Development of a HPLC method for the determination of selected anticancer drugs in Lebanese environmental water samples

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Introduction

The majority of the Lebanese water resources are polluted due to the absence of strict regulations from concerned parties. The main cause of water pollution in Lebanon is sewage and wastewater. These are being discharged directly into the aquatic environment or only after primary treatment by Wastewater Treatment Plants (WWTPs) (1). Environmental risk factors can be one of the reasons behind the increase and the appearance of numerous diseases, including cancer (2). In fact, the incidence of cancer in Lebanon, is still increasing from the time when the initial records on cancer occurrence were reported in 1966 (3). Hence, the consumption rate of anticancer drugs is on the rise.

According to oncologists, the top 6 most reoccurring types of cancer in Lebanon are: Breast Cancer, Prostate Cancer, Lung Cancer, Colon Cancer, Non-Hodgkin's Lymphoma and Bladder Cancer. Therefore, six anticancer drugs estimated to be among the most administered in Lebanon were selected for the method development (**Figure 1**).

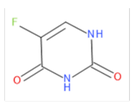

Figure 1 (a): 5-Fluorouracil

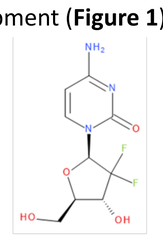

Figure 1 (b): Gemcitabine

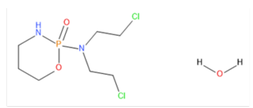

Figure 1 (c): Cyclophosphamide

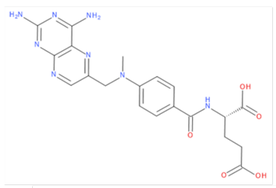

Figure 1 (d): Methotrexate

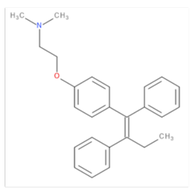

Figure 1 (e): Tamoxifen

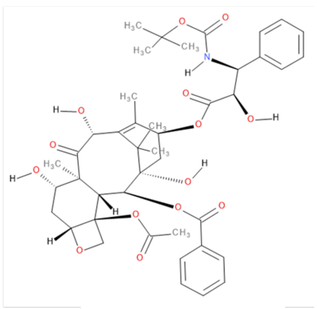

Figure 1 (f): Docetaxel

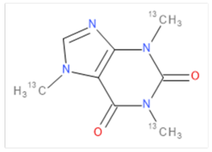

Figure 1 (g): $^{13}C_3$-Caffeine

Figure 1: Chemical structures of the selected anticancer drugs (a-f) and the internal standard (g)

Objectives

Develop and validate a method to evaluate the occurrence of selected anticancer drugs in hospital effluents, WWTPs influent and effluent, and in surface water using SPE, HPLC, LC/MS techniques.

Materials & Methods

The analytical standards: Cyclophosphamide (≥98%), Tamoxifen (≥98%), 5-fluorouracil (≥99%) and the European Pharmacopoeia (EP) Reference Standards: Gemcitabine Hydrochloride, Docetaxel Trihydrate, and Methotrexate were purchased from Sigma Aldrich, UK. Isotopically labelled caffeine, used as internal standard, 13C3-Caffeine (99atom %13C) in a methanol solution of 1mg/ml and formic acid reagent grade (≥95%), were supplied by Sigma Aldrich, UK. HPLC-grade methanol and LC-MS grade water were obtained from VWR Chemicals, UK.

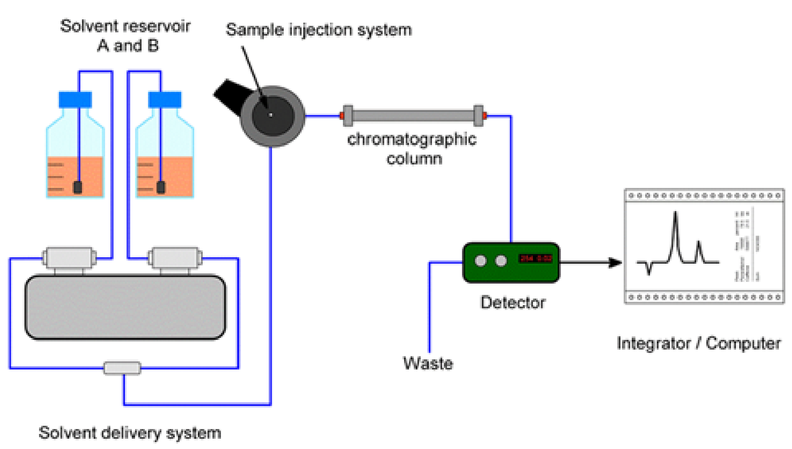

Figure 2: HPLC system

Stock solutions of each compound were prepared in LC-MS grade water. The separation of the compounds was achieved on an Agilent 1200 series HPLC (Infinity II) (**Figure 2**) using Kinetex 2.6 µm Phenyl-Hexyl column (100 x 3 mm) purchased from Phenomenex, UK. Gradient elution was performed with a binary mobile phase at a flow rate 0.3 ml/min using (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid.

Results & Discussion

The developed method (**Table 1**) allowed the separation of five anticancer drugs and caffeine as internal standard within 20 minutes.

Table 1: HPLC parameters selected for the separation of the 6 anticancer drugs and the internal standard

Selection of HPLC Parameters			
Column	Kinetex 2.6 µm Phenyl-Hexyl (100 x 3 mm)		
Temperature	Room Temperature (25°C)		
Flow Rate	0.3 ml/min		
Injection Volume	50 µl		
Wavelength	260 and 290 nm		
Run Time	20 minutes		
Gradient Elution	Time (min)	% (A)	% (B)
	0	100	0
	2	100	0
	5	20	80
	9	5	95
	13	5	95
	13.1	100	0
	19	100	0

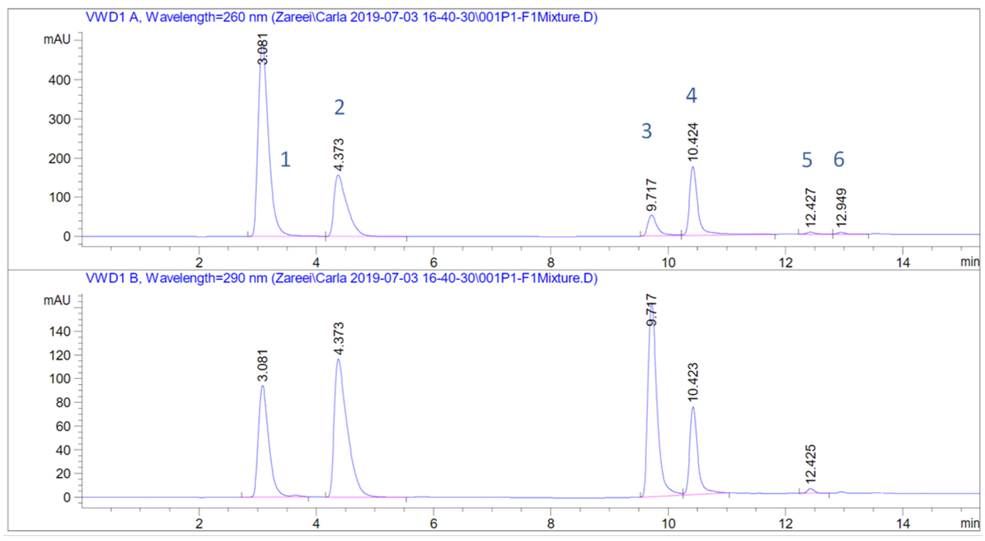

Figure 3: Chromatogram showing the separation of five anticancer drugs and the internal standard (A: λ= 260 nm; B: λ= 290 nm)

The compounds eluted were: (1) 5-Fluorouracil, (2) Gemcitabine, (3) Methotrexate, (4) Caffeine, (5) Tamoxifen and (6) Docetaxel (**Figure 3**).

However, the selection of the right wavelength was difficult as all these compounds have various structures and therefore, different spectra and molar absorbtivities. Also, Cyclophosphamide was not detected by this method due to its limited absorption of UV radiation (4).

Conclusion & Future Work

The developed method was found to be rapid and suitable for the determination of five anticancer drugs and caffeine. Continuing studies will include method validation on LC/MS and analysis of environmental water samples (**Figure 4**).

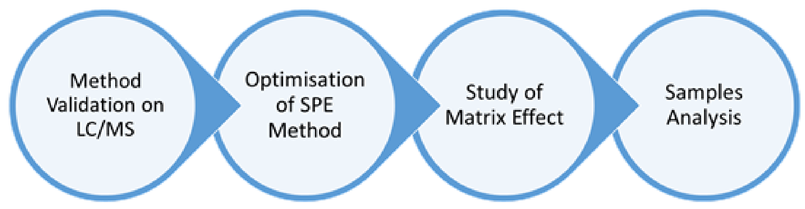

Figure 4: Future work

References

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