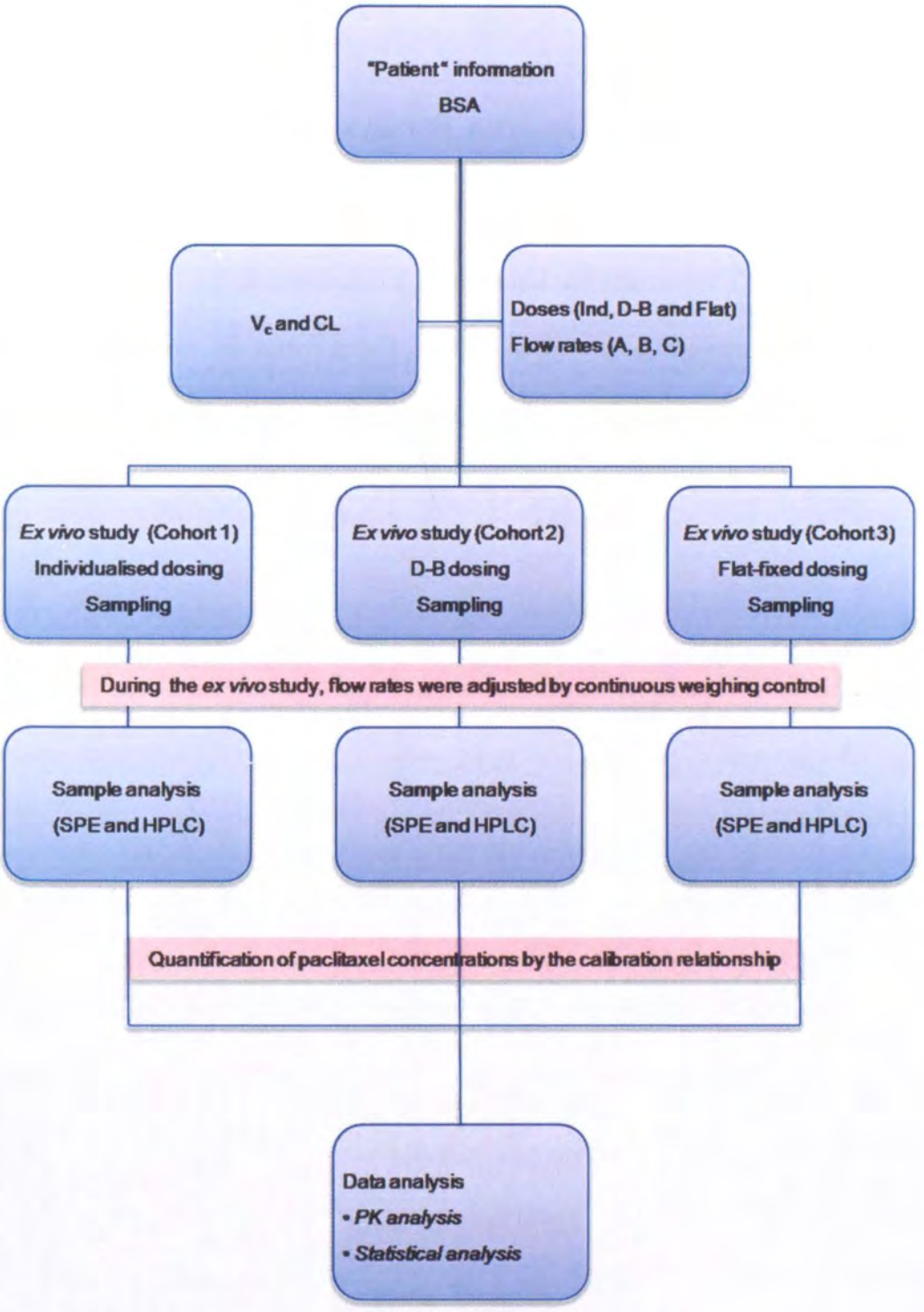

Figure 5-6 Flow chart for the *ex vivo* study

V_C, volume of distribution for the central compartment; CL, the total clearance; Ind, individualised dosing (BSA-based); D-B, dose-banding dosing; Flat, flat-fixed dosing.

5.4.4.2 "Patient" information

BSA values for "theoretical patients" were randomly generated from within the range of 1.50 m^2 to 1.95 m^2 (covering the average reference BSA values of 1.6 – 1.9 m^2 [254]) using Microsoft Excel 2007 Software (Microsoft Corporation, Washington, USA). They were: 1.79, 1.66, 1.62, 1.90, 1.60, 1.75, 1.50, 1.86 and 1.95 m^2.

Summarised information for "theoretical patients" is shown in Table 5-5. The individual volume of distribution of the central compartment (V_C) and the total clearance (CL) were calculated based on BSA values using the equation 5-6 and 5-7 (Section 5.2.3.1, p.182). This information would be applied to set up the *ex vivo* experiments.

Table 5-5 Summarised characteristics of "patient" information for the *ex vivo* experiments

BSA (m^2)	V_C (mL)	CL (mL/h)
1.79	388	1087
1.66	359	1008
1.62	351	983
1.90	411	1153
1.60	346	971
1.75	379	1062
1.50	325	911
1.86	403	1129
1.95	422	1184

V_C, volume of distribution of the central compartment; CL, the total clearance

V_C and CL were calculated based on the equation 5-6 and 5-7 (refer to Section 5.2.3.1, p.182)

5.4.4.3 Dose calculations, infusion preparation and pump flow rates

In this study, paclitaxel was administered by a 3-hour infusion schedule.

Individualised dose and infusion preparation: The scale-down individualised dose was calculated using Equation 5-5 (Section 5.2.3.1, p.182) and the required volume of paclitaxel concentrate (6 mg/mL) was obtained by the formula: Vol_{drug} = Dose (mg)/6. For an individual "patient", an equivalent volume (= Vol_{drug}) of infusion solution was removed from a 500 mL infusion bag and replaced by the required volume of paclitaxel concentrate (6 mg/mL) solution, giving a total volume of 500 mL.

D-B dose and infusion preparation: The D-B dose after scale-down was prepared referring to the chart of D-B scheme for paclitaxel chemotherapy (Table 5-2, p.185). The infusions were prepared according to Table 5-3 (p.186).

Flat-fixed dose and infusion preparation: A scaled-down flat-fixed dose of 15 mg was applied in this study. This was obtained by reducing the 300 mg used as the flat-fixed dose in the original protocol (Chapter 4) by a factor of 20. For preparation of an infusion bag, 2.5 mL of infusion solution was removed from a 500 mL infusion bag and replaced by an equal volume of paclitaxel concentrate solution (6 mg/mL).

All the details of the actual given doses, the total infusion volumes and the infusion rates for all theoretical patient BSA values are shown in Table 5-6.

Table 5-6 Dosage, infusion volume and flow rate of paclitaxel infusion under different dosing methods for individual "patients"

	Individualised Dose			D-B Dose			Flat-fixed Dose		
BSA (m^2)	D_{Ind} (mg)	Vol_{inf} (mL)	*F_B (mL/hr)	D_{DB} (mg)	Vol_{inf} (mL)	F_B (mL/hr)	D_{flat} (mg)	Vol_{inf} (mL)	F_B (mL/hr)
1.79	15.7	500	167	15.5	1050	350	15	500	167
1.66	14.5	500	167	14.5	700	233	15	500	167
1.62	14.2	500	167	14.5	700	233	15	500	167
1.90	16.6	500	167	16.5	1100	367	15	500	167
1.60	14.0	500	167	13.5	650	217	15	500	167
1.75	15.3	500	167	15.5	1050	350	15	500	167
1.50	13.1	500	167	13.5	650	217	15	500	167
1.86	16.3	500	167	16.5	1100	367	15	500	167
1.95	17.1	500	167	17.5	1100	367	15	500	167

BSA, body surface area; D_{Ind}, individualised dose; D_{DB}, dose-banded (D-B) dose; D_{flat}, flat-fixed dose; Vol_{inf}, the total volume of infusion; F_B, flow rate of infusion (Pump B) for D_{Ind}, D_{DB}, or D_{flat}.

**F_B was equal to $Vol_{inf}/3h$*

For individual "patients", the theoretical flow rates of pump B (F_B) for infusion are shown in the above table (Table 5-6).

The flow rate of pump C (F_C) for clearance was equal to the total clearance rate (refer to Table 5-5, pg.199).

The flow rate of pump A (F_A) connected to the Feed reservoir was decided by the difference of F_C and F_B ($F_A = F_C - F_B$) for the first 3 hours (infusion time). After infusion, the F_A would be equal to the F_C.

However, these flow rates were calculated theoretically. Due to the performance of these pumps, flow rates needed to be adjusted minutely in order to balance the weight of the central compartment (Body reservoir). The weight change of the body reservoir was controlled to be within ± 10% during experiments.

5.4.4.4 Study procedure

5.4.4.4.1 Experimental set-up of the *ex vivo* study

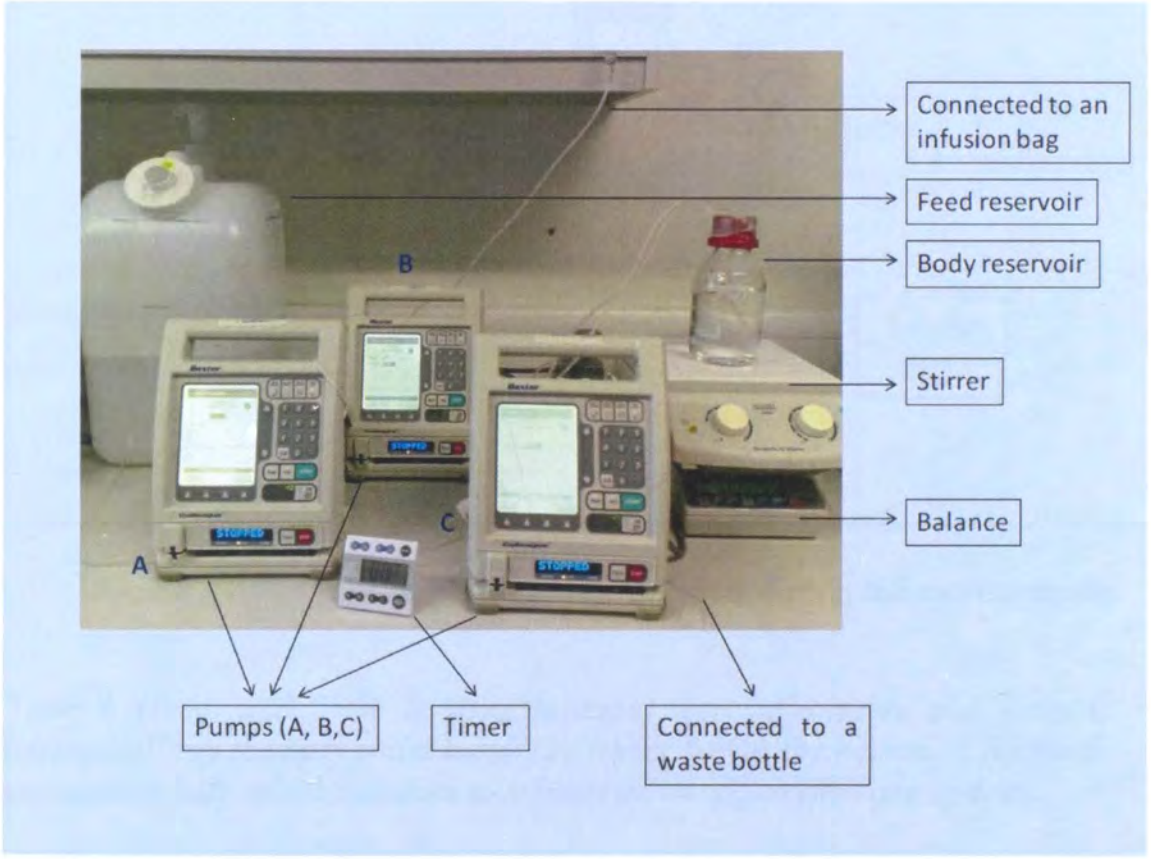

Figure 5-7 The experimental set-up of the *ex vivo* study

The drug infusion was passed through Pump B into the Body reservoir. The saline solution in the Feed reservoir (on the left side) was pumped into the Body reservoir by Pump A. Pump C was used for clearance, where the drug infusion was pumped out of the Body reservoir and into a large polypropylene waste bottle (30 L) invisible in this photograph. Sampling was conducted from a tube after Pump C. The Body reservoir (a 500 mL glass bottle) was set on the top of a stirrer and a top-pan balance. Two inlet tubes and one outlet tube were passed through the lid of the glass bottle (shown in Figure 5-8). The balance was connected to the PC with a programme to continuously log any weight change of the Body reservoir.

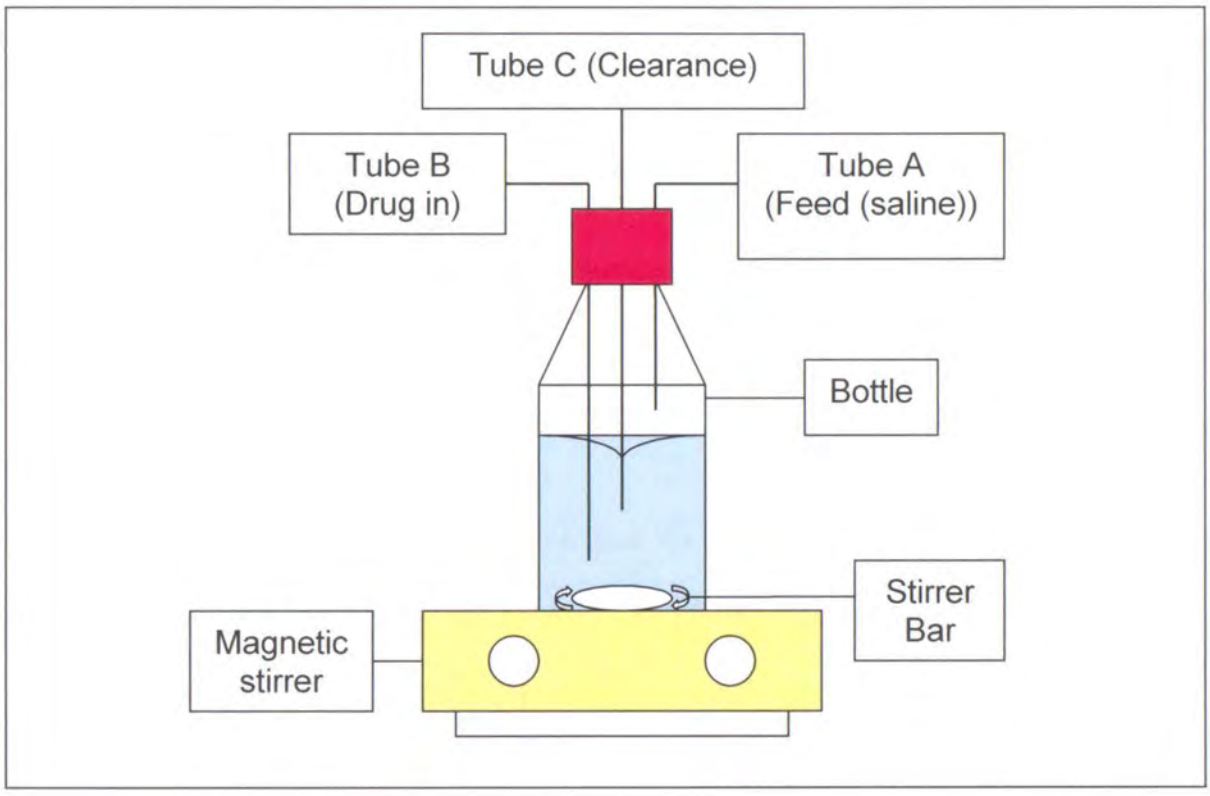

Figure 5-8 The tube set in the Body reservoir during the *ex vivo* study

Tube A (Feed) and Tube B (drug infusion) were inlet tubes and Tube C (clearance) was the only outlet tube. The stirrer bar at the bottom of the bottle was used to fully mix the solution to achieve an even concentration of drug.

5.4.4.4.2 Preparation of tubes

Infusion administration tube sets were chosen based on their purpose. The tubing sets were tailored for specific applications as shown below:

Tube A (Feed): normal infusion set used

The spike part was removed before use.

Tube B (Drug infusion): vented paclitaxel administration set used

The luer lock end was removed before use.

Tube C (Clearance): vented paclitaxel administration set used

Both the spike part and the luer lock end were removed before use.

5.4.4.4.3 Priming and connection of tubing

Using a needle and syringe, saline solution (0.9% sodium chloride) was drawn and used to remove all the air in all 3 tubes by filling saline solution into these tubes.

The tubes were closed by the stop cock and click to avoid any leakage. These tubes were connected to the system as shown in Figure 5-7 and Figure 5-8.

5.4.4.4.4 Start-up and sampling time

All pumps (A, B, C) were started at the same time. Weight data were logged on the PC. Approximately 10 mL of sample was collected in a 20 mL polypropylene container at different times: zero, 1 hr, 2 hr during the infusion and at the end of the infusion, 15 min, 30 min, 1 hr, 1.5 hr, 2 hr, 2.5 hr and 3 hr after infusion.

5.4.4.4.5 Study period

Based on the first two experiments of the *ex vivo* study, the drug (paclitaxel) could not be detected after 6 hours from the beginning of infusion. The study period for the following experiments was therefore set as 6 hours.

5.4.4.5 Labelling and storage of samples

All collected samples were clearly labelled with "patient" BSA, dose type, sampling time and experiment date. Samples were then kept at 2 – 8°C in a light protected overwrap and analysed within 24 hours.

5.4.4.6 Analysis of samples by SPE and HPLC

Each sample was subjected to SPE, followed by HPLC analysis. The details of the method have been described in Section 5.4.1 (p.187). Each sample was analysed in

duplicate. The average peak height ratio (paclitaxel/docetaxel) was used to estimate the concentration of paclitaxel at each sampling time.

5.4.4.7 Data analysis using the WinNonlin software

The paclitaxel concentration-time data of all "theoretical" patients were subjected to pharmacokinetic (PK) model analysis, non-compartmental analysis (NCA) and bio-equivalence analysis, all conducted by the WinNonlin 5.2 software (Pharsight Corporation, California, USA).

PK model analysis: This was to verify the design of the *ex vivo* model and define a best fit PK model for the data obtained.

NCA: All data for each "patient" were subjected to NCA to estimate the key PK parameters, especially C_{max} and AUC_{all}.

Bio-equivalence analysis: Data (C_{max} and AUC_{all}) were logarithmically transformed before this analysis. The log-transformed PK parameters (Ln (C_{max}) and Ln (AUC_{all})) in each test group (D-B dose or flat-fixed dose) were statistically compared to those in the reference group (individualised dose) using bioequivalence analysis. The standard bioequivalence criterion was 80% – 125% for the 90% confidence interval (C.I.) of the ratio (test/reference) of average C_{max} and AUC_{all}[283]. This means if the 90% C.I. for the ratio (test/reference) of averages lies in the range of 80 – 125%, the test dose will be bioequivalent to the reference dose.

5.5. Results

5.5.1 Description of data

All the raw data of paclitaxel concentrations with respect to time for the 9 "theoretical patients" are shown in Table 5-7. The "patients" were numbered according to

increased BSA values. Some typical plots of paclitaxel concentration versus time ("patient" BSA=1.75 m^2) are shown below. The 1st plot (Figure 5-9) shows data on a linear scale and the 2nd plot (Figure 5-10) on a semi-logarithmic scale. All other plots obtained in the *ex vivo* study are presented in Appendix 8 (p.306).

Table 5-7 Raw data of paclitaxel concentrations with time obtained in the *ex vivo* study

(Individualised dose; DB dose; Flat-fixed dose)

"Patient 1" (BSA = 1.5 m^2)				"Patient 2" (BSA = 1.6 m^2)				"Patient 3" (BSA = 1.62 m^2)			
Time (hr)	Concentration (μM)			Time (hr)	Concentration (μM)			Time (hr)	Concentration (μM)		
	IND	DB	FLAT		IND	DB	FLAT		IND	DB	FLAT
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.91	5.69	5.13	6.44	0.92	5.54	4.99	5.72	0.91	6.32	4.79	5.61
1.83	6.46	5.44	6.67	1.84	5.87	4.86	6.26	1.83	6.31	4.93	—
2.80	—	6.79	6.52	2.68	5.85	5.67	6.49	2.74	6.56	5.36	7.17
3.00	6.34	7.28	6.79	3.00	6.39	5.55	6.58	3.00	6.27	5.19	6.85
3.25	3.73	2.99	4.30	3.25	3.09	2.73	3.62	3.25	3.17	2.74	3.37
3.50	1.85	1.50	2.01	3.50	1.43	1.56	1.81	3.50	1.64	1.40	0.68
4.00	0.54	0.42	0.61	4.00	0.40	0.41	0.78	4.00	0.41	0.37	0.46
4.50	0.15	0.12	0.16	4.50	0.10	0.13	0.16	4.50	0.12	0.10	0.20
5.00	0.05	0.03	0.06	5.00	0.03	0.04	0.04	5.00	0.04	0.03	0.05
5.50	0.02	0.02	0.02	5.50	0.01	0.02	0.02	5.50	0.02	0.02	0.02
6.00	0.01	0.01	0.01	6.00	0.01	0.02	0.01	6.00	0.01	0.01	0.01
"Patient 4" (BSA = 1.66 m^2)				"Patient 5" (BSA = 1.75 m^2)				"Patient 6" (BSA = 1.79 m^2)			
Time (hr)	Concentration (μM)			Time (hr)	Concentration (μM)			Time (hr)	Concentration (μM)		
	IND	DB	FLAT		IND	DB	FLAT		IND	DB	FLAT
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.92	5.76	4.43	5.91	0.92	5.05	5.21	5.25	0.94	5.76	4.53	5.18
1.84	6.58	4.74	6.69	1.84	5.51	5.40	5.32	1.88	6.58	4.89	5.80
2.76	6.19	5.37	6.72	2.76	5.51	5.20	5.63	2.81	6.31	—	5.68
3.00	6.80	5.33	6.49	3.00	5.59	4.85	6.02	3.00	6.80	5.12	5.88
3.25	3.68	2.70	3.59	3.25	3.01	2.43	2.84	3.25	3.68	2.44	3.18
3.50	1.71	1.24	1.66	3.50	1.51	1.21	1.49	3.50	1.71	1.31	1.65
4.00	0.50	0.27	0.43	4.00	0.43	0.35	0.48	4.00	0.50	0.39	0.41
4.50	0.12	0.06	0.10	4.50	0.14	0.09	0.14	4.50	0.12	0.11	0.11
5.00	0.03	0.02	0.03	5.00	0.04	0.03	0.05	5.00	0.03	0.03	0.03
5.50	0.01	0.01	0.01	5.50	0.02	0.01	0.02	5.50	0.01	0.01	0.01
6.00	0.01	0.01	0.01	6.00	0.01	0.01	0.01	6.00	0.01	0.01	0.01

"Patient 7" (BSA = 1.86 m^2)				"Patient 8" (BSA = 1.90 m^2)				"Patient 9" (BSA = 1.95 m^2)			
Time (hr)	Concentration (μM)			Time (hr)	Concentration (μM)			Time (hr)	Concentration (μM)		
	IND	DB	FLAT		IND	DB	FLAT		IND	DB	FLAT
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.91	5.03	4.22	4.84	0.91	6.28	6.39	4.71	0.90	5.39	5.17	4.39
1.83	5.44	4.62	5.16	1.83	6.44	6.83	5.07	1.81	5.80	5.68	4.93
2.74	5.47	4.72	5.30	2.74	6.80	6.86	5.13	2.71	5.65	5.37	4.99
3.00	5.51	4.44	5.23	3.00	6.64	6.84	5.27	3.00	5.68	6.75	5.26
3.25	2.74	2.38	2.68	3.25	3.60	3.38	2.84	3.25	2.94	3.51	—
3.50	1.38	1.29	1.43	3.50	1.84	1.57	1.76	3.50	1.44	1.76	1.38
4.00	0.56	0.35	0.41	4.00	0.55	0.39	0.52	4.00	0.40	0.53	0.35
4.50	0.13	0.11	0.11	4.50	0.17	0.08	0.13	4.50	0.10	0.13	0.10
5.00	0.04	0.03	0.04	5.00	0.06	0.03	0.04	5.00	0.03	0.04	0.03
5.50	0.02	0.02	0.02	5.50	0.02	0.01	0.02	5.50	0.02	0.02	0.01
6.00	0.01	0.01	0.01	6.00	0.01	0.01	0.01	6.00	0.01	0.01	0.01

BSA, body surface area; IND, individualised dose; DB, dose-banded dose; FLAT, flat-fixed dose.

—Missing sample

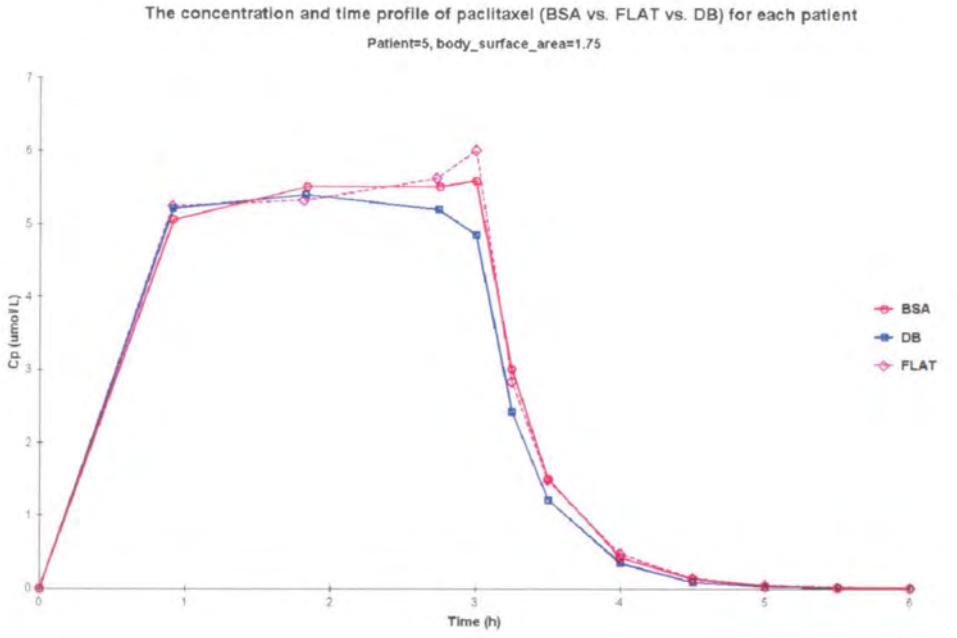

Figure 5-9 The linear plot of paclitaxel concentration versus time (BSA vs. DB vs. Flat dose) of a "patient" (BSA = 1.75 m^2) in the *ex vivo* study

BSA (IND), individualised dose; DB, dose-banded dose; Flat, flat-fixed dose

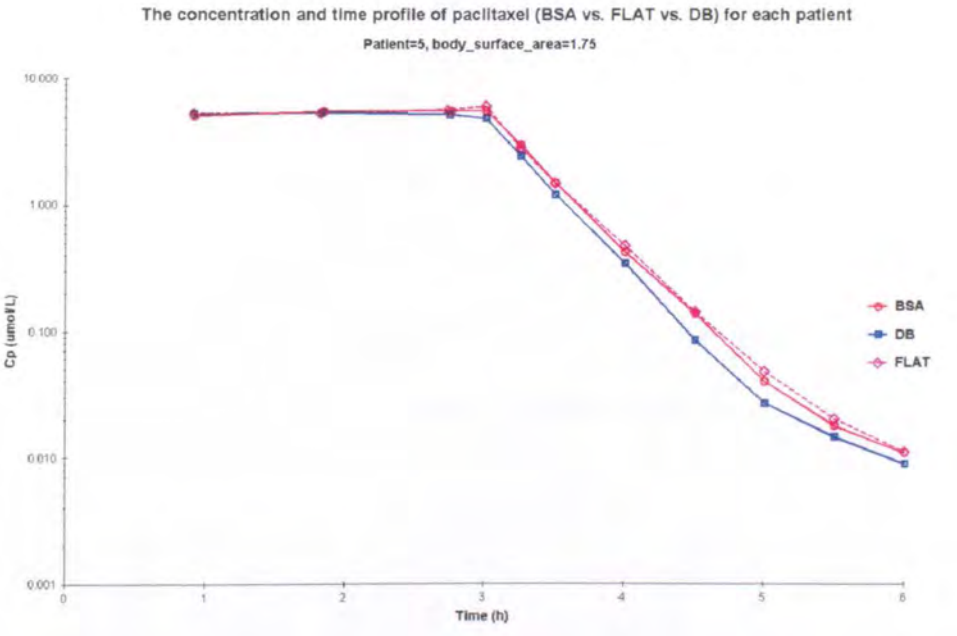

Figure 5-10 The semi-logarithmic plots of paclitaxel concentration versus time (BSA vs. DB vs. Flat dose) of a "patient" (BSA = 1.75 m^2) in the *ex vivo* study

BSA (IND), individualised dose; DB, dose-banded dose; Flat, flat-fixed dose

5.5.2 Verification of the *ex vivo* model via PK model analysis

Paclitaxel elimination behaviour is normally described by a 2- or more compartment model. However, in this *ex vivo* simulation study, only the central compartment (V_C) was considered and the influence of other compartments (the 2nd or 3rd compartment) was not considered, meaning that only the total clearance (CL) of the central compartment was studied while the distribution and metabolism of the drug were neglected. Although no accurate PK model was ideal for this study, the most likely PK model seemed to be the 1-compartment IV-infusion with 1st-order elimination as shown below.

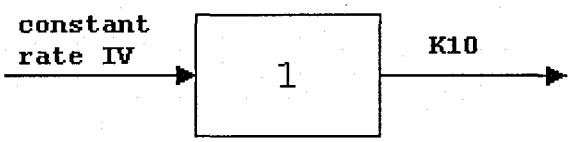

Figure 5-11 1-compartment IV-Infusion with 1st order elimination (no lag time)

Figure 5-12 and Figure 5-13 are typical plots of paclitaxel concentration versus time analysed using the 1-compartment IV-infusion model. From a visual inspection of these figures (linear scale and semi-logarithmic scale), the 1-compartment IV-infusion model seemed to be fit and describe the data obtained in this *ex vivo* study.

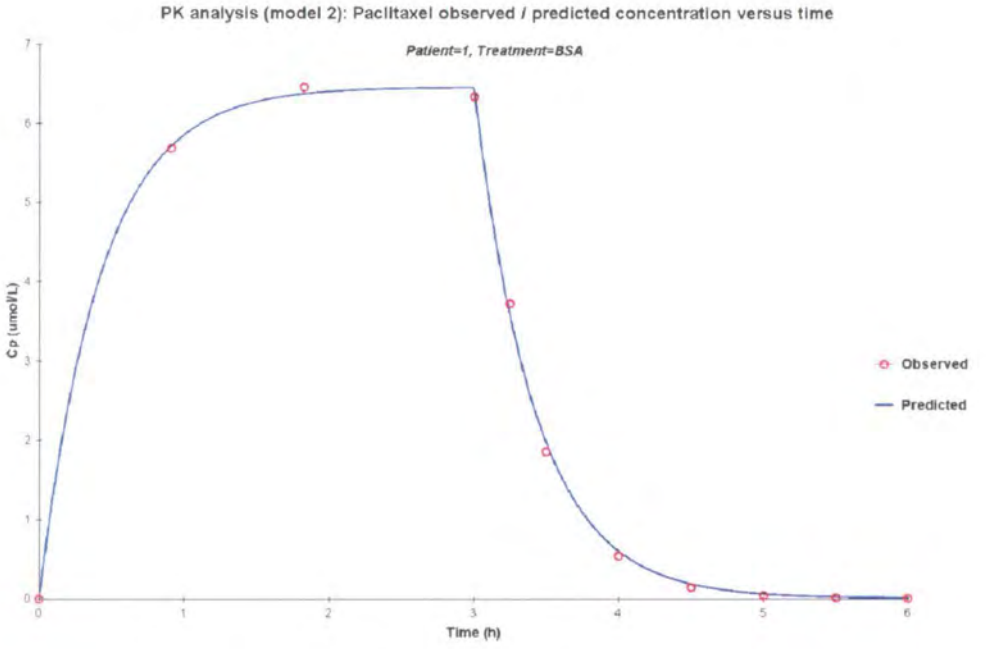

Figure 5-12 The linear scale plot of observed and predicted paclitaxel concentration versus time analysed by 1-compartment IV infusion model with 1st-order elimination for individualised dose (patient BSA = 1.5m^2, BSA = individualised dose)

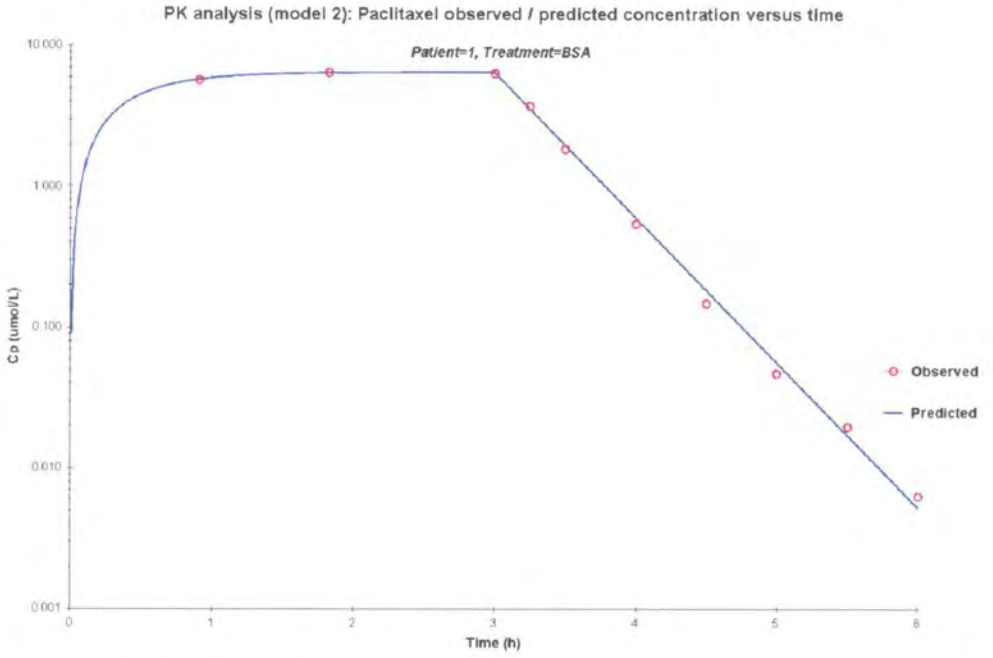

Figure 5-13 The semi-logarithmic plot of observed and predicted paclitaxel concentration versus time analysed by 1-compartment IV infusion model with 1st-order elimination for individualised dose (patient BSA = 1.5 m^2, BSA = individualised dose)

The paclitaxel dose is given as a short infusion and so is not strictly a constant rate IV infusion or a bolus dose. To confirm the best fit model for the data, another similar PK model was tested to analyse these data. This was 1-compartment IV-bolus with 1st order elimination as shown in Figure 5-14. The plots of concentration versus time with this model are shown below (Figure 5-15 and Figure 5-16). Visual inspection confirmed that the goodness-of-fit of this model was not as good as the former model.

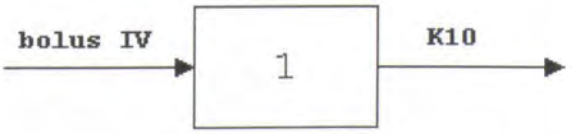

Figure 5-14 1-compartment IV-bolus with 1st order elimination (no lag time)

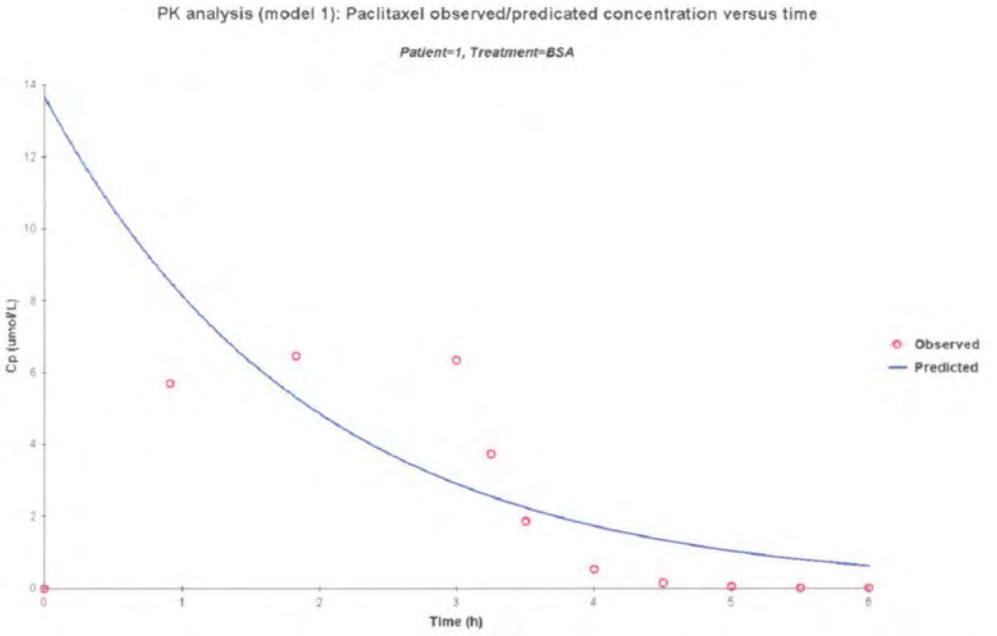

Figure 5-15 The linear scale plot of observed and predicted paclitaxel concentration versus time analysed by 1-compartment IV-bolus model (Model 1) with 1st order elimination for individualised dose (patient BSA = 1.5 m^2, BSA = individualised dose)

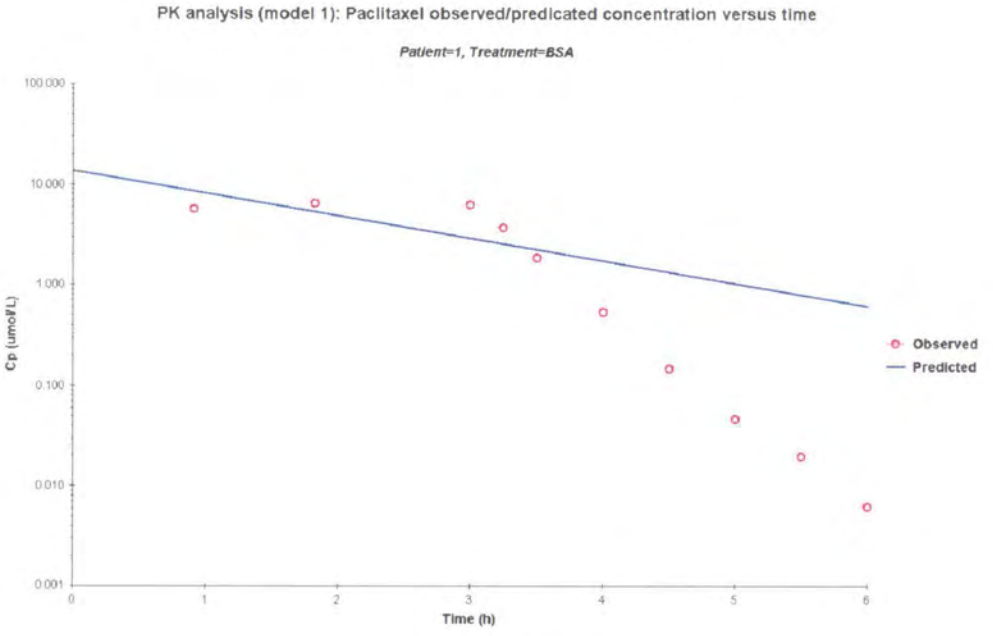

Figure 5-16 The semi-logarithmic plot of observed (symbol) and predicted (line) paclitaxel concentration versus time analysed by 1-compartment IV- bolus model (Model 1) with 1st-order elimination for individualised dose (patient BSA = 1.5 m^2, BSA = individualised dose)

The detail results of pharmacokinetic analysis using both models are displayed in Appendix 9 (p.316).

Table 5-8 The final estimates of pharmacokinetic (PK) parameters (AUC, C_{max}, $T_{1/2}$, K, CL, V_C) using 1-compartment IV-infusion and IV-bolus models compared to the initial estimates

PK parameters	*IV-bolus model Mean (±CV%)*	*IV-infusion model Mean (±CV%)*	*Initial parameters*
AUC	25.5 h*μmol/L (38.8)	17.7 h*μmol/L (1.2)	16.9 h*μmol/L
C_{max}	13.1μmol/L (31.7)	5.88μmol/L (1.2)	5.60 μmol/L
$T_{1/2}$	1.4 hr (46.2)	0.27 hr (4.1)	0.25 hr
K	0.5 (46.3)	2.6 (4.1)	2.8
CL	14.2 L/h (38.9)	20.4 L/h (1.2)	18.2-23.7 L/h
V_C	28.3 L (31.7)	7.8 L (4.3)	6.5-8.4 L
WSSR/AIC	210/66	0.3/–25	

AUC, the area under the drug concentration-time profile; C_{max}, peak plasma concentration; $T_{1/2}$, half-life of elimination; K, elimination rate constant from the central compartment; CL, total clearance; V_C, volume of distribution for the central compartment
AIC, the Akaike Information Criterion (refer to Appendix 10, p.321); WSSR, weighed sum of squared residuals (refer to Appendix 10, p.321)
Initial parameters were all calculated based on original V_C and CL values.

Table 5-8 compares the final parameter estimates for the above two PK models with the initial estimates. The initial parameters were obtained by calculation based on the original set parameters (V_C and CL) of the *ex vivo* model. Clearly, the final parameter estimates for the IV-infusion model were more closely comparable to the initial estimates, than the IV-bolus model. All the precisions of final estimates were better with the IV-infusion model (≤ 4.3%). Normally to compare different PK models, the average weighed sum of squared residuals (WSSR) and the average Akaike Information Criteria (AIC) are used as main measures of the goodness of fit of an estimated PK model. Lower values of these measures indicate a better goodness of fit of a model. In

this study the WSSR and AIC values were much lower for the IV-infusion model (WSSR = 0.3 and AIC = −25) than the bolus model (WSSR = 210 and AIC = 66).

5.5.3 The main PK parameters obtained via non-compartmental analysis (NCA)

The main pharmacokinetic parameters (T_{max}, C_{max}, AUC_{all} and CL_{obs}) were obtained after NCA as shown in Table 5-9. All the raw data are displayed in Appendix 11 (p.323).

Table 5-9 The final pharmacokinetic (PK) parameters obtained by non-compartmental analysis (NCA) in the *ex vivo* study

IND dose	Subject	T_{max} (hr)	C_{max} (μmol/L)	AUC_{all} (h*μmol/L)	Cl_{obs} (L/h)	CL_{obs} (L/h/m^2)
	1	1.83	6.46	18.46	16.69	11.13
	2	3.00	6.39	17.07	19.21	12.01
	3	2.74	6.56	18.69	17.81	10.99
	4	3.00	6.80	18.52	18.41	11.09
	5	3.00	5.59	15.93	22.47	12.84
	6	3.00	6.80	18.51	19.82	11.07
	7	3.00	5.51	15.75	24.18	13.00
	8	2.74	6.80	19.30	20.20	10.63
	9	1.81	5.80	16.57	24.07	12.34
	Mean	2.68	6.30	17.64	20.32	11.68
	Min	1.81	5.51	15.75	16.69	10.63
	Max	3.00	6.80	19.30	24.18	13.00
	S.D.	0.50	0.53	1.32	2.70	0.88
	CV%	18.65	8.38	7.50	13.28	7.55
DB dose	**Subject**	T_{max} (hr)	C_{max} (μmol/L)	AUC_{all} (h*μmol/L)	Cl_{obs} (L/h)	CL_{obs} (L/h/m^2)
	1	3.00	7.28	17.04	18.54	12.36
	2	2.78	5.67	15.25	20.71	12.94
	3	2.76	5.36	14.80	22.96	14.17
	4	2.76	5.37	14.18	23.97	14.44
	5	1.83	5.40	15.29	23.73	13.56
	6	3.00	5.12	14.20	25.55	14.27
	7	2.74	4.72	13.31	28.99	15.59
	8	2.77	6.86	19.50	19.79	10.42
	9	3.00	6.75	16.64	24.64	12.64
	Mean	2.74	5.84	15.58	23.21	13.38
	Min	1.83	4.72	13.31	18.54	10.42
	Max	3.00	7.28	19.50	28.99	15.59
	S.D.	0.36	0.89	1.89	3.19	1.50
	CV%	13.13	15.31	12.10	13.76	11.22
FLAT dose	**Subject**	T_{max} (hr)	C_{max} (μmol/L)	AUC_{all} (h*μmol/L)	Cl_{obs} (L/h)	CL_{obs} (L/h/m^2)
	1	3.00	6.79	19.71	17.80	11.87
	2	3.00	6.58	18.49	18.98	11.86
	3	2.77	7.17	18.33	19.14	11.81
	4	2.76	6.72	18.90	18.57	11.19

5	3.00	6.02	16.12	21.77	12.44
6	3.00	5.88	16.54	21.21	11.85
7	2.73	5.30	15.09	23.26	12.51
8	3.00	5.27	15.01	23.37	12.30
9	3.00	5.26	14.36	24.44	12.53
Mean	2.92	6.11	16.95	20.95	12.04
Min	2.73	5.26	14.36	17.80	11.19
Max	3.00	7.17	19.71	24.44	12.53
S.D.	0.12	0.74	1.95	2.42	0.44
CV%	4.24	12.03	11.52	11.55	3.67

IND dose, individualised dose; DB dose, dose-banded dose; FLAT, flat-fixed dose; T_{max}, time at which C_{max} is observed following administration of drug; C_{max}, peak concentration; AUC_{all}, the area under the drug concentration-time profile; Cl_{obs}, total observed clearance at L/h; CL_{obs}, total observed clearance at L/h/m²
All raw data in text file refer to Appendix 11 (p.323).

The T_{max} values were quite variable (from 1.81 hr to 3.00 hr) for all "patients" with different doses. The average T_{max} was 2.68 hr for IND dose, 2.74 hr for D-B dose, and 2.92 hr for FLAT dose. The C_{max} values were 6.30 ± 0.53 μmol/L (IND), 5.84 ± 0.89 μmol/L (DB), and 6.11 ± 0.74 μmol/L (FLAT). The AUC_{all} was 17.64 ± 1.32, 15.58 ± 1.89 and 16.95 ± 1.95 h*μmol/L for IND, D-B and FLAT dose, respectively. The total observed clearances (CL_{obs}) were 11.68 ± 0.88 L/h/m² (IND), 13.38 ± 1.50 L/h/m² (DB) and 12.04 ± 0.44 L/h/m² (FLAT).

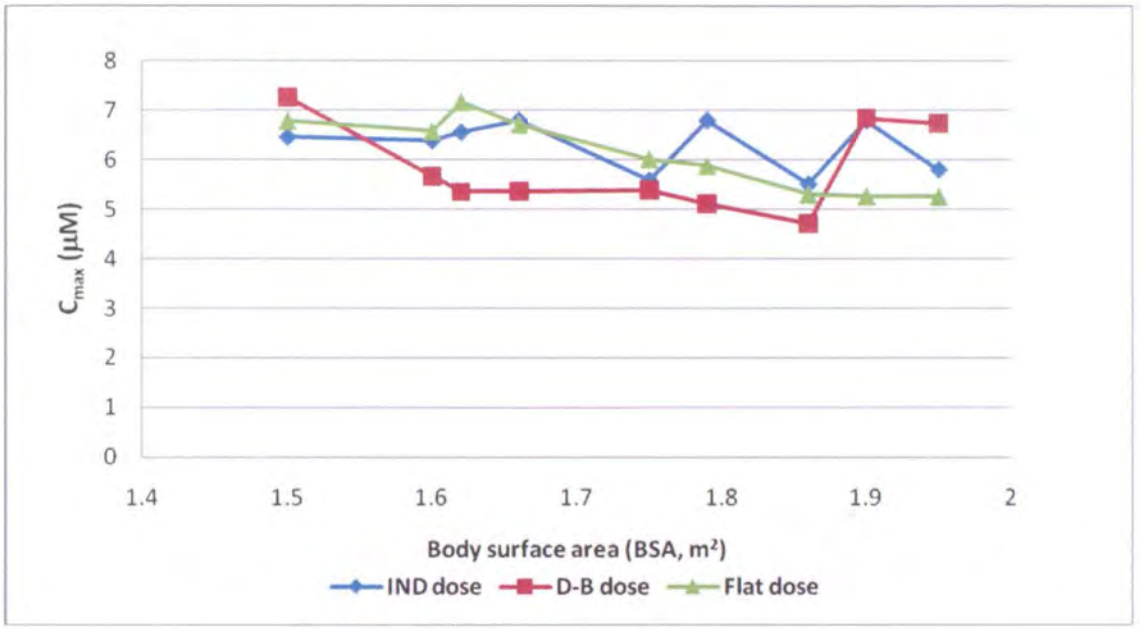

Figure 5-17 Plot of C_{max} versus body-surface area (BSA) for individualised (IND) dose, D-B dose and flat-fixed dose in the *ex vivo* study

Figure 5-17 is the plot of C_{max} versus body-surface area (BSA) for these three dosing methods. The C_{max} values for individualised (IND) dose were more stable than the other two doses. Most C_{max} points of D-B dose were lower than those of IND dose correspondingly. For the flat-fixed (Flat) dose, the C_{max} values decreased with increasing BSA values.

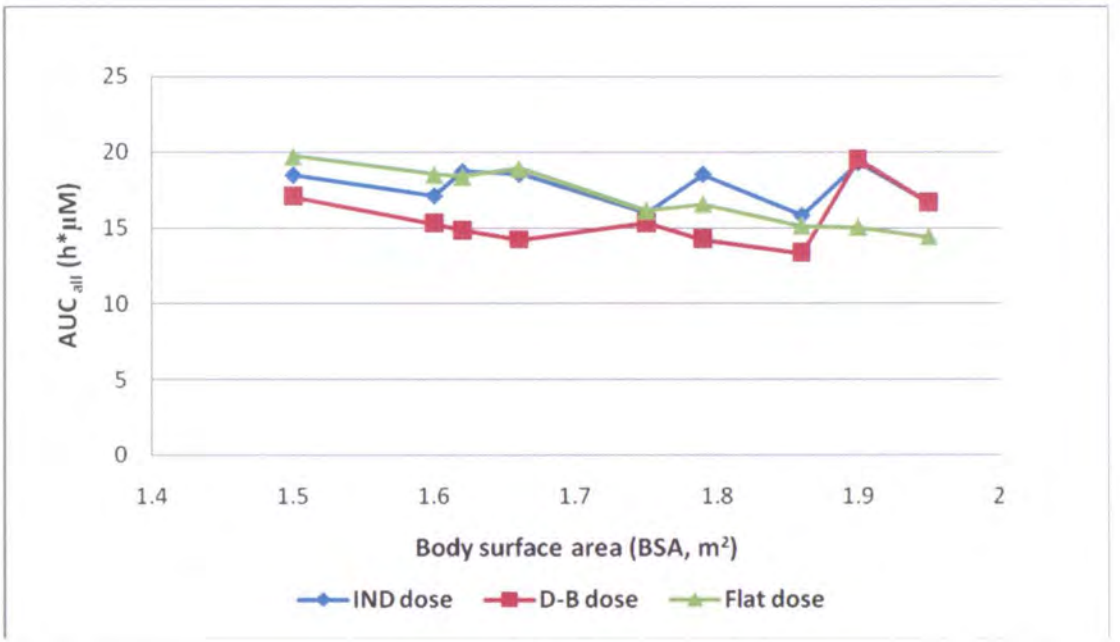

Figure 5-18 Plot of AUC_{all} versus body-surface area (BSA) for individualised (IND) dose, D-B dose and flat-fixed dose in the *ex vivo* study

Figure 5-18 shows the relationship of AUC and BSA for all three dosing strategies. The AUC values for IND dose seemed quite stable with low variability (17.64 ± 1.32 µmol/L). The AUC values for D-B dose seemed lower than those for IND dose. AUC values for flat-fixed (Flat) dose were decreasing with increasing BSA. At BSA ≤ 1.75 m^2, AUC (Flat) seemed to be equal to or higher than AUC (IND), but at BSA > 1.75 m^2, AUC (Flat) was lower than AUC (IND).

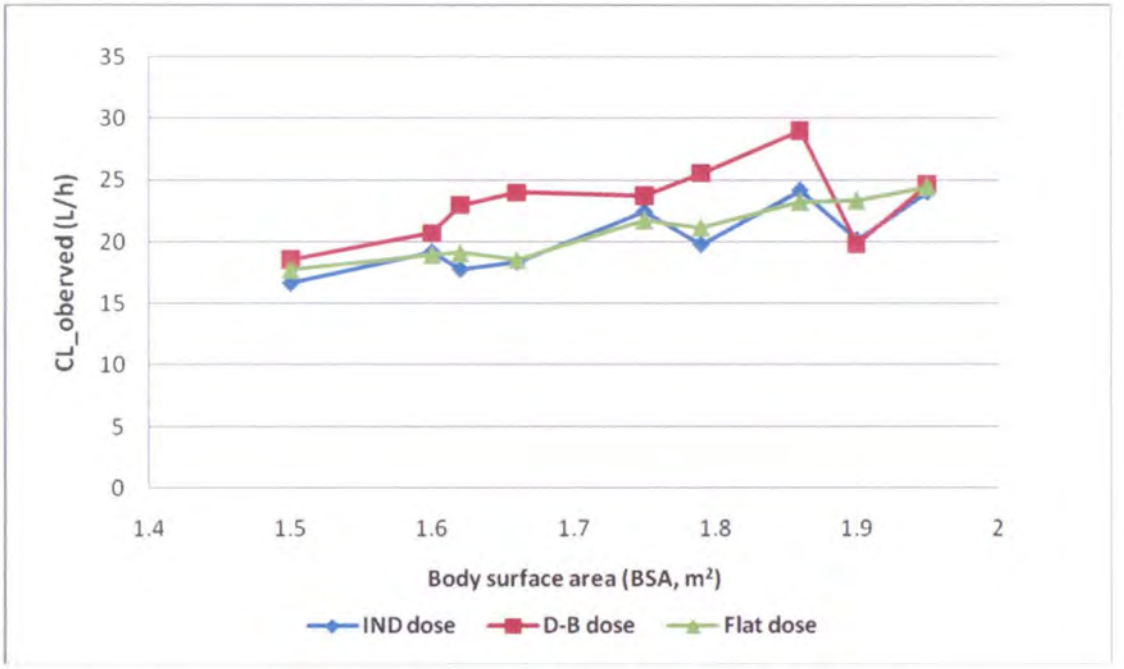

Figure 5-19 Plot of the total observed clearance (CL) versus body-surface area (BSA) for individualised (IND) dose, D-B dose and flat-fixed dose in the *ex vivo* study

Based on Figure 5-19, the total observed clearance (CL) seemed to increase with BSA in a linear fashion for all three types of doses. For flat-fixed dose, a good linear relationship was observed between CL and BSA. Comparatively, the variability of CL with D-B dose seemed higher than those with other two doses.

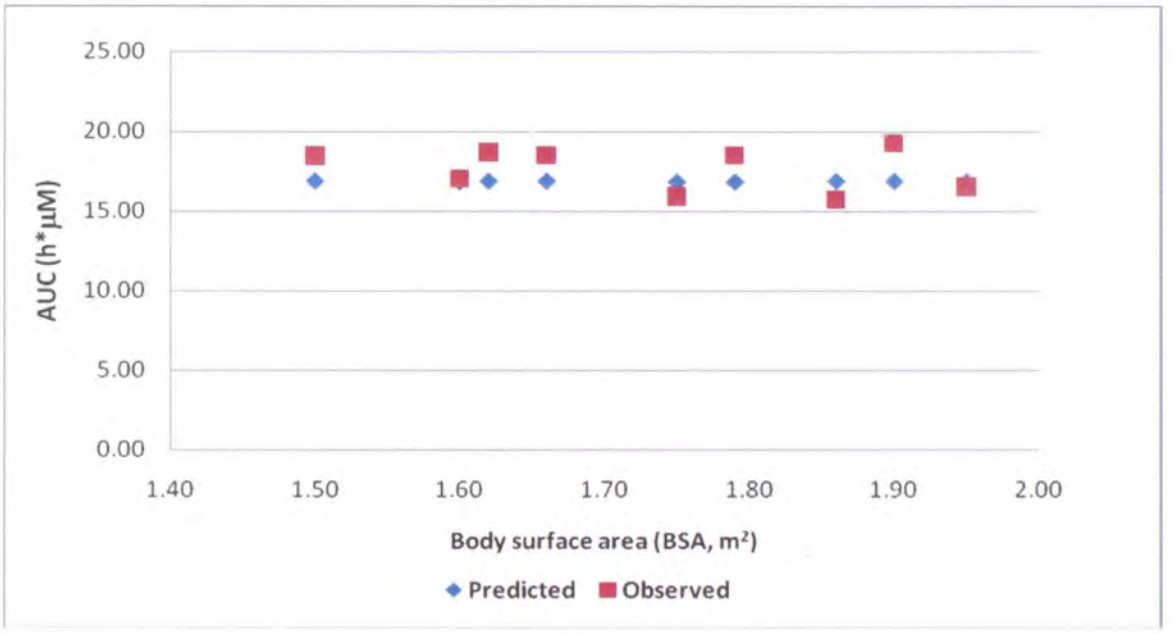

Figure 5-20 Plot of AUC (observed and predicted) versus body surface area (BSA) for individualised (IND) dosing in the *ex vivo* study

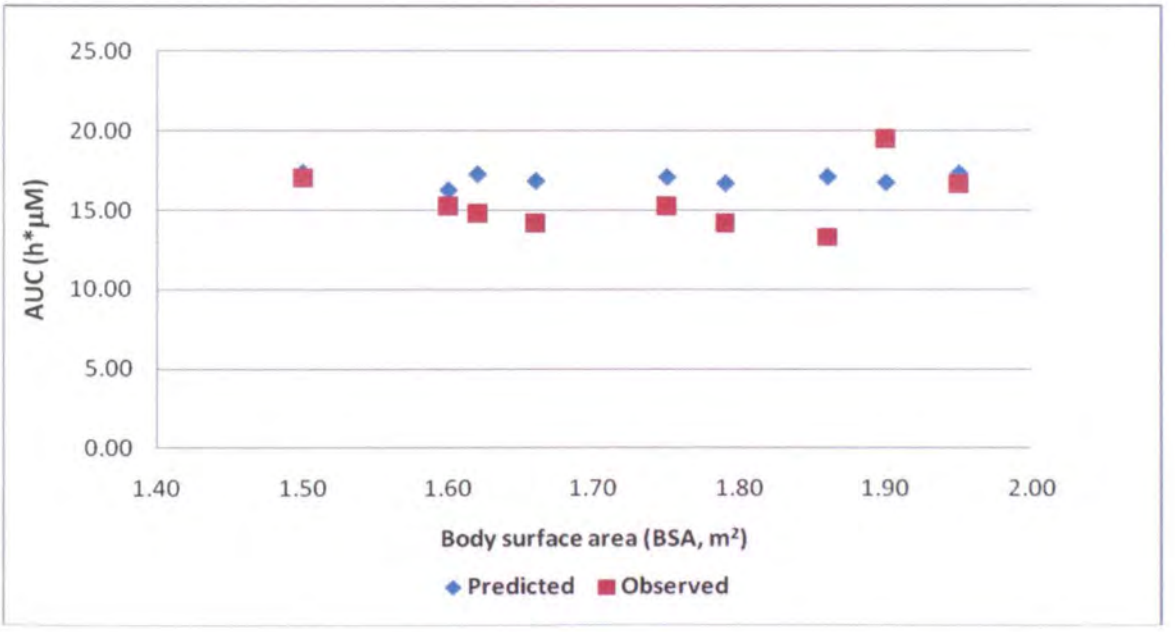

Figure 5-21 Plot of AUC (observed and predicted) versus body surface area (BSA) for D-B dosing in the *ex vivo* study

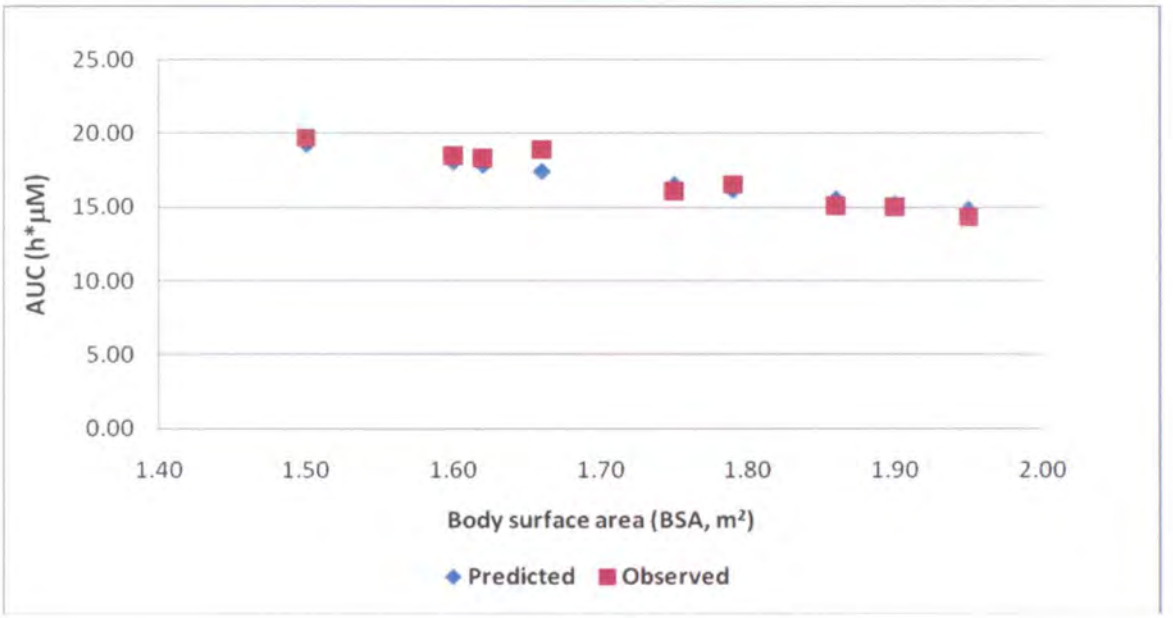

Figure 5-22 Plot of AUC (observed and predicted) versus body surface area (BSA) for flat-fixed dosing in the *ex vivo* study

The above 3 figures (Figure 5-20, Figure 5-21, Figure 5-22) describe the relationship of AUC_observed / AUC_predicted with BSA for all three dosing methods. For individualised (IND) dose (Figure 5-20), the AUC_observed values were as expected, quite stable with respect to BSA and quite close to those AUC_predicted values. Compared to IND dose, a larger difference between AUC_observed and AUC_predicted was observed for D-B dose (Figure 5-21) and most points of AUC_observed in the graph were lower than AUC_predicted except for the 2nd to last point (BSA = 1.90 m^2). For flat-fixed dose (Figure 5-22), it was found that the observed values were very close to the predicted values with the lowest variability, when compared with the other two dosing strategies.

5.5.4 Bioequivalence study

This bioequivalence study was conducted to determine if the D-B dose (D-B) and flat-fixed dose (FLAT) was significantly different to the individualised dose (IND) in

pharmacokinetics. The test group (D-B or FLAT) was compared to the reference group (IND). The log-transformed data of C_{max} and AUC_{all} were applied to conduct the bioequivalence analysis.

The results of the bioequivalence analysis are shown in Table 5-10. For D-B dose, the 90% confidence interval (C.I.) of the ratio (D-B/IND) of averages was 83.6 – 101.3% for C_{max} and 82.2 – 94.2% for AUC_{all}. For FLAT dose, the 90% C.I. of the ratio (FLAT/IND) of averages was 90.0 – 103.7% for C_{max} and 89.6 – 102.4% for AUC_{all}. These values were all within the acceptable bioequivalence range of 80 – 125% for both parameters.

Table 5-10 The average bioequivalence study comparing Dose-banded (D-B) dose and Flat-fixed (FLAT) dose to Individualised (IND) dose

Dependent	Units	Ref	RefLSM	RefLSM_SE	Test	TestLSM	TestLSM_SE	Difference	CI_90_ Lower	CI_90_ Upper	Prob <80.00	Prob >125.00	Power
$Ln(AUC_{all})$	h*µmol/L	IND	2.87	0.03	DB	2.74	0.03	-0.13	82.17	94.21	0.02	0	1
$Ln(C_{max})$	µmol/L	IND	1.84	0.04	DB	1.75	0.04	-0.08	83.55	101.26	0.01	0	0.98
Dependent	**Units**	**Ref**	**RefLSM**	**RefLSM_SE**	**Test**	**TestLSM**	**TestLSM_SE**	**Difference**	**CI_90_ Lower**	**CI_90_ Upper**	**Prob <80.00**	**Prob >125.00**	**Power**
$Ln(AUC_{all})$	h*µmol/L	IND	2.87	0.03	FLAT	2.82	0.03	-0.04	89.57	102.37	0	0	1
$Ln(C_{max})$	µmol/L	IND	1.84	0.03	FLAT	1.8	0.03	-0.03	90	103.66	0	0	1

Ref, reference group

Test, test group

LSM, the least square means

RefLSM, the least square means of the Reference

RefLSM_SE, the standard error of Reference LSM

TestLSM, the least square means of the Test

TestLSM_SE, the standard error of Test LSM

Difference, the difference of TestLSM and RefLSM (TestLSM –RefLSM)

CI_90_lower, the lower level of 90% confidence interval

CI_90_upper, the upper level of 90% confidence interval

5.6. Discussion

5.6.1 Pharmacokinetic (PK) analysis

The 1-compartment infusion model with 1st order elimination was the best model to describe the data obtained in this study. This was because the final estimates of PK parameters with this model were reproducible and also closely mirrored the initial parameters calculated based on the original parameters of the *ex vivo* model (Table 5-8, pg.215). The lower WSSR and AIC values also indicated this model was better fit compared with the other bolus model. This agreed with the initial study design that included a 3-hour drug infusion with first-order elimination. In other words, this *ex vivo* simulation model was approved valid to estimate main PK parameters with acceptable precision.

5.6.2 Non-compartmental analysis (NCA)

In this study, the observed time (T_{max}) of the maximum paclitaxel concentration (C_{max}) was quite variable. The large variability of T_{max} was not unusual. The half-life ($T_{1/2}$) of the drug in the simulated model was estimated at approximately 15 minutes (0.25 hr, refer to Table 5-8). In theory, a steady state should be achieved in this study after about $6 \times T_{1/2}$ (about 90 minutes) from the beginning of the infusion, when the drug concentration reached its maximum and remained stable until infusion was stopped. Based on the data obtained (Table 5-7, pg.207), a steady state was achieved between 1.8 hr (108 min) to 3 hr (infusion stopping). During the steady-state period, the peak paclitaxel concentration (C_{max}) only varied slightly, and thus the observed T_{max} had a large variability.

Table 5-11 lists some reference PK parameters for paclitaxel from different resources. The theoretical estimates were 5.6 μmol/L (C_{max}), 16.9 h*μmol/L (AUC) and 12.14 L/h/m^2 (CL) calculated based on the original parameters in the *ex vivo* study (refer to Table 5-8). In a previous study including a paclitaxel 175 mg/m^2 3-hr infusion schedule[47], the obtained PK parameters were 5.9 μmol/L (C_{max}), 18.5 h*μmol/L (AUC) and 11.4 L/h/m^2 (CL). The relationships between C_{max} and dose (Figure 5-23) and between AUC_{all} and dose (Figure 5-24) for the 3-hr infusion schedule have been plotted based on the available PK data in literature (Table 5-1). Both C_{max} and AUC_{all} increased with dose (mg/m^2) in an exponential manner with acceptable correlation coefficients (R^2) of 0.96. Based on the equations obtained, C_{max} and AUC_{all} were estimated to be 5.30 μmol/L and 17.50 h*μmol/L respectively for the 175 mg/m^2 dose.

The final PK parameters obtained in this *ex vivo* study were compared with the above reference values (Table 5-11). The estimates of C_{max}, AUC_{all} and CL through the *ex vivo* model were quite reproducible, when compared with all reference values, especially for individualised (IND) dose.

Table 5-11 The final PK parameters estimated by non-compartmental analysis (NCA) in the *ex vivo* study with comparison to reference values

	[a]*theoretical value*	[b]*literature value*	[c]*estimate by relationship*	*IND dose (ex vivo)*	*D-B dose (ex vivo)*	*Flat dose (ex vivo)*
C_{max} (μmol/L)	5.6	5.90	5.30	*5.51-6.80*	4.72-7.28	5.26-7.17
AUC (h*μmol/L)	16.9	18.50	17.50	*15.75-19.30*	13.31-19.50	14.36-19.71
CL (L/h/m^2)	12.14	11.40		*10.63-13.00*	10.41-15.59	11.19-12.53

IND dose, individualised dose; D-B dose, dose-banded dose; Flat dose, flat-fixed dose

[a]Theoretical value: calculated based on the experimental design of the ex vivo study (e.g.CL, V_C)

[b]literature value: obtained in a clinical study (following paclitaxel 175 mg/m^2 3-hr infusion schedule) [47]

[c]estimate by relationship: estimated based on the relationships of C_{max} and dose (Figure 5-23) and AUC_{all} and dose (Figure 5-24), which were produced based on PK data in literature

The C_{max} and AUC_{all} obtained with D-B dose were both generally lower than those with individualised dose. It was because for D-B dose, where the dose was given as a combination of infusions, one pre-made infusion bag had to be replaced by another during administration. This resulted in small time-delays during administration which may have influenced the C_{max} and AUC_{all}. For flat-fixed dose, a fixed dose (mg) was given to all "patients" with different BSA values so that a lower dose (mg/m^2) was achieved at higher BSA. This explained why the C_{max} and AUC_{all} values were reducing with increasing BSA.

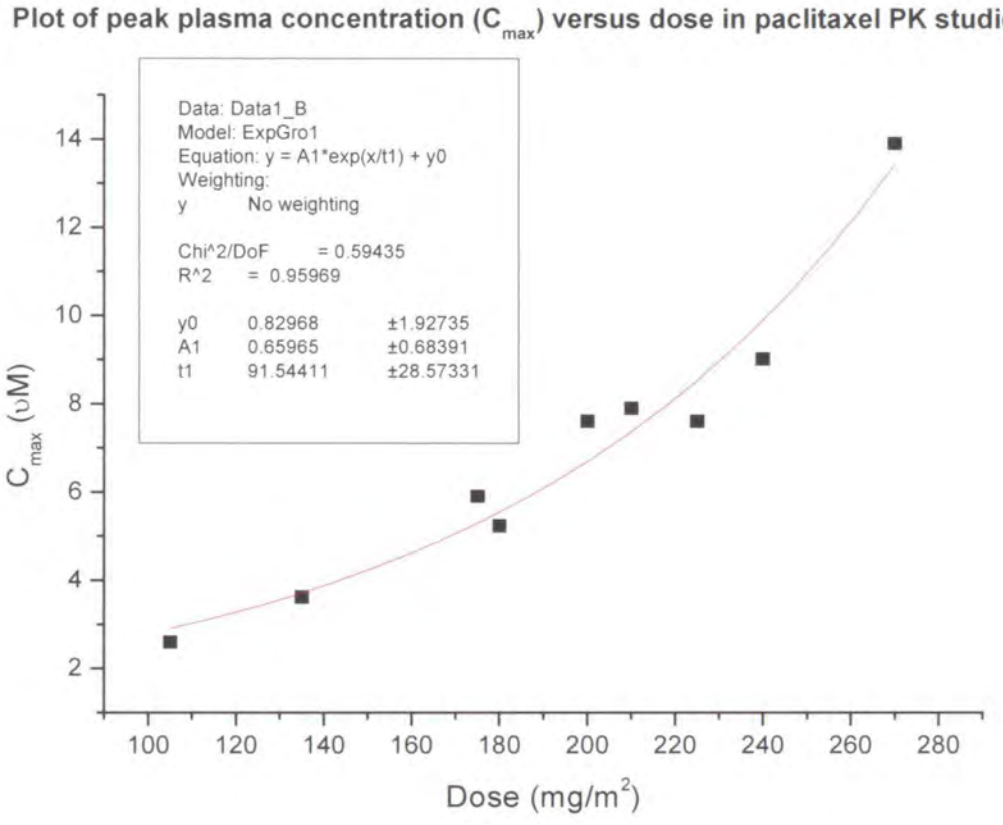

Figure 5-23 The plot of paclitaxel peak plasma concentration (C_{max}) and the given dose (mg/m^2) by a 3-hr infusion schedule in previous clinical studies based on literature (data selected from Table 5-1)

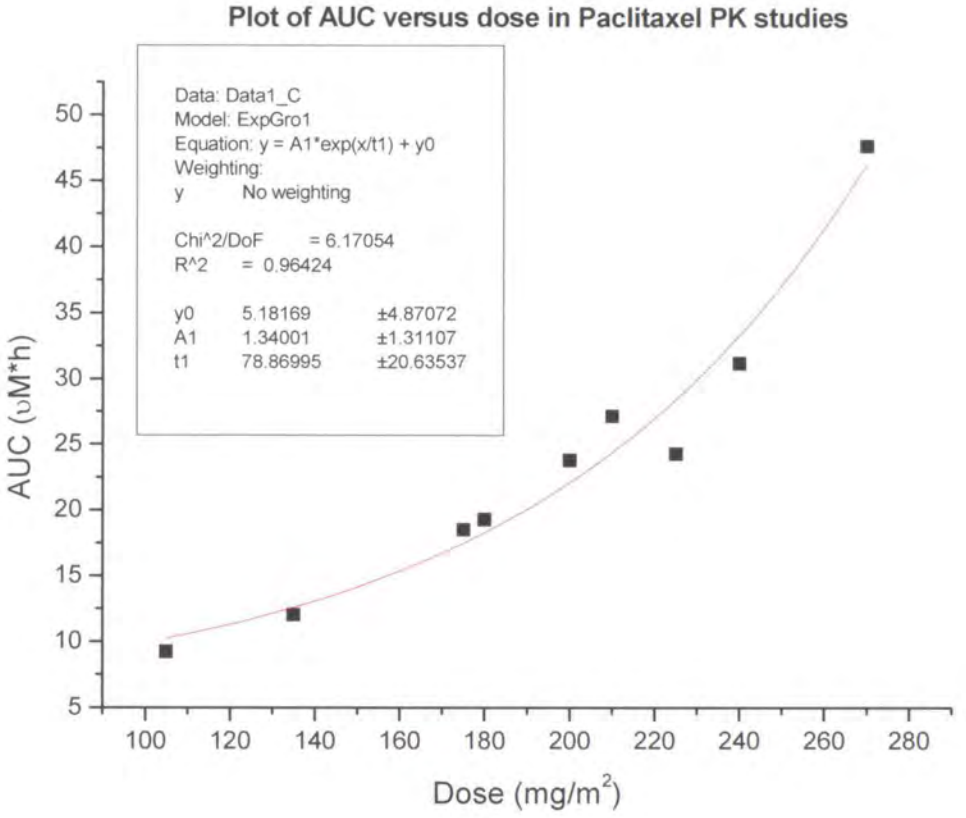

Figure 5-24 The plot of area under paclitaxel concentration-time profile (AUC) and the given dose (mg/m^2) by a 3-hr infusion schedule in previous clinical studies based on literature (data selected from Table 5-1)

5.6.3 The bio-equivalence analysis

The bio-equivalence analysis using WinNonlin software was conducted according to the guidance of "Statistical procedure for bioequivalence studies using a standard two-treatment crossover design" as recommended by the FDA in 1992. Although the *ex vivo* study was not a real clinical study, the experimental design of this study was representative of the clinical protocol (Chapter 4). The bio-equivalence analysis was done by comparing D-B dose or flat-fixed dose with the standard therapy (individualised dose).

As mentioned previously in the FDA guidance to establish bioequivalence, the bioequivalence criterion was 80 – 125% at the 90% confidence interval. In this study the 90% confidence intervals of the ratio (test/reference) of average C_{max} and AUC_{all} values were all within the acceptable range for both D-B dose and flat-fixed dose. Based on the results obtained in this *ex vivo* study, both D-B dose and Flat-fixed dose were statistically bioequivalent to the standard individualised dose.

In a clinical study the intra- and inter-individual variability in paclitaxel pharmacokinetics (especially distribution and metabolism) must be considered. This may influence the conclusion of whether a D-B dose or flat-fixed dose is equivalent to an individualised dose in a clinical study. However, the intra- and inter-individual variability in pharmacokinetics, for example, the variability in the expression of liver enzymes, is due to patients themselves rather than dosing methods. Therefore the final conclusion of this *ex vivo* simulation study is that regardless of pharmacokinetic variability, both D-B dose and flat-fixed dose are bioequivalent to the standard individualised dose. On this basis, adoption of either of these alternative dose strategies would not be expected to alter either the therapeutic outcome or side-effect profile, with the respect to the conventional individualised dosing based on BSA.

5.6.4 The ex vivo simulation model

The potential of the *ex vivo* PK simulation model was proven in this study because the main PK parameters of paclitaxel were estimated with acceptable accuracy and precision. The application of this model is subjected to two conditions: the pharmacokinetic variability between individuals and within individuals can be neglected; the PK behaviour of the drug is well known and the population PK information is available in literature. This *ex vivo* simulation model is particularly useful

for dose comparison studies, for example, to evaluate D-B dosing compared to the standard dosing for a drug.

Compared with a conventional clinical study, this *ex vivo* PK model offers the following advantages:- (a) for a dose comparison study, it can accurately estimate PK parameters such as AUC, C_{max} and CL because this model avoids intra- and inter-individual PK variability that can induce errors in PK parameter estimation; (b) it avoids considerable effort in the designing and conducting of a clinical trial, for example, patient recruitment and patient care from medical, nursing and pharmacy personnel; (c) it spares both cost and time; (d) it is possible to develop multi-compartment models if the complicated pharmacokinetics of a target drug is well known and there is sufficient equipment available; (e) most importantly of all, patients are spared on invasive procedure (no blood sampling) by not serving as trial subjects.

However, this *ex vivo* PK model is still associated with certain limitations: - (a) it is only applicable for drugs with well known pharmacokinetics where key parameters are available to set up the model; (b) its application is very limited and it can only be used for dose comparison studies, for example, to evaluate D-B strategy with the conventional individualised dosing based on BSA; (c) this model fails to explain the pharmacokinetic behaviour of a drug in the body, resulting from metabolic processes, for example, enzyme induction (e.g. CYP3A4 and CYP2C8).

In terms of the experimental design of the *ex vivo* model (refer to Figure 5-7), this model was associated with certain limitations which may be further optimised in the future:

(a). In the *ex vivo* experiment, it was difficult to simultaneously start all three pumps utilising one operator, which was a disadvantage of this *ex vivo* system. This

could be resolved by connecting these pumps to the PC and using software control rather than manual control.

(b). The balance used in this study should be replaced with one that has a large surface area. This would buffer the vibrations caused by the movement of the stirrer during experiment and maintain a more stable system.

(c). To minimise occupational exposure to cytotoxics, the cap of the body reservoir should be modified or specially made with customised tube connectors, which are well sealed. This can prevent the exposure of cytotoxics and loss of solution from the connection ports of the body reservoir.

6. CHAPTER 6: CONCLUDING DISCUSSION AND PROPOSALS FOR FUTURE WORK

6.1. Concluding Discussion

On the basis of work presented in this thesis, it is possible to assign extended stability of 15 – 29 days for paclitaxel infusions (0.3 – 1.0 mg/m^2) in 0.9% sodium chloride. This is in agreement with a previous report[244] and the collective evidence supports shelf-life assignments of the duration required for dose-banding. The stability of paclitaxel infusions was mainly decided by physical stability, which was limited by the formation of microcrystalline precipitation. This was dependent on concentration and temperature[244,245,246,247,252]. A lower concentration (0.3 mg/mL) and refrigerated storage (2 – 8°C) contributed to improved stability of paclitaxel infusions. In addition, this study indicated obvious changes in sub-visual particle counts may be used as a predictive indication of the physical stability of paclitaxel infusions.

This study has provided adequate stability data (> 14 days), which facilitates the application of D-B strategy on paclitaxel chemotherapy. Though, this is still limited compared with other drugs that have been successfully dose banded, for example, carboplatin, where infusions have a validated shelf-life of over 84 days when stored at 2 – 8°C[284]. A feasible D-B scheme (Table 2-7, p.93) for paclitaxel chemotherapy was successfully devised using infusion concentrations within the range of stability studies, covering all current available dosage regimens (80 – 225 mg/m^2) and a broad range of BSA values (1.6 – 2.0 m^2)[254] for adults. Dose-banding of paclitaxel would require more stringent management to avoid wastage of infusions due to the limited shelf-life. Also in order to prolong physical stability of paclitaxel infusions, more investigation should

be conducted to understand the mechanism of the formation of crystallisation and other factors which contribute to physical instability of infusions. This work may inform methods to better control physical stability in these infusions.

The finding of this study that physical stability of pre-made paclitaxel infusions is unpredictable requires that paclitaxel infusions are re-inspected for any sign of paclitaxel precipitation immediately before use to ensure patient safety.

To conduct a pharmacokinetic study, an analytical method with good sensitivity and selectivity was required. Although a number of analytical methods have been published for quantification of paclitaxel in biological fluids (refer to Section 1.1.5.1, p.36), HPLC combined with UV detection is still the most commonly used method mainly because of its wide availability and convenience. In this study the experimental conditions were initially adopted from some published studies but it was soon found that these methods were not optimal. For example, Huizing et al[137] introduced a sensitive HPLC assay and a detailed SPE method but failed to incorporate an internal standard during the analytical process. Among the HPLC-UV methods available in literature, not all of them demonstrated sufficient selectivity and sensitivity in the presence of co-administered drugs[137,140,255,270]. Many reports failed to publish the detailed procedure of method development including method optimisation and validation, which made it difficult to reproduce the authors' claims for sensitivity and selectivity. Furthermore, there may be large variability in performance between different HPLC instruments, which would make it difficult to transfer previously published methods from different laboratories. Therefore, chromatographic and sample preparation methods in this study were optimised and the validation of this paclitaxel assay in human plasma was finally completed, as shown in Chapter 3.

One of the main challenges during the development of the paclitaxel assay in human plasma was to achieve good sensitivity and selectivity at the same time. It was found previously that the paclitaxel peak was suffering from interference due to co-administered drugs even after extraction (refer to Section 3.3.1.2.3, p.114). By optimising buffer pH used during SPE, the interference from the co-administered drug was successfully removed with good and reproducible recovery of both paclitaxel and the internal standard. However, this did compromise the sensitivity of the assay at the same time. In response to this, the sensitivity of this assay was greatly improved by optimisation of the mobile phase (the addition of 2% THF) and the tubing size (7/1000 inch) between the autosampler and detector, with improved peak efficiency obtained.

Another main challenge during method development was the frequent occurrence of the blockage of SPE cartridges, which seriously increased experimental time and sometimes caused experimental failure. The addition of a protein precipitation step prior to SPE by pH adjustment successfully resolved this problem and obviously increased the sample loading speed during SPE.

As for the validation study, a linear range of 10 – 300 ng/mL was produced for paclitaxel in human plasma with an average correlation coefficient (R^2) of 0.9997. The LLOQ was defined as 10 ng/mL (11.7 nM), which was comparable with previous studies[136,137,138,140]. Considering the concentration of interest for paclitaxel in clinical studies was usually above the plasma concentration of 50 nM[47], this method was sufficiently sensitive. Also, this method showed excellent intra- and inter-day accuracy (100 ± 5%) and precision (< 6.4%). Compared with other published studies[136,137,138,139,140], a higher average recovery (%) was produced for paclitaxel (100.8%

with a CV of < 8%) and docetaxel internal standard (97.3% with a CV of < 6.5%) in this study. In addition, this assay was free from interference due to 11 co-administered drugs and endogenous products. This method was considered a sensitive and selective assay with acceptable accuracy and precision.

Following the development of the assay for paclitaxel in human plasma, paclitaxel stability in human plasma was evaluated after both long and short-term storage and during processes involved in collecting and analysing samples. Although stability of paclitaxel in human plasma had been previously reported, there was a lack of detailed data and information on the experimental procedures used. Paclitaxel in plasma demonstrated good stability after being stored at −20°C for up to 2 months, and also when kept at room temperature for over 5 hours before analysis and after undergoing 3 × freeze-thaw cycles. These results agreed with previous studies[138,255,267,268,269]. Paclitaxel was stable over 14 days when kept in the elution solvent at 2 – 8°C. Compared with previously published data (24 hours)[268,269,270], superior stability of up to 72 hours was determined in this study for reconstituted paclitaxel samples when stored in the autosampler at room temperature. These stability data demonstrated the robustness of the paclitaxel assay, developed above, and provided confidence during sample analysis in the event of any delay or failure during each step of the assay process. These data are particularly useful in the subsequent design of clinical and pharmacokinetic studies and the establishment of sampling times.

The clinical and pharmacokinetic study was designed to compare the dose-banding (D-B) strategy and flat-fixed dosing with individualised dosing according to BSA for paclitaxel chemotherapy by introducing limited sampling strategy (LSS). Clinically measured outcomes for cancer chemotherapy include a group of targets such as

survival rates, symptoms, tumour size and toxicity. These outcomes would be too variable for evaluation in this pharmacokinetic study, and would require 5-10 year follow-up and huge patient numbers. Therefore instead of using clinical outcomes, AUC was considered as a surrogate of therapeutic outcome and toxicity as it represents the exposure of tissues to drug. Both anti-tumour activity and drug-toxicity are likely to be related to AUC. The study protocol is presented in Chapter 4 (p.153).

After designing this clinical study, some national and international clinical trials recruited almost all cancer patients eligible to receive paclitaxel chemotherapy in the UK, which made it impossible for this planned clinical study to be conducted in the limited time period available. Therefore, this clinical study has been replaced by an *ex vivo* simulation study explained in Chapter 5.

The novel *ex vivo* simulation model was designed to reproduce the main pharmacokinetic parameters for paclitaxel including the peak "plasma" concentration (C_{max}) and the area under the paclitaxel concentration-time profile (AUC). Development of the *ex vivo* simulation PK model was based on population pharmacokinetic information available in literature such as V_C and CL. In accordance with pharmacokinetics theory, the total clearance was considered only from the central compartment. In this *ex vivo* model, only the central compartment (systemic circulation in the human body) was considered rather than the other compartments (e.g. the 2nd or 3rd compartment). Only the in- and out- kinetics of the central compartment were measured, for example, infusion (in) and clearance (out), and no distribution and metabolism effects were measured directly.

PK analysis using WinNonlin 5.2, the 1-compartment infusion model with 1st order elimination provided a good model to describe the data obtained in the *ex vivo* study,

which is understandable as the study included a 3-hour infusion and first-order elimination. By non-compartmental analysis, the main PK parameters (C_{max}, AUC_{all} and CL) have been estimated for each theorectical "patient" (each BSA). The estimates of C_{max}, AUC_{all} and CL obtained for individualised BSA dosing through the *ex vivo* model were reproducible, compared favourably with all reference values (Table 5-11, p.228). This proved that this *ex vivo* model can estimate main PK parameters for different doses of paclitaxel with good accuracy and precision.

Lower estimates in C_{max} and AUC_{all} with the D-B dose were obtained compared with individualised dose, mainly because loss of drug may have occurred through the use of multiple infusion bags to provide the dose. For flat-fixed dosing, both C_{max} and AUC_{all} were reduced with increased BSA values, which was due to a fixed dose given to all "patients" (from low to high BSA values). All these observations were predicted for the designed clinical pharmacokinetic study, if this clinical study had been able to proceed as planned.

The bioequivalence study showed D-B dose and flat-fixed dose both bioequivalent to the individualised dose according to BSA in terms of C_{max} and AUC_{all}. The bioequivalence study was conducted according to FDA guidance using WinNonlin 5.2 software. Results showed the 90% confidence intervals of the ratios of average C_{max} and AUC_{all} were all within the acceptable limit for both D-B dose and flat-fixed dose. Therefore, there was no statistically significant difference in both C_{max} and AUC_{all} between the D-B dose and individualised dose, and between the flat-fixed dose and individualised dose. In other words, the D-B dose and flat-fixed dose were both bioequivalent to the individualised dose.

The *ex vivo* PK simulation model has been proven effective to compare different dosing strategies for paclitaxel. It could potentially be applied to any given drug to evaluate different dosing strategies. The application requirements of this model, its advantages and disadvantages have been discussed in Chapter 5. When compared with a conventional clinical and pharmacokinetic study, this novel *ex vivo* PK simulation offers many advantages such as saving effort in the design and conducting of the clinical study and reducing both patient inconvenience and cost involved. However, this model cannot fully explain the pharmacokinetic behaviour of a drug in the body, for example drug distribution and metabolism, and it would not account for patients with large interindividual variability in gene expression of relevant CYP enzymes (e.g. CYP2C8 and CYP3A4 for paclitaxel). Therefore this simple *ex vivo* model cannot replace the important status of a clinical trial for dose comparison studies, but it is a useful tool for the estimation of results in a clinical study and helps gather more valuable information to improve the design of clinical studies.

6.2. Proposals for Future Work

Future drug stability studies should focus on improving the physical stability of paclitaxel infusions. More rigid temperature control at storage should be tested, for example 3 – 5°C used instead of 2 – 8°C, to evaluate the shelf-life of paclitaxel (solvent-based formulation, e.g. Taxol) in pre-made infusion containers. Infusion stability studies on nano-particulate paclitaxel (e.g. Abraxane) should also be conducted to compare with solvent-based paclitaxel (e.g. Taxol) with a view to considering the nano-particulate paclitaxel for dose banding. Different container types should be studied and compared to determine the effects of particle counts of the diluent solution. For example, low density polyethylene (LDPE) containers exhibited lower

sub-visual particle counts of diluent solution in this study, which may have contributed to longer shelf-life of paclitaxel infusions in those containers. Given the need for a more robust method to assess physical stability of infusions, other methods such as turbidimetric method should be evaluated.

Further development and optimisation of sample preparation for the assay of paclitaxel in plasma may focus on reducing the experimental time and minimising the required volume of plasma samples. These developments would be beneficial in clinical studies and would reduce the blood volumes taken from patients. The work may focus on minimising the size of the CN-E SPE cartridges, thus correspondingly reducing the volume of washes and required volume of plasma samples. All these would therefore reduce the experimental time.

- The clinical and pharmacokinetic study, as designed in Chapter 4, should be conducted to confirm the results of the *ex vivo* study and provide further clinical evidence to support the application of a dose-banding strategy on paclitaxel. The main barrier to such a study is currently the availability of patients requiring paclitaxel chemotherapy who are not enrolled in major national clinical trials. The recent availability of nano-particulate formulations of paclitaxel may make available small groups of patients for study (up to 10 patients). However, data from such a study could not be applied to solvent-based paclitaxel formulations, unless control arms using conventional paclitaxel formulations were also included.

The limited sampling strategy (LSS) for paclitaxel (described in Section 4.2.6.2.1, p.168) should be evaluated and validated in the future clinical and pharmacokinetic study. This could be carried out on 4 patients randomly selected from 10 patients, and would enable full validation of the LSS. Full sets of blood samples (10 to 12 samples) would

be collected at different times before and post infusion from these 4 patients for all three courses (Individualised, D-B and flat-fixed doses). The whole 12 sets of data from these 4 patients would be used to test the strategy. Bias and precision of the limited sampling strategy would be determined by mean prediction error and the root mean square error. The limited sampling points may be optimised by Bayesian estimation.

The *ex vivo* model may be further developed to be a 3- or more compartment model based on PK information in literature. This means the further developed models may account for drug distribution and metabolism apart from elimination.

Different infusion systems (e.g. multiple bags connected by connectors) for administration of standard D-B infusions could be evaluated through the *ex vivo* model. The results could be compared with doses provided from single infusion bags to establish the extent of drug loss arising from dose-banding using multiple (2 – 4) standard infusions to provide the D-B dose.

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Kingston University of London

Appendices

FOR THE THESIS OF STUDIES ON THE DOSE BANDING OF PACLITAXEL

Jing Xu 2009

Appendix List

Appendix 1: Typical HPLC chromatograms obtained during the validation of the stability-indicating HPLC assay used in Chapter 2

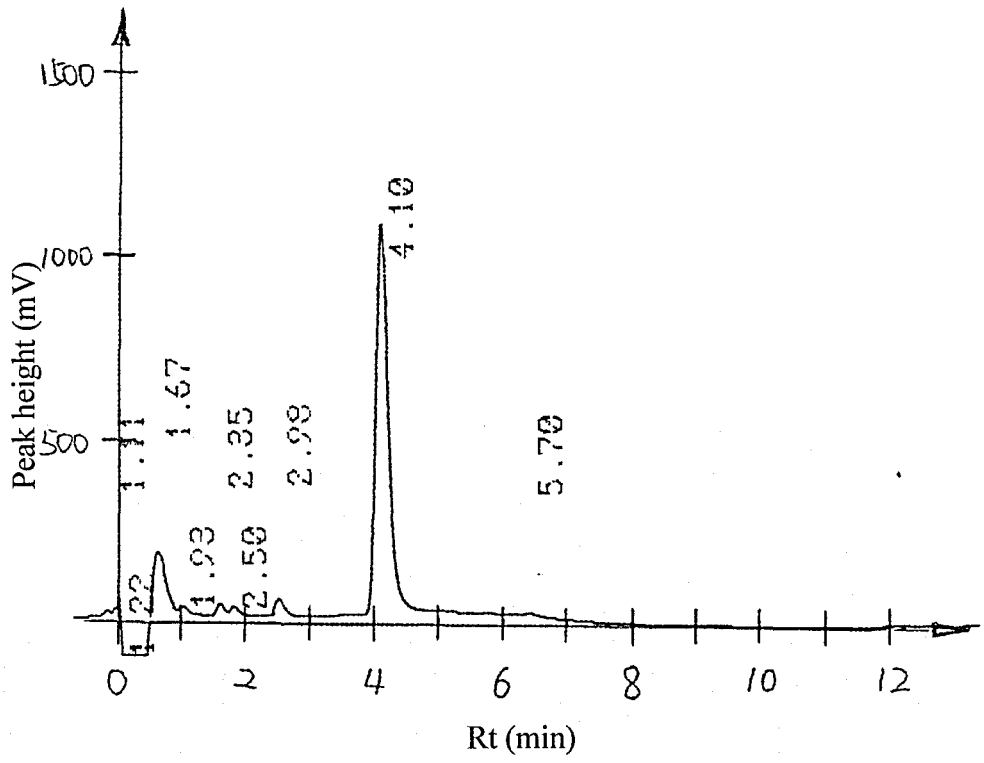


Figure 1. A typical chromatogram of paclitaxel control sample (15 μg/mL) after storage at 5°C for 1 hour (retention time of paclitaxel = 4.10 min)

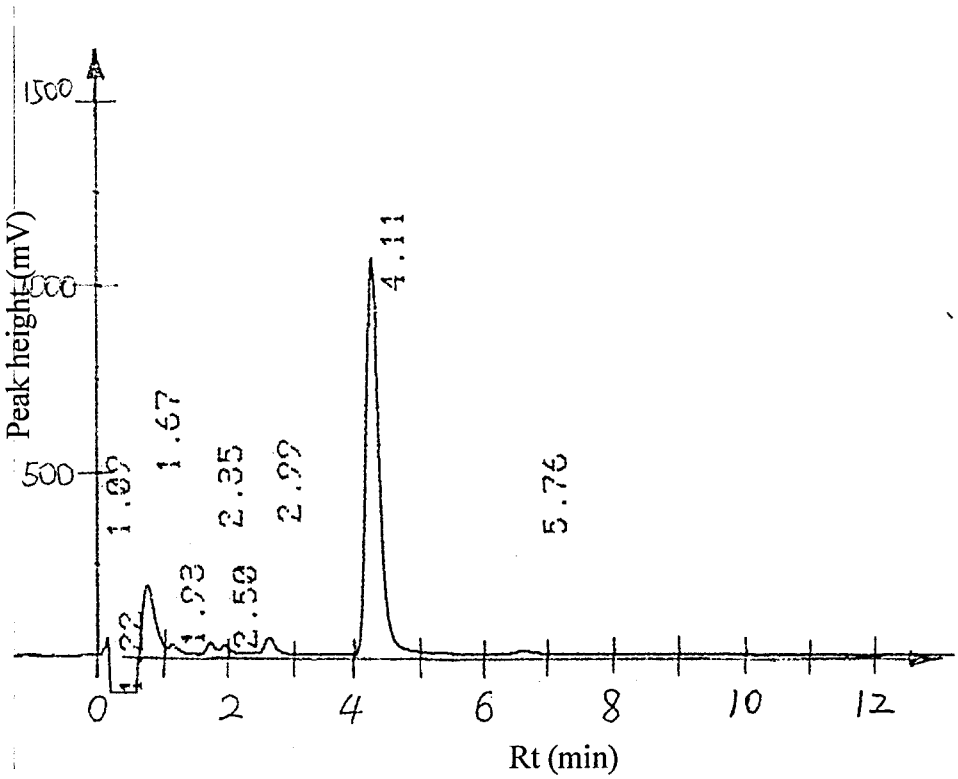


Figure 2. A typical chromatogram of paclitaxel sample (15 μg/mL) on exposure to heating (55°C) for 1 hour prior to analysis

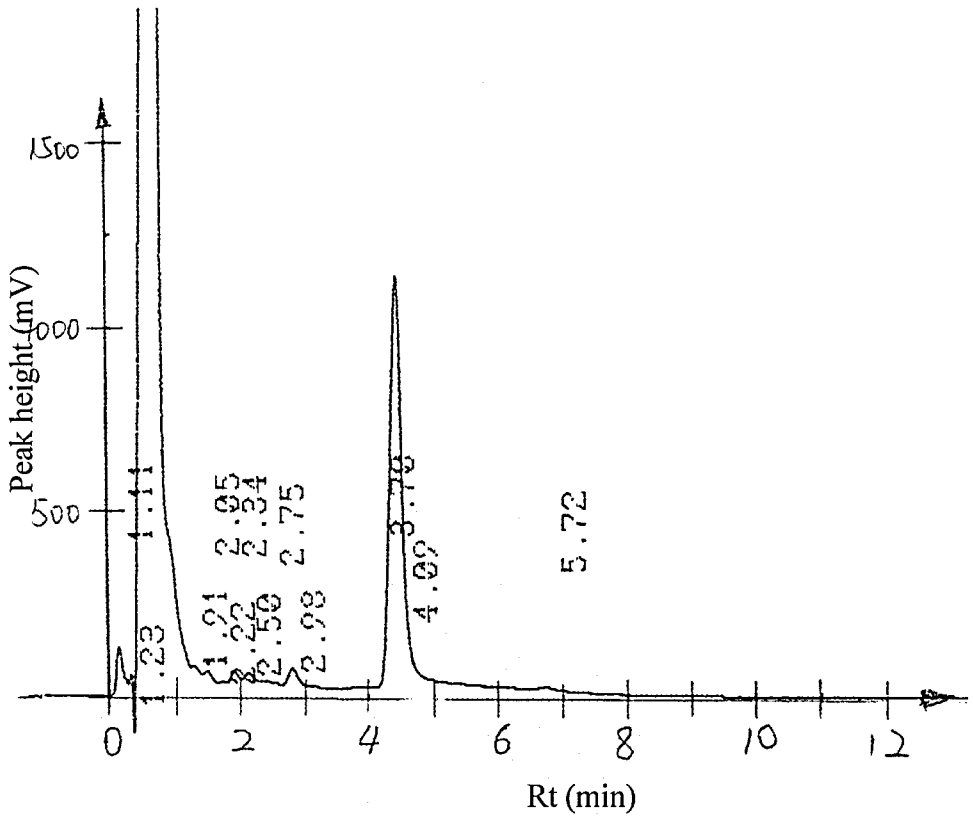


Figure 3. A typical chromatogram of paclitaxel sample (15 μg/mL) on exposure to oxidative degradation (6% hydrogen peroxide) for 1 hour prior to analysis

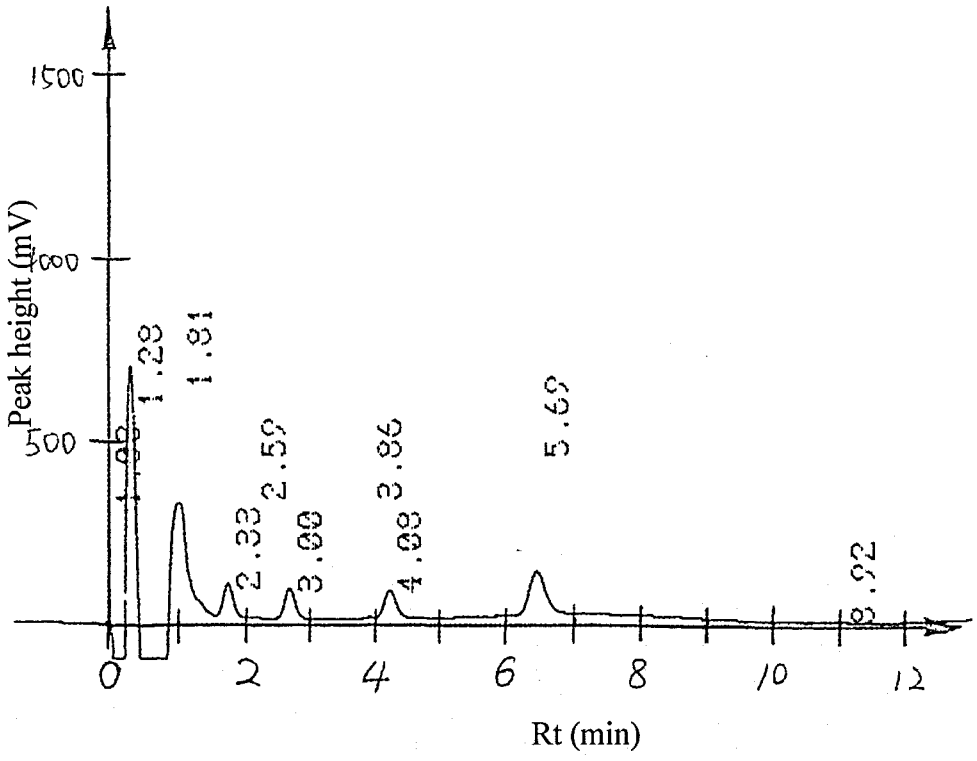


Figure 4. A typical chromatogram of paclitaxel sample (15 μg/mL) on exposure to acid hydrolysis (1M hydrochloric acid) for 1 hour prior to analysis

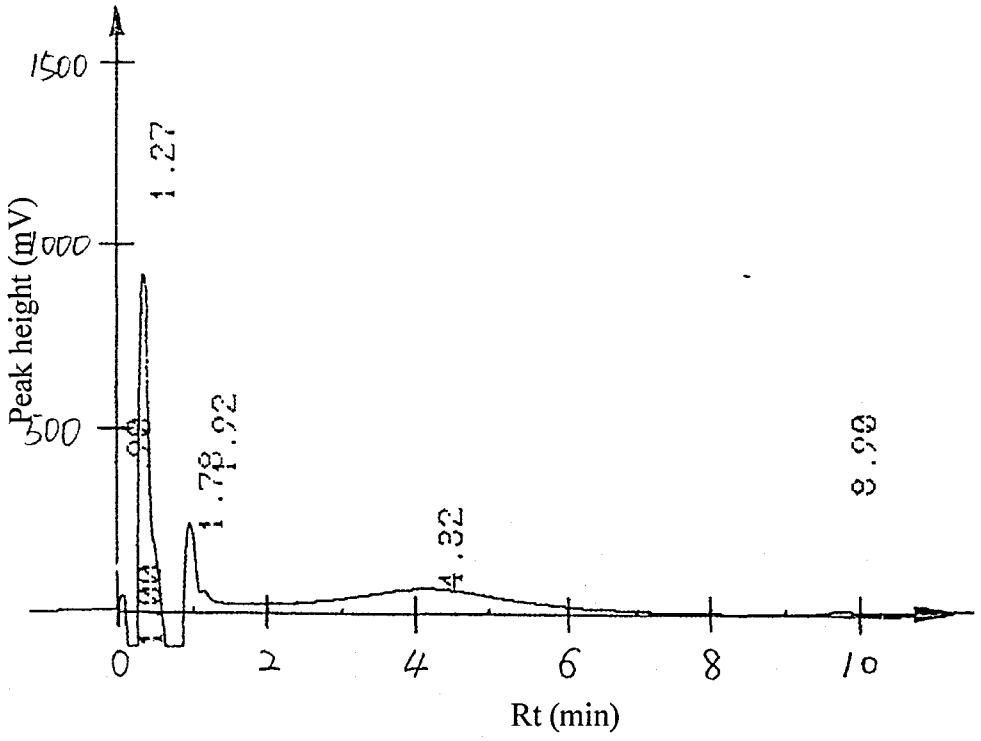
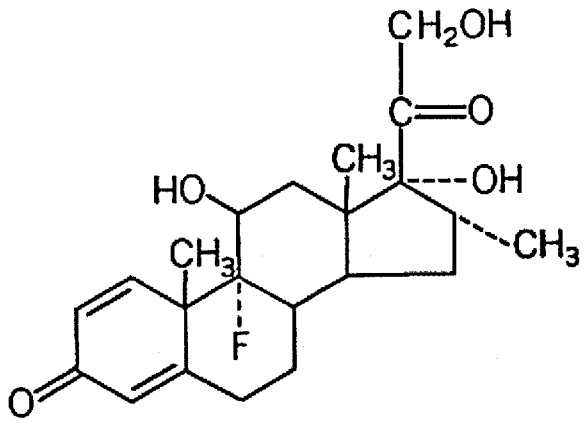


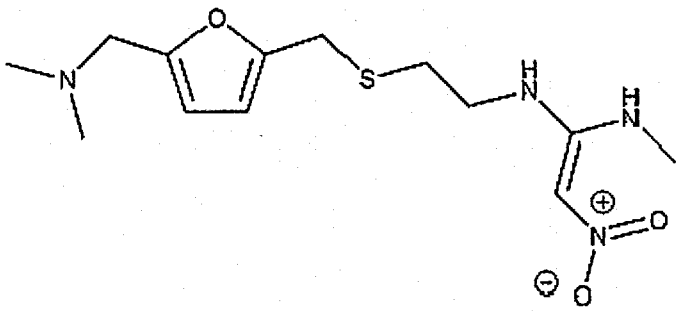
Figure 5. A typical chromatogram of paclitaxel sample (15 µg/mL) on exposure to alkaline hydrolysis (1M sodium hydroxide) for 1 hour prior to analysis

Appendix 2: Chemical structures of all co-administered drugs used during method development in Chapter 3

1. **Dexamethasone**

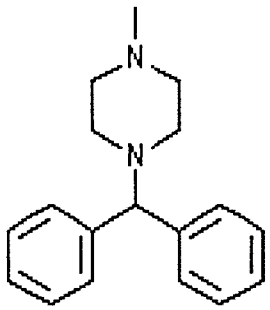


2. **Ranitidine**

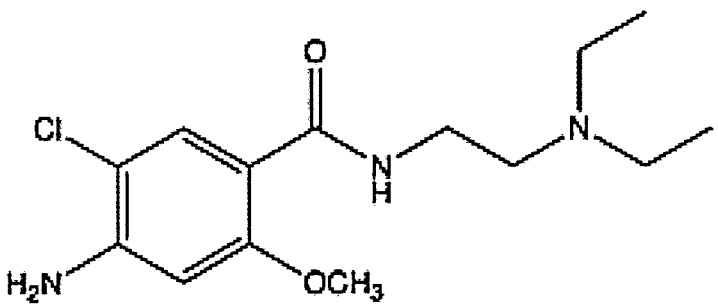


Chemical Formula: $C_{13}H_{22}N_4O_3S$

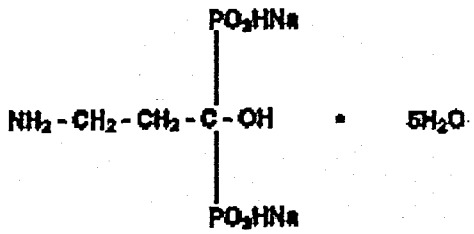
3. **Cyclizine**



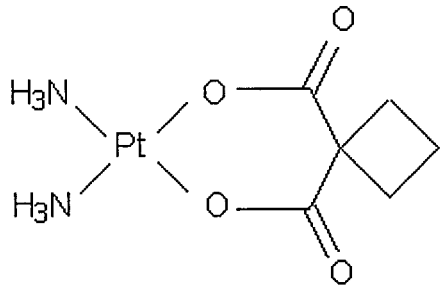
4. **Metoclopramide**



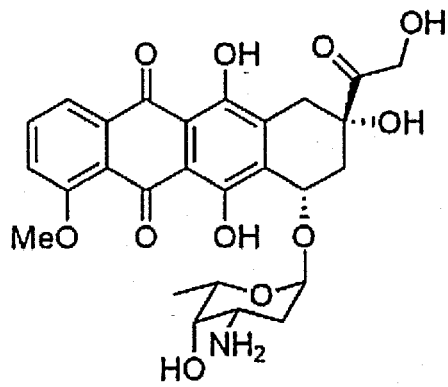
5. **Disodium pamidronate (Aredia)**



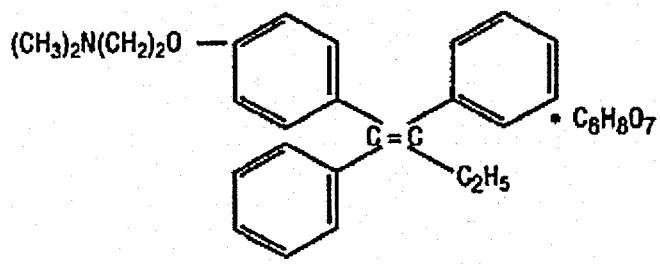
6. **Carboplatin**



7. **Doxorubicin**

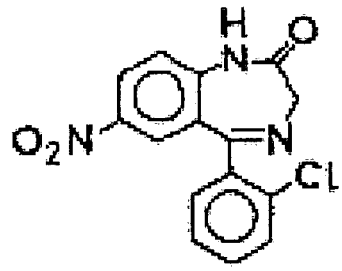


8. **Tamoxifen**

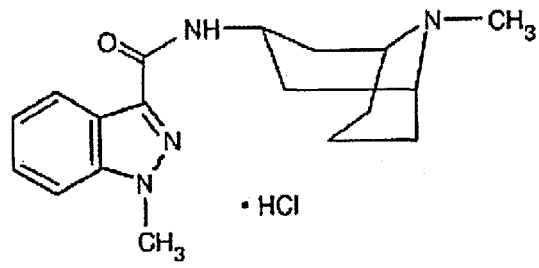


$(C_{32}H_{37}NO_8)$

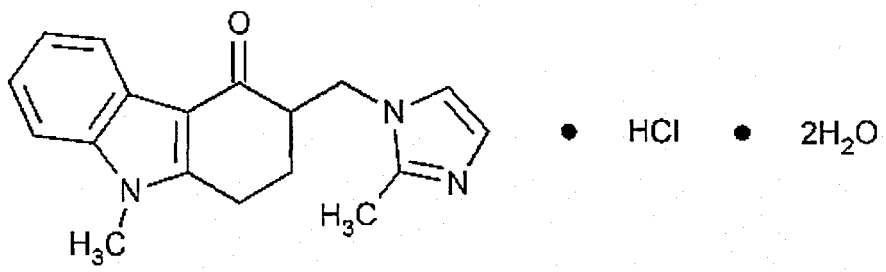
9. **Clonazepam**



10. Kytril Ampoules (Granisetron)



11. Zofran (Ondansetron Hydrochloride)



All structures of drug molecules were downloaded from the website of the Internet Drug Index: http://www.rxlist.com ; accessed on 01-June-2009.

Appendix 3: Optimisation of mobile phase for paclitaxel HPLC assay during method development in Chapter 3

Mobile phase composition (%, v/v)	Rt (Docetaxel), mins	Rt (paclitaxel), mins	Peak height (paclitaxel)	Resolution (R)	α (separation factor)
ACN/0.02M AA (50/50)	8.17	10.20	4242[a]	2.93	1.30
ACN/MeOH/0.02M AA (50/5/45)	7.16	8.34	4606[a]	1.83	1.20
ACN/MeOH/0.02M AA (45/10/45)	10.24	11.78	3654[a]	1.93	1.20
ACN/MeOH/0.02M AA (45/5/50)	14.20	17.40	3378[a]	3.45	1.27
ACN/THF/0.02M AA (45/5/50)	10.40	12.46	3720[a]	2.59	1.26
ACN/THF/0.02M AA (50/2.5/47.5)	7.32	8.70	4612[a]	2.47	1.25
ACN/THF/0.02M AA (50/5/45)	6.10	6.86	5125[a]	1.29	1.19
ACN/THF/0.02M AA (50/3/47)	6.95	8.10	478[b]	1.64	1.24
ACN/THF/0.02M AA (50/2/48)	7.50	9.05	457[b]	1.99	1.29
ACN/THF/0.02M AA (50/1/49)	7.90	9.64	415[b]	2.31	1.30

CAN, Acetonitrile; THF, Tetrohydrofenran; 0.02M AA, 0.02M ammonium acetate buffer (pH5.0)
[a] concentration = 600 ng/mL; [b] concentration = 50 ng/mL

Appendix 4: The chromatograms obtained in the experimental selection of SPE cartridges during method development in Chapter 3

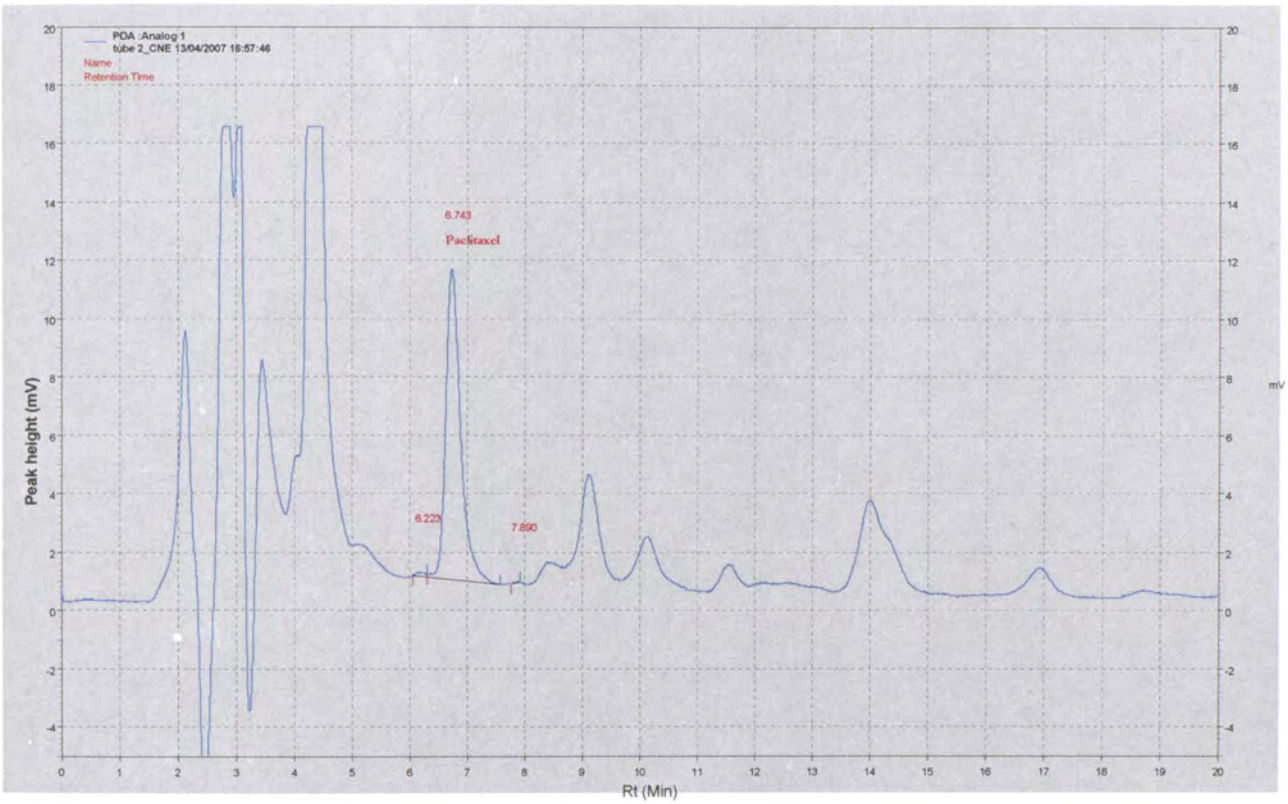


Figure 1. Chromatogram of paclitaxel plasma sample (200 ng/mL) after extraction from a Cyno-bond Elut (CNE) SPE cartridge

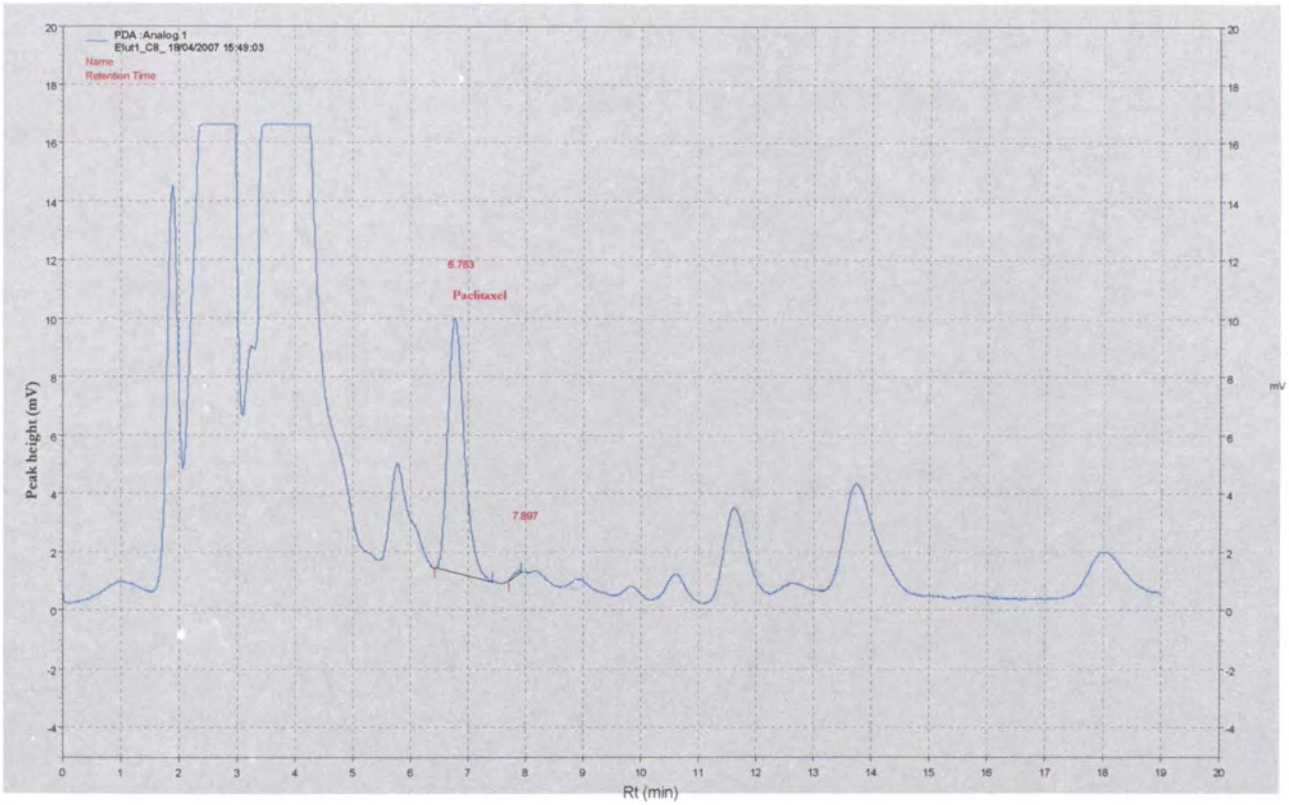


Figure 2. Chromatogram of paclitaxel plasma sample (200 ng/mL) after extraction from a C8 SPE cartridge

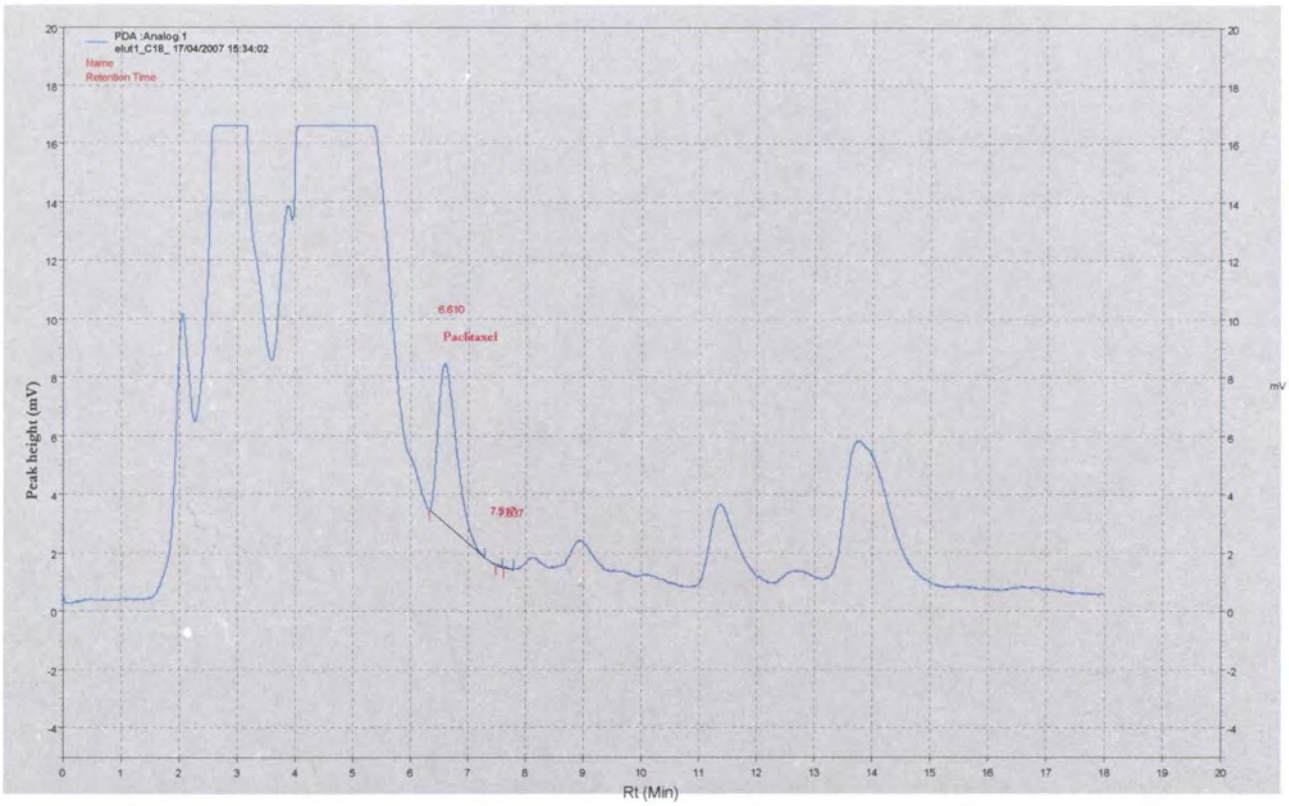


Figure 3. Chromatogram of paclitaxel plasma sample (200 ng/mL) after extraction from a C18 SPE cartridge

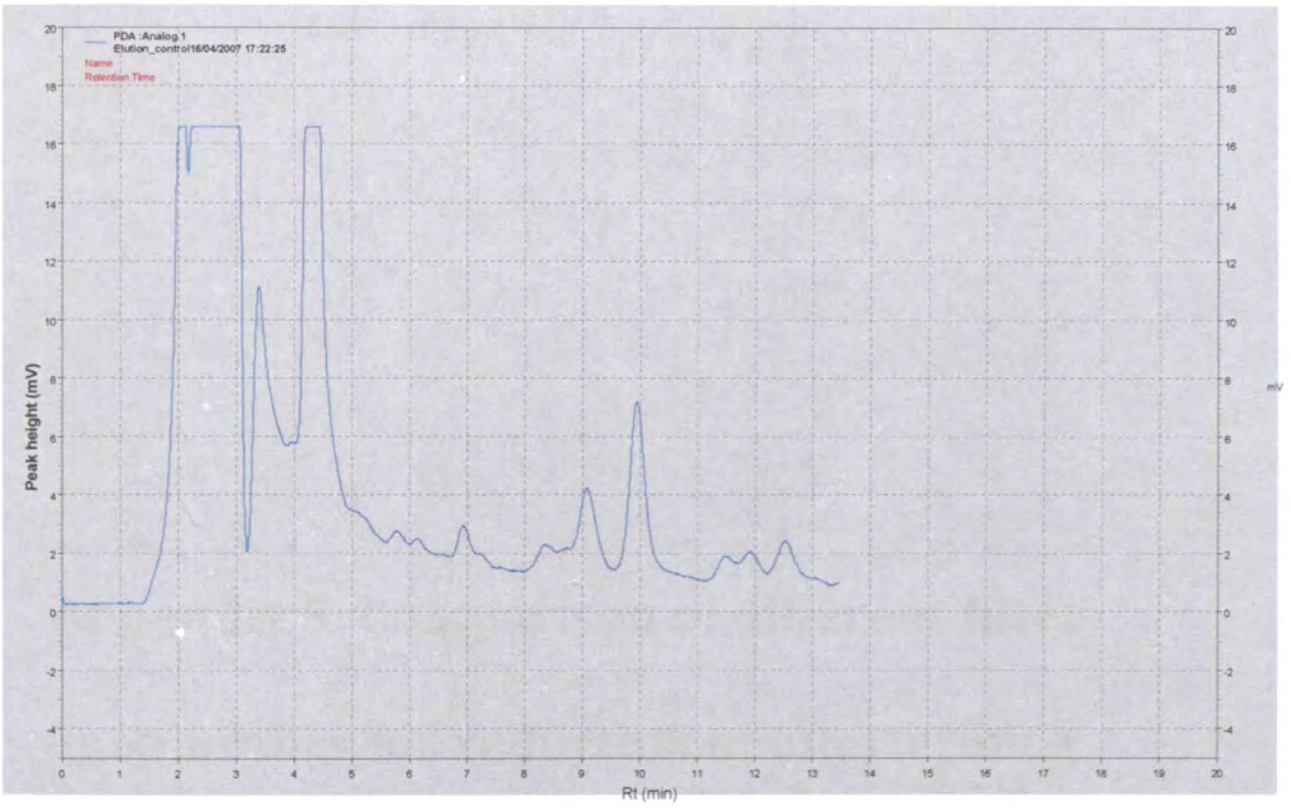


Figure 4. Chromatogram of a blank plasma sample (no drug) after extraction from a Cyno-bond Elut SPE cartridge

Appendix 5: Comparison of different filter membranes for reduction of interference during method development in Chapter 3

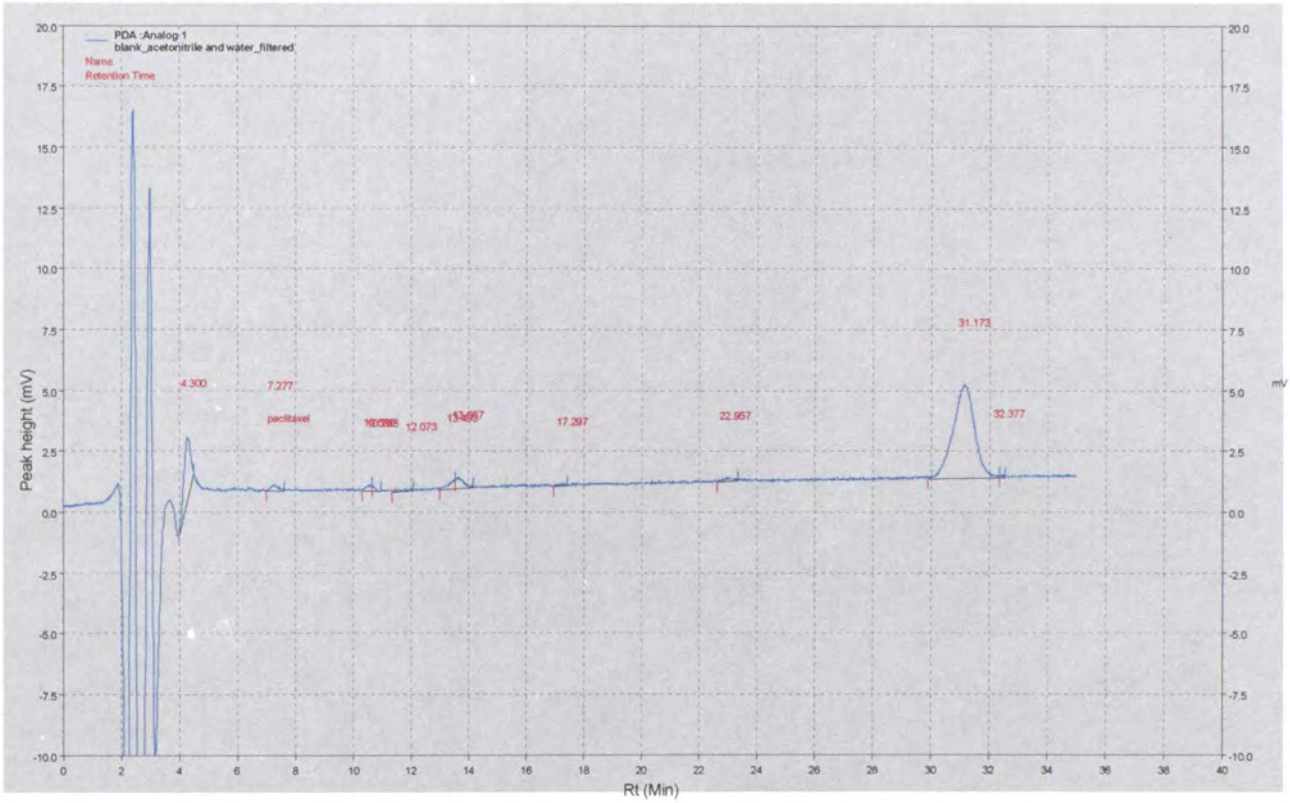


Figure 1. Chromatogram of acetonitrile and water (50/50, v/v) after passing a Polyvinylidenefluoride (PVDF) membrane

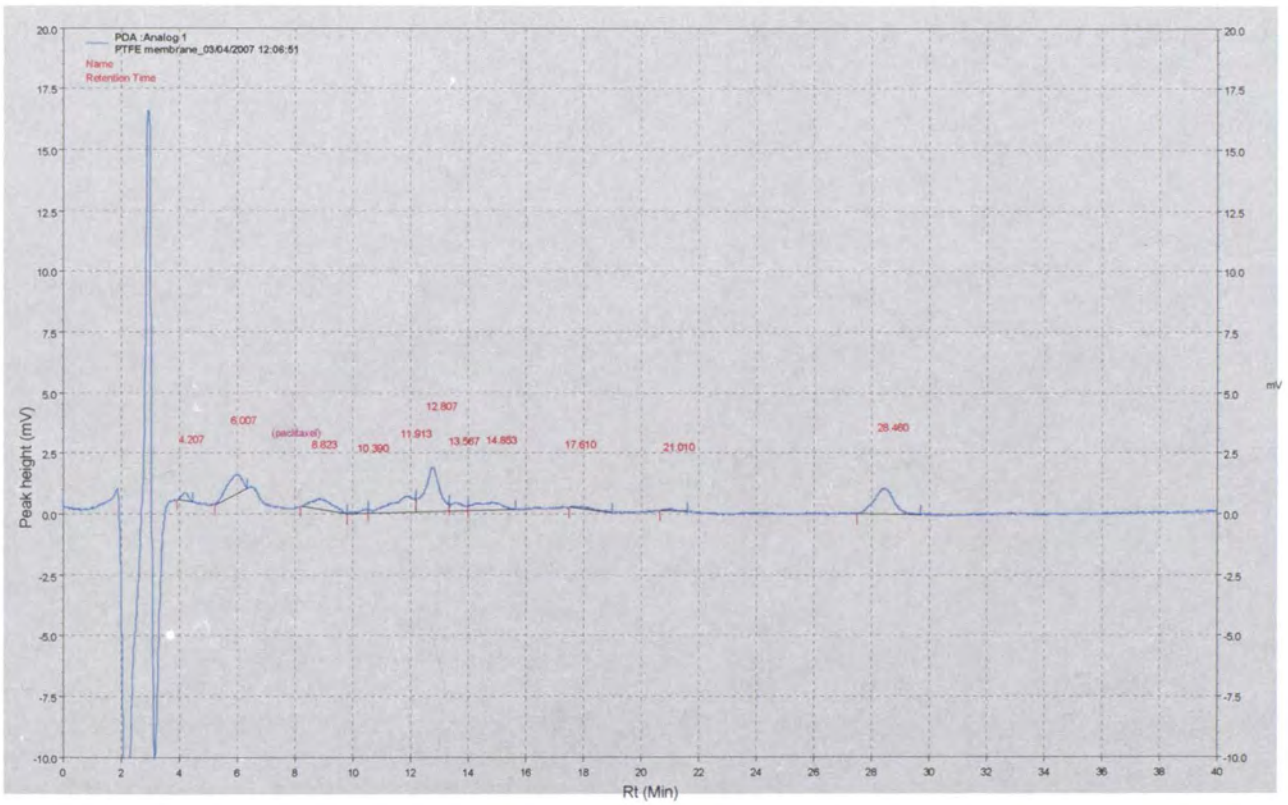


Figure 2. Chromatogram of acetonitrile and water (50/50, v/v) after passing a polytetrafluoroethylene (PTFE) membrane

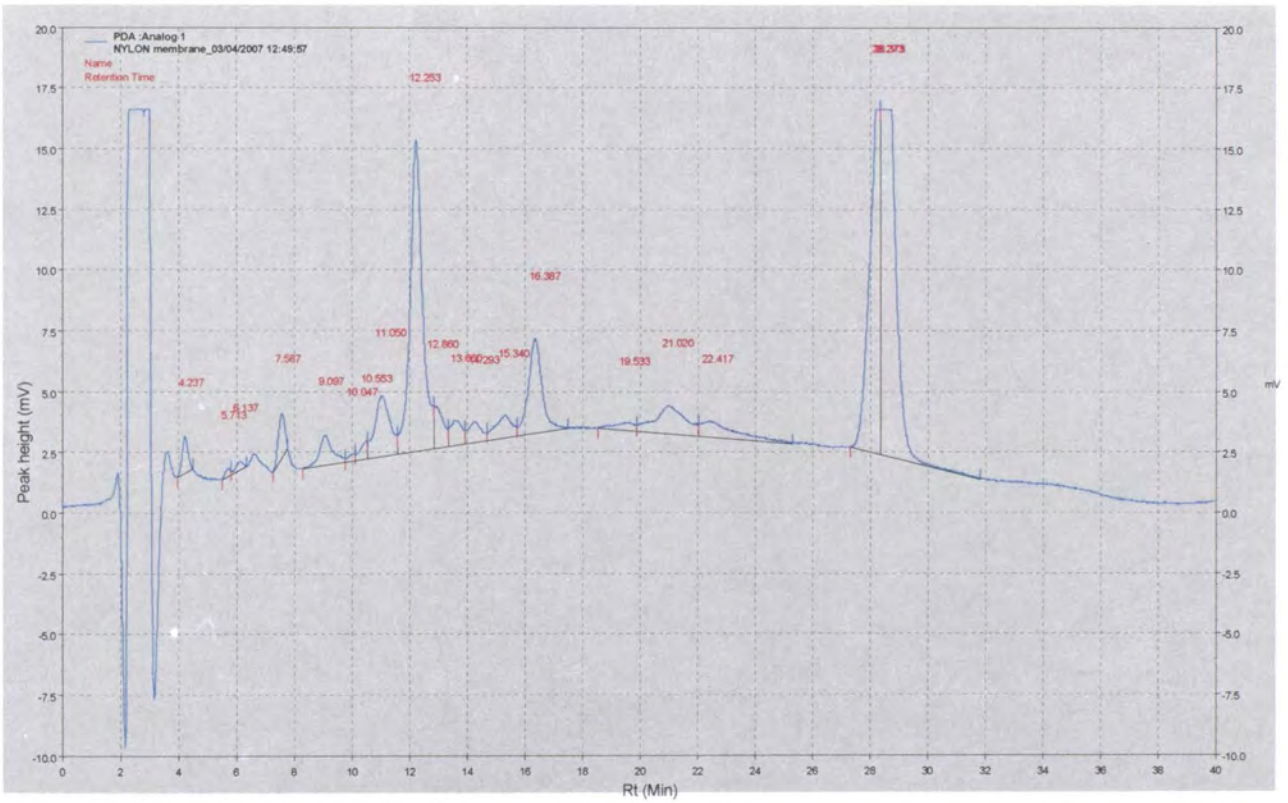


Figure 3. Chromatogram of acetonitrile and water (50/50, v/v) after passing a Nylon membrane

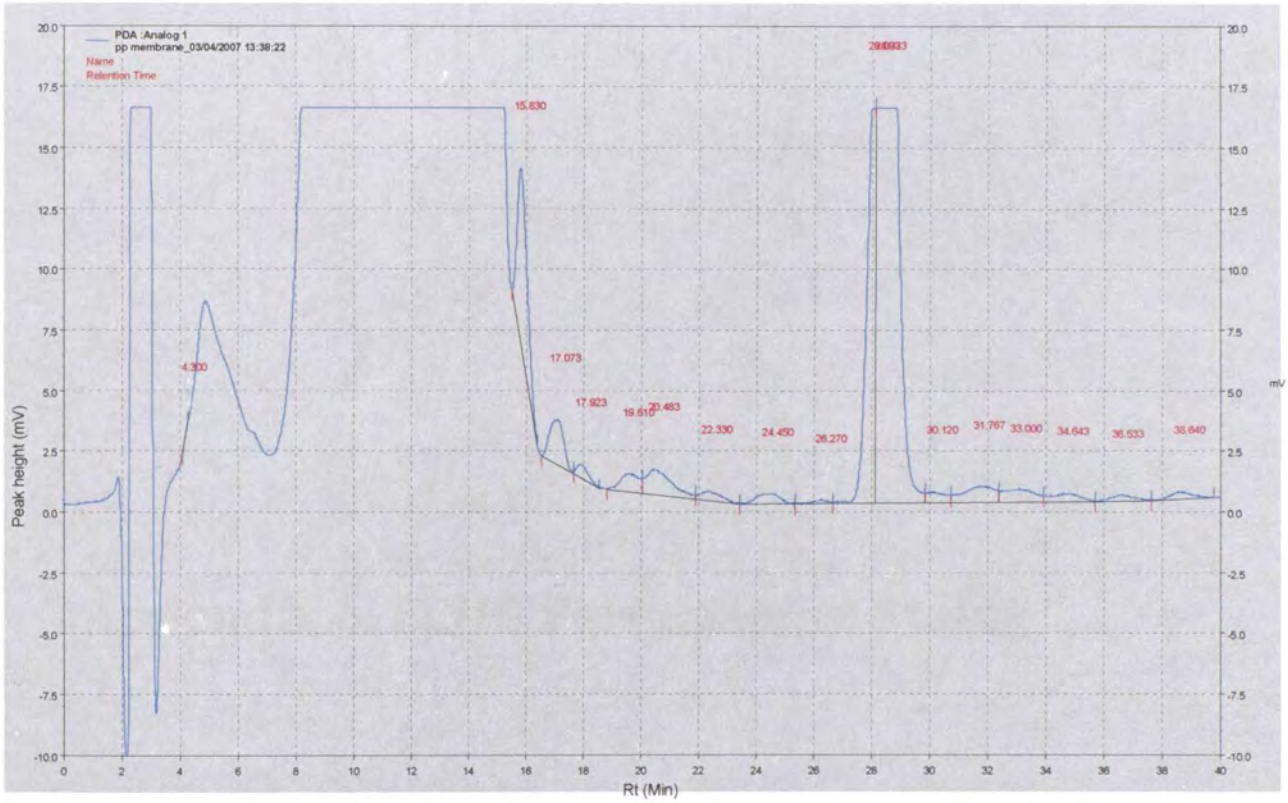


Figure 4. Chromatogram of acetonitrile and water (50/50, v/v) after passing a polypropylene (PP) membrane

Appendix 6: ECOG Performance Status

These scales and criteria are used by doctors and researchers to assess how a patient's disease is progressing, assess how the disease affects the daily living abilities of the patient, and determine appropriate treatment and prognosis. They are included here for health care professionals to access.

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

*Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Appendix 7: A typical HPLC chromatogram of paclitaxel standard sample obtained during validation of HPLC method for the *ex vivo* study (Chapter 5)

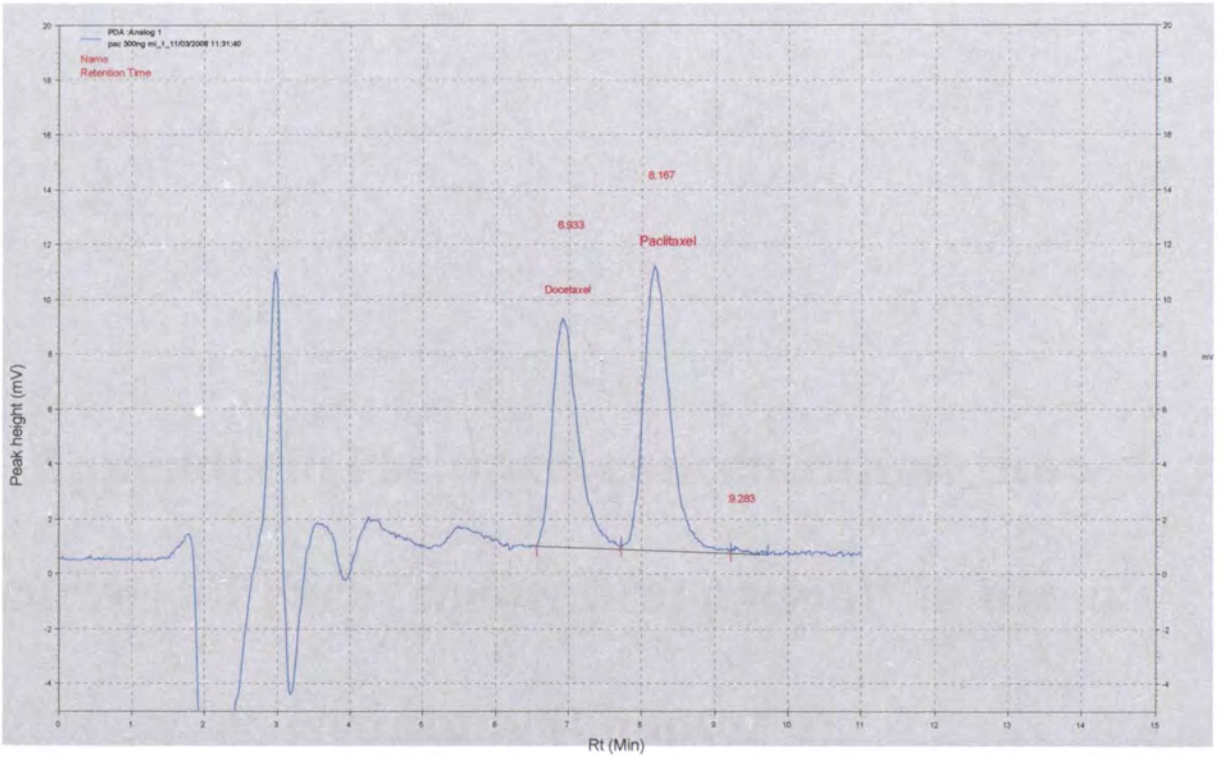


Figure 1. A typical HPLC chromatogram of paclitaxel standard sample (300 ng/mL in saline solution) during validation of HPLC assay for the *ex vivo* study (Retention time of paclitaxel = 8.1 min and retention time of docetaxel = 6.9 min)

Appendix 8: Paclitaxel concentration-time curves for each "theoretical patient" in the *ex vivo* study (Chapter 5)

All following curves were generated by WinNonlin 5.2 software.

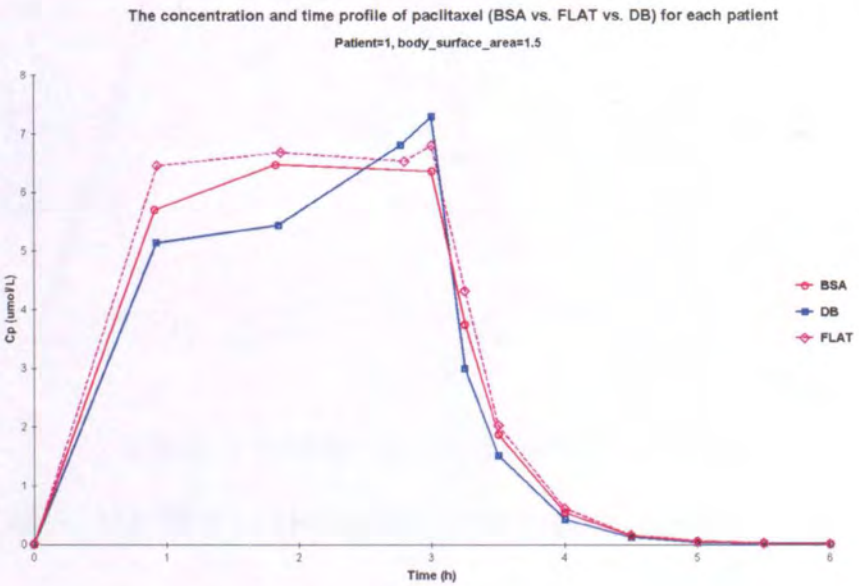


Figure 1. Paclitaxel concentration-time curves

(Patient 1, BSA = 1.5m^2) in the *ex vivo* study (on a linear scale)

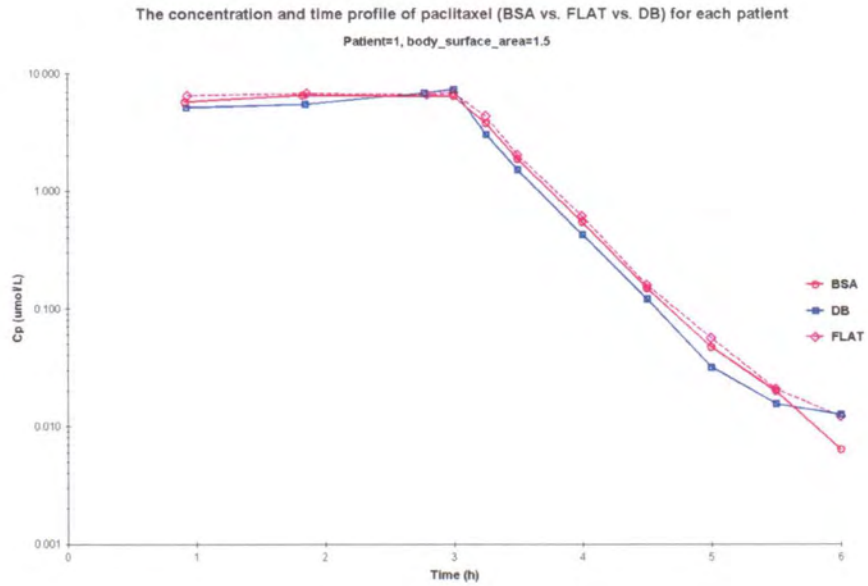


Figure 2. Paclitaxel concentration-time curves

(Patient 1, BSA = 1.5m^2) in the *ex vivo* study (on a semi-log scale)

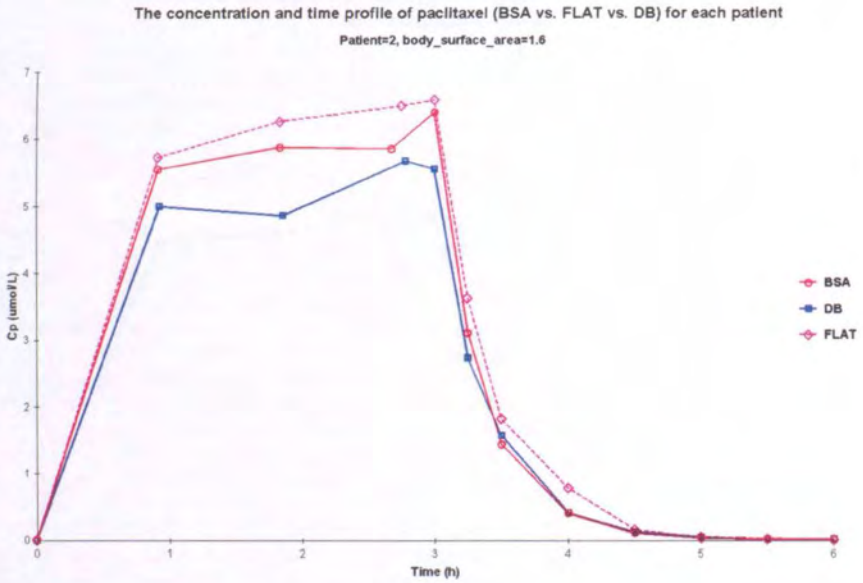


Figure 3. Paclitaxel concentration-time curves

(Patient 2, BSA = 1.6m^2) in the *ex vivo* study (on a linear scale)

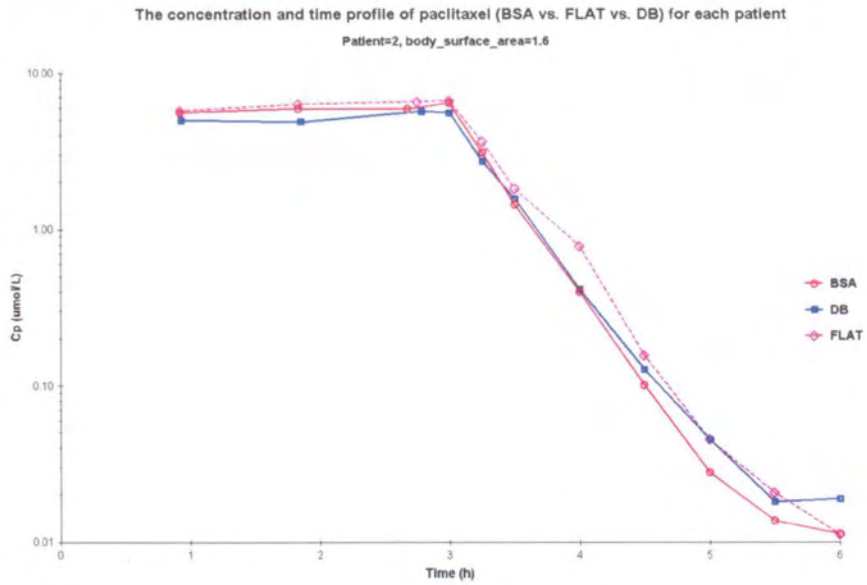


Figure 4. Paclitaxel concentration-time curves

(Patient 2, BSA = 1.6m^2) in the *ex vivo* study (on a semi-log scale)

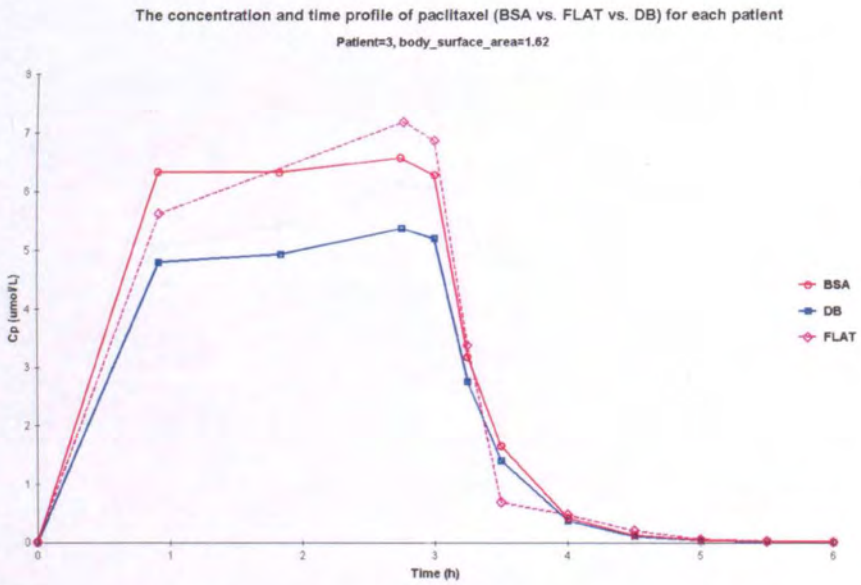


Figure 5. Paclitaxel concentration-time curves

(Patient 3, BSA = 1.62 m^2) in the *ex vivo* study (on a linear scale)

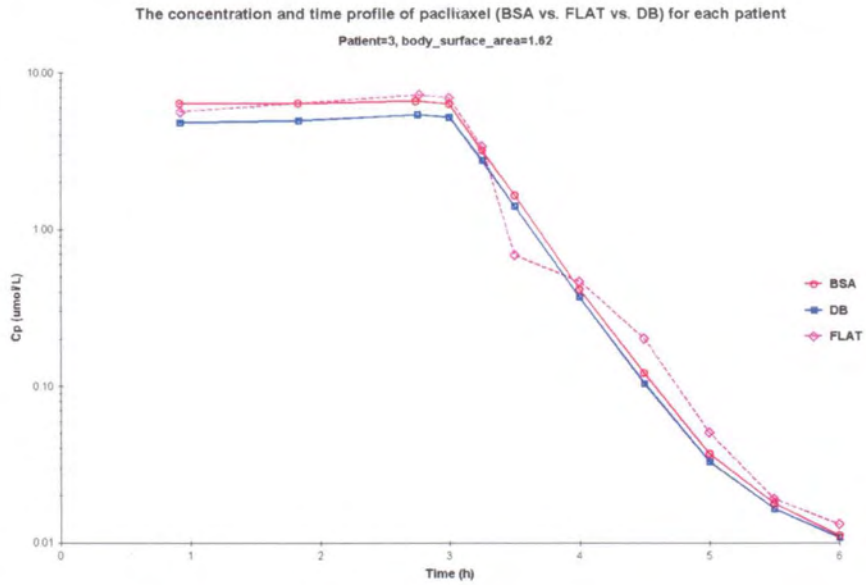


Figure 6. Paclitaxel concentration-time curves

(Patient 3, BSA = 1.62 m^2) in the *ex vivo* study (on a semi-log scale)

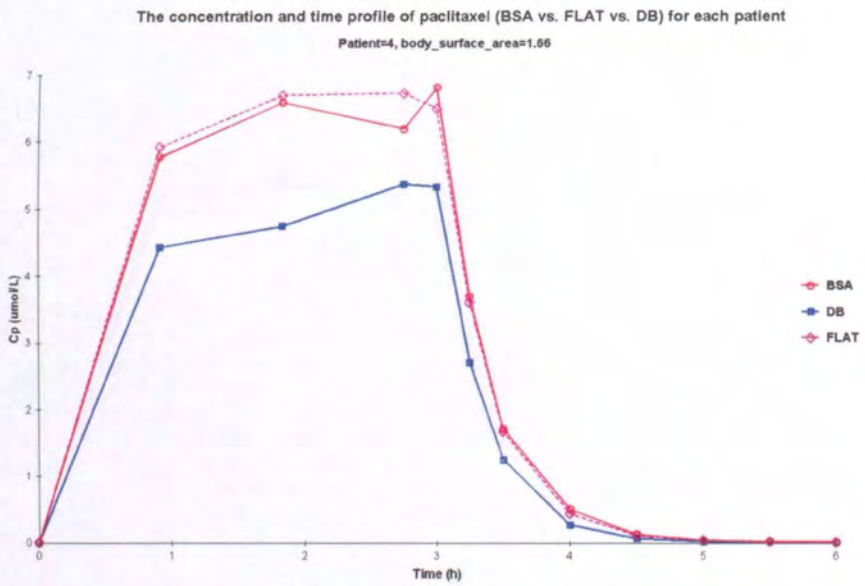


Figure 7. Paclitaxel concentration-time curves

(Patient 4, BSA = 1.66 m^2) in the *ex vivo* study (on a linear scale)

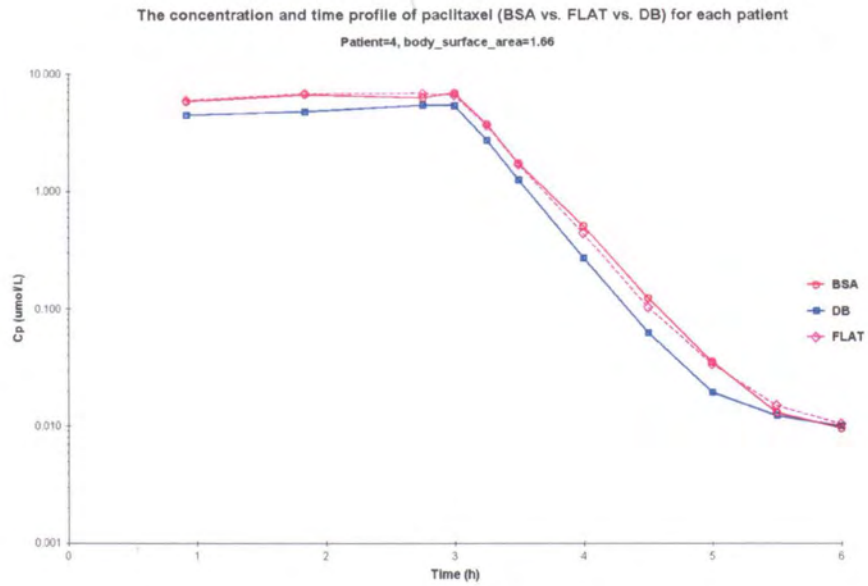


Figure 8. Paclitaxel concentration-time curves

(Patient 4, BSA = 1.66 m^2) in the *ex vivo* study (on a semi-log scale)

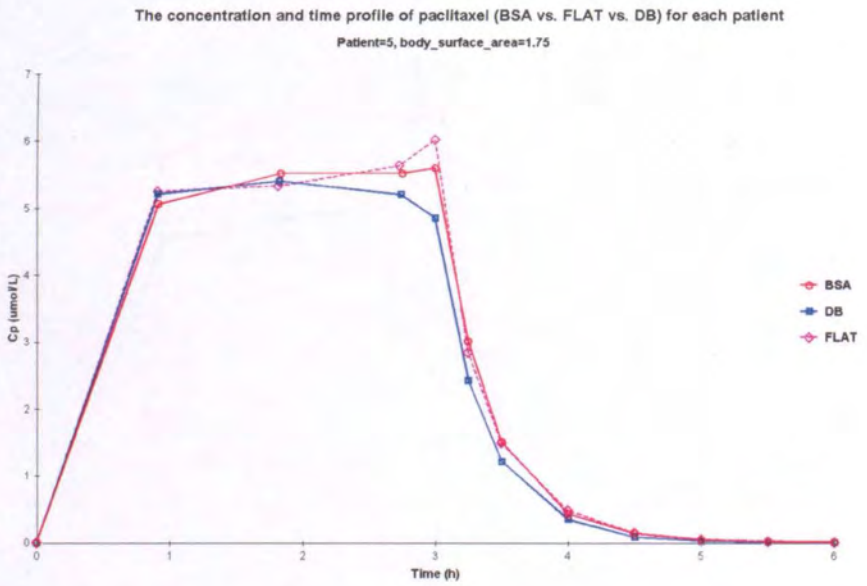


Figure 9. Paclitaxel concentration-time curves

(Patient 5, BSA = 1.75 m^2) in the *ex vivo* study (on a linear scale)

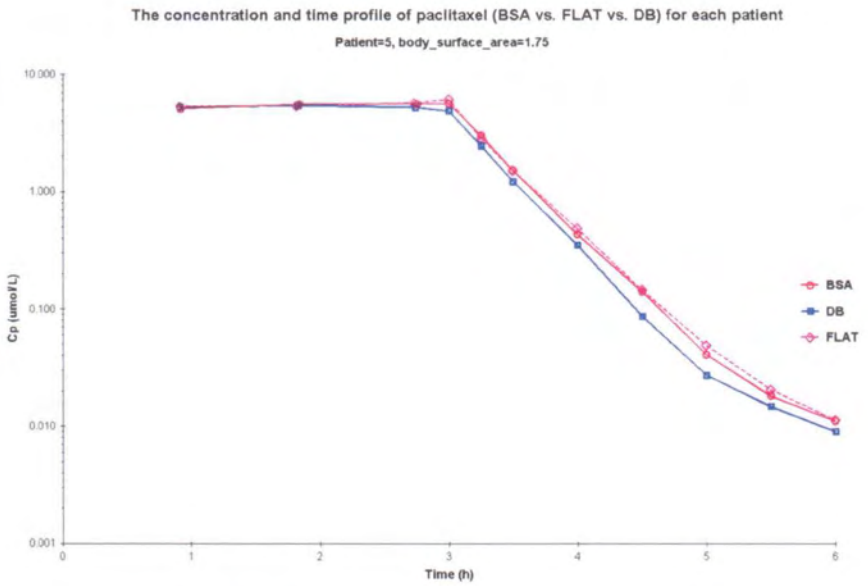


Figure 10. Paclitaxel concentration-time curves

(Patient 5, BSA = 1.75 m^2) in the *ex vivo* study (on a semi-log scale)

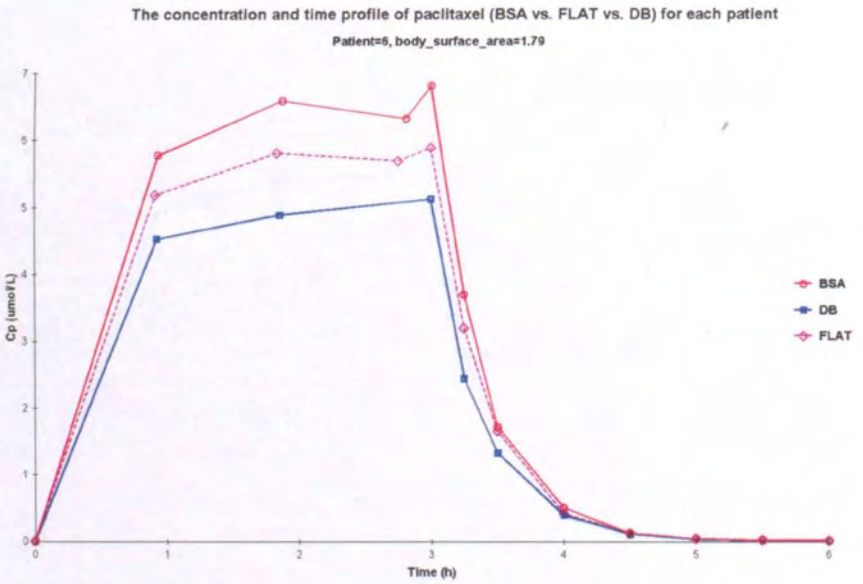


Figure 11. Paclitaxel concentration-time curves (Patient 6, BSA = 1.79 m^2) in the *ex vivo* study (on a linear scale)

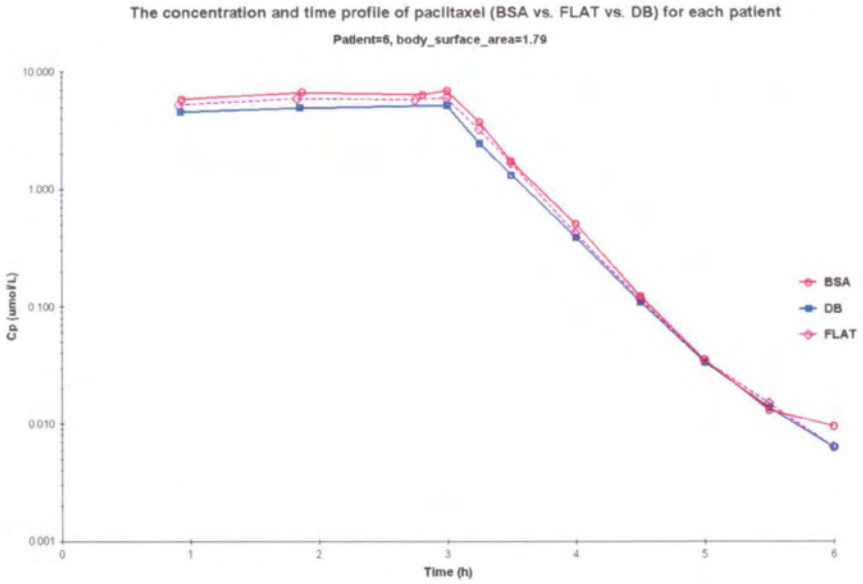


Figure 12. Paclitaxel concentration-time curves (Patient 6, BSA = 1.79 m^2) in the *ex vivo* study (on a semi-log scale)

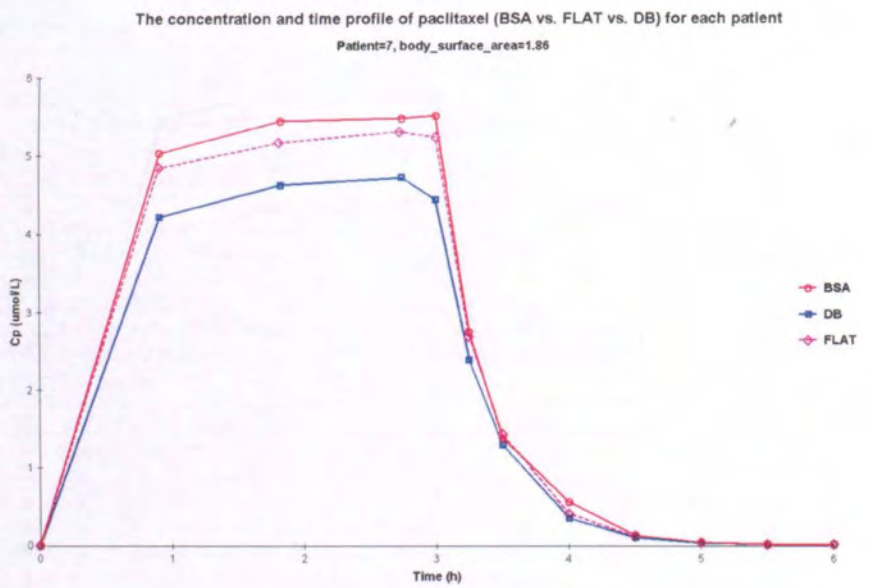


Figure 13. Paclitaxel concentration-time curves (Patient 7, BSA = 1.88 m^2) in the *ex vivo* study (on a linear scale)

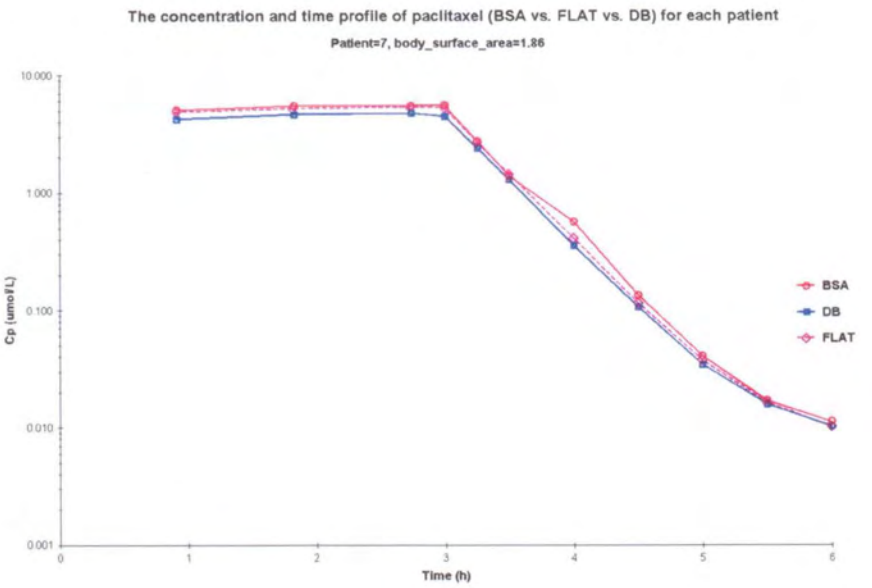


Figure 14. Paclitaxel concentration-time curves (Patient 7, BSA = 1.88 m^2) in the *ex vivo* study (on a semi-log scale)

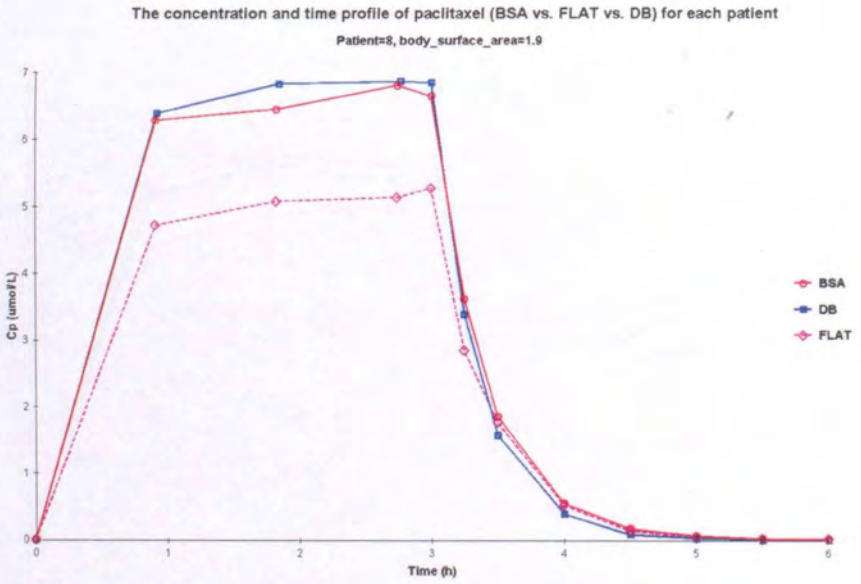


Figure 15. Paclitaxel concentration-time curves

(Patient 8, BSA = 1.9 m^2) in the *ex vivo* study (on a linear scale)

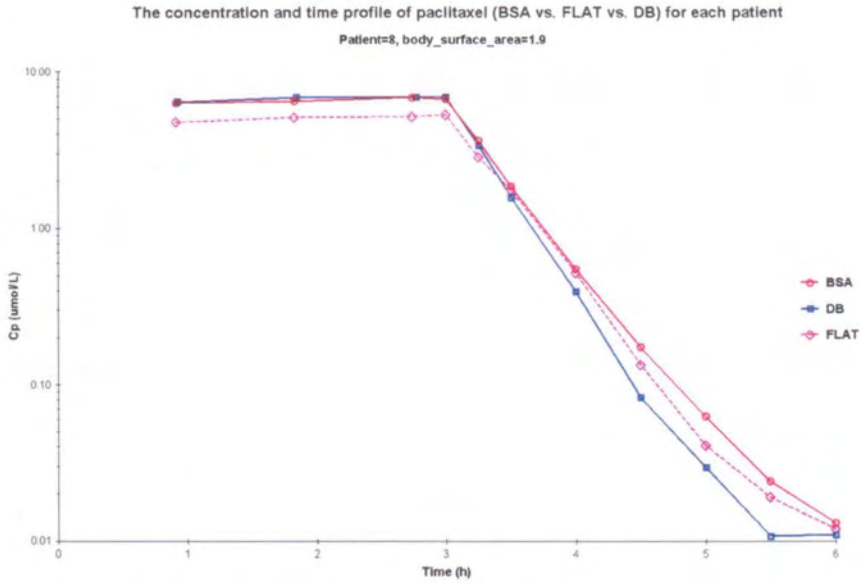


Figure 16. Paclitaxel concentration-time curves

(Patient 8, BSA = 1.9 m^2) in the *ex vivo* study (on a semi-log scale)

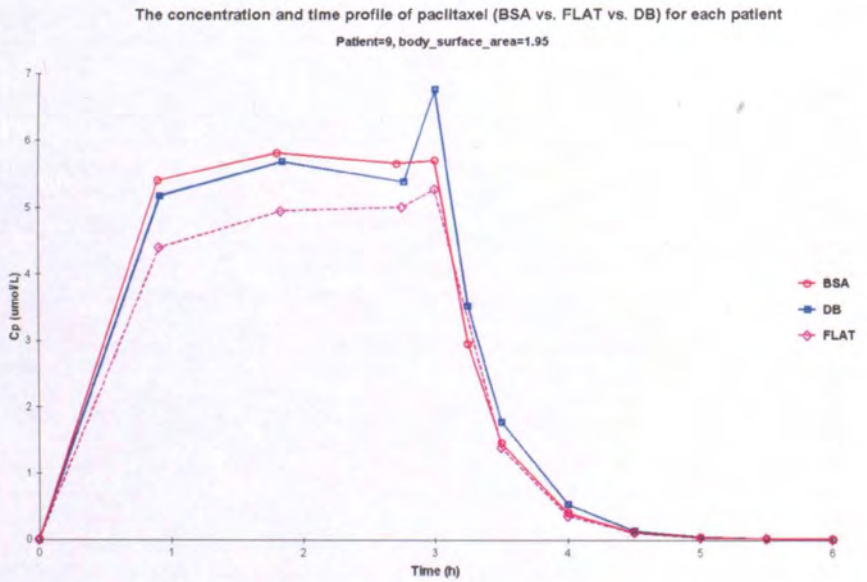


Figure 17. Paclitaxel concentration-time curves (Patient 9, BSA = 1.95 m^2) in the *ex vivo* study (on a linear scale)

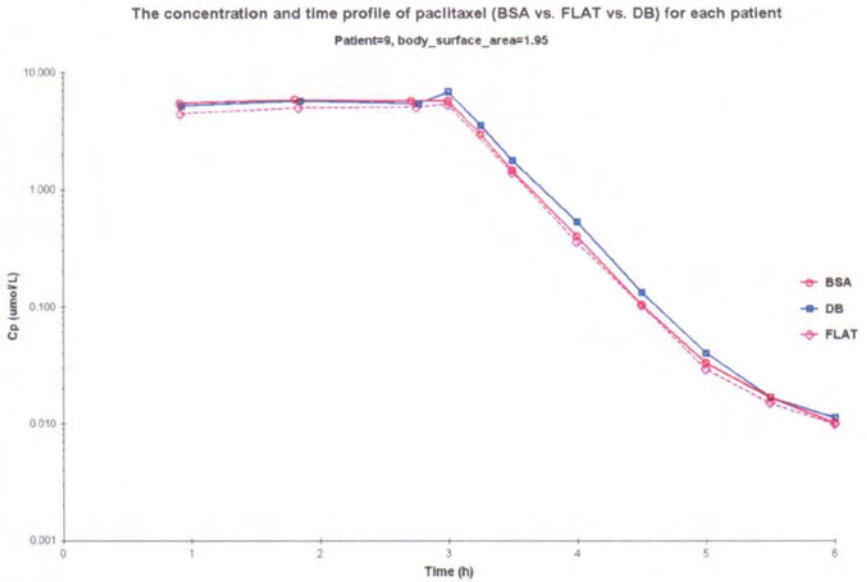


Figure 18. Paclitaxel concentration-time curves (Patient 9, BSA = 1.95 m^2) in the *ex vivo* study (on a semi-log scale)

Appendix 9: Pharmacokinetic (PK) analysis data from the *ex vivo* study (Chapter 5)

For 1-compartment IV infusion model

Patient	Treatment	V	V_StdError	V_CV%	K10	K10_StdError	K10_CV%	AUC (h*umol/L)	AUC_StdError	AUC_CV%
1	IND	6.69	0.16	2.36	2.37	0.05	2.22	19.37	0.14	0.75
	DB	6.35	0.90	14.22	2.64	0.36	13.55	18.84	0.72	3.80
	FLAT	7.33	0.42	5.71	2.32	0.13	5.44	20.61	0.33	1.60
2	IND	6.56	0.28	4.28	2.75	0.11	4.08	18.14	0.20	1.12
	DB	7.54	0.49	6.52	2.59	0.16	6.22	16.19	0.28	1.75
	FLAT	7.64	0.20	2.58	2.37	0.06	2.47	19.40	0.14	0.72
3	IND	5.93	0.23	3.92	2.88	0.11	3.74	19.48	0.20	1.01
	DB	8.34	0.27	3.24	2.61	0.08	3.09	15.60	0.14	0.87
	FLAT	5.68	0.68	11.96	3.09	0.35	11.19	20.04	0.67	3.36
4	IND	7.01	0.32	4.57	2.48	0.11	4.36	19.60	0.25	1.25
	DB	8.51	0.53	6.27	2.61	0.16	5.98	15.28	0.26	1.68
	FLAT	6.79	0.18	2.67	2.60	0.07	2.55	19.89	0.14	0.72
5	IND	8.41	0.09	1.05	2.55	0.03	1.01	16.72	0.05	0.29
	DB	7.46	0.47	6.34	3.09	0.19	6.05	15.76	0.25	1.59
	FLAT	7.66	0.38	4.98	2.69	0.13	4.75	17.05	0.23	1.33
6	IND	7.54	0.30	4.01	2.48	0.09	3.82	19.66	0.22	1.10
	DB	8.99	0.22	2.50	2.70	0.06	2.34	14.93	0.11	0.74
	FLAT	8.11	0.14	1.76	2.49	0.04	1.68	17.42	0.08	0.48
7	IND	8.61	0.19	2.23	2.70	0.06	2.13	16.45	0.10	0.59
	DB	10.69	0.30	2.76	2.61	0.07	2.64	13.85	0.10	0.74
	FLAT	8.38	0.10	1.23	2.65	0.03	1.17	15.78	0.05	0.33
8	IND	7.50	0.21	2.81	2.58	0.07	2.68	20.17	0.15	0.76
	DB	6.49	0.04	0.65	2.89	0.02	0.62	20.58	0.03	0.17
	FLAT	9.62	0.23	2.40	2.33	0.05	2.29	15.65	0.11	0.68
9	IND	8.34	0.17	2.04	2.77	0.05	1.95	17.30	0.09	0.54
	DB	10.04	0.95	9.43	2.28	0.21	9.00	17.88	0.48	2.68
	FLAT	9.28	0.37	3.96	2.51	0.10	3.99	15.09	0.15	0.98

Patient	Treatment	K10_HL (h)	K10_HL_StdError	K10_HL_CV%	Cmax (umol/L)	Cmax_StdError	Cmax_CV%	CL (L/h)	CL_StdError	CL_CV%
1	IND	0.29	0.01	2.22	6.45	0.05	0.75	15.90	0.12	0.75
	DB	0.26	0.04	13.54	6.28	0.24	3.80	16.77	0.64	3.80
	FLAT	0.30	0.02	5.44	6.86	0.11	1.61	17.03	0.27	1.61
2	IND	0.25	0.01	4.07	6.05	0.07	1.12	18.08	0.20	1.12
	DB	0.27	0.02	6.21	5.39	0.09	1.75	19.52	0.34	1.76
	FLAT	0.29	0.01	2.46	6.46	0.05	0.72	18.09	0.13	0.72
3	IND	0.24	0.01	3.73	6.49	0.07	1.01	17.09	0.17	1.01
	DB	0.27	0.01	3.09	5.20	0.05	0.87	21.79	0.19	0.87
	FLAT	0.22	0.03	11.18	6.68	0.22	3.36	17.52	0.59	3.36
4	IND	0.28	0.01	4.35	6.53	0.08	1.26	17.40	0.22	1.26
	DB	0.27	0.02	5.97	5.09	0.09	1.69	22.25	0.38	1.69
	FLAT	0.27	0.01	2.54	6.63	0.05	0.72	17.65	0.13	0.72
5	IND	0.27	0.00	1.00	5.57	0.02	0.29	21.42	0.06	0.29
	DB	0.22	0.01	6.04	5.25	0.08	1.59	23.04	0.37	1.59
	FLAT	0.26	0.01	4.75	5.68	0.08	1.33	20.59	0.27	1.33
6	IND	0.28	0.01	3.82	6.55	0.07	1.10	18.67	0.20	1.10
	DB	0.26	0.01	2.34	4.98	0.04	0.74	24.31	0.18	0.74
	FLAT	0.28	0.00	1.68	5.80	0.03	0.48	20.15	0.10	0.48
7	IND	0.26	0.01	2.13	5.48	0.03	0.59	23.23	0.14	0.59
	DB	0.27	0.01	2.63	4.62	0.03	0.75	27.87	0.21	0.75
	FLAT	0.26	0.00	1.17	5.26	0.02	0.33	22.24	0.07	0.33
8	IND	0.27	0.01	2.68	6.72	0.05	0.76	19.33	0.15	0.76
	DB	0.24	0.00	0.62	6.86	0.01	0.17	18.75	0.03	0.17
	FLAT	0.30	0.01	2.29	5.21	0.04	0.68	22.42	0.15	0.68
9	IND	0.25	0.00	1.95	5.76	0.03	0.54	23.07	0.12	0.54
	DB	0.30	0.03	9.00	5.95	0.16	2.68	22.93	0.62	2.68
	FLAT	0.28	0.01	3.98	5.03	0.05	0.97	23.26	0.23	0.98

For 1-compartment IV bolus model

		V	V_SE	CV%	K10	K10_SE	K10_CV%	AUC	AUC_SE	AUC_CV%
1	BSA	22.52	7.34	32.61	0.52	0.26	49.85	26.42	11.23	42.51
1	DB	22.21	7.14	32.17	0.51	0.23	46.07	28.09	10.84	38.60
2	BSA	21.94	6.88	31.37	0.54	0.25	45.65	27.45	10.67	38.88
2	DB	30.90	9.69	31.35	0.44	0.19	44.20	23.49	8.33	35.45
2	FLAT	23.13	7.23	31.24	0.51	0.23	44.88	29.64	11.17	37.69
5	DB	27.92	8.65	30.97	0.55	0.25	45.19	23.78	9.17	38.56
5	FLAT	30.05	9.36	31.16	0.47	0.21	44.34	24.94	9.05	36.28
6	DB	35.44	11.59	32.70	0.51	0.25	49.61	20.06	8.47	42.22
7	DB	39.53	12.27	31.04	0.48	0.21	44.19	20.46	7.44	36.38
8	DB	19.29	6.09	31.59	0.62	0.30	47.85	32.32	13.59	42.05
9	BSA	29.58	9.18	31.06	0.52	0.23	44.93	25.89	9.80	37.84
9	DB	29.12	9.17	31.49	0.51	0.23	45.22	27.38	10.42	38.04
9	FLAT	36.61	12.15	33.19	0.44	0.22	49.28	21.55	8.65	40.16

		K10_HL	K10_HL_SE	K10_HL_CV%	Cmax	Cmax_SE	Cmax_CV%	CL	CL_SE	CL_CV%
1	BSA	1.34	0.67	49.80	13.68	4.46	32.58	11.66	4.96	42.55
1	DB	1.37	0.63	46.02	14.23	4.57	32.14	11.25	4.35	38.64
2	BSA	1.27	0.58	45.60	14.95	4.68	31.34	11.95	4.65	38.92
2	DB	1.59	0.70	44.15	10.23	3.20	31.32	13.45	4.77	35.48
2	FLAT	1.35	0.61	44.84	15.17	4.74	31.21	11.84	4.47	37.73
5	DB	1.27	0.57	45.14	13.00	4.02	30.94	15.27	5.89	38.60
5	FLAT	1.48	0.66	44.29	11.68	3.64	31.13	14.07	5.11	36.32
6	DB	1.36	0.67	49.56	10.24	3.35	32.66	18.10	7.65	42.26
7	DB	1.45	0.64	44.14	9.76	3.03	31.01	18.87	6.87	36.41
8	DB	1.12	0.54	47.80	20.01	6.31	31.56	11.94	5.03	42.09
9	BSA	1.33	0.60	44.89	13.49	4.19	31.02	15.41	5.84	37.88
9	DB	1.35	0.61	45.17	14.08	4.43	31.46	14.98	5.70	38.08
9	FLAT	1.56	0.77	49.23	9.59	3.18	33.16	16.29	6.55	40.20

Appendix 10: Calculations of the Akaike Information Criterion (AIC) and weighed sum of squared residuals (WSSR) in Chapter 5

Akaike Information Criterion (AIC): A measure of goodness of fit based on maximum likelihood. When comparing several models for a given data set, the model associated with the smallest AIC is regarded as giving the best fit. Appropriate only for comparing models that use the same weighting scheme.

AIC = N log ($WSSR$) + 2P for modeling in WinNonlin. N is the number of observations with positive weight. $WSSR$ is the weighed sum of squared residuals. P is the number of parameters.

Weighed sum of squared residuals (WSSR): an estimate of the variance of the residuals. The equation of WSSR calculation is:

$$\text{WSSR} = \sum_{i=1}^{i=n} (\text{Y\_}observed, i - \text{Y\_}calculated, i)^2 * \text{W}i$$

Y_observed is the observed Y value in the study and Y_calculated is the calculated Y value by the model. (Y_observed – Y_calculated)^2 is the residual. Wi is a weight for each residual.

During modeling, the WinNonlin program minimizes the value for WSSR which represents the best fit according to the least squares criteria.

(All above information refer to the user's guide of WinNonlin software 5.2.)

Appendix 11: Raw data of non-compartmental analysis in the *ex vivo* study (Chapter 5)

NCA Text - [summary_NCA_533425.pto] (Read-only) (Derived)

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Patient=1
Treatment=IND

Date: 7/07/2008
Time: 13:36:14

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 262.50
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9137		5.691		2.600	2.375		
1.827		6.460		8.151	10.14		
2.741		7.997		14.75	25.55		
3.000	*	6.342	5.919	0.4233	16.61	30.85	1.000
3.250	*	3.730	3.306	0.4237	17.87	34.74	1.000
3.500	*	1.853	1.847	0.006749	18.57	37.07	1.000
4.000	*	0.5399	0.5762	-0.03633	19.17	39.23	1.000
4.500	*	0.1481	0.1798	-0.03171	19.34	39.94	1.000
5.000	*	0.04670	0.05609	-0.009384	19.39	40.16	1.000
5.500	*	0.01959	0.01750	0.002092	19.40	40.25	1.000
6.000	*	0.006255	0.005460	0.0007956	19.41	40.29	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9972
Rsq_adjusted		0.9968
Corr_XY		-0.9986
No_points_lambda_z		8
Lambda_z	1/h	2.3295
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.2976
Tmax	h	2.7411
Cmax	umol/L	7.9966
Cmax_D	umol/L/mg	0.0305
Tlast	h	6.0000
Clast	umol/L	0.0063
AUClast	h*umol/L	19.4100
AUCall	h*umol/L	19.4100
AUCINF_obs	h*umol/L	19.4127
AUCINF_D_obs	h*umol/L/mg	0.0740
AUC_%Extrap_obs	%	0.0138
Vz_obs	mg/(umol/L)	5.8047
Cl_obs	mg/(h*umol/L)	13.5221
AUCINF_pred	h*umol/L	19.4124
AUCINF_D_pred	h*umol/L/mg	0.0740
AUC_%Extrap_pred	%	0.0121
Vz_pred	mg/(umol/L)	5.8048
Cl_pred	mg/(h*umol/L)	13.5223
AUMClast	h*h*umol/L	40.2853
AUMCINF_obs	h*h*umol/L	40.3025
AUMC_%Extrap_obs	%	0.0428
AUMCINF_pred	h*h*umol/L	40.3003
AUMC_%Extrap_pred	%	0.0374
MRTlast	h	0.5755
MRTINF_obs	h	0.5761
MRTINF_pred	h	0.5760
Vss_obs	mg/(umol/L)	7.7899
Vss_pred	mg/(umol/L)	7.7890

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=1
Treatment=DB

Date: 7/07/2008
Time: 13:36:14

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 270.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

	Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
	h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
	0.0000	@	0.0000			0.0000	0.0000	
	0.9231		5.133			2.369	2.187	
	1.846		5.437			7.248	9.006	
	2.769		6.794			12.89	22.32	
	3.000	*	7.282	4.704	2.578	14.52	27.01	1.000
	3.250	*	2.989	2.699	0.2907	15.80	30.96	1.000
	3.500	*	1.503	1.548	-0.04488	16.36	32.83	1.000
	4.000	*	0.4220	0.5096	-0.08755	16.84	34.57	1.000
	4.500	*	0.1200	0.1677	-0.04769	16.98	35.12	1.000
	5.000	*	0.03165	0.05520	-0.02355	17.02	35.30	1.000
	5.500	*	0.01536	0.01817	-0.002806	17.03	35.36	1.000
	6.000	*	0.01250	0.005979	0.006516	17.04	35.40	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9711
Rsq_adjusted		0.9663
Corr_XY		-0.9854
No_points_lambda_z		8
Lambda_z	1/h	2.2226
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3119
Tmax	h	3.0000
Cmax	umol/L	7.2822
Cmax_D	umol/L/mg	0.0270
Tlast	h	6.0000
Clast	umol/L	0.0125
AUClast	h*umol/L	17.0354
AUCall	h*umol/L	17.0354
AUCINF_obs	h*umol/L	17.0410
AUCINF_D_obs	h*umol/L/mg	0.0631
AUC_%Extrap_obs	%	0.0330
Vz_obs	mg/(umol/L)	7.1286
Cl_obs	mg/(h*umol/L)	15.8441
AUCINF_pred	h*umol/L	17.0381
AUCINF_D_pred	h*umol/L/mg	0.0631
AUC_%Extrap_pred	%	0.0158
Vz_pred	mg/(umol/L)	7.1299
Cl_pred	mg/(h*umol/L)	15.8469
AUMClast	h*h*umol/L	35.3999
AUMCINF_obs	h*h*umol/L	35.4362
AUMC_%Extrap_obs	%	0.1023
AUMCINF_pred	h*h*umol/L	35.4173
AUMC_%Extrap_pred	%	0.0490
MRTlast	h	0.5780
MRTINF_obs	h	0.5795
MRTINF_pred	h	0.5787
Vss_obs	mg/(umol/L)	9.1811
Vss_pred	mg/(umol/L)	9.1708

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Date: 7/07/2008
Time: 13:36:15

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9326		6.445		3.005	2.803		
1.865		6.667		9.120	11.41		
2.798		6.516		15.27	25.71		
3.000	*	6.786	6.061	0.7255	16.61	29.61	1.000
3.250	*	4.302	3.478	0.8235	18.00	33.90	1.000
3.500	*	2.012	1.996	0.01632	18.79	36.53	1.000
4.000	*	0.6075	0.6574	-0.04996	19.44	38.89	1.000
4.500	*	0.1581	0.2165	-0.05839	19.63	39.68	1.000
5.000	*	0.05571	0.07131	-0.01561	19.69	39.93	1.000
5.500	*	0.02052	0.02349	-0.002967	19.71	40.03	1.000
6.000	*	0.01203	0.007736	0.004295	19.71	40.07	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9895
Rsq_adjusted		0.9878
Corr_XY		-0.9947
No_points_lambda_z		8
Lambda_z	1/h	2.2212
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3121
Tmax	h	3.0000
Cmax	umol/L	6.7860
Cmax_D	umol/L/mg	0.0226
Tlast	h	6.0000
Clast	umol/L	0.0120
AUClast	h*umol/L	19.7140
AUCall	h*umol/L	19.7140
AUCINF_obs	h*umol/L	19.7194
AUCINF_D_obs	h*umol/L/mg	0.0657
AUC_%Extrap_obs	%	0.0275
Vz_obs	mg/(umol/L)	6.8491
Cl_obs	mg/(h*umol/L)	15.2134
AUCINF_pred	h*umol/L	19.7175
AUCINF_D_pred	h*umol/L/mg	0.0657
AUC_%Extrap_pred	%	0.0177
Vz_pred	mg/(umol/L)	6.8498
Cl_pred	mg/(h*umol/L)	15.2149
AUMClast	h*h*umol/L	40.0713
AUMCINF_obs	h*h*umol/L	40.1062
AUMC_%Extrap_obs	%	0.0871
AUMCINF_pred	h*h*umol/L	40.0938
AUMC_%Extrap_pred	%	0.0560
MRTlast	h	0.5326
MRTINF_obs	h	0.5338
MRTINF_pred	h	0.5334
Vss_obs	mg/(umol/L)	8.1216
Vss_pred	mg/(umol/L)	8.1159

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=2
Treatment=IND

Date: 7/07/2008
Time: 13:36:15

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 280.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

	Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
	h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
	0.0000	@	0.0000			0.0000	0.0000	
	0.9184		5.542			2.545	2.337	
	1.837		5.872			7.786	9.626	
	2.679		5.847			12.72	20.76	
	3.000	*	6.394	4.448	1.946	14.69	26.36	1.000
	3.250	*	3.094	2.534	0.5595	15.87	30.01	1.000
	3.500	*	1.432	1.444	-0.01249	16.44	31.90	1.000
	4.000	*	0.3967	0.4688	-0.07215	16.89	33.55	1.000
	4.500	*	0.1005	0.1522	-0.05171	17.02	34.06	1.000
	5.000	*	0.02774	0.04942	-0.02168	17.05	34.20	1.000
	5.500	*	0.01363	0.01605	-0.002413	17.06	34.26	1.000
	6.000	*	0.01124	0.005210	0.006029	17.07	34.29	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9697
Rsq_adjusted		0.9647
Corr_XY		-0.9847
No_points_lambda_z		8
Lambda_z	1/h	2.2499
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3081
Tmax	h	3.0000
Cmax	umol/L	6.3938
Cmax_D	umol/L/mg	0.0228
Tlast	h	6.0000
Clast	umol/L	0.0112
AUClast	h*umol/L	17.0674
AUCall	h*umol/L	17.0674
AUCINF_obs	h*umol/L	17.0724
AUCINF_D_obs	h*umol/L/mg	0.0610
AUC_%Extrap_obs	%	0.0293
Vz_obs	mg/(umol/L)	7.2896
Cl_obs	mg/(h*umol/L)	16.4007
AUCINF_pred	h*umol/L	17.0698
AUCINF_D_pred	h*umol/L/mg	0.0610
AUC_%Extrap_pred	%	0.0136
Vz_pred	mg/(umol/L)	7.2908
Cl_pred	mg/(h*umol/L)	16.4033
AUMClast	h*h*umol/L	34.2919
AUMCINF_obs	h*h*umol/L	34.3241
AUMC_%Extrap_obs	%	0.0938
AUMCINF_pred	h*h*umol/L	34.3069
AUMC_%Extrap_pred	%	0.0435
MRTlast	h	0.5092
MRTINF_obs	h	0.5105
MRTINF_pred	h	0.5098
Vss_obs	mg/(umol/L)	8.3726
Vss_pred	mg/(umol/L)	8.3625

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=2
Treatment=DB

Date: 7/07/2008
Time: 13:36:15

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 270.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9278		4.995		2.317	2.150		
1.856		4.857		6.887	8.481		
2.784		5.669		11.77	19.98		
3.000	*	5.552	3.972	1.580	12.98	23.49	1.000
3.250	*	2.727	2.386	0.3411	14.02	26.68	1.000
3.500	*	1.564	1.433	0.1310	14.56	28.47	1.000
4.000	*	0.4112	0.5171	-0.1058	15.05	30.25	1.000
4.500	*	0.1269	0.1866	-0.05969	15.18	30.81	1.000
5.000	*	0.04493	0.06731	-0.02238	15.23	31.01	1.000
5.500	*	0.01799	0.02429	-0.006299	15.24	31.09	1.000
6.000	*	0.01880	0.008763	0.01003	15.25	31.14	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9673
Rsq_adjusted		0.9619
Corr_XY		-0.9835
No_points_lambda_z		8
Lambda_z	1/h	2.0388
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3400
Tmax	h	2.7835
Cmax	umol/L	5.6688
Cmax_D	umol/L/mg	0.0210
Tlast	h	6.0000
Clast	umol/L	0.0188
AUClast	h*umol/L	15.2521
AUCall	h*umol/L	15.2521
AUCINF_obs	h*umol/L	15.2613
AUCINF_D_obs	h*umol/L/mg	0.0565
AUC_%Extrap_obs	%	0.0604
Vz_obs	mg/(umol/L)	8.6775
Cl_obs	mg/(h*umol/L)	17.6918
AUCINF_pred	h*umol/L	15.2564
AUCINF_D_pred	h*umol/L/mg	0.0565
AUC_%Extrap_pred	%	0.0282
Vz_pred	mg/(umol/L)	8.6803
Cl_pred	mg/(h*umol/L)	17.6975
AUMClast	h*h*umol/L	31.1409
AUMCINF_obs	h*h*umol/L	31.2008
AUMC_%Extrap_obs	%	0.1918
AUMCINF_pred	h*h*umol/L	31.1688
AUMC_%Extrap_pred	%	0.0895
MRTlast	h	0.5418
MRTINF_obs	h	0.5444
MRTINF_pred	h	0.5430
Vss_obs	mg/(umol/L)	9.6321
Vss_pred	mg/(umol/L)	9.6098

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=2
Treatment=FLAT

Date: 7/07/2008
Time: 13:36:15

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9184		5.720			2.627	2.412	
1.837		6.257			8.126	10.10	
2.755		6.488			13.98	23.59	
3.000		6.580			15.58	28.19	
3.250		3.620			16.85	32.13	
3.500		1.812			17.53	34.39	
4.000		0.7779			18.18	36.76	
4.500		0.1556			18.41	37.71	
5.000	*	0.04499	0.04376	0.001234	18.46	37.94	1.000
5.500	*	0.02057	0.02175	-0.001177	18.48	38.03	1.000
6.000	*	0.01111	0.01081	0.0003048	18.49	38.07	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9953
Rsq_adjusted		0.9906
Corr_XY		-0.9976
No_points_lambda_z		3
Lambda_z	1/h	1.3982
Lambda_z_lower	h	5.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.4957
Tmax	h	3.0000
Cmax	umol/L	6.5795
Cmax_D	umol/L/mg	0.0219
Tlast	h	6.0000
Clast	umol/L	0.0111
AUClast	h*umol/L	18.4879
AUCall	h*umol/L	18.4879
AUCINF_obs	h*umol/L	18.4958
AUCINF_D_obs	h*umol/L/mg	0.0617
AUC_%Extrap_obs	%	0.0430
Vz_obs	mg/(umol/L)	11.6005
Cl_obs	mg/(h*umol/L)	16.2199
AUCINF_pred	h*umol/L	18.4956
AUCINF_D_pred	h*umol/L/mg	0.0617
AUC_%Extrap_pred	%	0.0418
Vz_pred	mg/(umol/L)	11.6006
Cl_pred	mg/(h*umol/L)	16.2201
AUMClast	h*h*umol/L	38.0705
AUMCINF_obs	h*h*umol/L	38.1239
AUMC_%Extrap_obs	%	0.1400
AUMCINF_pred	h*h*umol/L	38.1224
AUMC_%Extrap_pred	%	0.1362
MRTlast	h	0.5592
MRTINF_obs	h	0.5612
MRTINF_pred	h	0.5612
Vss_obs	mg/(umol/L)	9.1028
Vss_pred	mg/(umol/L)	9.1021

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=3
Treatment=IND

Date: 7/07/2008
Time: 13:36:15

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 283.50
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9137		6.321		2.888	2.639		
1.827		6.313		8.660	10.55		
2.741		6.559		14.54	24.03		
3.000	*	6.268	4.688	1.580	16.20	28.79	1.000
3.250	*	3.169	2.701	0.4682	17.38	32.43	1.000
3.500	*	1.642	1.556	0.08667	17.98	34.44	1.000
4.000	*	0.4128	0.5162	-0.1034	18.50	36.29	1.000
4.500	*	0.1201	0.1713	-0.05122	18.63	36.83	1.000
5.000	*	0.03678	0.05684	-0.02006	18.67	37.02	1.000
5.500	*	0.01773	0.01886	-0.001131	18.68	37.09	1.000
6.000	*	0.01108	0.006258	0.004825	18.69	37.13	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9805
Rsq_adjusted		0.9773
Corr_XY		-0.9902
No_points_lambda_z		8
Lambda_z	1/h	2.2063
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3142
Tmax	h	2.7411
Cmax	umol/L	6.5594
Cmax_D	umol/L/mg	0.0231
Tlast	h	6.0000
Clast	umol/L	0.0111
AUClast	h*umol/L	18.6887
AUCall	h*umol/L	18.6887
AUCINF_obs	h*umol/L	18.6937
AUCINF_D_obs	h*umol/L/mg	0.0659
AUC_%Extrap_obs	%	0.0269
Vz_obs	mg/(umol/L)	6.8737
Cl_obs	mg/(h*umol/L)	15.1655
AUCINF_pred	h*umol/L	18.6916
AUCINF_D_pred	h*umol/L/mg	0.0659
AUC_%Extrap_pred	%	0.0152
Vz_pred	mg/(umol/L)	6.8745
Cl_pred	mg/(h*umol/L)	15.1673
AUMClast	h*h*umol/L	37.1273
AUMCINF_obs	h*h*umol/L	37.1597
AUMC_%Extrap_obs	%	0.0872
AUMCINF_pred	h*h*umol/L	37.1456
AUMC_%Extrap_pred	%	0.0493
MRTlast	h	0.4866
MRTINF_obs	h	0.4878
MRTINF_pred	h	0.4873
Vss_obs	mg/(umol/L)	7.3980
Vss_pred	mg/(umol/L)	7.3909

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=3
Treatment=DB

Date: 7/07/2008
Time: 13:36:15

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 290.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9184		4.791		2.200	2.021		
1.837		4.929		6.664	8.198		
2.755		5.361		11.39	19.14		
3.000	*	5.189	3.919	1.270	12.68	22.85	1.000
3.250	*	2.742	2.281	0.4607	13.67	25.91	1.000
3.500	*	1.396	1.328	0.06789	14.19	27.64	1.000
4.000	*	0.3677	0.4499	-0.08215	14.63	29.23	1.000
4.500	*	0.1034	0.1524	-0.04901	14.75	29.71	1.000
5.000	*	0.03280	0.05165	-0.01885	14.78	29.87	1.000
5.500	*	0.01635	0.01750	-0.001147	14.79	29.93	1.000
6.000	*	0.01077	0.005929	0.004839	14.80	29.97	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9782
Rsq_adjusted		0.9746
Corr_XY		-0.9890
No_points_lambda_z		8
Lambda_z	1/h	2.1646
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3202
Tmax	h	2.7551
Cmax	umol/L	5.3614
Cmax_D	umol/L/mg	0.0185
Tlast	h	6.0000
Clast	umol/L	0.0108
AUClast	h*umol/L	14.8012
AUCall	h*umol/L	14.8012
AUCINF_obs	h*umol/L	14.8062
AUCINF_D_obs	h*umol/L/mg	0.0511
AUC_%Extrap_obs	%	0.0336
Vz_obs	mg/(umol/L)	9.0487
Cl_obs	mg/(h*umol/L)	19.5864
AUCINF_pred	h*umol/L	14.8040
AUCINF_D_pred	h*umol/L/mg	0.0510
AUC_%Extrap_pred	%	0.0185
Vz_pred	mg/(umol/L)	9.0501
Cl_pred	mg/(h*umol/L)	19.5893
AUMClast	h*h*umol/L	29.9702
AUMCINF_obs	h*h*umol/L	30.0023
AUMC_%Extrap_obs	%	0.1071
AUMCINF_pred	h*h*umol/L	29.9879
AUMC_%Extrap_pred	%	0.0590
MRTlast	h	0.5248
MRTINF_obs	h	0.5263
MRTINF_pred	h	0.5257
Vss_obs	mg/(umol/L)	10.3089
Vss_pred	mg/(umol/L)	10.2974

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=3
Treatment=FLAT

Date: 7/07/2008
Time: 13:36:15

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9231		5.613		2.591	2.391		
1.846		7.523		8.653	11.19		
2.769		7.171		15.43	26.77		
3.000		6.847		17.05	31.43		
3.250		3.368		18.33	35.36		
3.500	*	0.6805	0.8634	-0.1829	18.84	37.03	1.000
4.000	*	0.4644	0.3587	0.1056	19.12	38.09	1.000
4.500	*	0.1996	0.1491	0.05054	19.29	38.78	1.000
5.000	*	0.05033	0.06194	-0.01161	19.35	39.07	1.000
5.500	*	0.01901	0.02574	-0.006725	19.37	39.16	1.000
6.000	*	0.01304	0.01069	0.002347	19.38	39.20	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9724
Rsq_adjusted		0.9655
Corr_XY		-0.9861
No_points_lambda_z		6
Lambda_z	1/h	1.7565
Lambda_z_lower	h	3.5000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3946
Tmax	h	1.8462
Cmax	umol/L	7.5228
Cmax_D	umol/L/mg	0.0251
Tlast	h	6.0000
Clast	umol/L	0.0130
AUClast	h*umol/L	19.3755
AUCall	h*umol/L	19.3755
AUCINF_obs	h*umol/L	19.3829
AUCINF_D_obs	h*umol/L/mg	0.0646
AUC_%Extrap_obs	%	0.0383
Vz_obs	mg/(umol/L)	8.8117
Cl_obs	mg/(h*umol/L)	15.4775
AUCINF_pred	h*umol/L	19.3816
AUCINF_D_pred	h*umol/L/mg	0.0646
AUC_%Extrap_pred	%	0.0314
Vz_pred	mg/(umol/L)	8.8124
Cl_pred	mg/(h*umol/L)	15.4786
AUMClast	h*h*umol/L	39.2020
AUMCINF_obs	h*h*umol/L	39.2508
AUMC_%Extrap_obs	%	0.1243
AUMCINF_pred	h*h*umol/L	39.2420
AUMC_%Extrap_pred	%	0.1019
MRTlast	h	0.5233
MRTINF_obs	h	0.5250
MRTINF_pred	h	0.5247
Vss_obs	mg/(umol/L)	8.1260
Vss_pred	mg/(umol/L)	8.1217

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=4
Treatment=IND

Date: 7/07/2008
Time: 13:36:16

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 290.50
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		-
0.9184		5.765		2.647	2.431		
1.837		6.578		8.314	10.41		
2.755		6.186		14.18	23.78		
3.000	*	6.805	5.506	1.299	15.77	28.37	1.000
3.250	*	3.683	3.074	0.6083	17.08	32.42	1.000
3.500	*	1.708	1.717	-0.008840	17.75	34.66	1.000
4.000	*	0.5021	0.5353	-0.03318	18.30	36.66	1.000
4.500	*	0.1217	0.1669	-0.04522	18.46	37.30	1.000
5.000	*	0.03481	0.05204	-0.01723	18.50	37.48	1.000
5.500	*	0.01289	0.01623	-0.003332	18.51	37.54	1.000
6.000	*	0.009450	0.005059	0.004390	18.52	37.57	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9830
Rsq_adjusted		0.9802
Corr_XY		-0.9915
No_points_lambda_z		8
Lambda_z	1/h	2.3308
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.2974
Tmax	h	3.0000
Cmax	umol/L	6.8047
Cmax_D	umol/L/mg	0.0234
Tlast	h	6.0000
Clast	umol/L	0.0094
AUClast	h*umol/L	18.5160
AUCall	h*umol/L	18.5160
AUCINF_obs	h*umol/L	18.5201
AUCINF_D_obs	h*umol/L/mg	0.0638
AUC_%Extrap_obs	%	0.0219
Vz_obs	mg/(umol/L)	6.7298
Cl_obs	mg/(h*umol/L)	15.6857
AUCINF_pred	h*umol/L	18.5182
AUCINF_D_pred	h*umol/L/mg	0.0637
AUC_%Extrap_pred	%	0.0117
Vz_pred	mg/(umol/L)	6.7305
Cl_pred	mg/(h*umol/L)	15.6873
AUMClast	h*h*umol/L	37.5703
AUMCINF_obs	h*h*umol/L	37.5963
AUMC_%Extrap_obs	%	0.0693
AUMCINF_pred	h*h*umol/L	37.5842
AUMC_%Extrap_pred	%	0.0371
MRTlast	h	0.5291
MRTINF_obs	h	0.5300
MRTINF_pred	h	0.5296
Vss_obs	mg/(umol/L)	8.3140
Vss_pred	mg/(umol/L)	8.3078

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=4
Treatment=DB

Date: 7/07/2008
Time: 13:36:16

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 290.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9184		4.427		2.033	1.867		
1.837		4.739		6.242	7.731		
2.755		5.367		10.88	18.52		
3.000	*	5.329	3.489	1.839	12.19	22.29	1.000
3.250	*	2.696	1.987	0.7082	13.19	25.38	1.000
3.500	*	1.244	1.132	0.1124	13.69	27.02	1.000
4.000	*	0.2666	0.3671	-0.1006	14.07	28.37	1.000
4.500	*	0.06169	0.1191	-0.05739	14.15	28.71	1.000
5.000	*	0.01912	0.03863	-0.01951	14.17	28.80	1.000
5.500	*	0.01216	0.01253	-0.0003714	14.18	28.84	1.000
6.000	*	0.009879	0.004064	0.005815	14.18	28.87	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9528
Rsq_adjusted		0.9450
Corr_XY		-0.9761
No_points_lambda_z		8
Lambda_z	1/h	2.2517
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3078
Tmax	h	2.7551
Cmax	umol/L	5.3668
Cmax_D	umol/L/mg	0.0185
Tlast	h	6.0000
Clast	umol/L	0.0099
AUClast	h*umol/L	14.1807
AUCall	h*umol/L	14.1807
AUCINF_obs	h*umol/L	14.1850
AUCINF_D_obs	h*umol/L/mg	0.0489
AUC_%Extrap_obs	%	0.0309
Vz_obs	mg/(umol/L)	9.0792
Cl_obs	mg/(h*umol/L)	20.4441
AUCINF_pred	h*umol/L	14.1825
AUCINF_D_pred	h*umol/L/mg	0.0489
AUC_%Extrap_pred	%	0.0127
Vz_pred	mg/(umol/L)	9.0808
Cl_pred	mg/(h*umol/L)	20.4478
AUMClast	h*h*umol/L	28.8745
AUMCINF_obs	h*h*umol/L	28.9028
AUMC_%Extrap_obs	%	0.0978
AUMCINF_pred	h*h*umol/L	28.8861
AUMC_%Extrap_pred	%	0.0403
MRTlast	h	0.5362
MRTINF_obs	h	0.5376
MRTINF_pred	h	0.5368
Vss_obs	mg/(umol/L)	10.9898
Vss_pred	mg/(umol/L)	10.9754

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=4
Treatment=FLAT

Date: 7/07/2008
Time: 13:36:16

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9184		5.913			2.715	2.494	
1.837		6.686			8.501	10.63	
2.755		6.718			14.66	24.76	
3.000	*	6.492	4.997	1.495	16.27	29.42	1.000
3.250	*	3.594	2.824	0.7697	17.53	33.31	1.000
3.500	*	1.663	1.596	0.06672	18.19	35.50	1.000
4.000	*	0.4325	0.5097	-0.07720	18.71	37.38	1.000
4.500	*	0.1011	0.1628	-0.06171	18.85	37.93	1.000
5.000	*	0.03323	0.05199	-0.01876	18.88	38.09	1.000
5.500	*	0.01474	0.01660	-0.001863	18.89	38.15	1.000
6.000	*	0.01030	0.005303	0.004995	18.90	38.18	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9768
Rsq_adjusted		0.9729
Corr_XY		-0.9883
No_points_lambda_z		8
Lambda_z	1/h	2.2828
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3036
Tmax	h	2.7551
Cmax	umol/L	6.7183
Cmax_D	umol/L/mg	0.0224
Tlast	h	6.0000
Clast	umol/L	0.0103
AUClast	h*umol/L	18.8998
AUCall	h*umol/L	18.8998
AUCINF_obs	h*umol/L	18.9043
AUCINF_D_obs	h*umol/L/mg	0.0630
AUC_%Extrap_obs	%	0.0239
Vz_obs	mg/(umol/L)	6.9519
Cl_obs	mg/(h*umol/L)	15.8694
AUCINF_pred	h*umol/L	18.9022
AUCINF_D_pred	h*umol/L/mg	0.0630
AUC_%Extrap_pred	%	0.0123
Vz_pred	mg/(umol/L)	6.9527
Cl_pred	mg/(h*umol/L)	15.8712
AUMClast	h*h*umol/L	38.1831
AUMCINF_obs	h*h*umol/L	38.2122
AUMC_%Extrap_obs	%	0.0760
AUMCINF_pred	h*h*umol/L	38.1981
AUMC_%Extrap_pred	%	0.0392
MRTlast	h	0.5203
MRTINF_obs	h	0.5213
MRTINF_pred	h	0.5208
Vss_obs	mg/(umol/L)	8.2734
Vss_pred	mg/(umol/L)	8.2662

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=5
Treatment=IND

Date: 7/07/2008
Time: 13:36:16

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 306.30
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9231		5.055		2.333	2.154		
1.846		5.511		7.210	9.003		
2.769		5.514		12.30	20.75		
3.000	*	5.595	4.449	1.146	13.58	24.45	1.000
3.250	*	3.008	2.589	0.4186	14.66	27.77	1.000
3.500	*	1.510	1.507	0.002956	15.22	29.65	1.000
4.000	*	0.4290	0.5103	-0.08130	15.70	31.40	1.000
4.500	*	0.1383	0.1728	-0.03455	15.85	31.98	1.000
5.000	*	0.04035	0.05853	-0.01818	15.89	32.19	1.000
5.500	*	0.01790	0.01982	-0.001918	15.91	32.26	1.000
6.000	*	0.01095	0.006713	0.004237	15.91	32.30	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9864
Rsq_adjusted		0.9841
Corr_XY		-0.9932
No_points_lambda_z		8
Lambda_z	1/h	2.1655
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3201
Tmax	h	3.0000
Cmax	umol/L	5.5947
Cmax_D	umol/L/mg	0.0183
Tlast	h	6.0000
Clast	umol/L	0.0109
AUClast	h*umol/L	15.9130
AUCall	h*umol/L	15.9130
AUCINF_obs	h*umol/L	15.9181
AUCINF_D_obs	h*umol/L/mg	0.0520
AUC_%Extrap_obs	%	0.0318
Vz_obs	mg/(umol/L)	8.8859
Cl_obs	mg/(h*umol/L)	19.2423
AUCINF_pred	h*umol/L	15.9161
AUCINF_D_pred	h*umol/L/mg	0.0520
AUC_%Extrap_pred	%	0.0195
Vz_pred	mg/(umol/L)	8.8870
Cl_pred	mg/(h*umol/L)	19.2446
AUMClast	h*h*umol/L	32.3043
AUMCINF_obs	h*h*umol/L	32.3370
AUMC_%Extrap_obs	%	0.1010
AUMCINF_pred	h*h*umol/L	32.3243
AUMC_%Extrap_pred	%	0.0620
MRTlast	h	0.5301
MRTINF_obs	h	0.5315
MRTINF_pred	h	0.5309
Vss_obs	mg/(umol/L)	10.2265
Vss_pred	mg/(umol/L)	10.2173

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=5
Treatment=DB

Date: 7/07/2008
Time: 13:36:16

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 310.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9231		5.214			2.406	2.221	
1.846		5.402			7.306	9.045	
2.769		5.202			12.20	20.30	
3.000		4.854			13.36	23.64	
3.250		2.426			14.27	26.44	
3.500		1.215			14.73	27.96	
4.000		0.3482			15.12	29.37	
4.500		0.08567			15.22	29.82	
5.000	*	0.02680	0.02626	0.0005398	15.25	29.95	1.000
5.500	*	0.01457	0.01518	-0.0006051	15.26	30.00	1.000
6.000	*	0.008950	0.008770	0.0001802	15.27	30.03	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9959
Rsq_adjusted		0.9918
Corr_XY		-0.9979
No_points_lambda_z		3
Lambda_z	1/h	1.0968
Lambda_z_lower	h	5.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.6320
Tmax	h	1.8462
Cmax	umol/L	5.4020
Cmax_D	umol/L/mg	0.0174
Tlast	h	6.0000
Clast	umol/L	0.0090
AUClast	h*umol/L	15.2688
AUCall	h*umol/L	15.2688
AUCINF_obs	h*umol/L	15.2769
AUCINF_D_obs	h*umol/L/mg	0.0493
AUC_%Extrap_obs	%	0.0534
Vz_obs	mg/(umol/L)	18.5014
Cl_obs	mg/(h*umol/L)	20.2920
AUCINF_pred	h*umol/L	15.2768
AUCINF_D_pred	h*umol/L/mg	0.0493
AUC_%Extrap_pred	%	0.0523
Vz_pred	mg/(umol/L)	18.5016
Cl_pred	mg/(h*umol/L)	20.2922
AUMClast	h*h*umol/L	30.0340
AUMCINF_obs	h*h*umol/L	30.0904
AUMC_%Extrap_obs	%	0.1874
AUMCINF_pred	h*h*umol/L	30.0893
AUMC_%Extrap_pred	%	0.1837
MRTlast	h	0.4670
MRTINF_obs	h	0.4697
MRTINF_pred	h	0.4696
Vss_obs	mg/(umol/L)	9.5304
Vss_pred	mg/(umol/L)	9.5294

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=5
Treatment=FLAT

Date: 7/07/2008
Time: 13:36:16

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9231		5.246			2.421	2.235	
1.846		5.322			7.299	9.005	
2.769		5.626			12.35	20.73	
3.000	*	6.017	4.540	1.477	13.70	24.61	1.000
3.250	*	2.838	2.661	0.1764	14.80	28.02	1.000
3.500	*	1.486	1.560	-0.07390	15.34	29.82	1.000
4.000	*	0.4801	0.5361	-0.05592	15.83	31.60	1.000
4.500	*	0.1434	0.1842	-0.04080	15.99	32.24	1.000
5.000	*	0.04833	0.06329	-0.01496	16.04	32.47	1.000
5.500	*	0.02030	0.02175	-0.001449	16.06	32.55	1.000
6.000	*	0.01117	0.007474	0.003697	16.06	32.60	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9896
Rsq_adjusted		0.9879
Corr_XY		-0.9948
No_points_lambda_z		8
Lambda_z	1/h	2.1364
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3244
Tmax	h	3.0000
Cmax	umol/L	6.0171
Cmax_D	umol/L/mg	0.0201
Tlast	h	6.0000
Clast	umol/L	0.0112
AUClast	h*umol/L	16.0629
AUCall	h*umol/L	16.0629
AUCINF_obs	h*umol/L	16.0682
AUCINF_D_obs	h*umol/L/mg	0.0536
AUC_%Extrap_obs	%	0.0325
Vz_obs	mg/(umol/L)	8.7391
Cl_obs	mg/(h*umol/L)	18.6705
AUCINF_pred	h*umol/L	16.0664
AUCINF_D_pred	h*umol/L/mg	0.0536
AUC_%Extrap_pred	%	0.0218
Vz_pred	mg/(umol/L)	8.7400
Cl_pred	mg/(h*umol/L)	18.6725
AUMClast	h*h*umol/L	32.5993
AUMCINF_obs	h*h*umol/L	32.6331
AUMC_%Extrap_obs	%	0.1036
AUMCINF_pred	h*h*umol/L	32.6219
AUMC_%Extrap_pred	%	0.0694
MRTlast	h	0.5295
MRTINF_obs	h	0.5309
MRTINF_pred	h	0.5304
Vss_obs	mg/(umol/L)	9.9125
Vss_pred	mg/(umol/L)	9.9046

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=6
Treatment=IND

Date: 7/07/2008
Time: 13:36:16

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 313.30
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9375		5.765			2.702	2.533	
1.875		6.578			8.488	10.85	
2.813		6.310			14.53	24.95	
3.000	*	6.805	5.506	1.299	15.76	28.53	1.000
3.250	*	3.683	3.074	0.6083	17.07	32.57	1.000
3.500	*	1.708	1.717	-0.008840	17.74	34.82	1.000
4.000	*	0.5021	0.5353	-0.03318	18.30	36.81	1.000
4.500	*	0.1217	0.1669	-0.04522	18.45	37.45	1.000
5.000	*	0.03481	0.05204	-0.01723	18.49	37.63	1.000
5.500	*	0.01289	0.01623	-0.003332	18.50	37.69	1.000
6.000	*	0.009450	0.005059	0.004390	18.51	37.73	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9830
Rsq_adjusted		0.9802
Corr_XY		-0.9915
No_points_lambda_z		8
Lambda_z	1/h	2.3308
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.2974
Tmax	h	3.0000
Cmax	umol/L	6.8047
Cmax_D	umol/L/mg	0.0217
Tlast	h	6.0000
Clast	umol/L	0.0094
AUClast	h*umol/L	18.5082
AUCall	h*umol/L	18.5082
AUCINF_obs	h*umol/L	18.5122
AUCINF_D_obs	h*umol/L/mg	0.0591
AUC_%Extrap_obs	%	0.0219
Vz_obs	mg/(umol/L)	7.2611
Cl_obs	mg/(h*umol/L)	16.9239
AUCINF_pred	h*umol/L	18.5104
AUCINF_D_pred	h*umol/L/mg	0.0591
AUC_%Extrap_pred	%	0.0117
Vz_pred	mg/(umol/L)	7.2618
Cl_pred	mg/(h*umol/L)	16.9257
AUMClast	h*h*umol/L	37.7259
AUMCINF_obs	h*h*umol/L	37.7519
AUMC_%Extrap_obs	%	0.0690
AUMCINF_pred	h*h*umol/L	37.7398
AUMC_%Extrap_pred	%	0.0370
MRTlast	h	0.5383
MRTINF_obs	h	0.5393
MRTINF_pred	h	0.5388
Vss_obs	mg/(umol/L)	9.1270
Vss_pred	mg/(umol/L)	9.1204

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=6
Treatment=DB

Date: 7/07/2008
Time: 13:36:16

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 310.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9278		4.526			2.100	1.948	
1.856		4.885			6.466	8.102	
2.784		5.116			11.11	18.92	
3.000		4.407			12.14	21.89	
3.250		2.436			12.99	24.53	
3.500		1.315			13.46	26.09	
4.000		0.3876			13.89	27.63	
4.500		0.1087			14.01	28.14	
5.000	*	0.03298	0.03231	0.0006747	14.05	28.31	1.000
5.500	*	0.01350	0.01407	-0.0005698	14.06	28.37	1.000
6.000	*	0.006255	0.006127	0.0001280	14.06	28.39	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9981
Rsq_adjusted		0.9963
Corr_XY		-0.9991
No_points_lambda_z		3
Lambda_z	1/h	1.6625
Lambda_z_lower	h	5.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.4169
Tmax	h	2.7835
Cmax	umol/L	5.1165
Cmax_D	umol/L/mg	0.0165
Tlast	h	6.0000
Clast	umol/L	0.0063
AUClast	h*umol/L	14.0627
AUCall	h*umol/L	14.0627
AUCINF_obs	h*umol/L	14.0665
AUCINF_D_obs	h*umol/L/mg	0.0454
AUC_%Extrap_obs	%	0.0267
Vz_obs	mg/(umol/L)	13.2564
Cl_obs	mg/(h*umol/L)	22.0382
AUCINF_pred	h*umol/L	14.0664
AUCINF_D_pred	h*umol/L/mg	0.0454
AUC_%Extrap_pred	%	0.0262
Vz_pred	mg/(umol/L)	13.2565
Cl_pred	mg/(h*umol/L)	22.0383
AUMClast	h*h*umol/L	28.3933
AUMCINF_obs	h*h*umol/L	28.4182
AUMC_%Extrap_obs	%	0.0874
AUMCINF_pred	h*h*umol/L	28.4177
AUMC_%Extrap_pred	%	0.0856
MRTlast	h	0.5191
MRTINF_obs	h	0.5203
MRTINF_pred	h	0.5203
Vss_obs	mg/(umol/L)	11.4660
Vss_pred	mg/(umol/L)	11.4655

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=6
Treatment=FLAT

Date: 7/07/2008
Time: 13:36:17

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9184		5.179			2.378	2.184	
1.837		5.799			7.419	9.258	
2.755		5.684			12.69	21.34	
3.000		5.876			14.11	25.42	
3.250		3.179			15.24	28.91	
3.500		1.645			15.84	30.92	
4.000		0.4150			16.36	32.78	
4.500		0.1146			16.49	33.32	
5.000	*	0.03426	0.03435	-8.442e-005	16.53	33.49	1.000
5.500	*	0.01475	0.01468	7.241e-005	16.54	33.56	1.000
6.000	*	0.006255	0.006271	-1.541e-005	16.54	33.59	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		1.0000
Rsq_adjusted		0.9999
Corr_XY		-1.0000
No_points_lambda_z		3
Lambda_z	1/h	1.7006
Lambda_z_lower	h	5.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.4076
Tmax	h	3.0000
Cmax	umol/L	5.8765
Cmax_D	umol/L/mg	0.0196
Tlast	h	6.0000
Clast	umol/L	0.0063
AUClast	h*umol/L	16.5444
AUCall	h*umol/L	16.5444
AUCINF_obs	h*umol/L	16.5481
AUCINF_D_obs	h*umol/L/mg	0.0552
AUC_%Extrap_obs	%	0.0222
Vz_obs	mg/(umol/L)	10.6601
Cl_obs	mg/(h*umol/L)	18.1290
AUCINF_pred	h*umol/L	16.5481
AUCINF_D_pred	h*umol/L/mg	0.0552
AUC_%Extrap_pred	%	0.0223
Vz_pred	mg/(umol/L)	10.6601
Cl_pred	mg/(h*umol/L)	18.1290
AUMClast	h*h*umol/L	33.5862
AUMCINF_obs	h*h*umol/L	33.6104
AUMC_%Extrap_obs	%	0.0721
AUMCINF_pred	h*h*umol/L	33.6105
AUMC_%Extrap_pred	%	0.0723
MRTlast	h	0.5301
MRTINF_obs	h	0.5311
MRTINF_pred	h	0.5311
Vss_obs	mg/(umol/L)	9.6279
Vss_pred	mg/(umol/L)	9.6280

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=7
Treatment=IND

Date: 7/07/2008
Time: 13:36:17

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 325.50
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9231		5.029			2.321	2.142	
1.846		5.439			7.152	8.919	
2.769		5.471			12.19	20.55	
3.000	*	5.507	4.396	1.111	13.45	24.20	1.000
3.250	*	2.738	2.562	0.1758	14.48	27.38	1.000
3.500	*	1.377	1.493	-0.1165	15.00	29.09	1.000
4.000	*	0.5592	0.5071	0.05211	15.48	30.86	1.000
4.500	*	0.1323	0.1722	-0.03990	15.66	31.56	1.000
5.000	*	0.04035	0.05850	-0.01815	15.70	31.76	1.000
5.500	*	0.01679	0.01987	-0.003078	15.71	31.84	1.000
6.000	*	0.01107	0.006749	0.004323	15.72	31.88	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9860
Rsq_adjusted		0.9837
Corr_XY		-0.9930
No_points_lambda_z		8
Lambda_z	1/h	2.1597
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3209
Tmax	h	3.0000
Cmax	umol/L	5.5067
Cmax_D	umol/L/mg	0.0169
Tlast	h	6.0000
Clast	umol/L	0.0111
AUClast	h*umol/L	15.7200
AUCall	h*umol/L	15.7200
AUCINF_obs	h*umol/L	15.7252
AUCINF_D_obs	h*umol/L/mg	0.0483
AUC_%Extrap_obs	%	0.0326
Vz_obs	mg/(umol/L)	9.5844
Cl_obs	mg/(h*umol/L)	20.6993
AUCINF_pred	h*umol/L	15.7232
AUCINF_D_pred	h*umol/L/mg	0.0483
AUC_%Extrap_pred	%	0.0199
Vz_pred	mg/(umol/L)	9.5856
Cl_pred	mg/(h*umol/L)	20.7020
AUMClast	h*h*umol/L	31.8763
AUMCINF_obs	h*h*umol/L	31.9094
AUMC_%Extrap_obs	%	0.1038
AUMCINF_pred	h*h*umol/L	31.8964
AUMC_%Extrap_pred	%	0.0633
MRTlast	h	0.5277
MRTINF_obs	h	0.5292
MRTINF_pred	h	0.5286
Vss_obs	mg/(umol/L)	10.9540
Vss_pred	mg/(umol/L)	10.9437

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=7
Treatment=DB

Date: 7/07/2008
Time: 13:36:17

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 330.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9231		4.218			1.947	1.797	
1.846		4.625			6.028	7.535	
2.769		4.723			10.34	17.51	
3.000	*	4.436	3.506	0.9301	11.40	20.56	1.000
3.250	*	2.384	2.060	0.3234	12.25	23.19	1.000
3.500	*	1.287	1.211	0.07577	12.71	24.72	1.000
4.000	*	0.3513	0.4183	-0.06705	13.12	26.20	1.000
4.500	*	0.1052	0.1445	-0.03927	13.23	26.67	1.000
5.000	*	0.03400	0.04991	-0.01591	13.27	26.83	1.000
5.500	*	0.01561	0.01724	-0.001627	13.28	26.89	1.000
6.000	*	0.01015	0.005955	0.004193	13.29	26.93	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9830
Rsq_adjusted		0.9802
Corr_XY		-0.9915
No_points_lambda_z		8
Lambda_z	1/h	2.1260
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3260
Tmax	h	2.7692
Cmax	umol/L	4.7226
Cmax_D	umol/L/mg	0.0143
Tlast	h	6.0000
Clast	umol/L	0.0101
AUClast	h*umol/L	13.2874
AUCall	h*umol/L	13.2874
AUCINF_obs	h*umol/L	13.2922
AUCINF_D_obs	h*umol/L/mg	0.0403
AUC_%Extrap_obs	%	0.0359
Vz_obs	mg/(umol/L)	11.6778
Cl_obs	mg/(h*umol/L)	24.8267
AUCINF_pred	h*umol/L	13.2902
AUCINF_D_pred	h*umol/L/mg	0.0403
AUC_%Extrap_pred	%	0.0211
Vz_pred	mg/(umol/L)	11.6795
Cl_pred	mg/(h*umol/L)	24.8304
AUMClast	h*h*umol/L	26.9273
AUMCINF_obs	h*h*umol/L	26.9582
AUMC_%Extrap_obs	%	0.1146
AUMCINF_pred	h*h*umol/L	26.9454
AUMC_%Extrap_pred	%	0.0673
MRTlast	h	0.5265
MRTINF_obs	h	0.5281
MRTINF_pred	h	0.5275
Vss_obs	mg/(umol/L)	13.1116
Vss_pred	mg/(umol/L)	13.0972

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=7
Treatment=FLAT

Date: 7/07/2008
Time: 13:36:17

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9231		4.840			2.234	2.062	
1.846		5.162			6.850	8.523	
2.769		5.302			11.68	19.70	
3.000	*	5.232	4.094	1.138	12.90	23.20	1.000
3.250	*	2.677	2.377	0.3004	13.88	26.25	1.000
3.500	*	1.428	1.380	0.04851	14.40	27.96	1.000
4.000	*	0.4070	0.4651	-0.05817	14.86	29.62	1.000
4.500	*	0.1142	0.1568	-0.04255	14.99	30.16	1.000
5.000	*	0.03701	0.05285	-0.01583	15.02	30.33	1.000
5.500	*	0.01609	0.01781	-0.001725	15.04	30.40	1.000
6.000	*	0.009994	0.006004	0.003989	15.04	30.44	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9853
Rsq_adjusted		0.9829
Corr_XY		-0.9926
No_points_lambda_z		8
Lambda_z	1/h	2.1749
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3187
Tmax	h	2.7692
Cmax	umol/L	5.3016
Cmax_D	umol/L/mg	0.0177
Tlast	h	6.0000
Clast	umol/L	0.0100
AUClast	h*umol/L	15.0439
AUCall	h*umol/L	15.0439
AUCINF_obs	h*umol/L	15.0485
AUCINF_D_obs	h*umol/L/mg	0.0502
AUC_%Extrap_obs	%	0.0305
Vz_obs	mg/(umol/L)	9.1660
Cl_obs	mg/(h*umol/L)	19.9355
AUCINF_pred	h*umol/L	15.0467
AUCINF_D_pred	h*umol/L/mg	0.0502
AUC_%Extrap_pred	%	0.0183
Vz_pred	mg/(umol/L)	9.1671
Cl_pred	mg/(h*umol/L)	19.9379
AUMClast	h*h*umol/L	30.4374
AUMCINF_obs	h*h*umol/L	30.4670
AUMC_%Extrap_obs	%	0.0974
AUMCINF_pred	h*h*umol/L	30.4552
AUMC_%Extrap_pred	%	0.0586
MRTlast	h	0.5232
MRTINF_obs	h	0.5246
MRTINF_pred	h	0.5240
Vss_obs	mg/(umol/L)	10.4578
Vss_pred	mg/(umol/L)	10.4483

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=8
Treatment=IND

Date: 7/07/2008
Time: 13:36:17

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 332.50
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9231		6.282			2.899	2.676	
1.846		6.444			8.773	10.84	
2.769		6.799			14.88	25.02	
3.000	*	6.641	5.415	1.225	16.44	29.49	1.000
3.250	*	3.605	3.176	0.4290	17.72	33.45	1.000
3.500	*	1.844	1.862	-0.01773	18.40	35.72	1.000
4.000	*	0.5454	0.6403	-0.09492	18.99	37.88	1.000
4.500	*	0.1720	0.2202	-0.04814	19.17	38.62	1.000
5.000	*	0.06187	0.07571	-0.01385	19.23	38.89	1.000
5.500	*	0.02387	0.02603	-0.002163	19.25	39.00	1.000
6.000	*	0.01302	0.008952	0.004071	19.26	39.05	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9913
Rsq_adjusted		0.9899
Corr_XY		-0.9957
No_points_lambda_z		8
Lambda_z	1/h	2.1350
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3247
Tmax	h	2.7692
Cmax	umol/L	6.7986
Cmax_D	umol/L/mg	0.0204
Tlast	h	6.0000
Clast	umol/L	0.0130
AUClast	h*umol/L	19.2631
AUCall	h*umol/L	19.2631
AUCINF_obs	h*umol/L	19.2692
AUCINF_D_obs	h*umol/L/mg	0.0580
AUC_%Extrap_obs	%	0.0317
Vz_obs	mg/(umol/L)	8.0820
Cl_obs	mg/(h*umol/L)	17.2555
AUCINF_pred	h*umol/L	19.2673
AUCINF_D_pred	h*umol/L/mg	0.0579
AUC_%Extrap_pred	%	0.0218
Vz_pred	mg/(umol/L)	8.0828
Cl_pred	mg/(h*umol/L)	17.2572
AUMClast	h*h*umol/L	39.0516
AUMCINF_obs	h*h*umol/L	39.0911
AUMC_%Extrap_obs	%	0.1009
AUMCINF_pred	h*h*umol/L	39.0788
AUMC_%Extrap_pred	%	0.0694
MRTlast	h	0.5273
MRTINF_obs	h	0.5287
MRTINF_pred	h	0.5282
Vss_obs	mg/(umol/L)	9.1226
Vss_pred	mg/(umol/L)	9.1159

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=8
Treatment=DB

Date: 7/07/2008
Time: 13:36:17

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 330.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000		0.0000	0.0000		
0.9231		6.390		2.949	2.722		
1.846		6.827		9.049	11.26		
2.769		6.863		15.37	25.85		
3.000	*	6.843	4.771	2.072	16.95	30.41	1.000
3.250	*	3.379	2.672	0.7078	18.23	34.35	1.000
3.500	*	1.568	1.496	0.07146	18.84	36.41	1.000
4.000	*	0.3907	0.4692	-0.07846	19.33	38.17	1.000
4.500	*	0.08208	0.1471	-0.06505	19.45	38.65	1.000
5.000	*	0.02934	0.04614	-0.01680	19.48	38.78	1.000
5.500	*	0.01070	0.01447	-0.003769	19.49	38.83	1.000
6.000	*	0.01093	0.004537	0.006388	19.50	38.87	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9651
Rsq_adjusted		0.9592
Corr_XY		-0.9824
No_points_lambda_z		8
Lambda_z	1/h	2.3193
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.2989
Tmax	h	2.7692
Cmax	umol/L	6.8629
Cmax_D	umol/L/mg	0.0208
Tlast	h	6.0000
Clast	umol/L	0.0109
AUClast	h*umol/L	19.4957
AUCall	h*umol/L	19.4957
AUCINF_obs	h*umol/L	19.5005
AUCINF_D_obs	h*umol/L/mg	0.0591
AUC_%Extrap_obs	%	0.0242
Vz_obs	mg/(umol/L)	7.2964
Cl_obs	mg/(h*umol/L)	16.9227
AUCINF_pred	h*umol/L	19.4977
AUCINF_D_pred	h*umol/L/mg	0.0591
AUC_%Extrap_pred	%	0.0100
Vz_pred	mg/(umol/L)	7.2975
Cl_pred	mg/(h*umol/L)	16.9251
AUMClast	h*h*umol/L	38.8652
AUMCINF_obs	h*h*umol/L	38.8955
AUMC_%Extrap_obs	%	0.0779
AUMCINF_pred	h*h*umol/L	38.8778
AUMC_%Extrap_pred	%	0.0324
MRTlast	h	0.4935
MRTINF_obs	h	0.4946
MRTINF_pred	h	0.4940
Vss_obs	mg/(umol/L)	8.3699
Vss_pred	mg/(umol/L)	8.3604

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=8
Treatment=FLAT

Date: 7/07/2008
Time: 13:36:17

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9231		4.713			2.175	2.008	
1.846		5.070			6.690	8.336	
2.769		5.127			11.40	19.21	
3.000	*	5.265	4.522	0.7432	12.60	22.67	1.000
3.250	*	2.840	2.643	0.1963	13.61	25.80	1.000
3.500	*	1.759	1.545	0.2140	14.18	27.72	1.000
4.000	*	0.5159	0.5280	-0.01217	14.75	29.78	1.000
4.500	*	0.1328	0.1804	-0.04761	14.91	30.44	1.000
5.000	*	0.04058	0.06166	-0.02108	14.96	30.64	1.000
5.500	*	0.01894	0.02107	-0.002128	14.97	30.72	1.000
6.000	*	0.01188	0.007200	0.004683	14.98	30.76	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9853
Rsq_adjusted		0.9828
Corr_XY		-0.9926
No_points_lambda_z		8
Lambda_z	1/h	2.1475
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3228
Tmax	h	3.0000
Cmax	umol/L	5.2651
Cmax_D	umol/L/mg	0.0176
Tlast	h	6.0000
Clast	umol/L	0.0119
AUClast	h*umol/L	14.9806
AUCall	h*umol/L	14.9806
AUCINF_obs	h*umol/L	14.9861
AUCINF_D_obs	h*umol/L/mg	0.0500
AUC_%Extrap_obs	%	0.0369
Vz_obs	mg/(umol/L)	9.3217
Cl_obs	mg/(h*umol/L)	20.0185
AUCINF_pred	h*umol/L	14.9839
AUCINF_D_pred	h*umol/L/mg	0.0499
AUC_%Extrap_pred	%	0.0224
Vz_pred	mg/(umol/L)	9.3230
Cl_pred	mg/(h*umol/L)	20.0215
AUMClast	h*h*umol/L	30.7616
AUMCINF_obs	h*h*umol/L	30.7974
AUMC_%Extrap_obs	%	0.1162
AUMCINF_pred	h*h*umol/L	30.7833
AUMC_%Extrap_pred	%	0.0704
MRTlast	h	0.5534
MRTINF_obs	h	0.5551
MRTINF_pred	h	0.5544
Vss_obs	mg/(umol/L)	11.1116
Vss_pred	mg/(umol/L)	11.1003

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=9
Treatment=IND

Date: 7/07/2008
Time: 13:36:17

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 341.30
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
0.0000	@	0.0000			0.0000	0.0000	
0.9231		5.394			2.489	2.298	
1.846		5.803			7.657	9.540	
2.769		5.645			12.94	21.70	
3.000		5.685			14.25	25.47	
3.250		2.937			15.33	28.80	
3.500		1.443			15.87	30.62	
4.000		0.3966			16.33	32.28	
4.500		0.1024			16.46	32.79	
5.000	*	0.03252	0.03156	0.0009612	16.49	32.95	1.000
5.500	*	0.01653	0.01755	-0.001022	16.50	33.01	1.000
6.000	*	0.01006	0.009763	0.0002974	16.51	33.05	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9922
Rsq_adjusted		0.9844
Corr_XY		-0.9961
No_points_lambda_z		3
Lambda_z	1/h	1.1733
Lambda_z_lower	h	5.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.5908
Tmax	h	1.8462
Cmax	umol/L	5.8030
Cmax_D	umol/L/mg	0.0170
Tlast	h	6.0000
Clast	umol/L	0.0101
AUClast	h*umol/L	16.5105
AUCall	h*umol/L	16.5105
AUCINF_obs	h*umol/L	16.5191
AUCINF_D_obs	h*umol/L/mg	0.0484
AUC_%Extrap_obs	%	0.0519
Vz_obs	mg/(umol/L)	17.6098
Cl_obs	mg/(h*umol/L)	20.6610
AUCINF_pred	h*umol/L	16.5188
AUCINF_D_pred	h*umol/L/mg	0.0484
AUC_%Extrap_pred	%	0.0504
Vz_pred	mg/(umol/L)	17.6101
Cl_pred	mg/(h*umol/L)	20.6613
AUMClast	h*h*umol/L	33.0486
AUMCINF_obs	h*h*umol/L	33.1074
AUMC_%Extrap_obs	%	0.1775
AUMCINF_pred	h*h*umol/L	33.1056
AUMC_%Extrap_pred	%	0.1722
MRTlast	h	0.5017
MRTINF_obs	h	0.5042
MRTINF_pred	h	0.5041
Vss_obs	mg/(umol/L)	10.4171
Vss_pred	mg/(umol/L)	10.4157

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=9
Treatment=DB

Date: 7/07/2008
Time: 13:36:18

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 11
Dose time: 0.00
Dose amount: 350.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

	Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
	h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
	0.0000	@	0.0000		0.0000	0.0000		
	0.9231		5.171		2.387	2.203		
	1.846		5.677		7.393	9.243		
	2.769		5.375		12.49	20.95		
	3.000	*	6.754	5.354	1.400	13.89	25.01	1.000
	3.250	*	3.510	3.052	0.4584	15.18	28.96	1.000
	3.500	*	1.765	1.739	0.02544	15.84	31.16	1.000
	4.000	*	0.5280	0.5649	-0.03685	16.41	33.23	1.000
	4.500	*	0.1309	0.1835	-0.05259	16.57	33.91	1.000
	5.000	*	0.03985	0.05959	-0.01974	16.62	34.11	1.000
	5.500	*	0.01670	0.01936	-0.002660	16.63	34.18	1.000
	6.000	*	0.01110	0.006287	0.004815	16.64	34.22	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9837
Rsq_adjusted		0.9810
Corr_XY		-0.9918
No_points_lambda_z		8
Lambda_z	1/h	2.2491
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3082
Tmax	h	3.0000
Cmax	umol/L	6.7540
Cmax_D	umol/L/mg	0.0193
Tlast	h	6.0000
Clast	umol/L	0.0111
AUClast	h*umol/L	16.6377
AUCall	h*umol/L	16.6377
AUCINF_obs	h*umol/L	16.6426
AUCINF_D_obs	h*umol/L/mg	0.0476
AUC_%Extrap_obs	%	0.0297
Vz_obs	mg/(umol/L)	9.3507
Cl_obs	mg/(h*umol/L)	21.0304
AUCINF_pred	h*umol/L	16.6405
AUCINF_D_pred	h*umol/L/mg	0.0475
AUC_%Extrap_pred	%	0.0168
Vz_pred	mg/(umol/L)	9.3519
Cl_pred	mg/(h*umol/L)	21.0331
AUMClast	h*h*umol/L	34.2186
AUMCINF_obs	h*h*umol/L	34.2504
AUMC_%Extrap_obs	%	0.0929
AUMCINF_pred	h*h*umol/L	34.2366
AUMC_%Extrap_pred	%	0.0526
MRTlast	h	0.5567
MRTINF_obs	h	0.5580
MRTINF_pred	h	0.5574
Vss_obs	mg/(umol/L)	11.7348
Vss_pred	mg/(umol/L)	11.7245

Input File: Workbook - [C:\Documents and Set...\summary.PWO]
Patient=9
Treatment=FLAT

Date: 7/07/2008
Time: 13:36:18

WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM
Version 5.2 Build 200701231637
Core Version 18Sept2006

Settings

Model: Plasma Data, Constant Infusion Administration
Number of nonmissing observations: 10
Dose time: 0.00
Dose amount: 300.00
Length of Infusion: 3.00
Calculation method: Linear Trapezoidal with Linear Interpolation
Weighting for lambda_z calculations: Uniform weighting
Lambda_z method: Find best fit for lambda_z, Log regression

Summary Table

	Time		Conc.	Pred.	Residual	AUC	AUMC	Weight
	h		umol/L	umol/L	umol/L	h*umol/L	h*h*umol/L	
	0.0000	@	0.0000		0.0000	0.0000		
	0.9231		4.390		2.026	1.870		
	1.846		4.931		6.328	7.942		
	2.769		4.988		10.91	18.52		
	3.000	*	5.257	3.666	1.591	12.09	21.93	1.000
	3.500	*	1.380	1.236	0.1441	13.75	27.08	1.000
	4.000	*	0.3520	0.4165	-0.06447	14.18	28.64	1.000
	4.500	*	0.1015	0.1404	-0.03887	14.29	29.11	1.000
	5.000	*	0.02884	0.04732	-0.01848	14.33	29.26	1.000
	5.500	*	0.01474	0.01595	-0.001207	14.34	29.31	1.000
	6.000	*	0.009750	0.005376	0.004375	14.34	29.35	1.000

@) Note - the concentration at dose time was added for extrapolation purposes.

*) Starred values were included in the estimation of Lambda_z.

*** Warning 14511: MRT parameters are adjusted for length of infusion.

*** Warning 14504: Incompatible units for final parameter Vz_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vz_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Cl_pred; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_obs; using default units.

*** Warning 14504: Incompatible units for final parameter Vss_pred; using default units.

Final Parameters

Rsq		0.9741
Rsq_adjusted		0.9689
Corr_XY		-0.9870
No_points_lambda_z		7
Lambda_z	1/h	2.1750
Lambda_z_lower	h	3.0000
Lambda_z_upper	h	6.0000
HL_Lambda_z	h	0.3187
Tmax	h	3.0000
Cmax	umol/L	5.2571
Cmax_D	umol/L/mg	0.0175
Tlast	h	6.0000
Clast	umol/L	0.0098
AUClast	h*umol/L	14.3429
AUCall	h*umol/L	14.3429
AUCINF_obs	h*umol/L	14.3474
AUCINF_D_obs	h*umol/L/mg	0.0478
AUC_%Extrap_obs	%	0.0312
Vz_obs	mg/(umol/L)	9.6136
Cl_obs	mg/(h*umol/L)	20.9097
AUCINF_pred	h*umol/L	14.3454
AUCINF_D_pred	h*umol/L/mg	0.0478
AUC_%Extrap_pred	%	0.0172
Vz_pred	mg/(umol/L)	9.6149
Cl_pred	mg/(h*umol/L)	20.9126
AUMClast	h*h*umol/L	29.3485
AUMCINF_obs	h*h*umol/L	29.3775
AUMC_%Extrap_obs	%	0.0986
AUMCINF_pred	h*h*umol/L	29.3645
AUMC_%Extrap_pred	%	0.0544
MRTlast	h	0.5462
MRTINF_obs	h	0.5476
MRTINF_pred	h	0.5470
Vss_obs	mg/(umol/L)	11.4497
Vss_pred	mg/(umol/L)	11.4383

Appendix 12: Work published and presented from this thesis by author

Paper of "Paclitaxel stability in human plasma"

Submitted for publication in the Journal of Oncology Pharmacy Practice in 2009

Abstract

Objectives

Paclitaxel stability in human plasma is an important validation step in the development of bioanalytical methods and the design of pharmacokinetic studies. Drug instability in plasma can lead to erroneous estimation of pharmacokinetic parameters and potentially severe therapeutic consequences. To date only limited data have been published on paclitaxel stability in plasma, with many studies lacking validation data and specific details of storage conditions. We evaluated paclitaxel stability in plasma samples after frozen and room temperature storage, after freeze/thaw (F/T) cycles, and under different conditions during the analytical procedure. The effect of co-administered drugs was also determined.

Methods

Paclitaxel (30ng/mL and 300ng/mL) was prepared in human plasma. The analytical process included protein precipitation and solid phase extraction (SPE), followed by HPLC. Plasma samples were stored at -20°C ($\geq$ 3 months) and analyzed at regular intervals. Samples were assayed after storage at 23°C for over 5hrs and after 3 x F/T cycles. After extraction, paclitaxel stability was studied in elution solvent at 2-8 °C and also in reconstituted solution held in the HPLC autosampler during analysis.

Results

This study showed that paclitaxel was stable at -20°C in plasma at both concentrations for at least 2 months ($\leq$ 0.23 % loss). Paclitaxel was stable after 3 x F/T cycles and over 5hrs at 23°C prior to assay ($\leq$ 0.4% loss). After extraction, paclitaxel in elution solvent can be kept at 2-8°C for $\geq$ 14 days (< 7% loss). Reconstituted paclitaxel in acetonitrile/water did not degrade in the autosampler during analysis for 72hrs.

Conclusions

Paclitaxel showed sufficient stability in human plasma and analytical solvents to support proposed clinical and pharmacokinetic studies on paclitaxel.

Key words: Paclitaxel; human plasma; Assay; stability; HPLC; SPE;

Introduction

Paclitaxel is an important cancer chemotherapeutic agent and is currently licensed for the treatment of advanced ovarian cancer, non-small cell lung cancer (NSCLC), breast cancer, and AIDS-related Kaposi's sarcoma.

In order to study the pharmacokinetics of paclitaxel in humans, a number of analytical methods have been developed, including HPLC [1-4], LC-MS [5,6], electrophoresis [7], immunoassays and bioassays [8-10]. These are normally combined with sample preparation methods such as solid-phase extraction (SPE) [5,11], protein precipitation and liquid-liquid extraction (LLE) [6,7].

In studies on pharmaceutical formulations, paclitaxel exhibited limited stability [12,13], mainly because of physical instability and drug precipitation. Paclitaxel stability in human plasma is a crucial aspect in the development of bioanalytical methods and in the subsequent design of clinical studies. It is also important in the evaluation of bioavailability of new formulations of paclitaxel, such as nano-particulate albumin-bound paclitaxel [14,15]. Previous studies on this subject have been reported, but these have either used assay methods that are not fully validated, or have not considered the complete isolation of the drug from the biological matrices and the analytical procedure. According to previous reports, paclitaxel was stable in human plasma at -20°C for over 2 months [16] and for up to 2 years [11]. Also, it can tolerate up to 3 cycles of freezing and thawing [11,17,18]. It has also been suggested that paclitaxel plasma samples can be kept at room temperature for up to 4hrs before being analyzed without any loss [17,19]. The extracted paclitaxel sample was shown to be stable in the autosampler for up to 24hrs [18-20]. However, to date there are no published data about paclitaxel stability over the complete sample preparation and analytical process. Also many previous studies lack rigor and defined conditions.

This study evaluated paclitaxel stability in plasma samples after long and short term storage, after freeze/thaw cycles, and under different conditions to establish stability at each stage of the clean-up and assay process. The effect of 11 potentially co-administered drugs on the assay of paclitaxel was also determined. This work was undertaken as part of the method development and validation of a paclitaxel assay for clinical and pharmacokinetic studies on different paclitaxel dose strategies such as dose banding [21] and different paclitaxel formulations.

Experimental

1. Chemicals and reagents

Paclitaxel drug concentrate (6mg/mL) was obtained from Teva Ltd (Leeds, UK) and the internal standard (IS), docetaxel, was obtained from Sigma (Dorset, UK). Citrated mixed-pool human plasma was supplied by First Link (Birmingham, UK). Acetonitrile (ACN), Methanol (MeOH), Tetrahydrofuran (THF) were all HPLC grade from Fisher Scientific (Loughborough, UK). Deionised water was produced by Elga water purification system. Ammonium acetate (≥ 99.99%) was from Sigma. All other reagents were analytical grade from Fisher Scientific.

2. Preparation of stock solutions

Paclitaxel concentrate (6mg/mL) was kept at 2-8°C with light protection and used within the expiry date. Docetaxel powder was dissolved and diluted in pure ethanol to produce the concentrate stock (10mg/mL). The IS working stock (10μg/mL) was diluted in pure ethanol from the above stock every 2 months. Both IS concentrate stock and working stock were kept at – 20 °C with light protection. These two stocks solutions were found to be stable for at least 6 months under the above conditions. Standard samples were freshly prepared from the paclitaxel and the IS working stock solutions using ACN/water (1:1).

3. Preparation of paclitaxel plasma samples

Paclitaxel plasma samples (study samples) were prepared in citrated human plasma at concentrations of 30ng/mL and 300ng/mL in volumetric flasks, followed by vortexing

for over 1min and gently inverting the flasks 20 times. Samples of plasma (3mL) were then transferred into separate 15mL polypropylene tubes (Fisher, Loughborough, UK). All plasma samples were stored at - 20°C with light protection.

4. Protein precipitation and solid-phase extraction (SPE)

120 µl of IS solution (10µg/mL) was added to 3mL of plasma sample, followed by addition of 2.88 mL of pH 3 buffer (0.1M formic acid), mixed by vortexing and kept at 2-8 °C with light protection (for protein precipitation). After approximately one hour, the sample matrix was subjected to centrifugation at 4 °C at 3000 g for 10min. 5mL of the supernatant (equivalent to 2.5mL of paclitaxel plasma sample) was introduced onto a 500mg cyno Bond Elut SPE cartridge (Varian, Oxford, UK) which was pre-conditioned by 6mL MeOH and 6mL pH3 buffer. The cartridge was then washed with 5mL of pH3 buffer, MeOH/pH3 buffer (2:8, v:v) and 2mL of hexane. The cartridge was then dried under full vacuum and eluted using 1.2mL of ACN with 0.1% triethylamine. The elution sample was evaporated under a nitrogen stream at 30°C and finally the residue was reconstituted in 500 µl ACN/water (1:1). 20µl was injected on to HPLC column in replicates. Standard samples were analysed between test samples to calculate the individual recovery (%) for each sample.

5. HPLC conditions

The HPLC system consisted of a model PU-2080 pump, a model AS-2055 auto-sampler and a model MD- 2010 diode array detector (all from Jasco, Essex, UK). Data were collected and processed by the EZChrom software (Agilent, West Lothian, UK). A Spherisorb narrow-bore ODS2 column (5µm, 150 x 2mm) (Waters, Herts, UK) was applied in conjunction with a 4 × 2.0mm C18guard cartridge (Phenomenex, Macclesfield Cheshire, UK). The mobile phase consisted of ACN-THF-0.02M ammonium acetate buffer pH 5 (50/2/48, v/v) and was run at a flow rate of 0.2mL /min. The column temperature was maintained at 23-25°C. The UV detection was set at 227nm.

6. Stability indicating study and validation of HPLC method

This HPLC method has been validated to be stability-indicating by accelerated degradation of paclitaxel. Paclitaxel solutions were subjected to control (at 5°C), heating (at 55°C), oxidative (6% H_2O_2), acidic (1M hydrochloric acid) and alkaline (1M sodium hydroxide) conditions for 1hour before analysis. Typical chromatograms have been demonstrated in figure 1. Table 1 shows there was no significant loss of paclitaxel on exposure to heating (55°C) and oxidative conditions. A 48.2% loss of paclitaxel was observed under acidic conditions and no peak of interest was found at alkaline condition. No degradation products interfered with paclitaxel peak. Similar results have been reported previously [12,13,22].

Six calibration standards (10, 20, 40, 100, 200 and 300ng/mL) were prepared in citrated human plasma and at least 4 replicates at each level was analyzed using the above methods. The calibration plot was repeated on different weeks (n = 3). A mean calibration curve was produced based on the mean peak height ratios (paclitaxel: internal standard) and the known concentrations of paclitaxel: Ratio = 0.0036(Conc. Paclitaxel) + 0.0036, with an average correlation coefficient (R^2) of 0.9997. Example chromatograms are shown below: blank plasma (Figure 2) and paclitaxel spiked plasma sample (Figure 3). Eleven co-administered drugs including dexamethasone, ranitidine, cyclizine, metoclopramide, pamidranate disodium, clonazepam, granisetron, ondansetron, tamoxifen, carboplatin and doxorubicin were analyzed in aqueous solutions and in plasma for any interference. No interference due to endogenous product or from the above co-administered drugs was identified occurred within the retention time window of paclitaxel and internal standard (6.0-9.0min). Table 2 shows intra- and inter- day precision was from 1.8% to 6.4% and intra- and inter-day accuracy ranged from 97.4% to 104.8% at 10, 100 and 300ng/mL levels. The lower limit of quantification (LLOQ) was identified as 10ng/mL with an acceptable precision (CV%) of 6.4% (inter-day) and accuracy of 104.8% (inter-day). Average recovery of paclitaxel from human plasma was 100.8% (93.3% -111.7%) and individual mean recoveries were 111.7%, 97.5%, and 93.3% at 10, 100, and 300ng/mL levels. Average recovery of internal standard (docetaxel) was 97.3 % (93.1%-101.9%).

7. Paclitaxel stability in plasma

- *Long-term stability study*

At least three replicates of each plasma sample (30ng/mL and 300ng/mL) were thawed and analyzed at different time intervals during the study period (up to 3 months). The mean peak height ratios of paclitaxel vs. internal standard were used to calculate the observed concentrations of paclitaxel in the stability samples in comparison with first day results of the long term stability study.

- *Short-term temperature stability*

Paclitaxel plasma samples (30ng/mL and 300ng/mL, n= 4) were thawed from -20°C to room temperature (22-24°C) and kept at room temperature for over 5hrs before protein precipitation and SPE. The mean peak height ratios of paclitaxel vs. internal standard were compared with those samples analyzed immediately after thawing without standing at room temperature.

- *Freeze and thaw stability*

Three replicates at each 30ng/mL or 300ng/mL level were frozen at -20°C after preparation for 24hrs and then thawed at room temperature (22-24°C). The freeze/thaw (F/T) cycle was repeated three times. Samples were analyzed after the third cycle. The mean peak height ratios were compared with samples not subjected to F/T cycles.

- *Stability of elution samples in the refrigerator*

12 replicates of each plasma sample (30 and 300ng/mL) were subjected to SPE on the same day. Elution samples were then kept in the refrigerator (2-8°C). Two replicates of elution samples at each concentration were evaporated, reconstituted and analyzed on Day 0, 3, 7, 10 and 14 after refrigeration.

- *Stability of the reconstituted samples in the autosampler*

Reconstituted samples in ACN/water at both concentrations (30ng/mL and 300ng/mL) were analyzed on Day 0 and then kept in the autosampler at room temperature (20-

24°C). Stability was checked after different autosampler residence times (up to 72hrs). The % paclitaxel remaining was calculated with respect to the Day 0 concentration.

Results and Discussion

The aim of the stability study for paclitaxel in plasma was to support the experimental design of later clinical studies and to test the robustness of the methods used. In the FDA guidance of Bioanalytical Method Validation [23], the precision and accuracy determined at each standard concentration should not exceed 15% except for at the LLOQ, where it should not exceed 20%. Therefore, in this study, the acceptance criteria of accuracy (the remaining ratio %) and precision were defined to be within ± 15% at both test concentrations (30ng/mL and 300ng/mL).

1. Long-term stability study

Results are shown in the table 3. Paclitaxel (300ng/mL in plasma) was stable for up to 3 months with intra-day precision from 1.0% to 2.1% during the study period. Paclitaxel 30ng/mL was found stable for up to 2 months with intra-day precision from 2.5% to 6.7%. Paclitaxel remained at 107.2% (30ng/mL) and 105.7% (300ng/mL) after 2-month storage at -20°C.

This long-term stability study showed paclitaxel at 30ng/mL and 300ng/mL can be stored in plasma for at least 2 months without obvious degradation. This agreed with previous studies where paclitaxel at 44ng/mL and 750ng/mL were studied in glass tubes [16]. Although, paclitaxel was reported stable in frozen plasma for over 2 years [11], no specific data and concentrations were shown to support this conclusion. In addition, paclitaxel demonstrated good stability for up to 3 months when kept at -70°C [19]. Overall, storage of 2 months for paclitaxel in frozen plasma was considered sufficient for conducting normal clinical studies where samples would be batched for analysis.

2. Short-term temperature stability

After 5 hours at room temperature, paclitaxel in plasma remained at 99.6% of initial concentrations for 30ng/mL (intra-day precision 6.0%), and at 100.3% for 300ng/mL

(intra-day precision 1.5%), compared with samples analyzed immediately after being thawed (Table 4). All results met the acceptance criteria. Therefore, paclitaxel (30ng/mL and 300ng/mL) were stable when kept at room temperature for up to 5 hours prior to any treatment. This agreed with previous studies [11,17,19] and gave us a certain confidence if any delay happened during the sample preparation process.

3. Freeze and thaw stability

After 3 x F/T cycles, paclitaxel remaining was at 105.1% and 103.9% for 30ng/mL and 300 ng/mL respectively, with intra-day CV% of 3.9% and 1.3%, respectively, which means paclitaxel was stable for up to 3 × F/T cycles without loss compared with freshly made samples (Table 5). This confirms findings of other studies [11,17,18] and allows for the possibility thawed samples can be refrozen and subsequently re-thawed for analysis at a later date.

4. Stability in the elution solvent

Paclitaxel showed good stability in the elution solvent (ACN plus 0.1% triethylamine) after extraction for at least 2 weeks when kept at 2-8°C (Table 6.). After 14 days of storage, the average peak height ratios (paclitaxel/IS) were 0.095 and 1.135 with inter-day precisions of 5.3% and 3.2%, at 30 ng/mL and 300 ng/mL, respectively.
This offers the paclitaxel assay significant flexibility. Elution samples can be stored at 2-8° C for a short period (up to 2 weeks) and evaporated, reconstituted and analyzed with 14 days without any degradation.

5. Stability in the autosampler after preparation

The stability of paclitaxel in the autosampler was tested at room temperature to determine if samples could be re-injected in case of instrument or sample run failure. After reconstitution in ACN/water (1:1), paclitaxel samples were kept in the autosampler and stability was determined after 24, 48 and 72hrs separately. Paclitaxel was found stable for up to 72 hrs since it remained at 104.2% and 101.2% of initial concentrations, for 30ng/mL and 300ng/mL, respectively, with an average precision of 6.1% and 1.1% (Table 7).

In this study, the single run of each plasma sample by HPLC took 23 min. During each calibration study, there were 33 plasma samples in total for analysis and each one was repeated twice. Therefore, the total HPLC analysis time required for each calibration study was over 30 hrs including the analysis time of standard solutions (freshly made in ACN/water). The 72hrs stability provided confidence that samples could be left in the autosampler for lengthy batch analysis without compromising assay validation. This is a longer stability period than previous studies where a 24-hour stability period was reported [18-20]. This improvement in stability may have resulted from the use of different solvents for the reconstitution of the drug.

Conclusions

Paclitaxel stability in human plasma has been studied in the different stages and processes involved in collecting and analysing blood samples for clinical and pharmacokinetic studies. Paclitaxel was stable in human plasma at - 20°C for up to 2 months and can be left at room temperature for over 5 hrs prior to assay. Also, paclitaxel plasma samples can undergo at least 3 cycles of F/T cycles without drug loss. After extraction, paclitaxel can be kept in the elution solvent at 2-8°C for over 14 days and paclitaxel reconstituted samples can be stored in the autosampler for up to 72hrs without loss. The above results have been the basis for the design of methods for the determination of paclitaxel in plasma as part of clinical and pharmacokinetic studies to evaluate the effect of dose-banding of paclitaxel.

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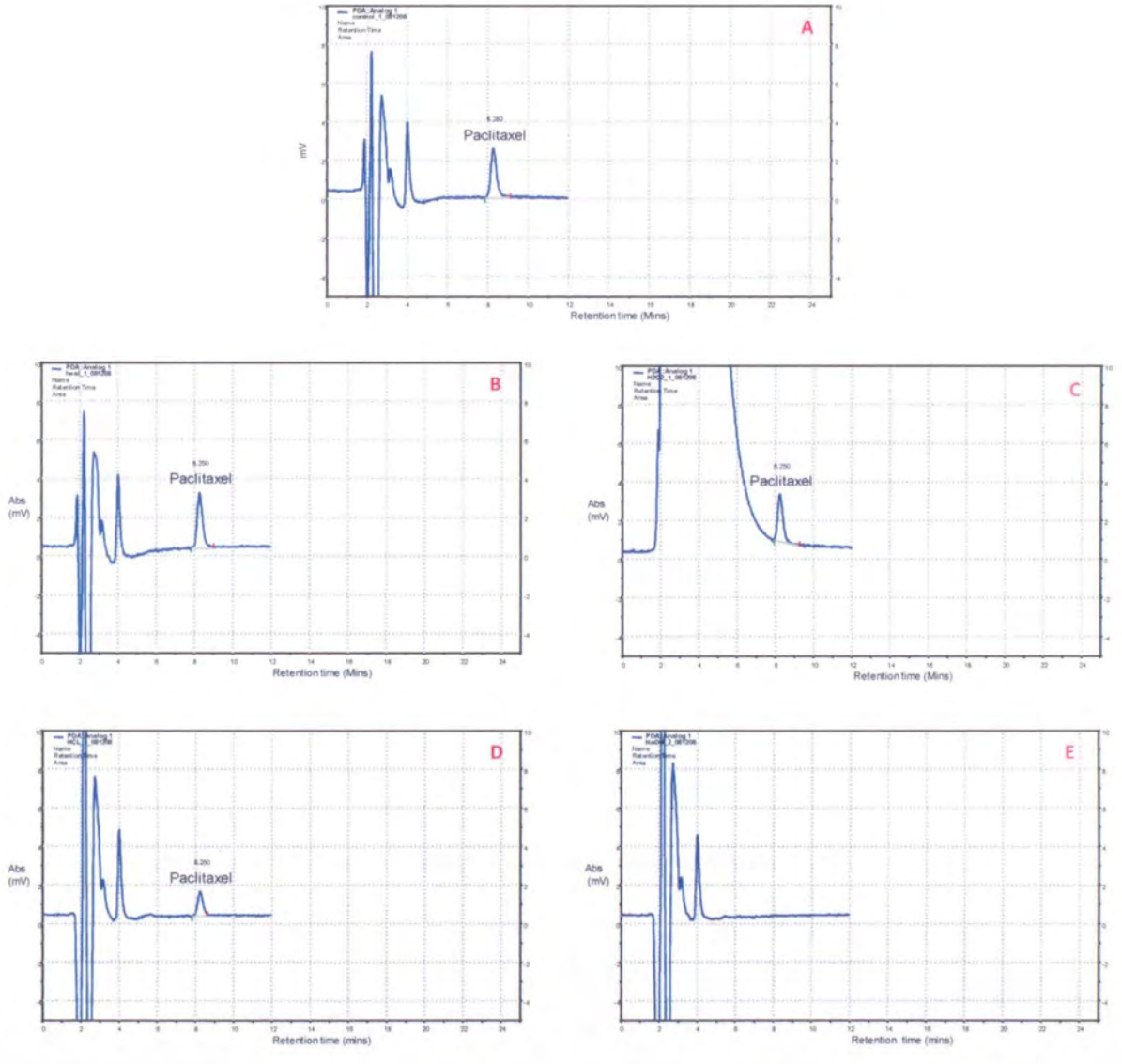

Figure 1. Typical chromatograms in stability indicating study (paclitaxel 500ng/mL)

A. Control (kept at 5°C for 1 hour); B. Heating (at 55°C for 1 hour); C. Oxidative condition (mixed with 6% H_2O_2 for 1hour); D. Acid hydrolysis (mixed with 1M HCL for 1hour); E. Alkaline hydrolysis (mixed with 1M NaOH for 1hour)

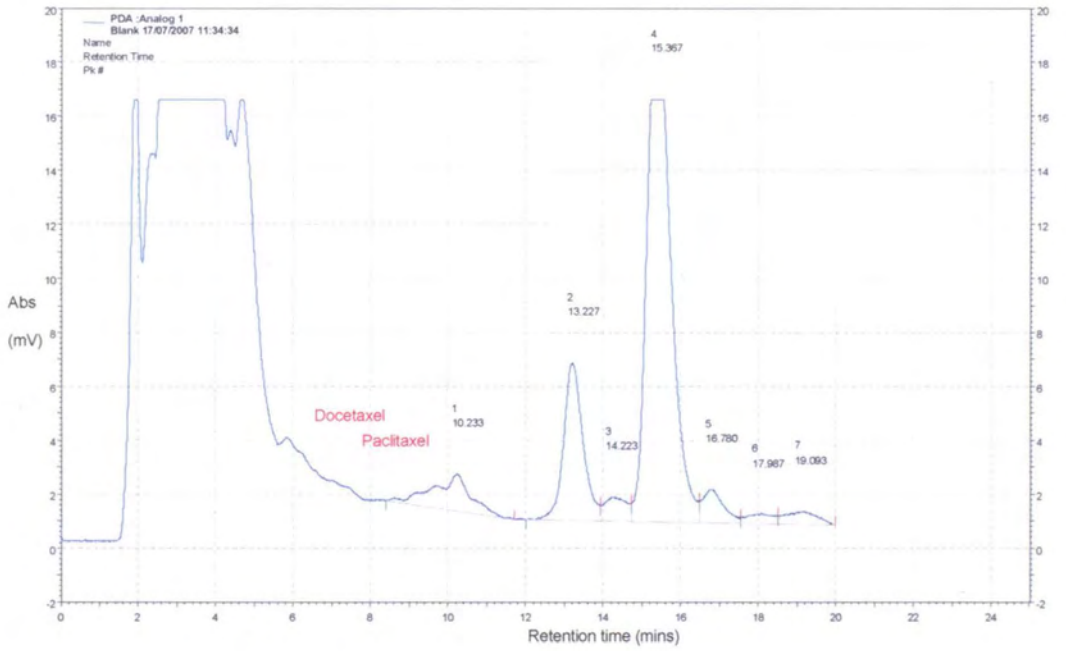

Figure 2. Chromatogram of a blank human plasma sample

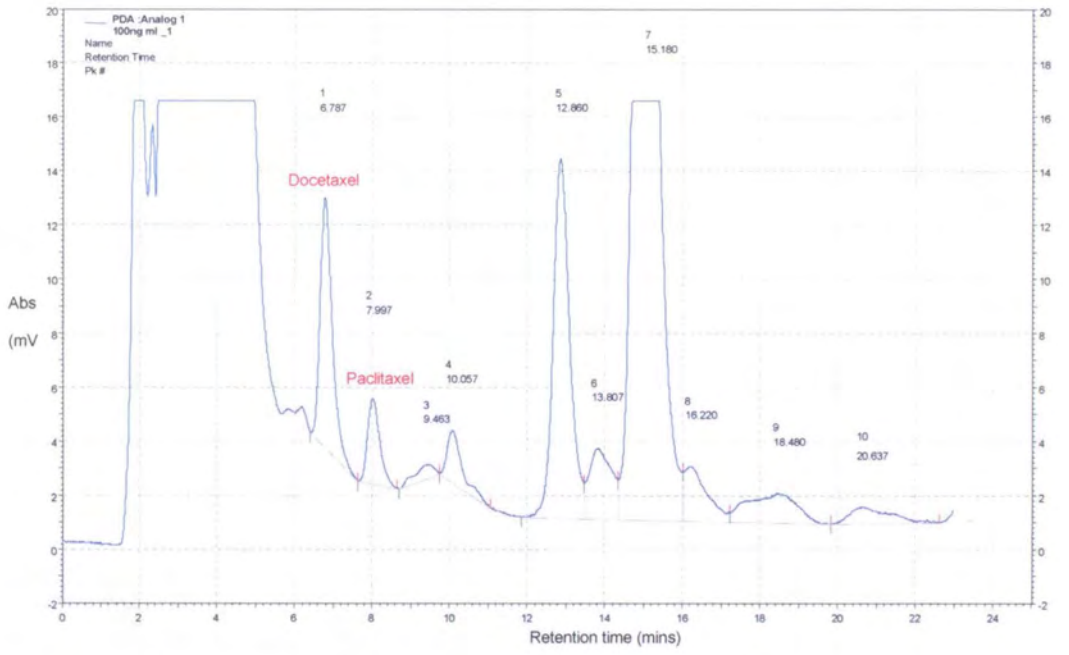

Figure 3. Chromatogram of human plasma spiked with paclitaxel (40ng/mL) and internal standard (docetaxel, 400 ng/mL): Retention time of paclitaxel = 8.0 mins; retention time of internal standard (docetaxel) = 6.8mins.

Table 1. Stability indicating capability of the LC assay under different conditions

(Heating, oxidative, acidic and alkaline)

Treatments	Retention time (min)	Quantity (ng/mL)	Quantity remaining (%)	Peak purity (%)
Control	8.28	500	100	100.00
heating (55°C)	8.27	509.02	101.8	99.36
Oxidative condition	8.27	487.76	97.6	99.94
Acid hydrolysis	8.25	259.18	51.8	___
Alkaline hydrolysis	___	___	___	___

N = 2 (each)

Control: kept at 5°C

Oxidative condition: H_2O_2 (6%, v/v)

Acid hydrolysis: 1M HCL (pH =0)

Alkaline hydrolysis: 1M NaOH (pH = 14)

Table 2. Validation characteristics of paclitaxel assay in human plasma

Nonimal conc. (ng/mL)	Measured Conc.(ng/mL)	Accuracy (%)		Precision (%)		Recovery %
		Intra-	Inter-	Intra-	Inter-	
10	10.48	104.77	104.77	3.61	6.38	111.7± 3.7
100	97.38	97.38	97.38	2.82	5.55	97.5 ± 1.9
300	299.56	99.81	99.85	1.78	1.91	93.3 ± 8.1

All values are mean, where n = 6.

Table 3. Long-term stability of paclitaxel in plasma at –20°C

Nominal concentration (ng/mL)	Day	Ratio [a]	Observed concentration (ng/mL) [b]	Remaining % [c]	N [d]	CV % [e]
30	0	0.096	30.00	100.00	3	2.49
	20	0.094	29.52	98.38	3	2.70
	35	0.093	29.27	97.56	6	2.49
	54	0.092	28.73	95.76	3	6.65
	60	0.103	32.14	107.15	4	4.78
300	0	1.106	300.00	100.00	3	1.25
	9	1.119	303.71	101.24	3	1.72
	16	1.076	292.02	97.34	3	1.29
	23	1.140	309.29	103.10	3	2.05
	32	1.099	298.13	99.38	3	4.00
	60	1.168	316.98	105.66	3	1.76
	71	1.158	314.25	104.75	3	1.00
	92	1.083	293.72	97.91	3	1.55

Storage conditions: -20℃ in polypropylene tubes and with light protection.

[a] *Ratio = mean peak height ratio of paclitaxel vs. docetaxel*

[b] *Observed concentration = [ratio on Day n/ ratio on Day 0] × Conc. on Day 0*

[c] *Remaining % = [observed conc. / Day 0 conc.] × 100 %*

[d] *N = number of replicates*

[e] *CV% = intra-day reproducibility*

Table 4. Short-term temperature stability of paclitaxel

Nominal concentration (ng/mL)	Ratio	Observed concentration (ng/mL)	Paclitaxel remaining %	N	CV %
30	0.10	29.88	99.60	4	5.96
300	1.11	301.01	100.34	4	1.46

Storage condition: room temperature (22-24℃) with light protection for over 5 hours before any treatment.

Table 5. Freeze and thaw stability of paclitaxel after 3 F/T cycles

Nominal concentration (ng/mL)	Ratio	Observed concentration (ng/mL)	Paclitaxel remaining %	N	CV%
30	0.10	31.52	105.07	3	3.91
300	1.13	307.80	102.60	3	1.11

Table 6. Paclitaxel stability (30ng/mL & 300ng/mL) in elution solvent at refrigerator (2-8°C)

Concentration 30ng/mL	Day	Ratio	Observed concentration (ng/mL)	Paclitaxel remaining %	CV%
	0	0.102	30.00	100.00	4.01
	3	0.098	28.67	95.58	4.41
	7	0.089	26.20	87.34	2.59
	10	0.092	27.06	90.19	2.42
	14	0.094	27.70	92.34	3.20
	Mean	0.095			
	SD	0.005			
	Inter-day CV%	5.262			
Concentration 300ng/mL	Day	Ratio	Observed concentration (ng/mL)	Paclitaxel remaining %	CV%
	0	1.109	300.00	100.00	0.6
	3	1.094	296.00	98.67	0.60
	7	1.185	320.61	106.87	0.94
	10	1.157	313.03	104.34	0.99
	14	1.129	305.26	101.75	0.41
	Mean	1.135			
	SD	0.037			
	Inter-day CV%	3.235			

N = 2 on each day

SD = standard deviation

Table 7. Summary of paclitaxel stability after different residence times in the autosampler

	Concentration (ng/mL)	Paclitaxel remaining %	N	CV%
Over 24hr	30	101.02	4	2.52
	300	98.52	12	1.04
Over 48hr	30	99.02	3	4.55
	300	101.80	6	2.75
Over 72hr	30	104.19	7	6.06
	300	101.15	6	1.12

Storage condition: kept in the autosampler at room temperature (20-24℃)

Poster of "Quantitative determination of Paclitaxel in human plasma using solid-phase extraction and liquid chromatography"

Presented at British Pharmaceutical Conference (BPC)
10^{th} -12^{th} September, 2007
Manchester, UK

Abstract

Objectives: To develop and validate a narrow-bore HPLC assay method for the quantification of paclitaxel in human plasma. This method is required for a pharmacokinetic study in the presence of other interference drugs to assess the likely clinical effect of dose-banding.

Methods: Paclitaxel and the internal standard, docetaxel, were extracted from human plasma by protein precipitation (pH change), followed by solid phase extraction (SPE) using cyano Bond Elut columns. Reconstituted samples were subject to reverse phase high performance liquid chromatography (HPLC) in combination with UV detection (at 227nm). A Waters narrow-bore ODS column (150 x 2.1mm id., 5um) was utilised in connection with a Phenomenex guard cartridge. The mobile phase was optimised to be ACN/THF/20mM Ammonium acetate (50/2/48, v/v) with a flow rate of 0.2ml/min. Eleven co-administered drugs including dexamethasone, ranitidine, cyclizine, metoclopramide, pamidranate disodium, clonazepam, granisetron, ondansetron, tamoxifen, carboplatin and doxorubicin were analyzed in aqueous solutions and in plasma for any interference. These drugs represent medicines that may be administered as supportive therapies or in combination chemotherapy, to patients receiving paclitaxel for carcinoma of the breast or ovary.

Results: Linear calibration curves were obtained over the concentration range from 10ng/ml to 300ng/ml in plasma with an average regression correlation coefficient > 0.999. A lower limit of quantification (LLOQ) of 10ng/ml was defined with an acceptable accuracy of 100.3% and precision of 7.5% (n = 7, 3 weeks). The overall recovery ranged from 91% to 123% with all CV% below 6.6%. The intra- and inter-day precisions were greater than 4.3% and 7.5%, respectively, and the accuracy ranged from 99.4 to100.3% at three different concentration levels. In the interference study, only pamidranate and granisetron were identified with potential interfering agents with the assay for paclitaxel. By optimisation of the pH buffer system used for SPE, the interference from the above two drugs was removed after extraction.

Conclusion: The analytical conditions have been optimised for paclitaxel quantification in human plasma in the presence of co-administered medicines. Accuracy and precision of this method complied with the acceptance criteria for validation of bio-analytical assays. The sensitivity and selectivity of this method was considered suitable for the purpose of planned pharmacokinetic studies to assess the effect of paclitaxel dose-banding schemes.

Quantitative determination of Paclitaxel in human plasma using solid-phase extraction and liquid chromatography

Jing Xu and Graham Sewell
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Introduction

Paclitaxel as an important cytotoxic agent, has been widely used in cancer chemotherapy, especially for breast and ovarian cancers. It exerts cytotoxic activity at concentrations as low as 50nM[1]. To study the pharmacokinetics of paclitaxel, a sensitive and selective analytical method is required. So far, HPLC-UV method has become the main tool for paclitaxel analysis in biological fluids in combination with sample preparation methods with LOD (5-10ng/ml) and LOQ (10-30ng/ml) published[2]. However, sample preparation in plasma is still a challenge since plasma is a complex matrix with many kinds of interference drugs, proteins and other plasma components.

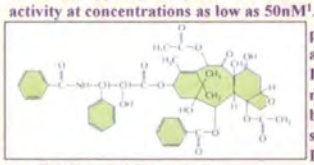

Fig.1. Structure of paclitaxel molecule

Objectives

To develop a narrow-bore HPLC assay method for the quantification of paclitaxel in human plasma in the presence of other interference drugs.

Methods

- **Protein precipitation :**
  - — Adjusting pH of sample matrix by addition of pH 3 buffer, followed by standing over one hour.
  - — Centrifuged at 3000g for 10mins (4°C)

- **Sample preparation — solid phase extraction (SPE)**

Conditioning:	MeOH and 0.1M formic acid (pH 3)
Loading:	2.5 ml plasma sample plus 2.5ml buffer (pH3)
Washing:	pH 3 buffer, MeOH/buffer (2:8, v/v) and hexane
Drying & elution:	under full vacuum and eluting with 1.2 ml of ACN/TEA (1000: 1)
Evaporation:	under nitrogen stream at 30°C
Reconstitution:	500 µl of ACN/water (50/50)

SPE cartridges: Bond Elut CN-E, 500mg (Varian)

- **High performance liquid chromatography**

HPLC system: Pump (PU-2080, Jasco)
Auto-sampler (AS-2055, Jasco)
Diode array detector (MD-2010, Jasco)

Chromatographic conditions:

Mobile phase: ACN/THF/0.02M Ammonium acetate (50/2/48)
Column: ODS2, 150 × 2.1mm, 5µm (Waters) combined with C18, 4 × 2.0mm guard cartridge (Phenomenex)
Detection wavelength: 227 nm

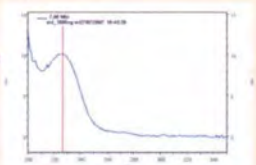

Fig.2. Spectrum of paclitaxel (1500 ng/ml): maximum absorption appeared at 227-230 nm

Results

- **Optimization of pH buffer system during SPE (previous data):** Different buffer systems (pH 1.0, 2.0, 3.0 ...11.0)during SPE were evaluated.

pH	Paclitaxel peak
≤ 2.0	not observed
= 3.0	fully separated (no interference observed)
≥ 5.0	not fully separated from Granisetron

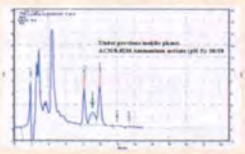

Fig.3. paclitaxel plus all *IDs (pH 5.0 buffer system): paclitaxel was not separated from granisetron (shown as in green arrow). *IDs - Interference Drugs

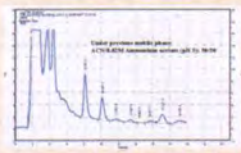

Fig.4. paclitaxel plus all IDs (Optimized under pH 3.0 buffer system): no interference was observed

- **Optimization of mobile phase and tubing system**

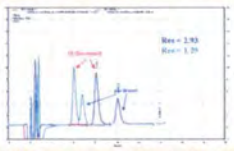

Fig.5. chromatograms under different mobile phase ACN/THF/0.02M ammonium acetate (50/5/45) (pale blue); ACN/0.02M ammonium acetate (50/50) (blue).

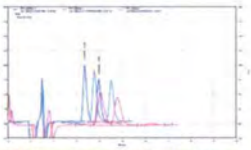

Fig.6. chromatograms under different tubing systems (between auto-sampler and detector): 7/1000 inch (blue); 10/1000 inch (pale blue); 15/1000 inch (red).

Tab.1. Optimization of % THF in the mobile phase

% THF in mobile phase	Res	% increase (Peak height)
0	2.93	0
1	2.31	49
2	2.01	63
3	1.64	71

Optimal conditions:

- 2% THF in the mobile phase → better separation and enough sensitivity
- 7/1000 inch tubing system → better sensitivity compared with others

- **Determination of paclitaxel in presence of co-administered drugs**

Tab.2. Chromatographic characteristics of drugs

Drugs	Rt (mins)
Paclitaxel	7.8
Docetaxel	6.7
Dexamethasone	2.2
Ranitidine	3.6
Cyclizine	—
Metoclopramide	8.4
Pamidronate	—
Carboplatin	1.9
Doxorubicin	4.8
Tamoxifen	—
Clonazepam	4.3
Granisetron	8.9
Ondansetron	9.7

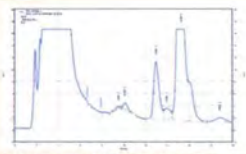

Fig.7. Chromatogram of plasma spiked with all interference drugs (after SPE under pH 3 buffer system)

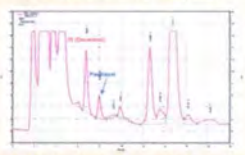

Fig.8. Chromatogram of paclitaxel in plasma (100ng/ml) with internal standard, docetaxel (400ng/ml)

- **Validation study**

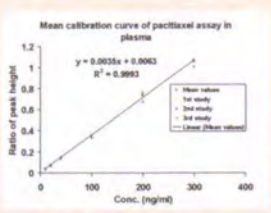

Fig.9. Mean calibration curve of paclitaxel assay in plasma (based on 6 points)

Linear range: 10 – 300ng/ml
LOD (limit of detection): 5 ng/ml
LLOQ (Lower limit of quantification): 10ng/ml (CV% < 8%)
Overall recovery %: 91% -123% (all CV% < 6.6%)

Tab.3. Validation characteristics of paclitaxel assay in plasma (n = 7)

Nominal conc. (ng/ml)	Mean measured conc. (ng/ml)	Accuracy (%)	Intra-day CV (%)	Inter-day CV (%)
10	9.9	99	4.3	7.6
100	99.3	99.3	2.9	5.6
300	300.9	100.3	2.6	3.7

Conclusion

This method has been optimized for paclitaxel quantification, in human plasma, in the presence of co-administered medicines. Accuracy and precision of this method complied with the acceptance criteria for validation of bio-analytical assays. The sensitivity and selectivity of this method was considered suitable for the purpose of planned pharmacokinetic studies to assess the effect of paclitaxel dose-banding schemes.

Reference:

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2. N. Martin, J. Catalin, M.F. Blachon, A. Durand. J. Chromatogr. B. 709(1998) 281-288.

Acknowledgement:

Special thanks to my supervisor, Prof Graham Sewell and Kingston University for all their support in my PhD.

Poster of "Paclitaxel Stability in Human Plasma Samples for Pharmacokinetic Studies"

Presented at the HOPA/ISOPP 2008 Conference

18^{th} – 21^{st} June, 2008

Anaheim, California, USA

Abstract published as:

Xu J, Sewell G. Paclitaxel stability in human plasma samples for pharmacokinetic studies. Journal of Oncology Pharmacy Practice, 2008, 14 (2): 99 (abstract TS3).

Abstract

Objectives

Paclitaxel stability in human plasma is important for the development of bioanalytical methods and the design of pharmacokinetic studies. Drug instability in plasma can lead to erroneous pharmacokinetic outcomes and potentially severe therapeutic consequences. To date only limited data have been published on paclitaxel stability in plasma, with many studies lacking validation data and specific details of storage conditions. We evaluated paclitaxel stability after frozen and room temperature storage, after freeze/thaw (F/T) cycles, and under different storage conditions.

Methods

Paclitaxel (30ng/ml and 300ng/ml) was prepared in human plasma. Analysis included protein precipitation and solid phase extraction (SPE), followed by HPLC. Plasma samples were stored at -20°C (≥3 months) and analyzed regularly. Samples were analyzed after storage at 23°C for over 5hrs and after 3 x F/T cycles. After extraction, paclitaxel stability was studied in elution solvent at 2-8 °C and also in reconstituted solution held in the HPLC autosampler prior to analysis.

Results

This study showed that paclitaxel in plasma at both concentrations was stable at -20°C for at least 2 months (≤ 0.23 % loss). Paclitaxel was stable after 3 x F/T cycles (no obvious loss) and over 5hrs at 23°C before the assay (≤ 0.4% loss). After extraction, paclitaxel in elution solvent can be kept at 2-8°C for ≥14 days (< 7% loss). Reconstituted paclitaxel in acetonitrile/water did not degrade in the autosampler for 72hrs.

Conclusions

Paclitaxel showed sufficient stability in human plasma and analytical solvents to support proposed clinical and pharmacokinetic studies on paclitaxel dose-banding.

Paclitaxel Stability in Human Plasma Samples for Pharmacokinetic Studies

Jing Xu, MD; Graham Sewell, PhD, MRPharmS
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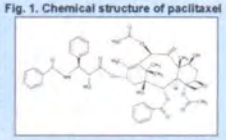
Abstract TS3

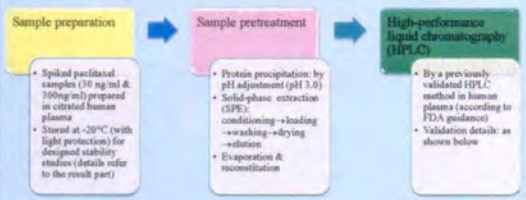
INTRODUCTION AND AIMS

Paclitaxel stability in human plasma is important for the development of bioanalytical methods and the design of clinical studies. Drug instability in plasma can lead to erroneous pharmacokinetic outcomes and potentially severe therapeutic consequences. To date only limited data have been published on paclitaxel stability in plasma, with many studies lacking validation data and specific details of storage conditions. This study evaluated paclitaxel stability after long- and short-term storage, after freeze/thaw (F/T) cycles, and under different storage conditions after preparation to establish stability at each stage of the clean-up and assay steps.

Fig. 1. Chemical structure of paclitaxel



METHODS



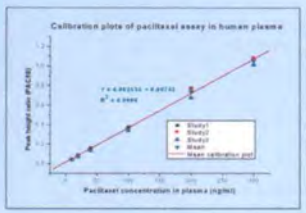


Fig. 2. Calibration plot of paclitaxel assay in human plasma

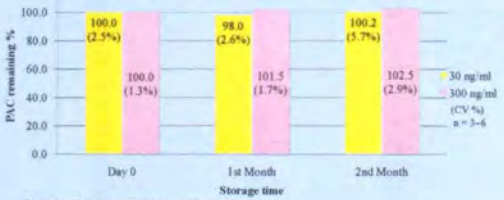
Internal standard (IS): docetaxel
Linear range :10 – 300ng/ml
LLOQ : 10ng/ml

Tab.1. Validation characteristics of paclitaxel assay in human plasma

Nominal conc. (ng/ml)	Accuracy (%)		Precision (%)		Recovery %
	Intra-	Inter-	Intra-	Inter-	
10	100.33	100.33	3.61	6.38	111.7± 3.7
100	99.42	99.42	2.82	5.55	97.5 ± 1.9
300	102.41	102.46	1.78	1.91	93.3 ± 8.1

RESULTS

Fig. 3. Long-term stability of paclitaxel in human plasma



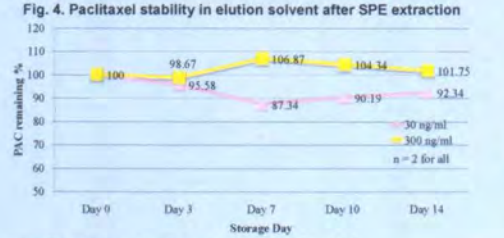
- *Plasma samples prepared on the same day*
- *Storage conditions: -20 °C with light protection*
- *Study period: 2 months*

Tab. 2. Short-term temperature stability & Freeze/thaw (F/T) stability

Nominal concentration	Control conditions	Remaining %	CV%
30 ng/ml	Short-term temperature [a]	99.60	5.96 (n=4)
	3 × F/T [b]	105.07	3.91 (n=3)
300 ng/ml	Short-term temperature	100.34	1.46 (n=4)
	3 × F/T	102.60	1.11 (n=3)

[a] samples after thawed at room temperature (from -20°C to 23°C) were kept at room temperature for over 5hrs before analysis;
[b] samples were subjected to 3 × F/T cycles (between -20°C and 23°C) before analysis.
• Remaining%s were calculated by the peak height ratio of test samples compared with those samples without control .

Fig. 4. Paclitaxel stability in elution solvent after SPE extraction



- *Freshly made plasma samples were subjected to SPE on Day 0.*
- *Elution solvent: acetonitrile (ACN) with 0.1% triethylamine*
- *Storage condition: at 2-8 °C in a pharmaceutical refrigerator (with light protection)*

Fig. 5. Post-preparative stability in the HPLC auto-sampler

Nominal concentration	Residence time (hr)	Remaining %	CV%
30 ng/ml	≥ 24	101.02	2.52 (n=4)
	≥ 48	99.02	4.55 (n=3)
	≥ 72	104.39	6.06 (n=7)
300 ng/ml	≥ 24	98.52	1.04 (n=12)
	≥ 48	101.80	2.75 (n=6)
	≥ 72	101.15	1.12 (n=6)

- *Reconstituted samples in ACN/water (50/50) were analyzed on Day 0 and then kept in the auto-sampler at 23°C. stability was checked at different residence time of 24, 48 and 72hrs.*
- *Remaining% was calculated with respect to the Day 0 result.*

DISCUSSION

- Paclitaxel showed sufficient stability (over 2 months) in human plasma when kept at -20°C;
- There was no obvious degradation produced after paclitaxel samples were kept for over 5hrs at room temperature before analysis;
- Paclitaxel can undergo at least 3 repeated F/T cycles without drug loss;
- Paclitaxel was stable in the SPE elution solvent for up to 2 weeks;
- Reconstituted samples can be kept in the HPLC auto-sampler at room temperature for up to 3 days without obvious loss.

CONCLUSIONS

Paclitaxel has shown sufficient stability in the different stages and processes involved in collecting and analysing blood samples for clinical and pharmacokinetic studies. The above results have been incorporated into the design of methods for the determination of paclitaxel in plasma as part of clinical and pharmacokinetic studies.

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2. Alexander M, et al. *J. Chromatogr. B.* 2003; 785: 253-61
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Presented at the HOPA/ISOPP 2008 Conference; June 18-21, 2008; Anaheim, California, USA.

Poster of "Validation of Paclitaxel Assay in Human Plasma"

Presented at the Analytical Research Forum 2008 (ARF08)

21st – 23rd July, 2008

University of Hull, UK

Abstract

Objectives
To develop and validate a sensitive narrow-bore HPLC assay method for the quantification of paclitaxel in human plasma. This method is required for a pharmacokinetic study in the presence of other interference drugs to assess the likely clinical effect of dose-banding.

Methods
Paclitaxel and the internal standard (docetaxel) were extracted from human plasma by protein precipitation, followed by solid phase extraction (SPE) on cyano Bond Elut columns. The HPLC system consisted of a Waters narrow-bore ODS column with a Phenomenex guard cartridge in combination with UV detection (at 227nm). The mobile phase of ACN/THF/20mM Ammonium acetate (50/2/48, v/v) was run at a flow rate of 0.2ml/min. Over 11 co-administered drugs including dexamethasone, ranitidine, cyclizine, metoclopramide, pamidranate disodium, clonazepam, granisetron, ondansetron, tamoxifen, carboplatin and doxorubicin were analyzed in aqueous solutions and in plasma for any interference. Calibration studies were repeated on three different weeks. Paclitaxel stability (10ng/ml and 300ng/ml) was evaluated for a long-term (>2 months) after frozen and at room temperature storage, after freeze/thaw (F/T) cycles, and under different storage conditions.

Results
Linear calibration curves were obtained over the concentration range from 10ng/ml to 300ng/ml in plasma with an average regression correlation coefficient > 0.999. A lower limit of quantification (LLOQ) of 10ng/ml was defined with an acceptable accuracy of 100.3% and precision of 7.5% (n = 7, repeated on 3 weeks). Average recoveries were 100.8% (93.3% -111.7 %) for paclitaxel and 97.3 % (93.1%-101.9%) for internal standard (docetaxel). The intra- and inter-day precisions were greater than 3.6%% and 6.4%%, respectively, and the accuracy ranged from 99.4 to102.5% at three different concentration levels. By optimization of the pH buffer system used for SPE, no interference was identified with paclitaxel and docetaxel peaks. Also, Paclitaxel showed a long-term stability over 2 months after frozen in plasma and found sufficient stability under different storage conditions.

Conclusion
The analytical conditions have been optimised for paclitaxel quantification in human plasma in the presence of co-administered medicines. Accuracy and precision of this method complied with the acceptance criteria for validation of bio-analytical assays by FDA. The sensitivity, selectivity and robustness of this method were considered suitable for the purpose of planned pharmacokinetic studies to assess the effect of paclitaxel dose-banding schemes.

Validation of Paclitaxel Assay in Human Plasma

Jing Xu, MD, MSc; Graham Sewell, PhD, MRPharmS
School of Pharmacy, Kingston University of London, Kingston upon Thames, KT1 2EE

INTRODUCTIONS AND AIMS

Paclitaxel as an important cytotoxic agent, has been widely used in cancer chemotherapy, especially for breast and ovarian cancers. It exerts cytotoxic activity at concentrations as low as 50nM[1]. To study the pharmacokinetics of paclitaxel, a sensitive and selective analytical method is required. So far, HPLC-UV method has become the main tool for paclitaxel analysis in biological fluids in combination with sample preparation methods with LOD (5-10ng/ml) and LOQ (10-30ng/ml) published[2]. However, sample preparation in plasma is still a challenge since plasma is a complex matrix with many kinds of interference drugs, proteins and other plasma components. This study aimed to develop a narrow-bore analytical HPLC method for the quantification of paclitaxel in human plasma in the presence of other interference drugs. Also, drug stability in human plasma has been evaluated under different storage conditions involved in clean-up and assay steps.

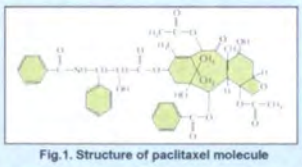

Fig.1. Structure of paclitaxel molecule

METHODS

- **Protein precipitation :**
  - Adjusting pH of sample matrix by addition of pH 3 buffer, followed by standing over one hour.
  - Centrifuged at 3000g for 10mins (4°C)

- **Sample preparation — solid phase extraction (SPE)**

Conditioning:	MeOH and 0.1M formic acid (pH 3)
Loading:	2.5 ml plasma sample plus 2.5ml buffer (pH3)
Washing:	pH 3 buffer, MeOH/buffer (2:8, v/v) and hexane
Drying & elution:	under full vacuum and eluting with 1.2 ml of acetonitrile (ACN) with 0.1% triethylamine
Evaporation:	under nitrogen stream at 30°C
Reconstitution:	500 µl of ACN/water (50/50)

SPE cartridges: Bond Elut CN-E, 500mg

- **High performance liquid chromatography**

HPLC SYSTEM:
Pump (PU-2080, Jasco)
Auto-sampler (AS-2055, Jasco)
Diode array detector (MD-2010, Jasco)

Chromatographic conditions:
Mobile phase: ACN/THF/0.02M Ammonium acetate (50/2/48)
Column: ODS2, 150 × 2.1mm, 5µm (Waters) combined with a C18, 4 × 2.0mm guard cartridge (Phenomenex)
Detection wavelength: 227 nm

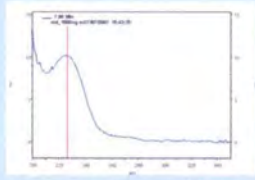

Fig.2. Spectrum of paclitaxel (1500 ng/ml): maximum absorption appeared at 227-230 nm

RESULTS

- **Determination of paclitaxel in presence of co-administered drugs**

Drugs	Rt (mins)
Paclitaxel	7.8
Docetaxel	6.7
Dexamethasone	2.2
Ranitidine	3.6
Cyclizine	—
Metoclopramide	8.4
Pamidronate	—
Carboplatin	1.9
Doxorubicin	4.8
Tamoxifen	—
Clonazepam	4.3
Granisetron	8.9
Ondansetron	9.7

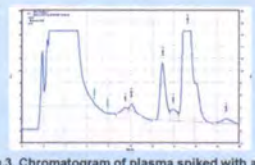

Fig.3. Chromatogram of plasma spiked with all interference drugs

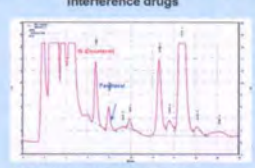

Fig.4. Chromatogram of paclitaxel in plasma (100ng/ml) with internal standard, docetaxel (400ng/ml)

- **Validation study**

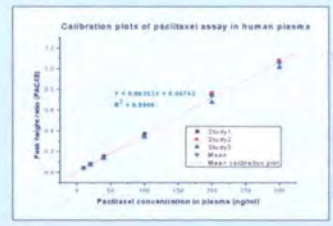

Fig.5. Calibration plot of paclitaxel assay in human plasma

Internal standard (IS): docetaxel
Linear range: 10 – 300ng/ml
LOD (limit of detection): 5 ng/ml
LLOQ (Lower limit of quantification): 10ng/ml (CV% < 7%)

Nominal conc. (ng/ml)	Accuracy (%)		Precision (%)		Recovery %
	Intra-	Inter-	Intra-	Inter-	
10	100.33	100.33	3.61	6.38	111.7± 3.7
100	99.42	99.42	2.82	5.55	97.5 ± 1.9
300	102.41	102.46	1.78	1.91	93.3 ± 8.1

- **Stability of paclitaxel in human plasma**

Nominal concentrations (ng/ml)	Study conditions	Stability period	Remaining %[f]	CV%
30	Long-term storage at -20 °C [a]	Up to 2 months	100.20	5.70 (n≥3)
	Short-term temperature [b]	Up to 5hr at room temperature	99.60	5.96 (n=4)
	Freeze/thaw [c]	Up to 3 cycles	105.07	3.91 (n=3)
	Stored in elution solvent at 2-8 °C [d]	Up to 2 weeks	93.09	5.26 (n=2)
	Post-preparation [e]	Up to 3 days	104.19	6.06 (n =7)
300	Long-term storage at -20 °C	Up to 2months	102.50	2.90 (n≥3)
	Short-term temperature	Up to 5hr at room temperature	100.34	1.46 (n=4)
	Freeze/thaw	Up to 3 cycles	102.60	1.11 (n=3)
	Stored in elution solvent at 2-8 °C	Up to 2 weeks	102.33	3.24 (n =2)
	Post-preparation	Up to 3 days	101.15	1.12 (n =6)

[a] *Plasma samples were prepared on the same day (study period = 2months).*
[b] *samples after thawed at room temperature (from -20°C to 23°C) were kept at room temperature for over 5hrs before analysis.*
[c] *samples were subjected to 3 × Freeze/Thaw cycles (between -20°C and 23°C) before analysis.*
[d] *Freshly made samples were subjected to SPE on Day 0.*
[e] *Reconstituted samples were analyzed on Day 0 and then kept in the auto-sampler at 23°C. stability was checked after different residence time of 24, 48 and 72hrs.*
[f] *Remaining %s were calculated by the peak height ratio of test samples compared with those samples without control .*

CONCLUSIONS

This method has been optimized for paclitaxel quantification, in human plasma, in the presence of co-administered medicines. Accuracy and precision of this method complied with the acceptance criteria for validation of bio-analytical assays. Paclitaxel was stable during sample collection and handling, after long- and short-term storage and at different conditions. The sensitivity and selectivity of this method was considered suitable for the purpose of planned pharmacokinetic studies to assess the effect of paclitaxel dose-banding schemes.

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Poster of "Physical and chemical stability of paclitaxel infusions for the application of dose banding strategy"

Presented at British Pharmaceutical Conference (BPC)

7^{th} – 9^{th} September, 2008

Manchester, UK

Abstract published as:

Xu J, Sewell G. Physical and chemical stability of paclitaxel infusions for the application of a dose-banding strategy. *Journal of Pharmacy and Pharmacology,* 2008, 59 (Supplement 1), A-49 (abstract 124).

Abstract

Objectives

Dose-banding (D-B) strategy, as a novel promising dosing method, has been successfully applied to many cancer treatments. There is stability requirement for any drug to be considered for D-B dosing, which is basis for the pre-preparation of drug (normally > 14days is sufficient). In order for the application of D-B strategy for paclitaxel chemotherapy, our study aimed to determine the physical and chemical stability of paclitaxel in 0.9% sodium chloride under normal hospital conditions.

Methods

Paclitaxel infusions in 0.9% sodium chloride (0.3mg/ml and 1.0mg/ml) were prepared in different containers (Freeflex® polyolefin infusion bags and Ecoflac® low-density polyethylene infusion bottles, n = 3) and stored at 2-8 °C. Samples were taken for physical and chemical stability at different time intervals for up to 35 days. Physical stability was inspected by weight loss (%), pH change, visual appearance and sub-visual particulate counting by a particulate counter. Chemical stability was conducted by a validated stability indicating HPLC method and counted as the remaining % of the initial concentrations.

Results

The main results of physical and chemical stability of paclitaxel infusions during the shelf-life periods are shown in the below table. In this study, the stability of paclitaxel infusions over storage was mainly decided by physical stability due to the formation of cloudy precipitation that may be caused by paclitaxel itself. Physical stability was increased with lower concentration (0.3mg/ml). The particulate counting study has shown satisfactory results based on regulations of the British Pharmacopeia within the shelf-life periods. An adequate stability period (≥ 20 days) has been produced with 0.3mg/ml paclitaxel infusions, which facilitates the dose-banding schema of paclitaxel chemotherapy.

Physical and chemical stability of paclitaxel infusions

Concentrations	0.3 mg/ml		1.0 mg/ml	
	Freeflex®	Ecoflac®	Freeflex®	Ecoflac®
Weight loss (≤%)	0.02	0.18	0.01	0.20
pH range	3.64 - 3.73	3.58 - 3.71	3.42 - 3.53	3.42 - 3.49
Visual appearance	20	35	17	31
Assay range (remaining %)	98.5-103.6	97.7-104.8	98.8-102.8	95.7-103.3

Conclusion

Paclitaxel has demonstrated robust stability in 0.9% sodium chloride, which facilitated the feasibility of D-B strategy. Further pharmacokinetic studies will be performed to evaluate the role of this D-B dosing strategy compared with standard BSA based dosing and flat-fixed dosing through ex vivo simulation studies and real clinical studies.

Physical and chemical stability of paclitaxel infusions for the application of dose banding strategy

Jing Xu, MD, MSc; Graham Sewell, PhD, MRPharmS
Department of Clinical Pharmacy, Kingston University of London

Objectives

Dose-banding (D-B) is a novel strategy to rationalise and expedite the provision of cancer chemotherapy. For any drug to be considered for D-B dosing there is a stability requirement to enable pre-preparation of the infusion (normally > 14days is required). In order for the application of D-B strategy to paclitaxel chemotherapy, our study aimed to determine the physical and chemical stability of paclitaxel in 0.9% sodium chloride under refrigerated storage conditions.

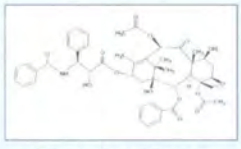

Figure 1. chemical structure of paclitaxel

Methods

Preparation of paclitaxel infusions and sampling

- Paclitaxel infusions in 0.9% sodium chloride (0.3mg/ml and 1.0mg/ml)
- Prepared in Freeflex® polyolefin bags and Ecoflac® low density polyethylene bottles (n = 3 each)
- Stored in a pharmaceutical refrigerator (2-8°C)
- Samples were taken for physical and chemical stability tests at different time intervals for over 20 days.

Determination of physical stability

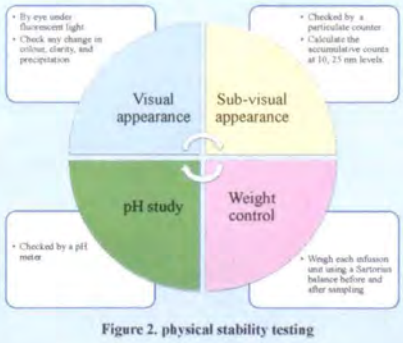

Figure 2. physical stability testing

Determination of chemical stability

- **Validation of a high-performance liquid chromatography (HPLC) method**

Linear range: 10 – 100 µg/ml
Average correlation coefficient (R^2) =1 (based on 3 replicate calibration curves)
Intra- and inter-day precisions (CV%): all ≤ 4.06% at both 10µg/ml & 80 µg/ml (n =6).

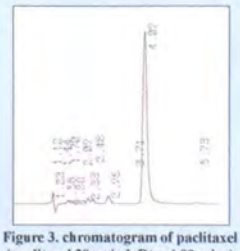

Figure 3. chromatogram of paclitaxel (paclitaxel 20 µg/ml, Rt = 4.09 mins)

- **Stability indicating study**

Table 1. results of stability indicating experiment

Treatments	Retention time (mins)	Quantity (µg/ml)	Peak purity (%)
Control	4.09	15.30	100.00
heating (55°C)	4.10	15.24	99.50
Oxidative condition	4.09	15.52	97.80
Acid hydrolysis	4.09	2.56	<0
Alkaline hydrolysis	—	—	—

Test concentration of paclitaxel: 15µg/ml

- **Chemical stability testing**

Samples were diluted and analyzed by the HPLC method in duplicates. The remaining % was decided by comparison with the initial concentration.

Results

Data for physical and chemical stability of paclitaxel infusions over the period where visual appearance remained satisfactory are shown in the table below:

Table 2. summary of physical and chemical stability of paclitaxel infusions

Concentrations	0.3 mg/ml		1.0 mg/ml	
	Freeflex®	Ecoflac®	Freeflex®	Ecoflac®
Weight loss (≤ %)	0.02	0.18	0.01	0.20
pH range	3.64 - 3.73	3.58 - 3.71	3.42 - 3.53	3.42 - 3.49
Visual appearance (days)	22	30	19	30
Sub-visual particulate count (10nm & 25nm)	Pass	Pass	Pass	pass
Assay range (remaining %)	96.8-103.6	97.7-104.8	98.5-102.8	95.7-103.3

Termination of this study: ≥ 1 particulates appearing in any container
Shelf-life period for each group corresponds to visual appearance (days).

Summary

- Stability of paclitaxel infusions was mainly limited by physical stability due to the formation of cloudy precipitation.
- Within the shelf-life periods, no obvious weight loss and pH change were observed and no significant change in sub-visual particulate counts at 10nm and 25nm levels were recorded based on British Pharmacopeia (BP) standard.
- No significant decrease of drug concentration occurred over the physical stability shelf-life.
- The maximum assigned shelf-life of 0.3mg/ml and 1.0mg/ml paclitaxel infusions in 0.9% sodium chloride was 30 days when kept at 2-8°C.

Table 3. dose-banding schema for paclitaxel chemotherapy

Dose bands (mg)	ml dose (mg)	Max. Dev (%)	Pre-prepared infusions (mg)	No. Pre-infusion	Total volume of infusions (ml)
125-135	*130*	4.00	100mg + 20mg +10mg	3	350
135-145	*140*	3.70	100mg + 20mg +20mg	3	350
145-155	*150*	3.45	100mg + 50mg	2	350
155-165	*160*	3.23	100mg + 50mg + 10mg	3	400
165-175	*170*	3.03	100mg + 50mg + 20mg	3	400
175-185	*180*	2.86	100mg + 50mg + 20mg + 10mg	4	450
185-200	*190*	5.00	100mg + 50mg + 20mg + 20mg	4	450
200-220	*210*	5.00	200mg + 10mg	2	550
220-240	*230*	4.55	200mg + 20mg + 10mg	3	600
240-260	*250*	4.17	200mg + 50mg	2	600
260-280	*270*	3.85	200mg + 50mg + 20mg	3	650
280-300	*290*	3.57	200mg + 50mg + 20mg + 20mg	4	700
300-320	*310*	3.33	300mg + 10mg	2	1050
320-340	*330*	3.13	300mg + 20mg + 10mg	3	1100
340-360	*350*	2.94	300mg + 50mg	2	1100
360-380	*370*	2.78	300mg + 50mg + 20mg	3	1150
380-400	*390*	2.63	300mg + 50mg + 20mg + 20mg	4	1200
400-420	*410*	2.50	200mg + 200mg + 10mg	3	1050
420-440	*430*	2.38	200mg + 200mg + 20mg + 10mg	4	1100
440-460	*450*	2.27	200mg + 200mg + 50mg	3	1100

Conclusions

Paclitaxel has demonstrated adequate stability in 0.9% sodium chloride, which facilitated the feasibility of D-B strategy. Table 3. shows a dose-banding schema for paclitaxel chemotherapy. Further pharmacokinetic studies will be performed to evaluate the clinical implications of this D-B dosing strategy compared with standard body-surface area (BSA) based dosing and flat-fixed dosing through *ex vivo* simulation studies and clinical studies.

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