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Study of the stability of 26 cytostatic drugs and metabolites in wastewater under different conditions

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HIGHLIGHTS

- Many cytostatics were unstable under typical storage conditions (4 and −20 °C).
- Degradation is influenced by two correlated factors: time and temperature.
- Samples should be analyzed as soon as possible to avoid erroneous results.
- Storage at −20 °C for up to 1 month is an effective option for some cytostatics.

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ABSTRACT

The stability of 26 cytostatic drugs (21 parent compounds and 5 metabolites) in wastewater was studied using liquid chromatography–electrospray–tandem mass spectrometry (LC–ESI–MS/MS). Wastewater aliquots were spiked with the target compounds at 1000 μg L^{-1} and stored in the dark under different temperature (−20, 4 and 25 °C) and pH (acid and neutral) conditions for different periods of time (up to 3 months). The influence of these factors (temperature, pH and time) on the stability of the compounds was evaluated through an experimental design. The most negative factor was the time of storage, but temperature also exerted a very important influence. Acidification of the samples is a good option for some cytostatics (e.g., temozolomide, tamoxifen and its metabolites, and chlorambucil) but it may have a negative effect on others (e.g. ifosfamide). The design also showed correlations between factors indicating that an increase of the storage time is more relevant at high temperatures, while an increase of the temperature is more detrimental in non-acidified samples. After 3 months at −20 °C, all compounds with the exception of temozolomide, vinorelbine, imatinib and erlotinib presented recoveries below 80%. The most unstable compounds were oxaliplatin, 5-(3-N-methyltriazen-1-yl)-imidazole-4-carboxamide and chlorambucil. To the authors' knowledge, the stability data reported in the present study is the first ever published for most of the target compounds in wastewater. The results obtained point out storage at −20 °C from collection to analysis as the best storage option.

1. Introduction

Among the various classes of pharmaceuticals, cytostatic drugs, used in the treatment of cancer (also called antineoplastic agents), are designed to damage DNA, inhibit DNA synthesis, and interrupt cell replication (Kovalova et al., 2009). In the last years, their demand in developed countries has grown considerably, and their administration in outpatient treatment departments is increasingly more common. Some of these drugs have been categorized as carcinogenic, mutagenic and teratogenic compounds (International Agency on the Research on Cancer, update 28 June 2012), even at low concentrations (Zounkova et al., 2010; Zounková et al., 2007). In recent studies, a significant increase of structural chromosomal aberrations has been observed among occupationally exposed nurses during the preparation and administration of anticancer drugs, which may result in an elevated risk of developing cancer (Bouraoui et al., 2011; Smerhovsky et al., 2001; Zhang et al., 2013). On the other hand, it is well known that many pharmaceuticals used in human medicine are not completely metabolized and hence they are excreted either unchanged or slightly transformed, frequently into more polar conjugated molecules (Zounkova et al., 2010). These compounds are most often directly discharged into the sewage system, without any specific control after being administered in hospitals, or by out-patients (Zhang et al., 2013). Once excreted their fate and behavior in the collector system on its way to the corresponding wastewater

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treatment plant (WWTP) and, thereafter, at the WWTP itself and in the receiving water compartment are largely unknown.

The information available on the removal of cytostatics from different environmental matrices, including surface and wastewater, has been reviewed by Zhang et al. (2013) putting into evidence that knowledge in this respect is very limited, with very few compounds having been investigated, in a limited number of conditions/treatments, and with different results. For instance, a number of studies on the biodegradation of cytostatics through the sewage treatment or under simulating processes using OECD mediums, have concluded that some compounds like cyclophosphamide (CP) (Bende et al., 2007; Steger-Hartmann et al., 1997), 5-fluorouracil (5-Fu) (Kümmerer and Al-Ahmad, 1997) and cisplatin (Kiffmeyer et al., 1998) are considerably stable, while other compounds like gemcitabine and cytarabine are more readily degradable (Kümmerer and Al-Ahmad, 1997). However, other studies conducted with 5-Fu found the compound to be completely biodegraded after 14 days (Kiffmeyer et al., 1998) or even after 14 h (Kosjek et al., 2013). Thus, further investigations covering more compounds and conditions are necessary to better know the fate and persistence of these drugs and their metabolites and transformation products (TPs) in the water cycle.

To this end, the availability of analytical methods for determination of cytostatics in aqueous and solid environmental matrices is essential. However, to date, very few analytical methodologies have been developed for this purpose. These methodologies have been reviewed in various recent papers that also addressed other related issues, such as environmental levels and toxicity (Kosjek and Heath, 2011; Nussbaumer et al., 2011; Turci et al., 2003). Cytostatic levels reported in waste and surface waters are relatively low, varying from ng L^{-1} to μg L^{-1} (Ferrando-Climent et al., 2013; Gomez-Canela et al., 2012; Mahnik et al., 2007; Martin et al., 2011; Mullot et al., 2009; Negreira et al., 2013a; Yin et al., 2010), in comparison to those of other groups of pharmaceuticals, but, due to their high potency, this specific group of drugs is conceived to be harmful to aquatic organisms and even humans, and hence high sensitivity analytical methods are required for appropriate exposure and risk assessment.

Another important issue often overlooked in the analysis of pharmaceuticals in the aquatic environment is the study of their stability during sample collection and storage. Cytostatics enter the water cycle via hospital or domestic wastewater after excretion in the urine and/or feces by patients under medical treatment (Zhang et al., 2013). Then, target compounds, on their way to the wastewater treatment facilities, and thereafter during integrative sampling, transport to the analytical laboratory, and eventual storage of the samples until analysis, may undergo different physical, chemical and biological processes that will vary in nature and intensity depending on the time, temperature, and preservation conditions existing in each stage. These processes involve a change in the original concentration of the compounds in the sample that can lead to erroneous, in general lower than real, results. This issue (together with other relevant sample preparation aspects, such as extract evaporation temperature, selection of extractant solvent, glassware silanization, and filtration) has been recently evaluated in a study conducted by Baker and Kasprzyk-Hordern (2011) on the analysis of pharmaceuticals (basically drugs of abuse) in waste and surface waters. However, in the case of cytostatics this stability issue that can become crucial in those cases where samples have to be stored for long periods of time (from days to months) due to instrument restrictions or practical impossibility posed by the large number of samples to be analyzed, has only been investigated in two previous works (Ferrando-Climent et al., 2013; Negreira et al., 2013b).

Ferrando-Climent et al. (2013) studied the stability of cyclophosphamide (CP), ifosfamide (IF), docetaxel (DOC), etoposide (ETP), tamoxifen (TAM), azathioprine (AZA) and ciprofloxacin (CIP) in wastewater samples stored in PET bottles without chemical preservative, in the wastewater samples preserved with formaldehyde, and in Oasis HLB cartridges previously used for extraction of the samples, after storage at −20 °C for 1 week, 1 month, 3 months and 6 months. The results showed that the presence of formaldehyde increased the degradation of the compounds, which were only stable for one week. In the case of the samples stored in the cartridges all the compounds, except ETP, remained stable until the third month. Finally, in the case of the samples stored at −20 °C without any pretreatment, the compounds CP, IF, ETP and CIP remained stable for at least 6 months, whereas DOC and AZA exhibited some degradation after 3 months, and TAM showed an odd behavior, with a decrease followed by an increase in concentration along the 6-month storage period.

Likewise, a previous study conducted by us to investigate the stability of 24 cytostatics in aqueous standard solutions prepared in HPLC water showed that all compounds, with the exception of carboplatin (Car-Pt), were degraded to a higher or lower extent when stored in aqueous solution for a certain time, which varied from hours to months depending on the compound and temperature (Negreira et al., 2013b).

Since compound instability is expected to be more pronounced in wastewater than in HPLC water the purpose of the present work was to comprehensively study the stability of 26 cytostatic compounds (the same as in the previous work (Negreira et al., 2013b) plus DOC and irinotecan, IRI) in wastewater samples stored under different conditions in order to, first, alert about the possible decomposition of the samples during storage, get an indication about the degradation/persistence of the compounds in the sewage system and during sewage treatment, and establish the best possible conditions for sample collection and storage until analysis.

2. Material and methods

2.1. Standards and solvents

All solvents were of HPLC grade and all chemicals were of analytical reagent grade.

Formic acid (98–100%), hydrochloric acid (HCL), methanol, and HPLC water were purchased from Merck (Darmstadt, Germany), while dimethyl sulfoxide (>99.9%) was acquired from Aldrich (Milwaukee, WI, USA).

Standards of the cytostatic compounds: capecitabine (CAP), Carb-Pt, chlorambucil (CHL), CP, DOC, doxorubicin hydrochloride (DOX), endoxifen or 4-hydroxy-N-desmethyl-tamoxifen (OH-D-TAM), erlotinib hydrochloride (ERL), ETP, 5-Fu, gemcitabine hydrochloride (GEM), hydroxymethotrexate (OH-MET), 6(α)-hydroxypaclitaxel (OH-PAC), (Z)-4-hydroxytamoxifen (OH-TAM), IF, imatinib mesylate (IMA), IRI hydrochloride trihydrate, 5-(3-N-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC), oxaliplatin (Oxa-Pt), methotrexate (MET), TAM, temozolomide (TMZ), vinblastine sulfate (VBL), vincristine sulfate (VCN), and vinorelbine ditartrate (VRB) were obtained from Santa Cruz Biotechnology (Heidelberg, Germany), whereas paclitaxel (PAC) was supplied by Aldrich at the highest available purity (>99%). The isotopically labeled standards: capecitabine-d_{11}, carboplatin-d_4, cyclophosphamide-d_4, etoposide-d_3, erlotinib-d_6 hydrochloride, 5-fluorouracil-^{13}C $^{15}N_2$, gemcitabine-^{13}C $^{5}N_2$ hydrochloride, 7-hydroxymethotrexate-d_3, 4-hydroxy-N-desmethyl-tamoxifen-d_5, 4-hydroxy-ethyl-tamoxifen-d_5, 6α-hydroxypaclitaxel-d_5, methotrexate-methyl-d_3, ifosfamide-d_4, irinotecan-d_{10} hydrochloride, MTIC-d_3, N-desmethyl imatinib-d_8, paclitaxel-d_5, temozolomide-d_3, vincristine-d_3 sulfate (VCN), and vinorelbine-d_3 ditartrate (VRB), were purchased from Santa Cruz Biotechnology.

The selected cytostatics and metabolites are shown in Table S1 (in Supplementary information), grouped into six families attending to their mode of action and chemical structure. The parent compounds were selected based on consumption data in the European Union (EU), and the metabolites on the basis of excretion rate and activity.

Individual solutions of each compound (ca. 1000 μg mL^{-1}) and a mixture of them (ca. 25 μg mL^{-1}) were prepared in dimethyl sulfoxide