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Determination of six chemotherapeutic agents in municipal wastewater using online solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry

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HIGHLIGHTS

- We analyzed six chemotherapy agents in wastewaters.
- We used 1 ml injections and an 11 min SPE-LC-MS/MS.
- Limits of detection ranged from 4 to 20 ng L^{-1}.
- Cyclophosphamide and methotrexate were found in wastewater at 17–60 ng L^{-1}.

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ABSTRACT

Due to the increased consumption of chemotherapeutic agents, their high toxicity, carcinogenicity, their occurrence in the aquatic environment must be properly evaluated. An analytical method based on online solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry was developed and validated. A 1 mL injection volume was used to quantify six of the most widely used cytotoxic drugs (cyclophosphamide, gemcitabine, ifosfamide, methotrexate, irinotecan and epirubicin) in municipal wastewater. The method was validated using standard additions. The validation results in wastewater influent had coefficients of determination (R^2) between 0.983 and 0.998 and intra-day precision ranging from 7 to 13% (expressed as relative standard deviation %RSD), and from 9 to 23% for inter-day precision. Limits of detection ranged from 4 to 20 ng L^{-1} while recovery values were greater than 70% except for gemcitabine, which is the most hydrophilic compound in the selected group and had a recovery of 47%. Matrix effects were interpreted by signal suppression and ranged from 55 to 118% with cyclophosphamide having the highest value. Two of the target anticancer drugs (cyclophosphamide and methotrexate) were detected and quantified in wastewater (effluent and influent) and ranged from 13 to 60 ng L^{-1}. The proposed method thus allows proper monitoring of potential environmental releases of chemotherapy agents.

1. Introduction

In recent years, an increased awareness about the occurrence of pharmaceutical compounds in the environment was observed and these substances have been identified as contaminants of emerging concern (Daughton and Ternes, 1999). Several classes of pharmaceuticals have been detected in wastewater treatment plant (WWTP) effluents, surface waters and groundwater as well as in drinking water (Kümmerer, 2008; Thomas, 2002). The high polarity and water solubility of the majority of these compounds make them less likely to be degraded or removed during the WWTP processes and thus more likely to reach the aquatic environment.

The number of cancer patients has increased during the last years with the Canadian Cancer Society reporting that the occurrence of many cancer types has increased by 2 to 7% per year in the decade spanning 1998–2007 (CCS, 2012). The higher incidence of cancer increases anticancer drug consumption as chemotherapy is one of the most commonly used treatment options (Shewach and Kuchta, 2009). The development of cancer chemotherapy began in the 1940s and involved the use of alkylating agents which opened the door for the development of a large number of anticancer drugs (Shewach and Kuchta, 2009). Chemotherapeutic agents, also called cytotoxic or antineoplastic agents are a group of compounds used to prevent or disrupt cell division. They are mainly used in hospitals and are administered for outpatients and inpatients (Allwood et al., 2002). According to the International Agency for Research on Cancer (IARC, 2011), anticancer drugs are toxic and some of them are carcinogenic as is the case of cyclophosphamide

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(CP) (Praga et al., 2005). Chemotherapy agents represent one of the most toxic compounds used as a medication. As such, their presence in the aquatic environment could have a significant impact on human and ecological health. It is therefore important to develop analytical methods which allow their detection at low nanogram-per-liter concentrations. For this study, a group of six cytotoxic agents (cyclophosphamide, ifosfamide, methotrexate, gemcitabine, epirubicin and irinotecan) were chosen. Several factors were considered in making this choice. First, the selected compounds are considered highly toxic and carcinogenic (Belfroid et al., 1993). Second, these compounds are among the most used in chemotherapy protocols in the hospitals of the province of Quebec (Table 1).[1] Third, the presence of some of these compounds in the aquatic environment has already been demonstrated (Garcia-Ac et al., 2009a; Kümmerer, 2008; Martín et al., 2011; Nussbaumer et al., 2011). Later work will certainly require a proper evaluation of the potential risks to human health for drug residues in drinking water or for biota exposed in receiving surface waters and in soils used for land-applied biosolids.

Cyclophosphamide (CP) is one of the most widely used drugs in cancer treatment since it was introduced in the 1950s (Thurston, 2007). Its structural isomer, ifosfamide (IF), has emerged as an important alkylating agent since the 1970s (Belfroid et al., 1993). They have both been found in hospital effluent samples by GC-MS analysis preceded by off-line SPE at concentrations of 146 ng L^{-1} and 24 ng L^{-1} respectively (Steger-Hartmann et al., 1996). The cytotoxic agents CP and IF were also quantified in biological samples (Sottani et al., 2008). Given the importance of methotrexate (MTX), several studies have been conducted for its detection and quantification in different matrices (mostly biological for medical purposes). This was related in an earlier review that described more than 70 studies between 1975 and 2000 (Rubino, 2001). In a study realized in China (Yin et al., 2010), MTX was quantified in hospital effluents at concentrations between 2 and 19 ng L^{-1}. Gemcitabine (GCA) is a cytotoxic nucleoside that has been used in chemotherapy in the last decade (Seo et al., 2007) and was quantified at concentrations varying from 2.4 ng L^{-1} to 9.3 ng L^{-1} in several water matrices (Martín et al., 2011). GCA has also been quantified in wipe samples used to monitor surface contamination in drug preparation and administration rooms at hospitals in Italy (Sottani et al., 2007). Few studies have been published for epirubicin (EPI). The published method in Italy for the quantification of EPI in urine samples based on off-line SPE and LC-MS/MS, revealed an LOD of 40 ng L^{-1} (Sottani et al., 2004). Furthermore, EPI has been quantified over a month in the effluent wastewater of the Vienna University Hospital (Austria) and reported concentrations of EPI vary between 100 ng L^{-1} and 1400 ng L^{-1} (Mahnik et al., 2006). Irinotecan (CPT-11) was studied but not detected in water as shown in the study carried out in Spain (Martín et al., 2011) and method limits of detection (MDL) were between 0.9 and 1.1 ng L^{-1} in different water matrices. Another study was performed for the determination of CPT-11 in human blood using LC combined with fluorescence detection (de Jong et al., 2003). Recently, several methods have included the use of online pre-concentration methods coupled to LC-MS/MS for the analysis of endocrine disrupters (López de Alda and Barceló, 2001), pharmaceutical compounds and pesticides in water matrices (Bones et al., 2006; Segura et al., 2007; Viglino et al., 2008). Both methods of SPE (off-line and online) follow the same steps and are governed by the same principles. The difference is that contrary to online SPE, the sample extraction steps in off-line SPE are completely independent of the chromatographic separation and quantitation. Published literature reviews (Chen et al., 2009; Hennion, 1999; Oliferova et al., 2006) confirm that online SPE is one of the most robust and promising techniques for the rapid extraction and preconcentration of pharmaceuticals in environmental matrices. In fact, online SPE coupled to LC allows high sensitivity and performance while showing good reproducibility (Oliferova et al., 2006). Additionally, standard additions, usually considered lengthy and arduous when combined with labor-intensive manual SPE, can be applied more easily when used with online SPE. Online SPE also lowers sample volume, limits sample loss (especially volatiles and semi-volatiles) and reduces the possibility of contamination when comparing traditional off-line SPE methods that require several manipulation steps and many more human resources. However, a key problem of online SPE remains achieving optimal recovery without affecting the coupling to liquid chromatography and atmospheric pressure ionization sources.

Table 1
Consumption of chemotherapeutic agents in Montreal's hospitals (Personal communication – President, advisory committee of pharmacists – Sigmasanté hospital group purchasing-2010).

Compounds	% mass	Kg/M people
5-Fluorouracil	45.85	21.10
Cyclophosphamide	12.45	5.72
Gemcitabine	11.12	5.11
Ifosfamide	5.00	2.30
Methotrexate	3.44	1.58
Carboplatin	2.95	1.36
Irinotecan	1.09	0.50
Epirubicin	0.26	0.12

The development of an online SPE-LC-MS/MS was challenging for the following reasons: i) the target analytes have a wide range of octanol–water partition coefficient values (a measure of hydrophobicity), varying from log $K_{ow} = -1.84$ for MTX to log $K_{ow} = 4.37$ for CPT-11; ii) hydrophilic compounds such as a GCA are poorly retained by conventional reversed phase columns (such as C_{18}) and require alternative stationary phases while reverse-wise we also want to integrate some compounds having high retention and that are more difficult to quantitatively desorb; iii) unlike off-line SPE, the optimization of the nature and percentage of solvents required for desorption (from SPE column towards analytical column) must be compatible with the MS source and must lead to a quantitative desorption since a non-quantitative desorption of compounds leads to loss of analytes that remain on the SPE column, induce a decrease of the signal and greatly affects the reproducibility (Oliferova et al., 2006) and iv) using different stationary phases between the SPE and the analytical columns could increase peak broadening and require further optimizations (Hennion, 1999).

The majority of methods developed for the analysis of chemotherapeutic agents focus on only a limited number of compounds, but a single method including several cytotoxic drugs is relevant and needed to properly evaluate potential environmental and human health risks. Furthermore, all published methods were based on off-line SPE. Up to 2012, there are only three published studies on chemotherapeutic agents that used online SPE (Garcia-Ac et al., 2009a, 2009b; Kovalova et al., 2012) in which the authors included two of the target chemotherapy agent compounds. In our project, the selected chemotherapeutic agents include some very polar compounds and have different physico-chemical properties and chemical structures which make their analysis within a single method a daunting analytical and chromatographic challenge especially when one integrates automated pre-concentration. We anticipate that this approach will be helpful to provide some much needed data to properly evaluate the risks caused by the environmental releases of chemotherapy agents.

The objective of this study was to develop a sensitive, rapid and reliable analytical method using online SPE coupled to LC-MS/MS for the analysis of the six selected chemotherapeutic agents with different physico-chemical structures and properties in wastewater matrices. Online SPE optimization (nature of sorbents, breakthrough volume and loading flow rate) and method validation will be presented.

[1] Personal communication – President, advisory committee of pharmacists – Sigmasanté hospital group purchasing-2010