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Degradation of the cytostatic etoposide in chlorinated water by liquid chromatography coupled to quadrupole-Orbitrap mass spectrometry: Identification and quantification of by-products in real water samples

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HIGHLIGHTS

- First study of the degradation of etoposide in chlorinated water.
- Etoposide reacts in chlorinated waters generating two by-products.
- The by-products formed were identified by their Q-Orbitrap MS/MS spectra.
- The by-product desmethyl etoposide has been found in river and waste waters.

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ABSTRACT

Once discharged into the sewage system, many pharmaceuticals may undergo degradation reactions in the presence of chemical disinfectants, generating by-products that may possess enhanced toxicity relative to the parent compounds. For this reason, the stability of the widely used cytostatic etoposide in chlorinated water has been investigated for the first time in the present work. Taking advantage of the high-resolution/accurate-mass capabilities of the hybrid quadrupole-Orbitrap mass spectrometer Q Exactive, two new oxidation by-products of etoposide were reliably identified. The time course of etoposide and its by-products was followed at different pH values, free chlorine concentrations and water matrices. Finally, the occurrence of etoposide and its major identified by-product (3′-*O*-desmethyl etoposide) was investigated in real water samples by on-line solid-phase extraction-liquid chromatography–tandem mass spectrometry using a 4000QTRAP hybrid quadrupole-linear ion trap mass spectrometer. The etoposide by-product was found in various river and wastewater samples at levels between 14 and 33 ng L^{-1}, whereas etoposide was not detected in any sample.

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1. Introduction

Cytostatic drugs have been widely used for chemotherapy for decades. However, concern about the occurrence of these compounds in the environment increased during recent years because of their cytotoxic, mutagenic and/or teratogenic properties (Zounkova et al., 2010; Ferk et al., 2009). These compounds are considered as one of the most hazardous contaminants in the water cycle (Seira et al., 2013) and are supposed to be harmful for human and wildlife even at very low doses (Mahnik et al., 2004).

Cytostatic drugs and their metabolites enter the aquatic environment mainly through excretion of urine and feces of patients undergoing chemotherapy (Köhler et al., 2012; Zounkova et al., 2010). Hospital effluents are rarely pretreated prior to their discharge into the public sewer system and are thereby considered potential hotspots (Zhang et al., 2013). In fact, elevated concentrations of cytostatics have been found in hospital effluents, where they have reached μg L^{-1} levels (Mahnik et al., 2004; Ferrando-Climent et al., 2013; Yin et al., 2010), and also in raw urban wastewater where levels have ranged from ng L^{-1} to μg L^{-1} (van Nuijs et al., 2012; Kosjek and Heath, 2011; Nussbaumer et al., 2011). Wastewater treatment plants (WWTPs) use chemical oxidants such as ozone, chlorine, chlorine dioxide and/or chloramines for both disinfection (elimination of microorganisms) and oxidation (elimination of micropollutants, taste and odor control, etc.)

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(Deborde and von Gunten, 2008). However, many compounds such as pharmaceuticals and their metabolites, excreted by the human body, pass this and other treatments applied in WWTPs and end up in the receiving water bodies (Zhang et al., 2013; Verlicchi et al., 2012).

Chlorine is globally the most used chemical oxidant in WWTPs and in the pretreatment of hospital effluents prior to their discharge into the public sewer system (Zhang et al., 2013; Verlicchi et al., 2010), basically because of its low cost. Since aqueous chlorine is not an oxidant strong enough to completely mineralize anthropogenic compounds, numerous transformation products may be formed due to oxidation/substitution reactions between the contaminant residues found in the water cycle and aqueous chlorine. However, still little is known about the stability and the biological effects of these compounds. In some cases, transformation products are fairly stable against further transformation and can persist from hours to days even in the presence of residual chlorine (Negreira et al., 2012). Moreover, some of these by-products have been identified to be even more toxic than the parent compounds (Bedner and MacCrehan, 2006; Canosa et al., 2006). For this reason, the chlorination of several organic contaminants, such as pharmaceuticals (Soufan et al., 2012), personal care products (Negreira et al., 2008) and pesticides (Rodríguez-Cabo et al., 2013), has been investigated. However, to the best of our knowledge, the stability of cytostatics in the presence of free chlorine has been only studied for methotrexate (Roig et al., 2014).

In the present work, the degradation of the highly used cytostatic drug etoposide (ETP) has been studied in chlorinated water. ETP is a semi-synthetic derivative of podophyllotoxin, a non-alkaloid toxin lignan extracted from the mandrake plant (*Podophyllum peltatum* L.) (Pang et al., 2001). It is commonly used in adults and children (Veal et al., 2006) for the treatment of a variety of malignancies, such as small-cell lung cancer, testicular carcinoma, lymphoma, other solid tumors, and several types of leukemia (Sachin et al., 2010).

ETP has been detected at levels from 15 (Martin et al., 2011) to 83 ng L^{-1} (Ferrando-Climent et al., 2013) in influent wastewater and ranging from 18 (Yin et al., 2010) to 406 ng L^{-1} (Ferrando-Climent et al., 2013) in hospital effluents. These levels are an important cause of concern since this cytostatic has been classified by the International Agency for Research on Cancer (IARC) as a carcinogen in humans (group 1) (International Agency on the Research on Cancer I).

On the other hand, data about the output levels of ETP from WWTPs are still scarce. ETP has only been reported to be present in an effluent from a municipal WWTP plant, at a level of 3.4 ng L^{-1} (Martin et al., 2011). This fact leads us to believe that it may be degraded during water treatment; however, nothing is known about its possible transformation into by-products which may be even more toxic than the parent compounds.

Thus, the aims of the present work were: (1) to investigate the stability of ETP in chlorinated water under different hypochlorite concentrations and pH values typically found in real water samples (from 6 to 8), (2) to identify the main by-products formed by accurate-mass measurements using a high-resolution/accurate-mass (HR/AM) hybrid quadrupole-Orbitrap mass spectrometer Q Exactive, and (3) to quantify the presence of those transformation products in the aquatic media. The target compound was selected on the basis of its extended use, mainly in the European Union, and as representative of cytostatics with different mechanisms of action.

2. Materials 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, 37%), methanol and ultrapure water were purchased from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO), ascorbic acid (>99%), sodium phosphate and sodium hydrogen phosphate were acquired from Sigma (Milwaukee, WI, USA), whereas sodium hydroxide was from Carlo Erba (Sabadell, Spain). A 10% (*w/v*) solution of sodium hypochlorite was purchased from Panreac (Barcelona, Spain). This solution was stored at 4 °C and its exact concentration was assessed using the *N,N*-diethyl-p-phenylenediamine method with photometric detection (Clesceri et al., 1998). The photometer (model HI 96710) was obtained from Hanna instruments (Guipuzkoa, Spain).

The cytostatic compound ETP and the corresponding isotopically labeled analogue etoposide-d_3, were obtained from Santa Cruz Biotechnology (Heidelberg, Germany) at the highest available purity (>99%). 3′-*O*-desmethyl etoposide was acquired from Toronto Research Chemicals (North York, ON, Canada). Individual stock solutions of each compound (ca. 1000 mg L^{-1}) were prepared in DMSO and stored in the dark at −20 °C. Different working standard solutions were made by appropriate dilution in DMSO (100 and 10 mg L^{-1}), ultrapure water and wastewater. Calibration standards (from 0.001 to 1 mg L^{-1}) were freshly prepared in ultrapure water on the day of the analyses.

2.2. Safety considerations on cytostatic drugs handling

As cytostatic drugs are highly toxic compounds, their handling requires strict safety precautions in order to guarantee the best possible protection of research workers. All stock solutions were prepared under a biological safety hood with laminar airflow, and absorbent paper was used to protect the work surfaces. All disposable material that was in contact with the tested compounds was treated as hazardous waste.

2.3. Chlorination experiments

The reactivity of ETP in the presence of free chlorine (10–200 mg L^{-1}) was investigated in ultrapure water spiked at 1 mg L^{-1}. All assays were performed at room temperature (20 ± 2 °C) in 16 mL amber glass vials, with samples buffered with sodium phosphate (10 mM) at pH values between 6.3 and 8.1. Vials were manually shaken for 2–3 s and then, at increasing times, 1 mL aliquots were immediately transferred to autosampler vials (2 mL volume) containing 0.01 mL of an aqueous ascorbic acid solution (60 mg mL^{-1}) to quench the excess of chlorine. The isotopically labeled compound was finally added at a level of 0.2 mg L^{-1}. At least six aliquots were taken from each chlorination experiment under the different considered experimental conditions. The thus obtained aliquots were directly analyzed in the same day by UHPLC coupled to hybrid quadrupole-Orbitrap mass spectrometry. Additional chlorination experiments were then performed in waste waters. The free chlorine content was determined by reaction with *N,N*-diethyl-p-phenylenediamine using photometric detection.

2.4. Chromatographic and detection conditions

The time course of the ETP degradation and the by-product formation was followed using an ultra-high performance liquid chromatography (UHPLC) system Acquity (Waters, Milford, MA, USA) consisting of a thermostated autosampler, a binary pump, a vacuum degasser, and a thermostated column compartment, coupled to a hybrid quadrupole-Orbitrap mass spectrometer Q Exactive from Thermo Fisher Scientific (San Jose, CA, USA) equipped with a heated-electrospray ionization source HESI II. Chromatographic separation was performed on a reversed-phase UHPLC column Hibar HR (50 × 2.1 mm, 2 μm) from Merck, maintained at room temperature, using a mobile phase consisting of ultrapure water (A) and methanol (B) with the following gradient: 0–1 min, 5% B; 3 min, 20% B; 7–9 min, 80% B; and 12–15 min, 5% B. The flow rate and the injection volume were set at 0.3 mL min^{-1} and 10 μL, respectively. The HESI interface was operated in both polarity modes under the following specific conditions: spray voltage, 2.5 kV; sheath gas flow rate, 40 arbitrary units; auxiliary gas,

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