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Determination of Anticancer Drugs in the Aquatic Environment by SPE–LC–MS/MS—A Lebanese Case Study

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Abstract: Compared to other countries in the Middle East and North Africa region, Lebanon is considered the richest in water resources. However, due to inadequate water management, Lebanese water resources are under stress. Previous studies in Lebanon were mainly focused on heavy metals and microbiological analysis. Following the increase in cancer rates in Lebanon, the occurrence of six anticancer drugs estimated to be the most administered in Lebanon was assessed in samples collected from wastewater treatment plants (WWTPs), sewage outfalls, and surface water. Two SPE–LC–MS/MS methods were developed and validated for the detection of the selected anticancer drugs with LOD values ranging between 0.1 and 0.9 ng/L and LOQ values ranging between 0.3 and 2.8 ng/L. Three anticancer drugs were detected using this technique, 5-fluorouracil, methotrexate, and cyclophosphamide, with an overall concentration range of 1 to 305 ng/L. The detection of these drugs in WWTP effluents, sewage outfalls, and rivers confirmed the weaknesses prevailing in the management of wastewater and the treatment technologies adopted by the few operational WWTPs in Lebanon.

Keywords: Lebanon; water pollution; anticancer drugs

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1. Introduction

1.1. Background

Although pharmaceuticals are essential for the survival of humankind and improvement of the quality of life, they actually represent an environmental threat. They are considered among the most critical emerging contaminants of the aquatic environment, with major concerns [1,2]. The upsurge in the production and consumption of pharmaceuticals in the last decade was due to the evolution of research and development, the growth of the world population, the improvement of life expectancy, and the ease of accessibility to healthcare and pharmaceuticals [3,4]. Since wastewater treatment plants (WWTPs) are not able to keep up with the production of pharmaceuticals, the complete removal of metabolites and parent compounds from domestic and hospital wastewater is still not achievable, making it the primary source of pharmaceuticals in the environment. Secondary sources include the manufacturing industry, agricultural activities, aquaculture, and veterinary practices [5].

The evolution of analytical techniques and increasing awareness of the potential adverse effects emerging from pharmaceutical contamination has led to the detection of several therapeutic groups in the aquatic environment. Yet monitoring programs still focus on conventional target pollutants while overlooking numerous emerging substances that can have environmental impacts, including anticancer drugs. Members of this pharmaceutical class, more precisely cyclophosphamide, daunorubicin, doxorubicin, fluorouracil, and mycophenolic acid, were considered substances of concern by the Joint Research Centre (JRC) and were listed in the priority three category to be included in the 4th EU Watch List. However, due to the lack of information on analytical methods and missing predicted

no-effect concentration (PNEC) values, these compounds were not included in the most recent Watch List [6,7].

1.2. Lebanon Case

Lebanon is a Middle Eastern country bounded by the Mediterranean Sea, with a coastline length of about 225 km. Despite its small area (10,452 km^2), Lebanon is privileged with plentiful water resources [8]. These consist of rivers, springs, groundwater, snow cover, lakes, reservoirs, and wetlands, with a flow ranging between 2000 and 2700 million m^3 per year [9]. These water resources are affected by several environmental stresses, which have deteriorated their quality. These stresses originate from human activities and have previously resulted in numerous waterborne disease outbreaks, such as cholera, typhoid fever, shigella infection, and hepatitis A & E [10]. In fact, only 8% of the total national wastewater generated is connected to WWTPs and is treated before discharge. The rest goes into septic tanks or is directly released into the environment without any treatment. In addition, more than 53 sewage outfalls covering the Lebanese coastline, accounting for approximately 65% of the total sewage, are directly discharged into the sea [11–15].

As a result, water resources are mainly contaminated by untreated sewage from households and industry, in addition to agricultural runoff, dumpsite leachate, and recreational activities [16]. These factors have drawn the attention of many researchers to evaluate the quality of Lebanese water resources, especially in terms of heavy metals and bacterial contamination. Regarding pharmaceutical contamination, only three studies were retrieved from the literature that assessed their presence in Lebanese surface water [17–19]. As shown in Table 1, mainly antibiotic analysis was conducted with concentrations reaching 4100 ng/L in river samples. The evaluation of other drug classes was limited in number and sampling locations (Kadicha River and Jeita Spring). Five pharmaceuticals were detected, including acetaminophen, carbamazepine, atenolol, caffeine, and diclofenac, with concentrations reaching 10,234 ng/L.

Table 1. Concentration levels of pharmaceuticals in Lebanese environmental water samples.

Compound		Concentration Range (ng/L)	Reference
Analgesic	Acetaminophen	n.d.–242	[17]
Antibiotic	Macrolide	n.d.–2806	[19]
	Fluoroquinolone	n.d.–190	[17,19]
	Tetracycline	n.d.	[19]
	Rifamycin	n.d.–542	[19]
	Sulfonamide	n.d.–4100	[17,19]
	Trimethoprim	n.d.	[19]
Anticonvulsant	Carbamazepine	n.d.–290	[17,18]
Benzodiazepine	Diazepam	n.d.	[17]
	Lorazepam	n.d.	[17]
β-Blocker	Atenolol	n.d.–93	[17]
	Propranolol	n.d.	[17]
CNS Stimulant	Caffeine	1–10,234	[19]
Mucolytic	Bromhexine	n.d.	[19]
Narcotic	Codeine	n.d.	[17]
	Norbuprenorphine	n.d.	[17]

Table 1. *Cont.*

Compound		Concentration Range (ng/L)	Reference
NSAID	Diclofenac	n.d.–1055	[17]
	Ibuprofen	n.d.	[17]
	Ketoprofen	n.d.	[17]
SNRI	Venlafaxine	n.d.	[17]
Statin	Pravastatin	n.d.	[17]

The scarcity of studies available in the literature that investigate the occurrence of pharmaceuticals in the Lebanese aquatic environment, in addition to the progressive increase of cancer incidence rates in Lebanon [20], were the motives to conduct this research. The aim of this study was to understand the fate of anticancer drugs in the aquatic environment by (1) optimizing two SPE methods and (2) developing and validating an LC–MS/MS method for the detection of 5-fluorouracil, gemcitabine, methotrexate, cyclophosphamide, tamoxifen, and docetaxel in WWTP, sewage outfall, and river samples from different locations in Lebanon.

2. Materials and Methods

2.1. Materials and Instrumentations

The analytical standards cyclophosphamide (≥98%), tamoxifen (≥98%), and 5-fluorouracil (≥99%) and the European Pharmacopoeia Reference Standards gemcitabine hydrochloride, docetaxel trihydrate, and methotrexate were purchased from Sigma-Aldrich (Gillingham, UK). Isotopically labelled caffeine, $^{13}C_3$-caffeine (99-atom % ^{13}C), used as internal standard in a methanol solution of 1 mg/mL, and formic acid for LC–MS (98–100%) were supplied by Sigma-Aldrich (Gillingham, UK). LC–MS grade water, methanol, and acetonitrile were obtained from VWR Chemicals (Lutterworth, UK).

All of the analytical experiments were performed on an Agilent 1260 Infinity coupled with a 6430 Triple Quad LC/MS. The LC column used for the analytical method was a Kinetex 2.6 µm Phenyl-Hexyl (100 × 3 mm) column from Phenomenex (Macclesfield, UK). The column was protected with a Phenomenex SecurityGuard ULTRA Holder with a suitable Phenyl 3 mm cartridge.

For the Solid-Phase Extraction (SPE) procedure, the SPEware Cerex 48 Sample Concentrator Positive Pressure Manifold was adopted. The SPE cartridges utilized were Isolute ENV+ (1 g/6 mL) from Biotage (Hengoed, UK) and Oasis HLB (60 mg/3 mL) from Waters (Wilmslow, UK). Two filter types for sample preparation were obtained from Sigma-Aldrich (Gillingham, UK): Whatman glass microfiber filters (GF/C—125 mm) and MF-Millipore membrane syringe filters (mixed cellulose esters membrane—0.45 µm).

2.2. Health and Safety Considerations

Strict handling procedures were applied to prevent occupational exposure to anticancer drugs and environmental samples. All of the solutions were prepared in a Class II microbiological biosafety cabinet, and the bench was constantly covered with a chemotherapy absorbent mat to avoid contamination in case of a spill. Moreover, complete personal protective equipment was worn at all times in the laboratory.

All materials that came into contact with the compounds, in addition to liquid waste, were adequately labelled and securely disposed of in appropriate containers and waste bins.

2.3. Stock Solutions Preparation

5-Fluorouracil, gemcitabine, and cyclophosphamide were dissolved in water; methotrexate was dissolved in water and methanol (50:50); and tamoxifen and docetaxel were dissolved in methanol. Working solutions of each compound were adjusted to a concentration of 600 ng/mL and were further diluted to obtain a mixture solution of

100 ng/mL. To achieve the concentration needed, stock solutions were diluted to volume with water. For the internal standard, $^{13}C_3$-caffeine, 6 µL was pipetted from the stock solution and diluted to 10 mL volume with water for a final concentration of 600 ng/mL. Working solutions were properly labelled and securely stored at 4 °C.

2.4. LC–MS/MS Method Development

The separation of the selected anticancer drugs was optimized on the LC–MS/MS system to identify a suitable column and mobile phase composition. Additional parameters were optimized, such as the column temperature, the choice of product ions, the ion source and its polarity, the collision energy, and the fragmentor voltage. Subsequently, the column efficiency (theoretical plate number), resolution, plate height, and asymmetry factor were calculated.

2.5. Solid-Phase Extraction Optimisation

Based on a previously conducted literature review [21], two SPE cartridges were selected for sample preparation and extraction of the compounds of interest: Isolute ENV+ (1 g/6 mL) and Oasis HLB (60 mg/3 mL). The parameters tested were: (1) filtration of the sample with the 0.45 µm and 1.22 µm filters; (2) pH of the sample: 4, 5, 6, and 7; (3) elution solvent: acetonitrile (100%), acetonitrile:water (95:5), methanol (100%), and methanol:water (95:5); (4) elution volume: 3, 6, 8, and 10 mL; (5) drying time for the Isolute ENV+ cartridge: 20 min and 2 h; and (6) breakthrough volume: 50, 100, and 200 mL.

To select the suitable conditions for each parameter, the obtained ratios of the compounds' responses to the internal standard's response (known as the response factor) were compared, and the conditions contributing to the best response were selected. Once all of the parameters were optimized, three samples were analyzed adopting the final developed method. Therefore, the percentage recovery was calculated for each compound by comparing the responses of three blank samples spiked with the compounds pre- and post-SPE, following the equation below:

$$\%\ Recovery = \frac{Peak\ Area\ of\ Pre-Spike}{Peak\ Area\ of\ Post-Spike} \times 100$$

2.6. SPE–LC–MS/MS Method Validation

Considering that the SPE methods were efficient for the recovery of most of the compounds, method validation was carried out in compliance with ICH guidelines [22]. The parameters evaluated were specificity, linearity, range, accuracy, precision, and limits of detection and quantification, as well as robustness of the analytical method.

2.7. Matrix Effect

The matrix effect was examined in order to identify whether the environmental samples suppressed or enhanced the responses of the compounds of interest. To verify that the SPE methods were removing any potential interferences, six samples underwent extraction: three blanks and three wastewater influent samples post-spiked with standards at three concentrations (low = 10 ng/L, medium = 40 ng/L, and high = 70 ng/L) and their responses were compared. This is known as the post-extraction addition method, and the matrix effect was calculated following the equation below:

$$\%\ ME = \frac{PA_{(extract)}}{PA_{(neatblank)}} \times 100$$

where $PA_{(extract)}$ = peak area of the compound post-spiked in the environmental sample, and $PA_{(neat\ blank)}$ = peak area of the compound post-spiked in the blank sample.

2.8. Method Application

The sampling period was between the 5th and 10th of January 2022. In total, 22 samples were collected covering all Lebanese regions, including 14 from rivers, 6 from WWTPs (3 influents and 3 effluents), and 2 from sewage outfalls to the sea. Sample bottles were soaked and washed with 5% nitric acid and triple rinsed with the sample water before collection.

Upon reaching the laboratory, the samples were filtered through 0.45 µm syringe filters and stored in the freezer at −27 °C until further preparation and analysis. The analysis of the samples was carried out by adopting the developed and validated SPE and LC–MS/MS methods. Nevertheless, to avoid any instrumental drift, a calibration curve was run before every sample analysis. In addition, quality control measures were run throughout the analysis at regular intervals, followed by two instrumental blank washes.

2.9. Analysis

The LC–MS/MS was operated by Agilent Masshunter Workstation Software. It was controlled by the LC–MS Data Acquisition system (version B.04.01), and the analytical data were obtained from the Qualitative Analysis system (version B.04.00). The Optimizer program was also operated through the same software (version B.04.01). All data analysis and calculations were completed in Microsoft Excel for Microsoft 365 MSO (version 2208 Build 16.0.15601.20148).

3. Results and Discussion

3.1. LC–MS/MS Method Development

Different columns were evaluated for their ability to separate the tested compounds, including the Spherisorb S5CN, Aqua C18, Prodigy 5ODS, and Luna Omega Polar C18 columns. Yet these columns demonstrated poor separation quality mainly for the two hydrophilic compounds, 5-fluorouracil and gemcitabine, which were not retained on the stationary phase and eluted at the solvent front. Greater retention and separation of all compounds were achieved using the Kinetex 2.6 µm Phenyl-Hexyl column (100 × 3 mm); hence, it was selected for the subsequent experiments.

Considering the different physicochemical properties of the selected anticancer drugs, gradient elution was preferred to isocratic elution. The suitability of a binary mobile phase was examined at a flow rate of 0.4 mL/min using (A) water with 0.1% formic acid and (B) methanol:acetonitrile (75:25) with 0.1% formic acid. Additionally, the column temperature was increased to 40 °C and the final run time was 12 min. The chromatographic parameters for the separation of the selected anticancer drugs and the internal standard are presented in Table 2.

Since this analytical method allowed the detection and characterization of analytes based on their molecular mass determination and mass spectra fragmentation [23], it was essential to identify the fragment ions of each compound and other associated parameters to achieve improved sensitivity. The optimization of the LC–MS/MS method parameters was completed using the Optimizer program. Each compound was separately injected by standard injection into the MS system via a loop. The same method was run in both polarities (negative and positive) using the electrospray ionization (ESI) technique.

After analysis, the report produced by the Optimizer program allowed the procurement of essential parameters required for detection by mass spectrometry, including the polarity of the ion source, the precursor ions, the fragmentor voltage, the product ions, their abundance, and the collision energy. Usually in the ionization process, the target compound becomes charged by losing or gaining atoms and electrons. The formation of the charged precursor ions, known as adducts, can be in the form of protonated $[M + H]^+$ or deprotonated $[M - H]^-$ molecules or other adduct ions, such as $[M + Na]^+$ [24–26]. Here, all of the selected compounds were ionized in positive mode and formed protonated molecules, except for docetaxel, which formed a sodium adduct that was probably influenced by the properties of the mobile phase [24]. The precursor ions were then fragmented into four

product ions for each compound and three for docetaxel. The precursor ion and the most abundant fragment ion were chosen for selective quantification of the compound of interest. The second most abundant product ion was only selected for confirmation purposes.

Table 2. LC parameters for the separation of the selected anticancer drugs and the internal standard.

LC Parameters			
Column	Kinetex 2.6 μm Phenyl-Hexyl (100 × 3 mm)		
Temperature	40 °C		
Flow rate	0.4 mL/min		
Injection volume	10 μL		
Run time	12 min		
Mobile phase timetable	Time	% (A)	% (B)
	0	100	0
	2	100	0
	4	25	75
	8	5	95
	8.5	5	95
	8.6	100	0
	12	100	0

The MRM acquisition parameters are presented in Table 3. The default source parameters of the mass spectrometer were set, as follows: the gas temperature was 350 °C with a flow rate of 13 L/min, the nebulizer pressure was set at 50 psi, and the capillary voltage was set at 4000 V. Moreover, the electron multiplier was set at ΔEMV = 400 V.

Table 3. Optimized MRM parameters for the selected anticancer drugs and internal standard.

Compound	Precursor Ion	Fragmentor (V)	Product Ions	Collision Energy (eV)	Dwell Time (ms)
5-Fluorouracil	131.01	99	114	13	100
			58.1	29	
Gemcitabine	264.08	99	112.1	17	100
			95.1	40	
Methotrexate	455.18	118	308.3	17	50
			175	40	
Cyclophosphamide	261.03	99	140	21	50
			63.1	40	
Tamoxifen	372.23	137	72.2	25	50
			70.2	40	
Docetaxel	830.34	137	549.3	21	100
			304.3	21	
$^{13}C_3$-Caffeine	198.1	99	140	17	50
			112.1	25	

As a result of the method development, the chromatogram below (Figure 1) showed the separation of the six anticancer drugs and the isotopically labelled internal standard. The compounds eluted as follows: 5-fluorouracil, gemcitabine, methotrexate, $^{13}C_3$-caffeine, cyclophosphamide, tamoxifen, and docetaxel.

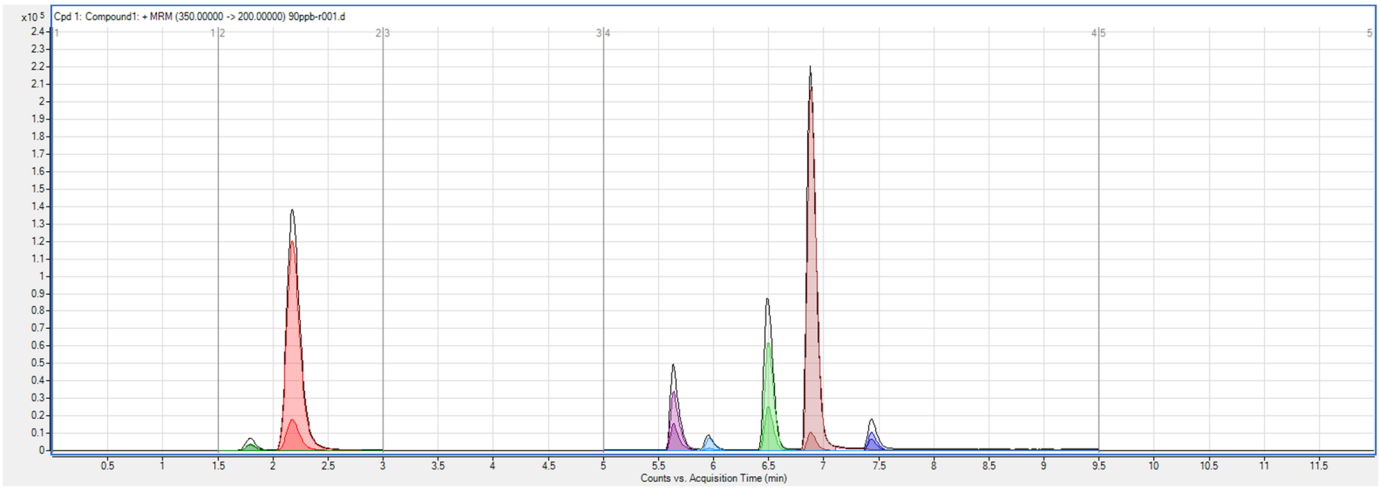

Figure 1. LC–MS/MS chromatogram showing the separation of the selected anticancer drugs with the internal standard (5-fluorouracil: dark green, gemcitabine: red, methotrexate: purple, $^{13}C_3$-caffeine: light blue, cyclophosphamide: light green, tamoxifen: brown, and docetaxel: dark blue).

3.2. System Suitability

The system suitability parameters of the LC–MS/MS method were tested and are presented in Table 4. The results ranged within the established acceptance criteria, except for some instances. The column efficiency for 5-fluorouracil and gemcitabine was below 2000, possibly due to the short retention times of both compounds and the moderate increase in the peak width attributed to the nature of the mobile phase. Moreover, tailing was observed in the obtained chromatographic peaks, as the asymmetry factors were not below two. Tailing could affect the separation of the compounds, hence compromising the resolution. However, good resolution (>1.5) was achieved for all of the compounds, which meant that the chromatographic separation was satisfactory for quantitative analysis [27–30]. Considering the diversity of the tested compounds, the accomplished separation by the developed method was deemed acceptable and adequate for the intended analysis.

Table 4. System suitability parameters of the developed LC–MS/MS method.

Compound	Retention Time (min)	Column Efficiency	Plate Height (cm)	Asymmetry	Resolution
5-FU	1.8	1886	5.30×10^{-3}	2	
GEM	2.2	1672	6.00×10^{-3}	1.5	1.78
MET	5.6	18,496	5.41×10^{-4}	2	16.22
$^{13}C_3$-CAF	5.9	20,736	4.82×10^{-4}	2	2.00
CP	6.5	33,367	2.99×10^{-4}	2	3.67
TAM	6.9	37,378	2.68×10^{-4}	3	2.67
DOC	7.4	43,820	2.28×10^{-4}	1.3	3.70

Nevertheless, more critical parameters were considered when using LC–MS/MS equipment to maintain accurate and reproducible analytical measurements. Commonly, these devices are more susceptible to having inconsistent or drifting responses due to their low limits of detection [31]. Therefore, additional system suitability parameters were controlled on the LC–MS/MS system, including signal stability, response, and carry-over. To achieve this, the instrument's performance was evaluated before, during, and at the end of every batch by: (1) carrying out automatic check-tunes before every analysis to ensure that the required sensitivity was maintained. In case the check-tune failed, auto-tuning was conducted to readjust the resolution; (2) allowing the equipment sufficient

time to equilibrate. This was achieved by injecting blank samples spiked with a constant concentration of the internal standard and starting the analysis only when achieving a consistent signal for the standard; (3) checking that no carry-over was detected in the blank injections; (4) introducing quality control checks at constant intervals during a batch analysis to review the signal stability and consistency of the response; (5) injecting blank samples after QC runs to check for any carry-over; (6) monitoring the signal of the internal standard (spiked at the same concentration in all samples) throughout the analysis to detect any significant drop in the instrumental response; and (7) troubleshooting the instrument before re-initiating the batch if a failure of any of the stated parameters was identified.

3.3. Solid-Phase Extraction Optimization

Two SPE methods were optimized for the extraction of the selected anticancer drugs: Oasis HLB was used for the extraction of methotrexate, cyclophosphamide, tamoxifen, and docetaxel, and the cartridge Isolute ENV+, which is more efficient for the extraction of polar analytes, was utilized for the extraction of 5-fluorouracil and gemcitabine. The preliminary methods generated in previous studies were adopted [32–43]; however, the optimization of some parameters was essential to improve the recoveries of the tested compounds when in a mixture and ensure that the methods were well adapted to the equipment used. The tested parameters included the effects of sample filtration and pH, the nature of the elution solvent and its volume, the time required for drying the cartridges, and the breakthrough volume of each cartridge. The isotopically labelled caffeine standard was added post-extraction to resolve any instrumental variations.

Taking into consideration all of the parameters optimized for each cartridge, the percentage of recovery of the selected compounds was calculated by comparing the responses of the standard when spiked before and after extraction. The outcomes are presented in Table 5. The optimized SPE methods enabled efficient extraction and satisfactory recovery of the tested anticancer drugs, with recovery percentages ranging between 40% and 104%. The low recovery rates of tamoxifen and docetaxel were similar to those achieved in previous studies [44–48]. This could have been due to the high hydrophobicity of these two compounds, which led to high affinity for the sorbent and negatively affected the desorption process [49]. This might be resolved by using an elution solvent with a stronger eluent strength. Moreover, a recovery slightly higher than 100% was observed for cyclophosphamide, indicating a positive error. This could have been due to contamination from materials released from the SPE cartridges that coeluted with cyclophosphamide or other experimental (e.g., volume spiked) or analytical errors (e.g., background noise).

Table 5. Recovery percentages of the selected anticancer drugs after extraction with the optimized SPE methods.

Compound	5-FU	GEM	MET	CP	TAM	DOC
Pre-spike response	3761	92,219	25,576	9046	238,716	2025
	3450	103,297	25,608	8163	222,362	2183
	3299	94,225	24,214	8339	221,330	1984
Post-spike response	4036	97,417	26,175	8324	566,720	3485
	3277	126,620	26,419	8039	584,059	3499
	3438	111,605	25,288	8092	557,580	3679
%Recovery	98.14	86.89	96.80	104.42	39.96	58.14
±SD	6.34	6.88	0.99	3.76	2.04	4.23

Accordingly, as the recoveries were considered acceptable for the intended analysis, the final methods of both SPE cartridges were generated as follows:

(1) Oasis HLB cartridge for the extraction of methotrexate, cyclophosphamide, tamoxifen, and docetaxel:

- Conditioning: 3 mL methanol
- Equilibrating: 3 mL water (pH 5)
- Sample loading: 50 mL (pH 5)
- Washing: 3 mL water (pH 5)
- Drying: 20 min
- Soaking and eluting: 3 mL methanol (soaking for 5 min before eluting)
- Reconstituting: 495 µL methanol and 5 µL internal standard (600 ng/mL)

(2) Isolute ENV+ cartridge for the extraction of 5-fluorouracil and gemcitabine:

- Conditioning: 3 mL × 2 methanol
- Equilibrating: 3 mL × 2 water (pH 5)
- Sample loading: 50 mL (pH 5)
- Washing: 3 mL × 2 water (pH 5)
- Drying: 2 h
- Soaking and eluting: 3 mL × 2 methanol (soaking for 5 min before eluting)
- Reconstituting: 495 µL water and 5 µL internal standard (600 ng/mL)

3.4. SPE–LC–MS/MS Method Validation

The methods were tested for their linearity, accuracy, precision, and limits of detection and quantification (Table 6). The linearity was checked over the estimated range of 5 to 100 ng/L. Acceptable regression coefficients were obtained for all of the compounds with R^2 values higher than 0.995, except for tamoxifen, which was probably due to its low recovery leading to inconsistency in the results. The accuracy and precision were measured from QC samples set at 10, 40, and 70 ng/L for 5-fluorouracil, gemcitabine, methotrexate, cyclophosphamide, and docetaxel and 10, 40, and 85 ng/L for tamoxifen. The overall accuracy of the methods ranged between 100.3% and 108.6%, and precision was between 1.3% and 3.7%; thus, they were in good agreement with the proposed criteria. The limits of detection and quantification were based on the standard deviation of the background responses of ten blank samples. The limit of detection ranged between 0.1 and 0.9 ng/L, and the limit of quantification ranged between 0.3 and 2.8 ng/L. Previous studies reported LOD and LOQ values in the range of 0.00002 and 200 ng/L [50,51]; hence, the developed methods provided satisfactory sensitivity. Finally, the proposed methods demonstrated robustness, as slight and deliberate variations in the mobile phase composition, temperature, and flow rate did not significantly affect the responses of the compounds nor the reliability of the results [52].

Table 6. Validation parameters of the developed SPE–LC–MS/MS methods.

Compound	Range (ng/L)	Regression Coefficient (R2)	Accuracy (%) (±SD)	Interday Precision (%) (±SD)	Intraday Precision (%) (±SD)	LOD (ng/L)	LOQ (ng/L)
5-FU	5–100	0.9975	100.3 (±7.74)	3.7 (±0.94)	2.7 (±1.09)	0.9	2.8
GEM	5–100	0.9994	101.5 (±2.90)	1.3 (±0.82)	2.1 (±1.23)	0.3	1.0
MET	5–100	0.9994	104.0 (±2.11)	2.6 (±0.37)	2.4 (±1.24)	0.4	1.1
CP	5–100	0.9997	101.7 (±2.70)	3.3 (±0.87)	3.0 (±0.87)	0.4	1.1
TAM	5–100	0.9943	100.7 (±8.53)	2.6 (±0.56)	2.5 (±1.62)	0.1	0.3
DOC	5–100	0.9983	108.6 (±2.08)	2.9 (±1.65)	2.8 (±1.36)	0.3	0.8

3.5. Matrix Effect

The specificity of the developed analytical method was assessed by evaluating the matrix effect. Environmental samples could potentially contain organic and/or inorganic molecules that could co-elute and interfere with the analyzed compounds during the ionization process, thus reducing or enhancing their responses' intensity. Although these matrix components are not detected, they could adversely affect the sensitivity of the method [53–55]. Considering the high variability in the occurrence of co-eluting compounds depending on the nature of the sample [54], the matrix effect was predicted from influent WWTP samples since they represented the worst possible matrix conditions among the collected samples.

The matrix effect was calculated for each compound at three different concentrations, and the results are presented in Table 7. In theory, values of 100% suggested no matrix effect was observed. Values higher than 100% indicated an ionization enhancement, and values lower than 100% indicated an ionization suppression. In this case, the findings fluctuated between the different compounds and ranged from weak (>51%), to medium (21–50%), to strong matrix effects (<20%) [56], though showing, in general, a signal suppression.

Table 7. Calculated matrix effect on the signal of the selected anticancer drugs and correction of the matrix effect by the internal standard.

Compound	Matrix Effect (%) (±SD)		IS Corrected Matrix Effect (%) (±SD)	
5-FU	L: 32 (±10)	Suppression	L: 62 (±15)	Suppression
	M: 39 (±4)	Suppression	M: 84 (±12)	Suppression
	H: 34 (±18)	Suppression	H: 64 (±37)	Suppression
GEM	L: 81 (±6)	Suppression	L: 161 (±13)	Enhancement
	M: 81 (±19)	Suppression	M: 177 (±38)	Enhancement
	H: 72 (±16)	Suppression	H: 146 (±56)	Enhancement
MET	L: 153 (±24)	Enhancement	L: 344 (±80)	Enhancement
	M: 95 (±2)	Suppression	M: 189 (±12)	Enhancement
	H: 88 (±3)	Suppression	H: 166 (±5)	Enhancement
CP	L: 51 (±28)	Suppression	L: 112 (±68)	Enhancement
	M: 41 (±4)	Suppression	M: 82 (±9)	Suppression
	H: 40 (±2)	Suppression	H: 76 (±8)	Suppression
TAM	L: 51 (±2)	Suppression	L: 114 (±16)	Enhancement
	M: 47 (±2)	Suppression	M: 93 (±7)	Suppression
	H: 49 (±2)	Suppression	H: 92 (±9)	Suppression
DOC	L: 17 (±2)	Suppression	L: 39 (±9)	Suppression
	M: 16 (±1)	Suppression	M: 32 (±4)	Suppression
	H: 16 (±2)	Suppression	H: 30 (±5)	Suppression
IS	Matrix Effect (%) (±SD)			
$^{13}C_3$-CAF	L: 44 (±5)		Suppression	
	M: 51 (±3)		Suppression	
	H: 53 (±3)		Suppression	

To achieve accurate results, matrix effects should be entirely eliminated. However, it is generally agreed upon that this cannot be experimentally accomplished due to the extreme variation of matrices. Therefore, additional effort was required to reduce these effects as much as possible. This could be achieved by lowering the matrix compounds in the samples (e.g., improving sample preparation, modifying chromatographic conditions) and by compensating for the matrix effects with analytical calibration methods (e.g., matrix-matched external standard calibration, internal standard calibration, standard addition calibration) [57].

In the case of sample preparation, filtration and SPE were formerly adopted and seemed to provide moderate selectivity to reduce the matrix effect. Nevertheless, to

overcome the loss of signal, an isotopically labelled internal standard was added to the calibration standards and samples at the same concentration. This method could enable matrix effect compensation as well as instrumental drift compensation throughout the analysis [57]. Since obtaining structural analogs of the selected compounds or several isotopically labelled standards was restricted by their availabilities and high costs, using a single labelled internal standard was preferred. As shown in the results in Table 7, it appeared that the $^{13}C_3$-caffeine internal standard was affected by the matrix in the same way as the target compounds and approximately to the same extent, which was recommended for matrix effect compensation [58]. Therefore, $^{13}C_3$-caffeine proved to be a reasonable alternative to recover the signal suppression for all of the compounds; thus, the matrix could account for up to 30% of suppression.

3.6. Method Application

The occurrence of the selected anticancer drugs was assessed in Lebanese water samples collected from 22 different locations by adopting the developed and validated method. Considering the high complexity of the matrices, especially in wastewater samples, it was expected that some interfering peaks would be detected at the same or close to the retention times of the target analytes [56]. Moreover, as different matrices could contain different interferences, the extraction technique could not be specific to all of the tested matrices and remove all interferences [57]. Therefore, to avoid false-positive detection, additional criteria were applied for confirmation: (1) the retention time shift should not be higher than ± 0.5 min; (2) both quantitative and confirmation ions should be detected at the same retention time; and (3) the ratio between the quantitative and confirmation ions (SRM1/SRM2) should not deviate by more than ± 20% in surface water and ± 50% in wastewater, as higher variation was estimated in more complex matrices [35,59–62].

As a result, the concentrations of the detected anticancer drugs were determined from standard calibration curves and are presented in Table 8. Three out of the six selected anticancer drugs were detected mainly in wastewater samples, except for 5-fluorouracil, which was also detected in two rivers at low concentrations. It was noticed that in some cases, the concentrations detected in effluent samples were higher than those detected in influent samples, as they could correspond to different wastewater collection dates.

Lebanese water resources have been chronically suffering from mismanagement and severe pollution from untreated sewage [8]. In fact, a report by Ali Karnib in 2016 revealed that 58.54% of the population was connected to sewerage systems. The rest of the population used on-site sanitation (e.g., septic tanks, pit latrines) or discharged the untreated wastewater into streams. Moreover, results showed that only 11.65% of the population in the North of Lebanon and only 6.87% of the population in Beirut and Mount Lebanon were connected to safely managed wastewater collection systems. Hence, almost 90% of the generated wastewater was discharged without any treatment into the aquatic environment, including rivers and coastal outfalls [63,64]. This could explain the occurrence of 5-fluorouracil in the Awali and Damour Rivers since previous studies demonstrated excessive fecal coliform contamination, thus confirming the discharge of raw sewage in these rivers [65,66]. Furthermore, the detection of 5-fluorouracil, methotrexate, and cyclophosphamide in influent as well as effluent and coastal sewage outfalls validated the current non-operational conditions of WWTPs in Lebanon [64].

Table 8. Concentration levels of the selected anticancer drugs in Lebanese environmental samples.

Sample Location	Concentration (ng/L) (±SD)					
	5-FU	**GEM**	**MET**	**CP**	**TAM**	**DOC**
Antelias River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Al Kalb River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Zahrani River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Hasbani River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Litani River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Wazzani River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Awali River	8 ± 3	<lod	n.d.	n.d.	n.d.	n.d.
Damour River	3 ± 1	n.d.	n.d.	n.d.	n.d.	n.d.
Orontes River	<loq	n.d.	n.d.	n.d.	n.d.	n.d.
Al Bared River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ibrahim River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Al Jawz River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ostouene River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Abou Ali River	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
WWTP Joub Jannine Influent	211 ± 41 *	n.d	n.d.	3 ± 2	n.d.	n.d.
WWTP Joub Jannine Effluent	15 ± 2	<lod	15 ± 3	1.4 ± 1	n.d.	n.d.
WWTP Saida Influent	118 ± 28 *	n.d.	20 ± 3	1.5 ± 1	n.d.	n.d.
WWTP Saida Effluent	128 ± 39 *	n.d.	21 ± 4	n.d.	n.d.	n.d.
WWTP Al Ghadir Influent	195 ± 59 *	n.d.	7 ± 2	4 ± 2	n.d.	n.d.
WWTP Al Ghadir Effluent	305 ± 95 *	n.d.	n.d.	12 ± 1	n.d.	n.d.
Ramlet Al Baida Outfall	6 ± 3	n.d.	n.d.	n.d.	n.d.	n.d.
Jiyeh Outfall	130 ± 30 *	n.d.	31 ± 3	2 ± 2	n.d.	n.d.

Notes: * Concentrations were higher than 100 ng/L; hence they were extrapolated from the calibration curve; n.d.: not detected.

The results obtained in this analysis were comparable with those of previous studies evaluating the occurrence of anticancer drugs in the aquatic environment (Table 9). For 5-fluorouracil, high concentrations were detected in hospital wastewater samples ranging between 27 and 4000 ng/L in Switzerland and France [33,37]. However, lower concentrations were obtained in WWTP influents as opposed to the concentrations found in Lebanon. The levels of 5-fluorouracil in wastewater influent samples ranged between 3.1 and 14 ng/L in Spain and Slovenia [35,41] and it was never detected in wastewater effluent nor surface water in any country.

Regarding methotrexate and cyclophosphamide, previous research achieved similar ranges of concentrations in wastewater influent and effluent samples [21], except for a few cases where higher concentrations were identified. For instance, methotrexate concentrations reached 303 and 433 ng/L in WWTP influents in Slovenia and Greece, respectively [35,67], and cyclophosphamide was detected at concentrations up to 13,100 ng/L in Spain [61].

Table 9. Previously reported 5-fluorouracil, methotrexate, and cyclophosphamide concentrations in environmental and wastewater samples.

Compound	Sample Source	Concentration (ng/L)	Ref.
5-FU	Switzerland—Hospital WW	27	[33]
	France—Hospital WW	90–4000	[37]
	Slovenia—WWTP influent	3.1–14	[35,41]
	Spain—WWTP influent	3.5	[35]
MET	Italy—WWTP effluent	12.6	[68]
	China—Hospital WW	4–4689	[69]
	China—WWTP influent	1.6–18.1	[70]
	Spain—WWTP influent	2.1–20.1	[51]
	Canada—WWTP influent	17–60	[71]
	Canada—WWTP effluent	13–53	[71]
	Spain—WWTP influent	7.3–55.8	[48]
	Slovenia—WWTP influent	303	[35]
	Greece—WWTP influent	433	[67]
CP	Italy—WWTP effluent	2.1–9.0	[68]
	Switzerland—Surface water	0.05–0.17	[72]
	China—Hospital WW	6–2000	[69]
	Canada—WWTP influent	17–22	[71]
	Canada—WWTP effluent	18–21	[71]
	Spain—WWTP influent	13,100	[61]

Although variations in concentrations were observed in different countries, these were expected as the levels of an analyte in the aquatic environment could be affected by several parameters. These include the consumption rate of the anticancer drug, population served by the WWTP, and type of treatment adopted in the operational plant [67], in addition to further variability factors such as the sample source, seasonal variability, and method sensitivity [21].

4. Conclusions

In summary, two SPE–LC–MS/MS methodologies were developed and validated in compliance with the ICH guidelines for the detection of six anticancer drugs in Lebanese wastewater and surface water. Three anticancer drugs were detected, 5-fluorouracil, methotrexate, and cyclophosphamide, in water samples collected between the 5th and 10th of January 2022. By detecting these anticancer drugs in effluent wastewater samples as well as river samples, this study reinforced previous research related to surface water pollution and contamination by raw sewage in addition to the mismanagement of wastewater and unsatisfactory treatment technologies adopted by the few operational WWTPs in Lebanon.

At the levels detected, these drugs alone would not cause any significant effects to the aquatic biota, except for 5-fluorouracil, for which adverse effects were reported in several instances at concentrations in the range of ng/L, including DNA damage, modification of enzymatic activities, and swimming behavior [73]. Nevertheless, the continuous discharge of untreated wastewater that contains recalcitrant compounds such as anticancer drugs can have a significant environmental impact. In developing countries such as Lebanon, waste management is often disregarded [74]. Indeed, if no stringent actions are taken, this can gradually lead to the deterioration of water resources, which affects the whole ecosystem and human health and extends to neighboring countries.

Finally, the results presented in this study offer scope for future research, such as the study of seasonal and geographical variability in correlation with the consumption rates of the selected anticancer drugs in Lebanon, in order to eliminate inaccuracies caused by these factors and reliably estimate the total concentration of anticancer drugs in the aquatic environment.

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References

1. Hernández-Tenorio, R.; González-Juárez, E.; Guzmán-Mar, J.L.; Hinojosa-Reyes, L.; Hernández-Ramírez, A. Review of Occurrence of Pharmaceuticals Worldwide for Estimating Concentration Ranges in Aquatic Environments at the End of the Last Decade. *J. Hazard. Mater. Adv.* **2022**, *8*, 100172. [CrossRef]
2. Courtier, A.; Cadiere, A.; Roig, B. Human Pharmaceuticals: Why and How to Reduce Their Presence in the Environment. *Curr. Opin. Green Sustain. Chem.* **2019**, *15*, 77–82. [CrossRef]
3. Adeleye, A.S.; Xue, J.; Zhao, Y.; Taylor, A.A.; Zenobio, J.E.; Sun, Y.; Han, Z.; Salawu, O.A.; Zhu, Y. Abundance, Fate, and Effects of Pharmaceuticals and Personal Care Products in Aquatic Environments. *J. Hazard. Mater.* **2022**, *424*, 127284. [CrossRef]
4. Li, W.C. Occurrence, Sources, and Fate of Pharmaceuticals in Aquatic Environment and Soil. *Environ. Pollut.* **2014**, *187*, 193–201. [CrossRef]
5. Branchet, P.; Arpin-Pont, L.; Piram, A.; Boissery, P.; Wong-Wah-Chung, P.; Doumenq, P. Pharmaceuticals in the Marine Environment: What Are the Present Challenges in Their Monitoring? *Sci. Total Environ.* **2021**, *766*, 142644. [CrossRef] [PubMed]
6. Dulio, V.; Slobodnik, J. NORMAN-Network of Reference Laboratories, Research Centres and Related Organisations for Monitoring of Emerging Substances. *Environ. Sci. Pollut. Res.* **2009**, *16*, 132–135. [CrossRef] [PubMed]
7. Gomez Cortes, L.; Marinov, D.; Sanseverino, I.; Navarro Cuenca, A.; Niegowska, M.; Porcel Rodriguez, E.; Stefanelli, F.; Lettieri, T. *Selection of Substances for the 4th Watch List under the Water Framework Directive*; Publications Office of the European Union: Luxembourg, 2022.
8. Geara-Matta, D.; Moilleron, R.; El Samarani, A.; Lorgeoux, C.; Chebbo, G. State of Art About Water Uses and Wastewater Management in Lebanon. *World Wide Workshop Young Environ. Sci.* **2010**, *11*, 139–152.
9. MOE/UNDP/ECODIT. State and Trends of the Lebanese Environment. 2011. Available online: https://www.undp.org/lebanon/publications/state-trends-lebanese-environment (accessed on 10 April 2023).
10. Houri, A.; El Jeblawi, S.W. Water Quality Assessment of Lebanese Coastal Rivers during Dry Season and Pollution Load into the Mediterranean Sea. *J. Water Health* **2007**, *5*, 615–623. [CrossRef] [PubMed]
11. Merhaby, D.; Ouddane, B.; Net, S.; Halwani, J. Assessment of Persistent Organic Pollutants in Surface Sediments along Lebanese Coastal Zone. *Mar. Pollut. Bull.* **2020**, *153*, 110947. [CrossRef]
12. Abboud-Abi Saab, M.; Hassoun, A.E.R. Effects of Organic Pollution on Environmental Conditions and the Phytoplankton Community in the Central Lebanese Coastal Waters with Special Attention to Toxic Algae. *Reg. Stud. Mar. Sci.* **2017**, *10*, 38–51. [CrossRef]
13. MOE/EU/UNDP. Lebanon Environmental Assessment of the Syrian Conflict & Priority Interventions. 2014. Available online: https://www.undp.org/lebanon/publications/lebanon-environmental-assessment-syrian-conflict (accessed on 10 April 2023).
14. World Bank. *Lebanon Country Water Sector Assistance Strategy*; World Bank: Washington, DC, USA, 2012.
15. Tokajian, S.; Moghnieh, R.; Salloum, T.; Arabaghian, H.; Alousi, S.; Moussa, J.; Abboud, E.; Youssef, S.; Husni, R. Extended-Spectrum β-Lactamase-Producing Escherichia Coli in Wastewaters and Refugee Camp in Lebanon. *Future Microbiol.* **2018**, *13*, 81–95. [CrossRef]
16. Nehme, N.; Haydar, C.; Koubaissy, B.; Fakih, M.; Awad, S.; Toufaily, J.; Villieras, F.; Hamieh, T. The Distribution of Heavy Metals in the Lower River Basin, Lebanon. *Phys. Procedia* **2014**, *55*, 456–463. [CrossRef]
17. Merhabi, F.; Gomez, E.; Castro, N.A.; Amine, H.; Rosain, D.; Halwani, J.; Fenet, H. Occurrence and Ecological Risk Assessment of Pharmaceutical Products in the Kadicha River in Lebanon. *Emerg. Contam.* **2021**, *7*, 196–203. [CrossRef]
18. Doummar, J.; Geyer, T.; Baierl, M.; Nödler, K.; Licha, T.; Sauter, M. Carbamazepine Breakthrough as Indicator for Specific Vulnerability of Karst Springs: Application on the Jeita Spring, Lebanon. *Appl. Geochem.* **2014**, *47*, 150–156. [CrossRef]

19. Mokh, S.; El Khatib, M.; Koubar, M.; Daher, Z.; Al Iskandarani, M. Innovative SPE-LC-MS/MS Technique for the Assessment of 63 Pharmaceuticals and the Detection of Antibiotic-Resistant-Bacteria: A Case Study Natural Water Sources in Lebanon. *Sci. Total Environ.* **2017**, *609*, 830–841. [CrossRef] [PubMed]
20. Mohty, R.; Tfayli, A. General Oncology Care in Lebanon. In *Cancer in the Arab World*; Springer: Singapore, 2022; pp. 115–132; ISBN 9789811679445.
21. Nassour, C.; Barton, S.J.; Nabhani-Gebara, S.; Saab, Y.; Barker, J. Occurrence of Anticancer Drugs in the Aquatic Environment: A Systematic Review. *Environ. Sci. Pollut. Res.* **2020**, *27*, 1339–1347. [CrossRef] [PubMed]
22. ICH Harmonised Triopartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1). Available online: https://database.ich.org/sites/default/files/Q2%28R1%29Guideline.pdf (accessed on 13 July 2022).
23. Barbarin, N.; Henion, J.D.; Wu, Y. Comparison between Liquid Chromatography-UV Detection and Liquid Chromatography-Mass Spectrometry for the Characterization of Impurities and/or Degradants Present in Trimethoprim Tablets. *J. Chromatogr. A* **2002**, *970*, 141–154. [CrossRef] [PubMed]
24. Kruve, A.; Kaupmees, K. Adduct Formation in ESI/MS by Mobile Phase Additives. *J. Am. Soc. Mass Spectrom.* **2017**, *28*, 887–894. [CrossRef] [PubMed]
25. Mortier, K.A.; Zhang, G.-F.; Van Peteghem, C.H.; Lambert, W.E. Adduct Formation in Quantitative Bioanalysis: Effect of Ionization Conditions on Paclitaxel. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 585–592. [CrossRef]
26. Blumer, M.R.; Chang, C.H.; Brayfindley, E.; Nunez, J.R.; Colby, S.M.; Renslow, R.S.; Metz, T.O. Mass Spectrometry Adduct Calculator. *J. Chem. Inf. Model.* **2021**, *61*, 5721–5725. [CrossRef]
27. Ravisankar, P.; Naga Navya, C.; Pravallika, D.; Sri, D.N. A Review on Step-by-Step Analytical Method Validation. *IOSR J. Pharm.* **2015**, *5*, 2250–3013.
28. Awasthi, A.; Kumar, A.; Kumar, R.; Vishwas, S.; Khursheed, R.; Kaur, J.; Corrie, L.; Kumar, B.; Gulati, M.; Kumar, D.; et al. RP-HPLC Method Development and Validation for Simultaneous Estimation of Mesalamine and Curcumin in Bulk Form as Well as Nanostructured Lipid Carriers. *South African, J. Bot.* **2022**, *151*, 529–537. [CrossRef]
29. Huang, C.; Liu, C.; Tang, J.; Fang, Y.; Ji, L.; Hu, Q.; Li, Q.; Xie, M.; Chen, Z. Development and Validation of High-Performance Liquid Chromatography Method for Analysis of β-CCT and Its Related Substances. *Arab. J. Chem.* **2022**, *15*, 103725. [CrossRef]
30. Berruex, L.G.; Freitag, R. Separation and Purification of Biochemicals. In *Encyclopedia of Physical Science and Technology*; Elsevier: Amsterdam, The Netherlands, 2003; pp. 651–673.
31. Briscoe, C.J.; Stiles, M.R.; Hage, D.S. System Suitability in Bioanalytical LC/MS/MS. *J. Pharm. Biomed. Anal.* **2007**, *44*, 484–491. [CrossRef]
32. Tauxe-Wuersch, A.; De Alencastro, L.F.; Grandjean, D.; Tarradellas, J. Trace Determination of Tamoxifen and 5-Fluorouracil in Hospital and Urban Wastewaters. *Int. J. Environ. Anal. Chem.* **2006**, *86*, 473–485. [CrossRef]
33. Kovalova, L.; McArdell, C.S.; Hollender, J. Challenge of High Polarity and Low Concentrations in Analysis of Cytostatics and Metabolites in Wastewater by Hydrophilic Interaction Chromatography/Tandem Mass Spectrometry. *J. Chromatogr. A* **2009**, *1216*, 1100–1108. [CrossRef] [PubMed]
34. Petrie, B.; Youdan, J.; Barden, R.; Kasprzyk-Hordern, B. Multi-Residue Analysis of 90 Emerging Contaminants in Liquid and Solid Environmental Matrices by Ultra-High-Performance Liquid Chromatography Tandem Mass Spectrometry. *J. Chromatogr. A* **2016**, *1431*, 64–78. [CrossRef] [PubMed]
35. Isidori, M.; Lavorgna, M.; Russo, C.; Kundi, M.; Žegura, B.; Novak, M.; Filipič, M.; Mišík, M.; Knasmueller, S.; de Alda, M.L.; et al. Chemical and Toxicological Characterisation of Anticancer Drugs in Hospital and Municipal Wastewaters from Slovenia and Spain. *Environ. Pollut.* **2016**, *219*, 275–287. [CrossRef] [PubMed]
36. Gros, M.; Petrović, M.; Barceló, D. Tracing Pharmaceutical Residues of Different Therapeutic Classes in Environmental Waters by Using Liquid Chromatography/Quadrupole-Linear Ion Trap Mass Spectrometry and Automated Library Searching. *Anal. Chem.* **2009**, *81*, 898–912. [CrossRef] [PubMed]
37. Mullot, J.U.; Karolak, S.; Fontova, A.; Huart, B.; Levi, Y. Development and Validation of a Sensitive and Selective Method Using GC/MS-MS for Quantification of 5-Fluorouracil in Hospital Wastewater. *Anal. Bioanal. Chem.* **2009**, *394*, 2203–2212. [CrossRef]
38. Ort, C.; Lawrence, M.G.; Rieckermann, J.; Joss, A. Sampling for Pharmaceuticals and Personal Care Products (PPCPs) and Illicit Drugs in Wastewater Systems: Are Sampling for PPCPs in Wastewater Systems: Comparison of Different Sampling Modes and Optimization Strategies. *Environ. Sci. Technol.* **2010**, *44*, 6024–6035. [CrossRef] [PubMed]
39. López-Serna, R.; Petrović, M.; Barceló, D. Development of a Fast Instrumental Method for the Analysis of Pharmaceuticals in Environmental and Wastewaters Based on Ultra High Performance Liquid Chromatography (UHPLC)-Tandem Mass Spectrometry (MS/MS). *Chemosphere* **2011**, *85*, 1390–1399. [CrossRef]
40. Verlicchi, P.; Al Aukidy, M.; Galletti, A.; Petrovic, M.; Barceló, D. Hospital Effluent: Investigation of the Concentrations and Distribution of Pharmaceuticals and Environmental Risk Assessment. *Sci. Total Environ.* **2012**, *430*, 109–118. [CrossRef]
41. Kosjek, T.; Perko, S.; Žigon, D.; Heath, E. Fluorouracil in the Environment: Analysis, Occurrence, Degradation and Transformation. *J. Chromatogr. A* **2013**, *1290*, 62–72. [CrossRef]
42. Česen, M.; Kosjek, T.; Laimou-Geraniou, M.; Kompare, B.; Širok, B.; Lambropolou, D.; Heath, E. Occurrence of Cyclophosphamide and Ifosfamide in Aqueous Environment and Their Removal by Biological and Abiotic Wastewater Treatment Processes. *Sci. Total Environ.* **2015**, *527–528*, 465–473. [CrossRef] [PubMed]

43. Česen, M.; Kosjek, T.; Busetti, F.; Kompare, B.; Heath, E. Human Metabolites and Transformation Products of Cyclophosphamide and Ifosfamide: Analysis, Occurrence and Formation during Abiotic Treatments. *Environ. Sci. Pollut. Res.* **2016**, *23*, 11209–11223. [CrossRef]
44. Zhang, Z.L.; Zhou, J.L. Simultaneous Determination of Various Pharmaceutical Compounds in Water by Solid-Phase Extraction-Liquid Chromatography-Tandem Mass Spectrometry. *J. Chromatogr. A* **2007**, *1154*, 205–213. [CrossRef]
45. Zhou, J.L.; Zhang, Z.L.; Banks, E.; Grover, D.; Jiang, J.Q. Pharmaceutical Residues in Wastewater Treatment Works Effluents and Their Impact on Receiving River Water. *J. Hazard. Mater.* **2009**, *166*, 655–661. [CrossRef] [PubMed]
46. Martín, J.; Camacho-Muñoz, D.; Santos, J.L.; Aparicio, I.; Alonso, E. Simultaneous Determination of a Selected Group of Cytostatic Drugs in Water Using High-Performance Liquid Chromatography-Triple-Quadrupole Mass Spectrometry. *J. Sep. Sci.* **2011**, *34*, 3166–3177. [CrossRef]
47. Ferrando-Climent, L.; Rodriguez-Mozaz, S.; Barceló, D. Development of a UPLC-MS/MS Method for the Determination of Ten Anticancer Drugs in Hospital and Urban Wastewaters, and Its Application for the Screening of Human Metabolites Assisted by Information-Dependent Acquisition Tool (IDA) in Sewage Samples. *Anal. Bioanal. Chem.* **2013**, *405*, 5937–5952. [CrossRef]
48. Martín, J.; Camacho-Muñoz, D.; Santos, J.L.; Aparicio, I.; Alonso, E. Occurrence and Ecotoxicological Risk Assessment of 14 Cytostatic Drugs in Wastewater. *Water. Air. Soil Pollut.* **2014**, *225*, 1896. [CrossRef]
49. Majors, R.E. Chapter 7—Solid-Phase Extraction. In *Handbook of Sample Preparation*; Wiley-Blackwell: Hoboken, NJ, USA, 2011; ISBN 9780470099346.
50. Biel-Maeso, M.; Baena-Nogueras, R.M.; Corada-Fernández, C.; Lara-Martín, P.A. Occurrence, Distribution and Environmental Risk of Pharmaceutically Active Compounds (PhACs) in Coastal and Ocean Waters from the Gulf of Cadiz (SW Spain). *Sci. Total Environ.* **2018**, *612*, 649–659. [CrossRef]
51. Negreira, N.; López de Alda, M.; Barceló, D. On-Line Solid Phase Extraction-Liquid Chromatography-Tandem Mass Spectrometry for the Determination of 17 Cytostatics and Metabolites in Waste, Surface and Ground Water Samples. *J. Chromatogr. A* **2013**, *1280*, 64–74. [CrossRef]
52. Swartz, M.E.; Krull, I.S. *Handbook of Analytical Validation*; CRC Press: Boca Raton, FL, USA, 2012; ISBN 9781420014488.
53. Taylor, P.J. Matrix Effects: The Achilles Heel of Quantitative High-Performance Liquid Chromatography-Electrospray-Tandem Mass Spectrometry. *Clin. Biochem.* **2005**, *38*, 328–334. [CrossRef]
54. Kloepfer, A.; Quintana, J.B.; Reemtsma, T. Operational Options to Reduce Matrix Effects in Liquid Chromatography- Electrospray Ionisation-Mass Spectrometry Analysis of Aqueous Environmental Samples. *J. Chromatogr. A* **2005**, *1067*, 153–160. [CrossRef]
55. Matuszewski, B.K.; Constanzer, M.L.; Chavez-Eng, C.M. Strategies for the Assessment of Matrix Effect in Quantitative Bioanalytical Methods Based on HPLC-MS/MS. *Anal. Chem.* **2003**, *75*, 3019–3030. [CrossRef] [PubMed]
56. Ferrer Amate, C.; Unterluggauer, H.; Fischer, R.J.; Fernández-Alba, A.R.; Masselter, S. Development and Validation of a LC-MS/MS Method for the Simultaneous Determination of Aflatoxins, Dyes and Pesticides in Spices. *Anal. Bioanal. Chem.* **2010**, *397*, 93–107. [CrossRef] [PubMed]
57. Raposo, F.; Barceló, D. Challenges and Strategies of Matrix Effects Using Chromatography-Mass Spectrometry: An Overview from Research versus Regulatory Viewpoints. *TrAC—Trends Anal. Chem.* **2021**, *134*, 116068. [CrossRef]
58. Benijts, T.; Dams, R.; Lambert, W.; De Leenheer, A. Countering Matrix Effects in Environmental Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry Water Analysis for Endocrine Disrupting Chemicals. *J. Chromatogr. A* **2004**, *1029*, 153–159. [CrossRef] [PubMed]
59. Angeles, L.F.; Aga, D.S. Establishing Analytical Performance Criteria for the Global Reconnaissance of Antibiotics and Other Pharmaceutical Residues in the Aquatic Environment Using Liquid Chromatography-Tandem Mass Spectrometry. *J. Anal. Methods Chem.* **2018**, *2018*, 7019204. [CrossRef]
60. Kovács, R.; Csenki, Z.; Bakos, K.; Urbányi, B.; Horváth, Á.; Garaj-Vrhovac, V.; Gajski, G.; Gerić, M.; Negreira, N.; López de Alda, M.; et al. Assessment of Toxicity and Genotoxicity of Low Doses of 5-Fluorouracil in Zebrafish (Danio Rerio) Two-Generation Study. *Water Res.* **2015**, *77*, 201–212. [CrossRef] [PubMed]
61. Gómez-Canela, C.; Cortés-Francisco, N.; Oliva, X.; Pujol, C.; Ventura, F.; Lacorte, S.; Caixach, J. Occurrence of Cyclophosphamide and Epirubicin in Wastewaters by Direct Injection Analysis-Liquid Chromatography-High-Resolution Mass Spectrometry. *Environ. Sci. Pollut. Res.* **2012**, *19*, 3210–3218. [CrossRef] [PubMed]
62. Gómez-Canela, C.; Ventura, F.; Caixach, J.; Lacorte, S. Occurrence of Cytostatic Compounds in Hospital Effluents and Wastewaters, Determined by Liquid Chromatography Coupled to High-Resolution Mass Spectrometry. *Anal. Bioanal. Chem.* **2014**, *406*, 3801–3814. [CrossRef] [PubMed]
63. Karnib, A. Assessing Population Coverage of Safely Managed Wastewater Systems: A Case Study of Lebanon. *J. Water Sanit. Hyg. Dev.* **2016**, *6*, 313–319. [CrossRef]
64. Dagher, L.A.; Hassan, J.; Kharroubi, S.; Jaafar, H.; Kassem, I.I. Nationwide Assessment of Water Quality in Rivers across Lebanon by Quantifying Fecal Indicators Densities and Profiling Antibiotic Resistance of Escherichia Coli. *Antibiotics* **2021**, *10*, 883. [CrossRef] [PubMed]
65. Korfali, S.I.; Jurdi, M. Suitability of Surface Water for Domestic Water Use: Awali River Case Study. *Eur. Water* **2011**, *35*, 3–12.
66. Massoud, M.A. Assessment of Water Quality along a Recreational Section of the Damour River in Lebanon Using the Water Quality Index. *Environ. Monit. Assess.* **2012**, *184*, 4151–4160. [CrossRef] [PubMed]

67. Ofrydopoulou, A.; Nannou, C.; Evgenidou, E.; Christodoulou, A.; Lambropoulou, D. Assessment of a Wide Array of Organic Micropollutants of Emerging Concern in Wastewater Treatment Plants in Greece: Occurrence, Removals, Mass Loading and Potential Risks. *Sci. Total Environ.* **2022**, *802*, 149860. [CrossRef]
68. Castiglioni, S.; Bagnati, R.; Calamari, D.; Fanelli, R.; Zuccato, E. A Multiresidue Analytical Method Using Solid-Phase Extraction and High-Pressure Liquid Chromatography Tandem Mass Spectrometry to Measure Pharmaceuticals of Different Therapeutic Classes in Urban Wastewaters. *J. Chromatogr. A* **2005**, *1092*, 206–215. [CrossRef]
69. Yin, J.; Shao, B.; Zhang, J.; Li, K. A Preliminary Study on the Occurrence of Cytostatic Drugs in Hospital Effluents in Beijing, China. *Bull. Environ. Contam. Toxicol.* **2010**, *84*, 39–45. [CrossRef]
70. Yin, J.; Yang, Y.; Li, K.; Zhang, J.; Shao, B. Analysis of Anticancer Drugs in Sewage Water by Selective SPE and UPLC-ESI-MS-MS. *J. Chromatogr. Sci.* **2010**, *48*, 781–789. [CrossRef] [PubMed]
71. Rabii, F.W.; Segura, P.A.; Fayad, P.B.; Sauvé, S. Determination of Six Chemotherapeutic Agents in Municipal Wastewater Using Online Solid-Phase Extraction Coupled to Liquid Chromatography-Tandem Mass Spectrometry. *Sci. Total Environ.* **2014**, *487*, 792–800. [CrossRef] [PubMed]
72. Buerge, I.J.; Buser, H.R.; Poiger, T.; Müller, M.D. Occurrence and Fate of the Cytostatic Drugs Cyclophosphamide and Ifosfamide in Wastewater and Surface Waters. *Environ. Sci. Technol.* **2006**, *40*, 7242–7250. [CrossRef] [PubMed]
73. Nassour, C.; Nabhani-Gebara, S.; Barton, S.J.; Barker, J. Aquatic Ecotoxicology of Anticancer Drugs: A Systematic Review. *Sci. Total Environ.* **2021**, *800*, 149598. [CrossRef] [PubMed]
74. Nassour, C.; Nabhani-Gebara, S.; Barton, S.J.; Barker, J. Anti-Cancer Drug Waste Disposal Practices and Wastewater Management in Hospitals: A Lebanese Survey. *J. Oncol. Pharm. Pract.* **2023**. [CrossRef] [PubMed]