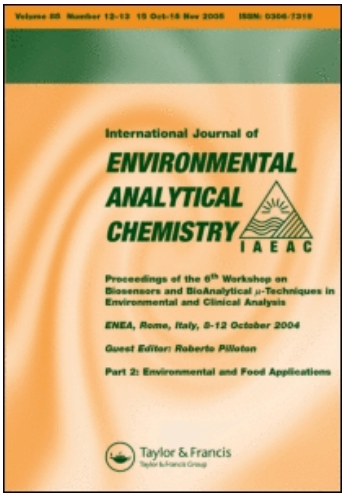

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Trace determination of tamoxifen and 5-fluorouracil in hospital and urban wastewaters

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To cite this Article Tauxe-Wuersch, Annick , De Alencastro, Luiz Felippe , Grandjean, Dominique and Tarradellas, Joseph(2006) 'Trace determination of tamoxifen and 5-fluorouracil in hospital and urban wastewaters', International Journal of Environmental Analytical Chemistry, 86: 7, 473 — 485
To link to this Article: DOI: 10.1080/03067310500291502
URL: http://dx.doi.org/10.1080/03067310500291502

Intern. J. Environ. Anal. Chem.
Vol. 86, No. 7, 15 June 2006, 473–485

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(*Received 7 April 2005; in final form 18 July 2005*)

Tamoxifen and 5-fluorouracil are widely used in cancer therapy. They are highly toxic (teratogenic, mutagenic, etc.), as are most of the anticancer drugs. Two methods were set up to analyse these drugs in wastewaters to evaluate the potential for environmental contamination by cytostatic agents. Liquid–liquid extraction followed by purification on OASIS® MCX cartridge and gas chromatography with mass spectrometry detection (GC-MS) was used for the analysis of tamoxifen. 5-Fluorouracil was extracted with an ENV+ (Isolute) cartridge (solid-phase extraction), derivatized with pentafluorobenzyl bromide (PFBBr) and detected by GC-MS. Both methods showed good recoveries (>70%), repeatability (RSD < 10%) and limits of detection (LOD 6–15 ng/L). Wastewaters from a residential area, a hospital, and sewage-treatment plants (STPs) were analysed using the analytical methods developed in this study. Tamoxifen was detected in wastewaters of the hospital, residential area, and influent of STPs, but not in treated wastewaters. 5-Fluorouracil in all wastewaters was below the LOD of the analytical method.

Keywords: Tamoxifen; 5-Fluorouracil; Cytostatic; Chemotherapy; Cancer; Pharmaceuticals; Wastewaters; Sewage-treatment plants

1. Introduction

Many drugs are detected in aquatic environments [1, 2]. Most studies are based on widely used pharmaceutical compounds such as anti-inflammatory drugs [3, 4], which have a low toxicity. Few cytostatic substances have been studied [5–7], and no ecotoxicological data on cytostatic have been published. However, antineoplastic drugs are very toxic (mutagenic, carcinogenic, or teratogenic) for humans [8], and this is a group of potential concern for environmental effects [9].

Our study focused on tamoxifen and 5-fluorouracil, since they are two of the most commonly used anticancer drugs (see table 1). Tamoxifen is a non-steroidal anti-oestrogen that is used as adjuvant therapy for breast cancer, and it is undergoing several clinical trials as a chemo-preventive agent in healthy women at risk of breast cancer.

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International Journal of Environmental Analytical Chemistry
ISSN 0306-7319 print/ISSN 1029-0397 online © 2006 Taylor & Francis
DOI: 10.1080/03067310500291502

Table 1. Antineoplastic drugs sold the most in Switzerland: quantities of substances sold from July 2001 to June 2002 by hospitals, pharmacies, and self-dispensing doctors.

	Amount sold (kg/yr)[a]			
Substances	Total	Hospitals	Pharmacies	Doctors
Capecitabine	455	145	207	103
Hydroxycarbamide	352	44	216	92
Tamoxifen	156	10	108	38
5-Fluorouracil	81	60	11	10
Cyclophosphamide	34	22	8	5
Methotrexate	13	6	4	3
Ifosfamide	12	12	0.1	0.1

[a]From Institut für Haushaltsanalysen (IHA)-IMS in Switzerland.

According to Adjei [10], colorectal cancer is the third leading cause of cancer deaths in the United States, and standard therapy is 5-fluorouracil modulated with folinic acid. Capecitabine, metabolized to 5-fluorouracil in the tumour, is approved in the treatment of colorectal and breast cancer. Capecitabine allows more convenient administration (oral), provides a quality-of-life and economic advantage, and offers the potential of less gastrointestinal toxicity as compared with intravenous 5-fluorouracil chemotherapy [10–12]. These advantages induce a higher consumption of capecitabine than 5-fluorouracil (see table 1).

Tamoxifen causes liver cancer in rats [13]. In tamoxifen-treated women, there is an increase in endometrial abnormalities [14] and in the incidence of uterine endometrial tumours [15]. Tamoxifen and its metabolite, 4-hydroxytamoxifen, exhibit both oestrogenic and anti-oestrogenic activities [16, 17]. 5-Fluorouracil is mutagenic, genotoxic and teratogenic [8, 18].

There are several methods described in the literature for the analysis of tamoxifen. From wastewater, tamoxifen has been extracted with a SPE column but with a low recovery reported (42%) and a high standard deviation (40%) [19]. Tumor tissues were analysed with a C2 (Bond-Elut) solid-phase extraction (SPE) cartridge [20]. Liquid–liquid extraction (LLE) methods have been used for the extraction of tamoxifen from plasma and horse serum [21, 22]. High-performance liquid chromatography (HPLC)–UV [23], HPLC with fluorescence detection [20, 24], LC-MS/MS [19], gas chromatography (GC), mass spectrometry (MS) [25, 26], and GC with flame ionization detection (FID) [27] have been described for measuring levels of tamoxifen.

Several methods have been reported for the quantitative analysis of 5-fluorouracil in various matrices. For environmental samples, Kiffmeyer *et al.* [28] proposed SPE with various sorbents (Amberlyst, C18, and ENV+). The limit of detection (LOD), however, was much higher than environmental concentrations of drugs. Occupational environmental samples (air, glove) were analysed with an SPE cartridge (Isolute ENV+). LLE methods were used for the extraction of 5-fluorouracil from human or rat plasma and urine [29–32]. The extraction was followed either by HPLC and UV detection [28, 33–35] or by GC-MS where appropriate derivatization increases the sensitivity [31, 36, 37].

The first aim of our work was to set up efficient methods for the analysis of tamoxifen and 5-fluorouracil in wastewater. The second aim was to analyse the contamination of hospital wastewater and municipal sewage (residential area, sewage treatment

plant (STP) influents and effluents) with these drugs, and to evaluate the removal efficiency by STPs for the two compounds.

2. Experimental

2.1 *Standards and reagents*

Tamoxifen, 5-fluorouracil and pentafluorobenzyl bromide (PFBBr) were purchased from Sigma-Aldrich (Steinheim, Germany). OASIS® HLB and MCX (150 mg, 6 mL, 30 μm) cartridges were purchased from Waters (Rupperswil, Switzerland). The OASIS® HLB sorbent is a poly(divinylbenzeneco-*N*-vinylpyrrolidone) copolymer. The OASIS® MCX sorbent is OASIS® HLB sorbent with sulphonic groups. Isolute ENV+ (200 mg, 500 mg, 1 g, 6 mL) and C2 cartridges were purchased from Separis (Grellingen, Switzerland). SiOH cartridges (3 mL) containing 500 mg of unmodified silica were obtained from Macherey-Nagel Chromabond (Düren, Germany). Supelclean ENVI-18 (6 mL, 1 g) solid-phase extraction (SPE) cartridges were purchased from Supelco (Bellefonte, PA). All solvents were of super-pure quality from Romil (Cambridge, UK) or analytical-grade from Merck (Dietikon, Switzerland). Stock solutions of both compounds were prepared in methanol.

2.2 *Handling cytostatic drugs*

Since cytostatic drugs are (geno)toxic, their handling requires a number of organizational and technical precautions in order to guarantee the best-possible protection of research workers. The workers wore special protective clothing (Chemoprotect® gloves and gowns from CODAN, Germany). All stock solutions were prepared under a biological safety cabinet with laminar airflow. An absorbent paper (BenchGuard®) was used to protect the work surfaces. Waste materials were collected in appropriate sealed containers and were disposed of as contaminated material from hospital pharmacies.

2.3 *Tamoxifen (extraction and purification)*

2.3.1 Method development. Several SPE cartridges were tested with spiked bi-distilled water ($n = 1$) for the extraction of tamoxifen: MCX and HLB (OASIS®), ENVI-18 (Supelco), C2 (Isolute), ENV+ (Isolute). Many different conditions of column conditioning, elution, sample pretreatment, sample volumes, and quantities of sorbent in the cartridge were tested.

The preferred MCX (OASIS®) cartridge was tested with spiked and filtered (0.45 μm) wastewater ($n = 1$). Since tamoxifen is lipophilic, the addition of methanol (1–2% of final volume) was tested to desorb this compound of wastewater particles.

LLE was performed on spiked bi-distilled water ($n = 1$) using both dichloromethane and diethyl ether.

2.3.2 Final method. One hundred grams of sodium chloride was mixed with 1 L of raw wastewater in a LLE separating funnel. The extraction was performed three times with 60 mL of dichloromethane. The dichloromethane emulsion was centrifuged at 2500 rpm

for 10 min. The dichloromethane phase (bottom layer) was passed through a funnel filled with sodium sulphate and collected in a flask. Two millilitres of methanol were added, and the solution was evaporated to 0.2–0.4 mL in a rotary evaporator (850 mbar, 40°C). The addition of methanol is essential to avoid losses of tamoxifen during rotary evaporation.

The purification was performed on an OASIS® MCX (150 mg, 6 mL, 30 μm) cartridge conditioned with 6 mL of methanol and 1 mL of MQ water. Acidified water (10 mL at pH 2) was added to the sample extract and was loaded onto the cartridge at a flow rate of 1 drop/s (5–10 min for 10 mL). The flask was cleaned three times with 5 mL of acidified water, which was passed through the cartridge. The cartridge was washed with 4 mL of 0.1 N HCl and dried for 2 min under vacuum. The cartridge was washed a second time with 4 mL of methanol and 4 mL of methanol : acetonirile (30 : 70, v : v). The analyte was eluted (soak the sorbent for 4 min and then elute dropwise) with 3 mL of methanol : NH_4OH (95 : 5, v : v) and collected in an SPE tube. The eluate volume was evaporated to dryness under a gentle stream of nitrogen and resuspended in toluene.

2.4 *5-Fluorouracil (extraction, derivatization, purification)*

2.4.1 Method development. Several SPE cartridges were tested with spiked bi-distilled water for the extraction of 5-fluorouracil: ENVI-18 (Supelco), C2 (Isolute), MCX and HLB (OASIS®), ENV+ (Isolute). Many different conditions of column conditioning, elution, sample pretreatment, sample volume and quantity of sorbent in the cartridge were tested.

Various conditions were tested to obtain an optimal and repeatable derivatization. Three catalysts (triethylamine [31, 38], K_2CO_3 [39], and K_2HPO_4 [36]), various final concentrations of pentafluorobenzyl bromide (PFBBr), and different temperatures (20–100°C) and durations (0.5–3 h) for the reaction were tested.

2.4.2 Final method. The pH of the raw wastewater sample (150 mL) was adjusted to 5 with one (or two) drops of HCl (32%) and phosphate buffer (0.01 mol/L KH_2PO_4 adjusted with 0.1 mol/L of phosphoric acid to pH 3).

The cartridge (ENV+, 6 mL, 1 g) was conditioned with 12 mL of methanol and 12 mL of phosphate buffer (0.01 mol/L KH_2PO_4 adjusted with 0.01 mol/L KOH solution to pH 5). The sample was loaded with a flow rate of 3–5 mL/min (30–50 min for 150 mL) by applying a low vacuum. After drying the solid phase for 2–3 h under vacuum, the analyte was eluted dropwise with 4×3 mL of methanol. The sorbent was soaked for 4 min with each 3 mL. The methanol extract was evaporated to dryness under a gentle stream of nitrogen.

The derivatization was performed by adding 1 mL of acetonitrile and 100 μL of K_2CO_3 solution (25% in MQ-water; w/w). This solution was mixed (vortex) for 30 s, and 100 μL PFBBr solution in acetonitrile (20 : 80, v : v) was added. The tubes were capped and incubated at 80°C for 1 h.

One millilitre of toluene was added. The solution was evaporated to 200 μL under a stream of nitrogen before adding 1 mL of isooctane. Purification was performed on an SiOH cartridge with 0.5 cm of Na_2SO_4 and conditioned with 5 mL of hexane : acetone (80 : 20, v : v) followed by 5 mL of hexane. After adding the extract,

the cartridge was washed with 8 mL of toluene : hexane (15 : 85, v : v). The cartridge was dried for 1 min under vacuum. Then, the cartridge was washed with 2 mL of hexane : acetone (80 : 20, v : v). The analyte was eluted dropwise with the next 2 mL of hexane : acetone (80 : 20, v : v) and collected in an SPE tube. Toluene (0.7 mL) was added, and the eluate volume was reduced under a gentle stream of nitrogen to 100 µL.

2.5 *Gas chromatography and quantification*

A GC/MS system (Varian CP 3800 gas chromatograph/Varian 1200L mass spectrometer) was used for the quantitative analysis.

The gas chromatograph was equipped with a 60 m × 0.25 mm i.d. × 0.25 µm RTX-5 capillary column connected to a 5 m deactivated fused silica pre-column. A constant column flow mode was chosen (1 mL/min).

- *GC injection parameters*. 1 µL with a septum-equipped programmable injector (on-column); injection port: 85°C for 0.2 min; 100°C/min to 250°C.
- *GC oven-temperature programme*. 90°C for 4 min; 50°C/min to 180°C for 1 min; 1.5°C/min to 270°C for 5 min; 50°C/min to 300°C; 300°C isothermal 30 min.
- *MS parameters*. Transfer line temperature: 250°C; EI mode, electron energy: 70 eV; NCI mode; gas: methane. Tamoxifen was detected in the EI mode. Mass spectra are shown in figure 1. Derivative 5-fluorouracil was detected in NCI and EI mode. Figure 2 shows the mass spectra in both modes. For identification of the substance in SIM mode, three to four characteristic ions were selected for each compound (see table 2) and scanned for 10 min (delay times depending on the retention time of the substance) with a scan (dwell) time of 0.5 s. External standards were used for quantification. Calibration curves were obtained with four to seven standard concentrations (linear regression: $R^2 > 0.99$). The identity of substances in samples was confirmed by checking the relative abundances of the characteristic ions.

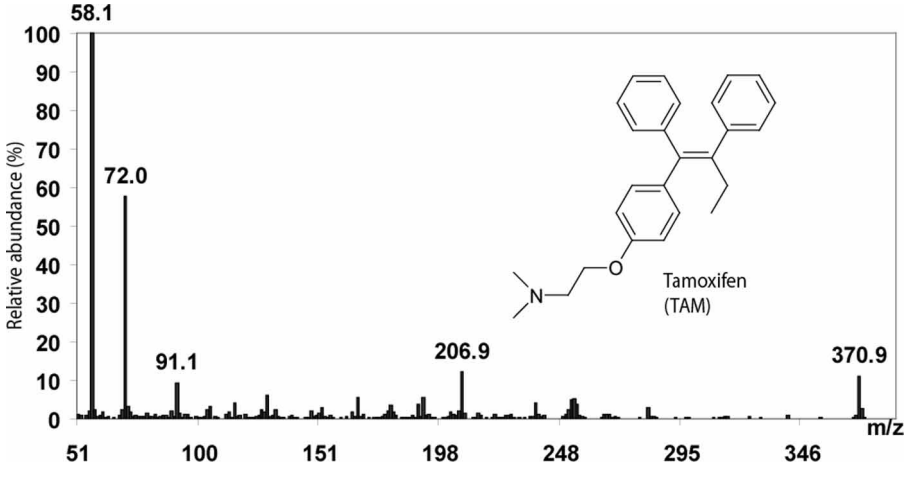

Figure 1. Structure and mass spectra of tamoxifen (EI mode).

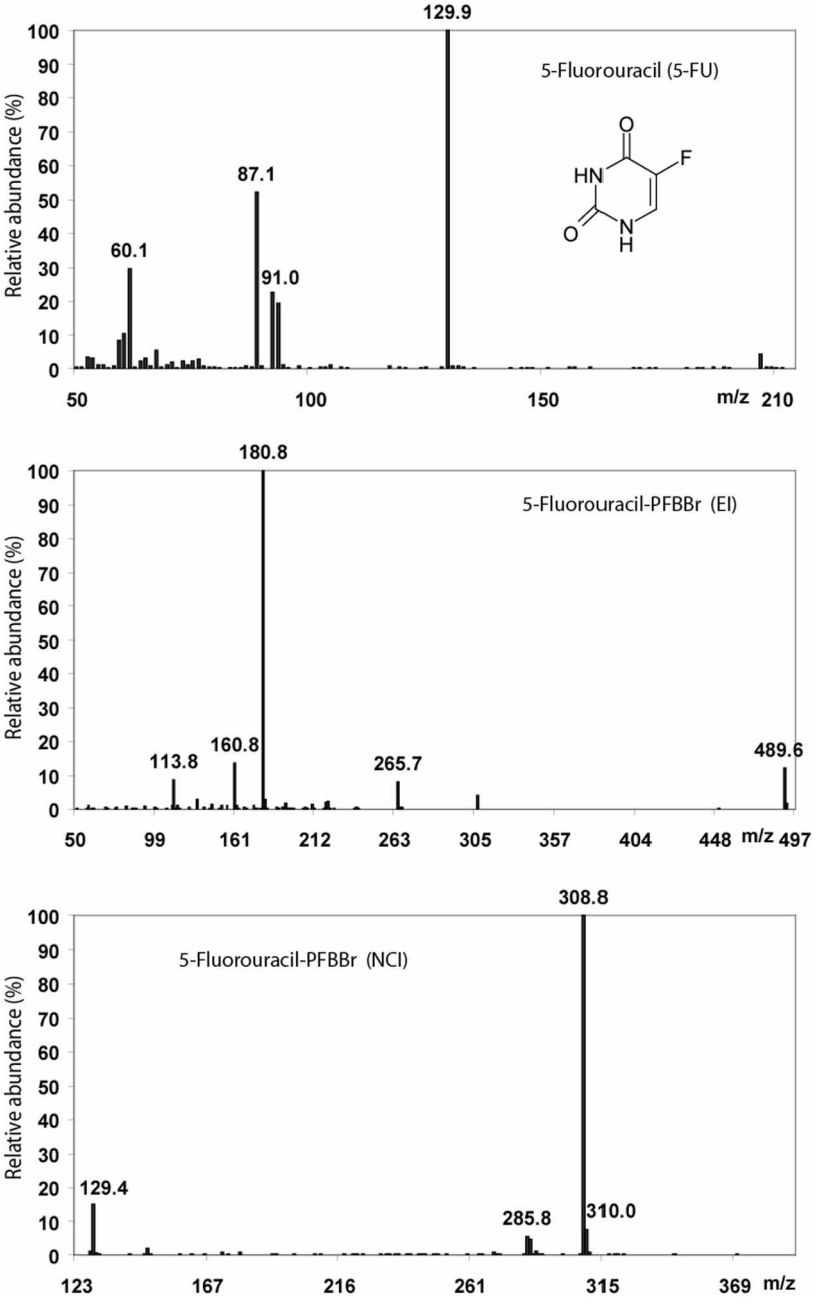

Figure 2. Structure and mass spectra of 5-fluorouracil (EI mode) and mass spectra of derivatised 5-fluorouracil (EI and NCI mode).

2.6 *Repeatability, determination of recoveries, and detection limits*

To quantify the repeatability of the whole method, a spiked sample was analysed four times. The relative standard deviations are shown in table 3.

Table 2. GC/MS data for the detection of tamoxifen and 5-fluorouracil-PFBBr.

Substances	Retention time (min)	Characteristic ions (m/z)
Tamoxifen	63.5	58/72/371
5-Fluorouracil-PFBBr (NCI)	42.9	308–311
5-Fluorouracil-PFBBr (EI)		114/181/266/490

Table 3. Relative standard deviations (RSD) of the method repeatability ($n=4$), recoveries and their standard deviations (SD), limits of detection (LOD), and limits of quantification (LOQ) per litre of wastewater for tamoxifen and 5-fluorouracil.

Substances	Repeatability (RSD)	Recovery ± SD	LOD (ng/L)	LOQ (ng/L)
Tamoxifen	3%	81% ± 4%	1	4
5-Fluorouracil	9%	73% ± 4%	NCI: 15	50
			EI: 30	90

To determine the recoveries, samples of wastewater were spiked with the pharmaceutical substances at four concentrations: about 5, 10, 15, and 20 times the limit of detection. Samples were then taken through the analytical procedure. The experimental quantities expressed as a function of the theoretical quantities enabled a regression with the slope indicating the recovery. Deviation standards of slopes were calculated with the method of least-squares and are also shown in table 3. Recoveries after SPE or LLE, derivatization, and clean-up exceeded 70% for both compounds. Relative standard deviations on the repeatability and standard deviations on recoveries varied from 3 to 9%. These results indicate that the procedures are suitable for the analyses of both substances.

The LODs (signal-to-noise ratio of 3) and limits of quantification (signal-to-noise ratio of 10) of the entire analytical procedure were calculated from spiked samples and were corrected for recovery (table 3).

2.7 *Sampling*

Thirty-seven samples of wastewater were collected: in June and July 2004 at the University hospital (1200 beds) of Lausanne (CHUV) and at the STP of Lausanne (220 000 equivalent inhabitants), in July 2004 at the STP of Morges (29 000 equivalent inhabitants; Western Switzerland, on Lake Geneva), and in July and August 2004 in a residential area (RA) of Lausanne. Both STPs have a similar treatment process (activated sludge and chemical precipitation with $FeCl_3$ followed by a secondary clarifier). A more precise description of these STPs has already been published [38].

The samples (24 h composites) were collected each day during 6–7 consecutive days, with a flow proportional automatic sampler for the STP of Morges and with a time-related automatic sampler for the Lausanne STP (30 mL every 15 min) and for the hospital and residential wastewaters (70 mL every 15 min). The samples were analysed immediately.

From the STPs of Morges and Lausanne, two samples (influent and effluent) per day of the sampling period were analysed.

2.8 *Calculation of predicted environmental concentrations*

The environmental concentrations (PECs) in Switzerland were estimated from the following equation, modified from several authors [38, 40–42]:

$$\mathrm{PEC} = \frac{A \times (100 - R) \times E}{365 \times P \times V \times D \times 10\,000}, \quad (1)$$

where A is the predicted amount used per year (kg/yr) (table 1), R the removal rate in percent (due to loss by adsorption to sludge particles, by hydrolysis, by biodegradation during sewage treatment, etc.), E the maximal excretion of unchanged drug in percent, P the number of inhabitants of the geographic area considered (in Switzerland: 7 261 000 in 2001), V the volume of wastewater per capita and day (0.3 m^3/capita-day), and D the factor for dilution of wastewater by surface water flow.

To estimate concentrations in wastewater (influent) and to be able to compare with analytical measurements and limits of detection, two scenarios were chosen:

- PEC_{infa}: without metabolizing. The excretion (E) was set to 100, the removal rate (R) to zero, and the dilution factor (D) to 1.
- PEC_{infb}: scenario considering the metabolization rate (excretion). The excretion is the percentage of the dose (ingested or injected) that is found in urine or faeces. We calculated a more realistic influent concentration which was compared with the measured influent concentrations.

3 Results and discussion

3.1 *Method development*

3.1.1 Tamoxifen. Solid-phase extraction (SPE) cartridges were tested for the extraction of tamoxifen. ENV+ (Isolute) gave a recovery below 5% ($n = 1$). ENVI-18 (Supelco) provided a slightly higher recovery ($< 30\%$, $n = 1$). Eighty percent of extraction was achieved with a C2 cartridge (1 g, isolute), but only with a mixture of eluants (either methanol : NH_4OH or methanol : NaCl). MCX cartridge (OASIS®) gave a recovery of up to 100% ($n = 1$) in bidistilled water with methanol : NH_4OH (95 : 5, v : v) elution. Because of the high lipophilicity of tamoxifen, losses of 50% were observed with filtered (0.45 μm) wastewater, and the filtration step was absolutely necessary to avoid clogging the cartridge. The addition of methanol (1–2%) before filtration did not improve the recovery. Thus, SPE cartridges could not be adapted to obtain acceptable recovery of tamoxifen. For this reason, LLE was tested. Seventy-five percent of tamoxifen was extracted from bidistilled water with diethyl ether (1×120 mL and 2×60 mL) and 100% with dichloromethane (3×60 mL). Before injection into the GC/MS, the LLE extract needed a purification step. Since wash steps could be introduced in the extraction with a MCX cartridge (OASIS®), we decided to combine the LLE and a SPE with several washing steps. More details on the method development are available [3].

The final conditions were applied to the wastewater samples, and the whole methodology was tested for repeatability and recovery (see table 3). The recovery ($81\% \pm 4\%$) and repeatability (RSD = 3%) are better than previously published results [43].

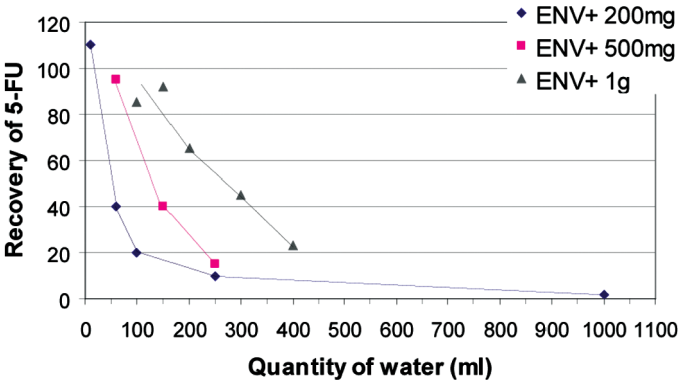

Figure 3. Recoveries of 5-fluorouracil using solid-phase extraction with ENV+ (200 mg , 500 mg and 1 g).

A mass spectrum (in EI mode) is presented in figure 1 and is similar to that previously published [25].

3.1.2 5-Fluorouracil. Most of the tested cartridges gave 0% recovery ($n = 1$) with bidistilled water: ENVI-18 (Supelco), C2 (Isolute), MCX and HLB (OASIS®). ENV+ (Isolute) showed the highest recoveries (2–110%). Losses of 5-fluorouracil were observed with high sample volumes (see figure 3). The sorption of this drug on the sorbent was weak, and even water could elute 5-fluorouracil. These losses could be reduced by using a cartridge with more sorbent. The best compromise was to use a cartridge of 1 g with only 150 mL of water (see figure 3).

5-Fluorouracil can be detected without derivatization, but the peak shape is poor. Its mass spectra are provided in figure 2.

Testing of derivatization revealed that the quantity of PFBBr is a key parameter for a complete and repeatable derivatization. Four hundred microlitres of the solution of PFBBr (2%) was not sufficient. Six hundred microlitres of PFBBr (4%) showed a significant amelioration. An increase in temperature was necessary for complete derivatization. With the catalyst K_2CO_3 [39], 60°C could be adequate with a reaction time of 3 h. To decrease the duration of this method, 1 h of reaction was necessary at 80°C or 100°C. Derivatization with K_2CO_3 or K_2HPO_4 catalysts worked better than with triethylamine. K_2HPO_4 seemed very efficient, but an impurity was detected very close to 5-fluorouracil in the GC/MS analysis. Mass spectra in EI and NCI modes are presented in figure 2. The spectrum in the NCI mode is similar to that previously published [37]. The EI spectra demonstrated molecular and fragment ions consistent with the addition of two PFB groups ($CH_2C_6F_5$; $m/z = 181$). The NCI spectra showed one major fragment (M-$C_7H_2F_5$). Again, a detailed description of the results on the method development are available [3].

The selected conditions were applied to the wastewater samples, and the whole methodology was tested for repeatability (RSD = 9%) and recovery (73% ± 4%) (table 3). The sensitivity was better in NCI (LOD = 15 ng/L; table 3) than in EI mode (LOD = 30 ng/L), but this difference was lower than expected. The signal was higher in NCI than in EI mode. Nevertheless, the baseline noise was also more important

in NCI mode that decreased the sensitivity of this mode. The sensitivity of our method is 100 times greater than that of a previously published technique [44].

3.2 *Wastewater contamination*

3.2.1 Tamoxifen. Tamoxifen was detected in the wastewaters from the hospital, residential area, and both STPs (see table 4). The concentrations of this drug were between the limit of quantification and the limit of detection (1 and 4 ng/L) of the technique. This range of concentration is below the predicted environmental concentrations PEC_{infa} and PEC_{infb} (see table 5). This difference could be explained in different ways. First, tamoxifen could be degraded before the analysis. Indeed, tamoxifen is sensitive to UV light, and up to 90% is degraded in 5 days [23]. Our analyses were performed as soon as possible and were protected from light. But some degradation cannot be ruled out. Second, tamoxifen is adsorbed onto particles due to its high lipophilicity (estimated value: $\log K_{ow} = 6.3$ [45]). These particles would settle in sewer systems and would not be analysed. Another explanation could be that the proposed value for excretion of unchanged drug is too high, so the PEC_{infb} value is overestimated.

Other authors have tried to detect this drug in wastewaters but in most samples, concentrations were below the limit of detection with the exception of two samples [43].

Our results indicate that the hospital effluent samples for the Saturday and Sunday were not contaminated by tamoxifen, possibly because of decreased work/treatment

Table 4. Percentage of wastewaters of a hospital (CHUV) and of two sewage-treatment plants where tamoxifen (TAM) and 5-fluorouracil (5-FU) were detected[a].

Substances	TAM (>1 ng/L; <4 ng/L)		5-FU
CHUV (seven samples)	70%	(5/7) (Saturday, Sunday: nd)	0%
Lausanne influents (seven samples)	100%	(7/7)	0%
Lausanne effluents (seven samples)	0%		0%
Morges influents (six samples)	100%	(6/6)	0%
Morges effluents (six samples)	0%		0%
RA Lausanne (four samples)	100%	(4/4)	0%

[a]nd: not detected.
RA: residential area.

Table 5. Excretion in the urine and in the bile of unchanged drugs and predicted environmentally concentrations (PECs) (ng/L) estimated from equation (1), comparing several cytostatic drugs.

Substances	Excretion (percentage of dose)[a]	PEC_{infa}[b] (ng/L)	PEC_{infb}[c] (ng/L)
Tamoxifen	20% (F)	196	39
5-Fluorouracil	<20% (U)	675	<23
Capecitabine	0.5% of 5-fluorouracil (U)		
Ifosfamide	12–90% dose-dependent (U)	15	2–14
Methotrexate	50–80% (U)	16	8–13
Hydroxycarbamide	30–60% (U)	443	133–266
Cyclophosphamide	50% (U)	43	22

[a]From rxlist (www.rxlist.com) or Swiss drug compendium (www.kompendium.ch).
F: faecal excretion. U: urinal excretion.
[b]Without metabolism and STP removal.
[c]With metabolism.

during the weekend. Indeed, patients were fewer during the weekend, and only a small amount of tamoxifen was distributed by the hospital. Since most of this substance is sold by pharmacies (see table 1), and patients ingest the drug at home. On that account, we observed no difference in the contamination of hospital wastewaters and municipal sewage.

Because of the high adsorption of tamoxifen on particles, this drug was removed from wastewater by both STPs (see table 4), avoiding surface water contamination. To our knowledge, no studies are available on the biodegradability of this compound.

3.2.2 5-Fluorouracil. 5-Fluorouracil was not detected in any of the wastewater samples (see table 4). Since only a small portion of this pharmaceutical is excreted in the same form, the PEC_{infb} was lower than the PEC_{infa} (see table 5). The predicted environmental concentrations using excretion (PEC_{infb}) were in the range of the limit of detection of our method ($PEC_{infb} = 23$ ng/L and $LOD = 15$ ng/L; see tables 3 and 5). Seeing that the calculation of PEC used an approximated value of excretion and that it did not take into consideration the degradation, the real concentration is below the LOD of the method. Contradictory results have been published concerning the biodegradability of 5-fluorouracil [6, 28]. According to Kümmerer and Al-Ahmad [6], it is not biodegradable in the closed bottle test (CBT) or in the Zahn–Wellens test (ZWT). On the other hand, Kiffmeyer *et al*. [28] found that 5-fluorouracil was completely removed from the spiked influent in a laboratory sewage plant within a few days, but the rate seemed dependent on the initial concentration. Nevertheless, this drug can be inactivated by ozonation [46].

5-Fluorouracil has been detected in effluents of the oncologic department in Vienna University Hospital [44]. Because of the absence of dilution with other sources of wastewaters (such as other medical departments), the detected concentrations were high (20–122 μg/L).

3.2.3 Potential contamination by other anticancer drugs. PECs for the seven most used chemotherapeutic agents are presented in table 5. These estimations showed that hydroxycarbamide could be a substance with a high contamination potential. Nevertheless, this risk is decreased by the fact that this compound is labile in water [47]. Cyclophosphamide PEC_{infb} is of the same level as 5-fluorouracil, which is in accordance with reported environmental concentrations (< 6 ng/L to 140 ng/L) [7]. Ifosfamide has also been detected in a few samples of wastewaters (< 6 ng/L to 30 ng/L) within the range of the PEC_{infb} [6]. Methotrexate was not detected in river and potable water samples but has been reported in one hospital effluent (1 μg/L) [5]. As no STP effluent was analysed, a comparison with the calculated PEC_{infb} is not possible.

The other antineoplastic drugs were administered in lower quantities in Switzerland, and thus the risk of a detectable contamination in the environment is low.

4. Conclusion

Cytostatic drugs are used less in comparison with other pharmaceutical substances such as anti-inflammatory drugs. Predicted environmental concentrations (PEC) are very low. Powerful methods are necessary to detect these compounds at such low

concentrations (ng/L level). The methods developed in our study showed good limits of detection and quantification, recoveries and repeatability.

Tamoxifen was detected in all wastewaters (hospital, residential area, and STPs) but was not detected in treated wastewaters. Thus, both STPs efficiently removed tamoxifen. 5-Fluorouracil was not detected in any of the wastewater samples.

Acknowledgments

The authors wish to thank the Swiss Agency for the Environment, Forest and Landscape, and Interpharma for the financial support of this work. We would like to thank the Institut für Haushaltlsanalysen (IHA-IMS) for supplying information on the quantity of substances sold. We also thank Frédéric Davoli, Pascal Blunier, and Alain Sciboz, who helped to collect samples, and the personnel of the hospital and sewage-treatment plants.

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