

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). 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 (2). Hence, the consumption rate of anticancer drugs is on the rise. The aim of this research project is to detect and quantify anticancer drugs in the aquatic environment of Lebanon.



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 (3). Therefore, six anticancer drugs estimated to be among the most administered in Lebanon were selected for the method development (Figure 1).

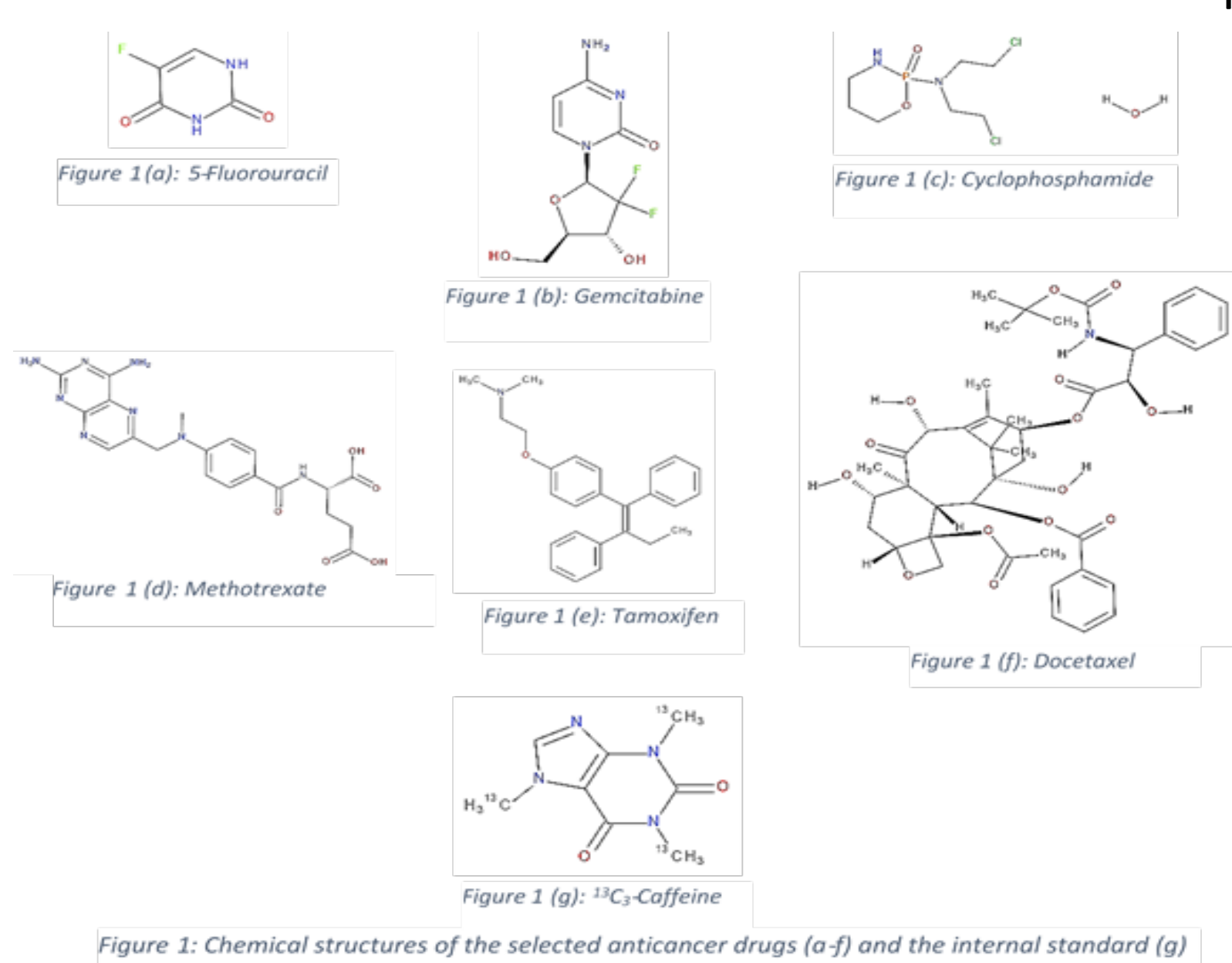


Figure 1: Chemical structures of the selected anticancer drugs (a-f) and the internal standard (g)

Objectives

Develop a specific 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 ($\geq 98\%$), Tamoxifen ($\geq 98\%$), 5-Fluorouracil ($\geq 99\%$) 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, $^{13}\text{C}_3$ -Caffeine (99atom % ^{13}C) in a methanol solution of 1mg/ml and formic acid reagent grade ($\geq 95\%$), were supplied by Sigma Aldrich, UK. HPLC-grade methanol and LC-MS grade water were obtained from VWR Chemicals, UK.

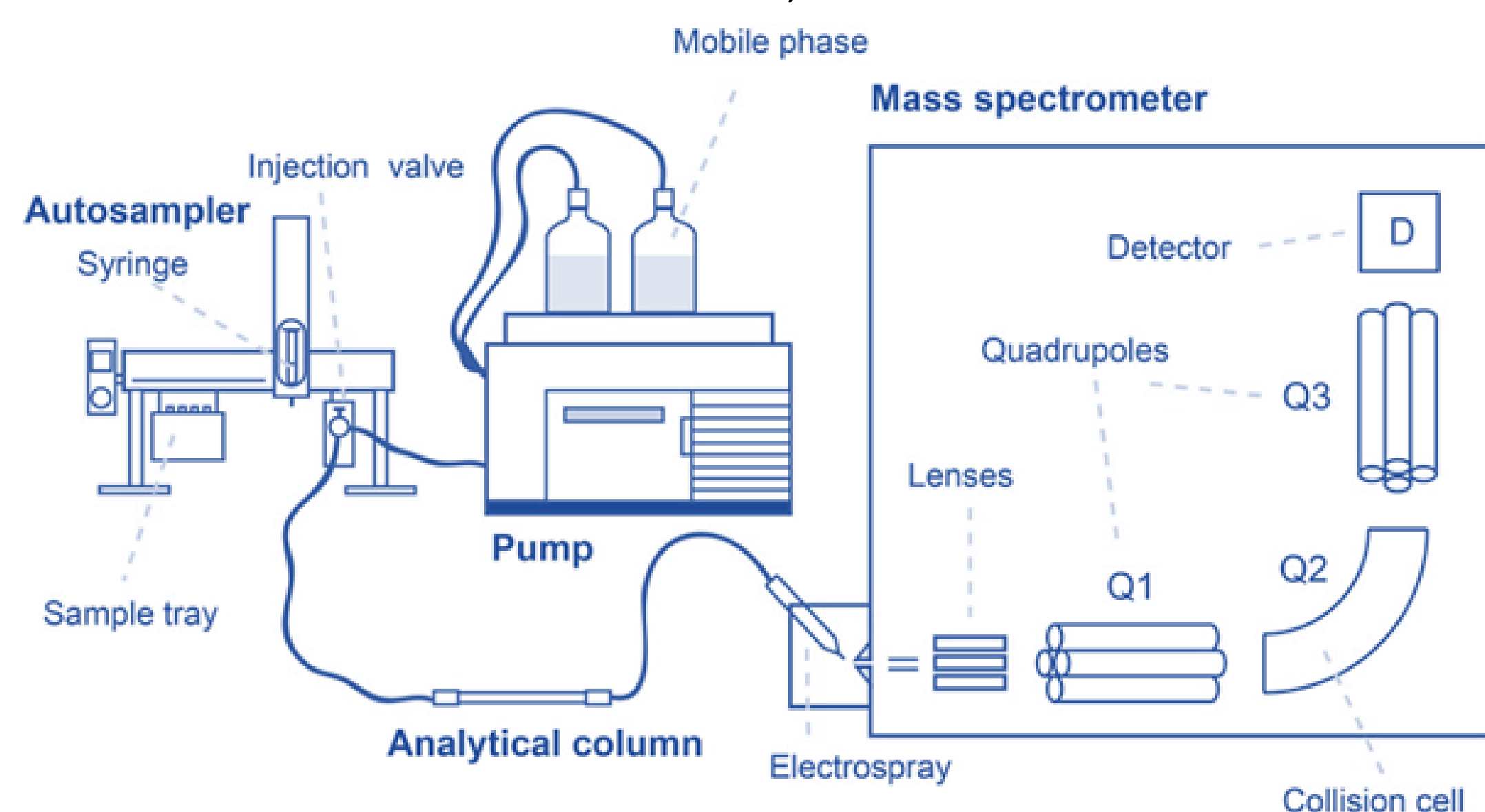


Figure 2: LC-MS/MS system

Stock solutions of each compound were prepared in LC-MS grade water. The separation of the compounds was achieved on an Agilent 1260 Infinity coupled with a 6430 Triple Quad LC/MS (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 allowed the separation of six anticancer drugs and caffeine as an internal standard within 15 minutes. All compounds were ionised in positive mode. Precursor ion and the most abundant fragment ion were chosen for the selective quantification of the compound of interest. Additionally, the second most abundant product ion was selected for confirmation purposes. The MRM acquisition settings are presented in Table 1.

Table 1: Optimised MRM parameters for the anticancer drugs and the internal standard

Compound	Precursor Ion	Product Ions	Fragmentor (V)	CE (eV)
5- Fluorouracil	131.01	114	99	13
		58.1	99	29
Gemcitabine	264.08	112.1	99	17
		95.1	99	40
Methotrexate	455.18	308.3	118	17
		175	118	40
Cyclophosphamide	261.03	140	99	21
		63.1	99	40
Tamoxifen	372.23	72.2	137	25
		70.2	137	40
Docetaxel	830.34	549.3	137	21
		304.3	137	21
$^{13}\text{C}_3$ -Caffeine	198.1	140	99	17
		112.1	99	25

The chromatogram below presents the separation of the six anticancer drugs and the isotopically internal standard. The compounds eluted were: (1) 5-Fluorouracil, (2) Gemcitabine, (3) Methotrexate, (4) $^{13}\text{C}_3$ -Caffeine, (5) Cyclophosphamide, (6) Tamoxifen and (7) Docetaxel (Figure 3).



Figure 3: Chromatogram showing the separation of six anticancer drugs and the internal standard

Conclusion & Future Work

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

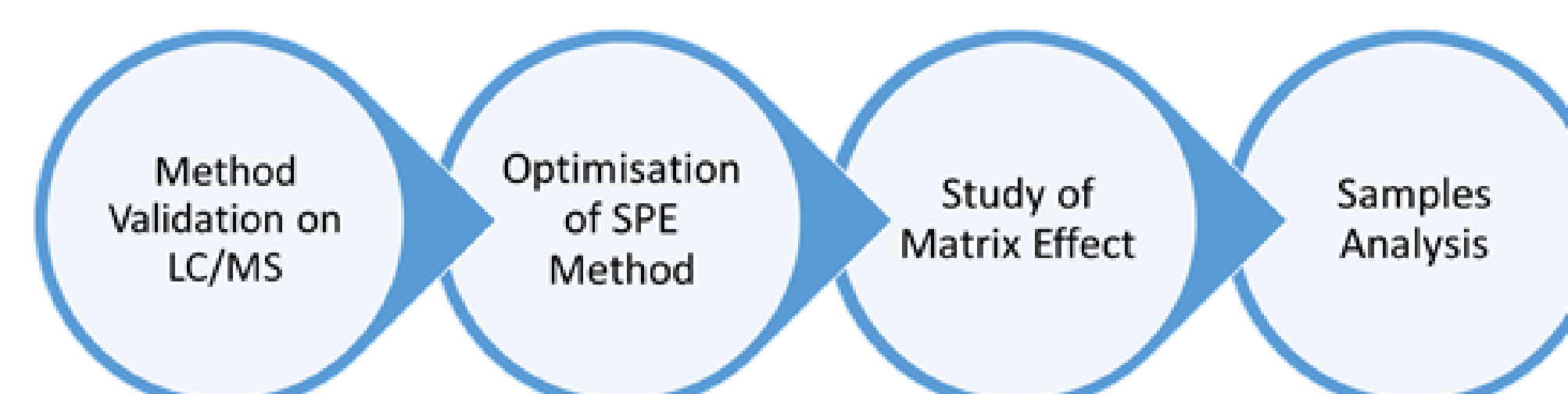


Figure 4: Future work

References

- (1) Geara-Matta, D. et al. (2010) 'State of Art About Water Uses and Wastewater Management in Lebanon', Lebanese Science Journal, 11(2), pp. 139–152. doi: 10.1016/1geoforum.2008.11.002.
- (2) Shamseddine, A.; Saleh, A.; Charafeddine, M.; Seoud, M.; Mukherji, D.; Temraz, S.; Sibai, A. M. Cancer Trends in Lebanon: A Review of Incidence Rates for the Period of 2003-2008 and Projections until 2018. Popul. Health Metr. 2014, 12 (1), 1–8.
- (3) Shamseddine, A. (2015) 'Cancer Trends in Lebanon & Projections to 2020', Human & Health, pp. 9–11.