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Development and validation of a UHPLC-MS/MS method for the identification of irinotecan photodegradation products in water samples☆

Fabio Gosetti*, Masho Hilawie Belay, Emilio Marengo, Elisa Robotti

Department of Science and Technological Innovation, University of Piemonte Orientale, Viale T. Michel 11, 15121 Alessandria, Italy

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ABSTRACT

Irinotecan (CPT-11) is a water-soluble anticancer drug widely used to treat several types of cancer.

Even if the metabolites of CPT-11 are well-known and investigated, only limited information is available in the literature about the formation of photo-degradation products that can naturally originate from sunlight irradiation when the drug is released in aqueous systems.

CTP-11 solutions at 10.0 mg L^{-1} were irradiated by simulated sunlight. The intensity of the drug decreased by 90% after 7.5 days of irradiation and no significant reduction of absorbance values was observed after 13 days.

A sensitive UHPLC-MS/MS method was developed employing a hybrid triple quadrupole/linear ion trap mass spectrometer, that is able to work in data-dependent acquisition mode and to obtain information about the compounds formed during the photoirradiation. Moreover, a selected reaction monitoring method was built using the MS/MS fragmentation pattern of the compounds previously investigated. The method was validated considering LOD, LOQ, linearity, precision, selectivity, recovery and matrix effect. LOD and LOQ values were 0.02 and 0.05 ng mL^{-1}, respectively, whereas MDL and MQL values in real water samples (river water, groundwater, well water, and wastewater) were lower than 0.05 and 0.2 ng mL^{-1}, respectively.

Eight photodegradation products were identified, among which five for the first time. Based on the MS and MS/MS fragmentation, the chemical structures of the degradation products were proposed. Hydrolysis experiments were carried out on the same solutions preserved in the dark, but no formation of other species was highlighted.

The method was applied to several real samples: CPT-11 was detected and quantified only in a hospital effluent sample at the concentration of 0.41 ± 0.2 ng mL^{-1} together with the occurrence of PDP3.

The outcomes of this study may be useful for updating the pollutant screening in water samples.

1. Introduction

Along the last decades, the detection of micropollutants such as pharmaceuticals, personal care products, endocrine disrupting compounds and their metabolites in drinking water sources, including surface water and groundwater, increased the attention on emerging concern contaminants (Negreira et al., 2013). Many studies have shown that the presence of pharmaceutical compounds in water is an emerging issue (Ferrando-Climent et al., 2013; Boix et al., 2015; Nannou et al., 2015). The increase in population density, in population ageing, the fast development of medical science and practitioners' prescription habits (Le Corre et al., 2012) have been the factors which caused the increase in levels of pharmaceuticals in urban wastewater, and this will likely continue to be the situation in the future. The major sources of pharmaceutical residues in wastewater include agricultural and pharmaceutical industries, households and hospitals (Gaw et al., 2014).

Recently, since highly sensitive analytical and bioassay methods have been developed, the persistence and toxicity of antitumoral drugs in aquatic environments and their potential impact on human health have continued to raise scientific and public concerns

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* Corresponding author.
E-mail address: fabio.gosetti@uniupo.it (F. Gosetti).

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(Frédéric and Yves, 2014; Isidori et al., 2016). Antitumoral drugs, also called chemotherapeutic agents or cytotoxic agents are a group of compounds mainly administered to outpatient and inpatient cancer therapy in hospitals and they represent one of the most toxic compounds used as a medication (Rabii et al., 2014). Municipal and hospital wastewaters, treated or untreated, are the main route for antitumoral drugs in the aquatic environment. What happens next in the aquatic environment is not fully investigated yet (Calza et al., 2013; Kosjek et al., 2013). In relation to this, the investigation of transformation products of emerging contaminants in water by employing different analytical techniques has been a focus of recent research (Franquet-Griell et al., 2017).

In this study, the photodegradation of the key anticancer drug irinotecan has been investigated. Irinotecan or 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy camptothecin (CPT-11, Supplementary Material, Fig. 1) is a semi-synthetic water-soluble analogue of the natural alkaloid camptothecin (Marangon et al., 2015).

According to the Anatomical Therapeutic Chemical (ATC) classification of cytotoxic compounds based on their mode of action, CPT-11 belongs to the class of other antineoplastic agents. CPT-11 is among the most widely used drugs in cytotoxic chemotherapy; in 2013, it was reported as one of the 200 most-prescribed off-patent active ingredients in the European Union (Wouters et al., 2017). It is mainly used to treat colon cancer and small cell lung cancer (Besse et al., 2012), but it also showed promising results in the treatment of non-small cell lung cancer, ovarian cancer, malignant brain tumors, gastric cancer, cervical cancer and pancreatic cancer (Babu et al., 2012). Many studies have demonstrated that an extension of overall survival by 30 months has been achieved by combining CPT-11 with 5-fluorouracil, oxaliplatin and several molecularly-targeted antitumoral drugs (Fujita et al., 2015). Moreover, other studies have recommended dose reduction since exposure to SN-38, the active metabolite of CPT-11, can cause severe CPT-11-related toxicities (Ando et al., 2000; Rivory, 2000; Minami et al., 2007).

The aminopentane carboxylic acid (APC), the NPC (a primary amine metabolite) and the SN-38G glucuronide are the other CPT-11 metabolites in humans. APC and SN-38G are the most significant excretion products in urine and bile, whereas SN-38 and APC showed to be most significant in faeces (Slatter et al., 2000). After 4 h of the administration, the CTP-11 concentration in urine is ten times greater than that of its metabolites, whereas after 24 h the CTP-11 excretion due to the faeces is equal to about twice the concentration of its metabolites (Basu et al., 2017). Therefore, not all administered CPT-11 is metabolized, but an amount between 45% and 63% is excreted as parent drug by the human body (Slatter et al., 2000), an amount that typically enters the sewerage system ultimately reaching groundwater and surface water.

For this reason, we focused this study on the possible presence of CPT-11 in the aquatic system, given its relatively high abundance with respect to that of its metabolites.

The occurrence of CPT-11 in the environment has been reported by several studies. CPT-11 was determined in 10 out of 14 hospital wastewater treatment plant (WWTP) effluents in Brazil in concentrations from 1.21 $\mu g\,L^{-1}$ up to 2.03 $\mu g\,L^{-1}$ (Souza et al., 2018). Moreover, CPT-11 was quantified in Spain in hospital effluents at concentration levels up to 0.73 $\mu g\,L^{-1}$ (Gómez-Canela et al., 2014), from 0.042 $\mu g\,L^{-1}$ up to 0.273 $\mu g\,L^{-1}$ (Ferre-Aracil et al., 2016), and from 0.085 $\mu g\,L^{-1}$ up to 0.273 $\mu g\,L^{-1}$ (Olalla et al., 2018), whereas it was found in Norway hospital effluents and in sewage treatment plants at concentrations in the range of 0.015–0.035 $ng\,mL^{-1}$ and 0.015–0.030 $ng\,mL^{-1}$, respectively (Schlabach et al., 2008). In addition, CPT-11 was quantified in wastewater samples up to 49 $ng\,L^{-1}$ from Slovenia (Isidori et al., 2016).

This implies that the anticancer drug was not effectively removed using the technologies employed in the current WWTPs. In agreement with this, a study carried out by Chen et al. (2008) on the removal of CPT-11 in water by both physical and chemical treatment processes indicated that removal of the drug could be partially achieved by physical processes such as powdered activated carbon. The removal of CPT-11 in WWTPs was successfully carried out by direct ozonation (Ferre-Aracil et al., 2016) and by UV-C/H_2O_2 able to remove the drug after 1 min of treatment and with oxidation rates three orders of magnitude higher than ozonation, but the possible formation of more toxic or recalcitrant degradation by-products have to be still investigated (Franquet-Griell et al., 2017). Once entered the aquatic environment, like all other pharmaceutical compounds, the parent drug generally will undergo various transformations such as hydrolysis reactions, sunlight photodegradation, microbial degradation, interactions with dissolved organic matter etc., that have not been fully investigated yet (Calza et al., 2013; Kosjek et al., 2013; Gosetti et al., 2018). Therefore, the monitoring of these species in water samples is fundamental, as they could have strong effects on ecological and human health. The scope of this study was the developing of an ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method for the identification of photodegradation products (PDPs) of CPT-11 in water. To the best of our knowledge, only a few studies concerning the photodegradation of CPT-11 in clinical (Dodds et al., 1998) and water samples (Dodds et al., 1997) have been reported. High-performance liquid chromatography coupled with triple quadrupole mass spectrometry (LC-MS/MS) was used for the detection of five PDPs of CPT-11 by exposing a high concentration of the drug (20 $g\,L^{-1}$ in 0.9% saline solution, pH 8.5) to laboratory fluorescent light at ambient temperature (Dodds et al., 1997). The α-hydroxy δ-lactone ring of the CPT-11 structure has been shown to be photolabile, hydrolyzed reversibly in aqueous solutions, chemically reactive and easily fragmented in MS spectrometry experiments. The present study was focused on investigating the natural photodegradation of CPT-11 for the identification of novel degradation products in water samples. We carried out the degradation of CPT-11 under simulated solar irradiation in a solarbox equipped with a xenon lamp, fixing radiation intensity and temperature values to reproduce the average conditions of surface water for the period between May and September in Alessandria, Italy. This approach was successfully used in previous studies and allowed the identification of several transformation products of different micropollutants in water (Bottaro et al., 2008; Gosetti et al., 2010a, 2010b; Gosetti et al., 2015b; Gosetti et al., 2018; Gosetti et al. 2019). In this paper, we describe the development of a rapid and sensitive UHPLC-MS/MS method suitable for the identification of CPT-11 and its PDPs in different real water samples.

2. Materials and methods

2.1. Reagents and samples

Irinotecan hydrochloride (purity ≥97%), methanol (LC-MS Ultra CHROMASOLV, >99.9%), water (LC-MS grade), and formic acid for LC-MS were purchased from Sigma-Aldrich (Milan, Italy).

The standard stock solution of CPT-11 was prepared in methanol at 50.0 $mg\,L^{-1}$ and was used after proper dilution for the LC-MS method development and validation. The solution was preserved at −20 °C in dark glass vials. On the contrary, CPT-11 aqueous solutions at 10.0 $mg\,L^{-1}$ were used for the irradiation experiments and were freshly prepared.

Some water samples were collected close to the outlet of the Turin depurator, others come from the Po river (close to the depurator exit and near to the Molinette Hospital) and from the

hospital effluent. The groundwater and well water (before and after chlorination) were obtained from the countryside area on the outskirts of the Turin city. All the blank samples were collected near the Noasca, Orco Valley in the protected area of Gran Paradiso National Park (Piedmont, Italy), where we assumed CPT-11 was not present. All the water samples were filtered through 0.2 mm polytetrafluoroethylene (PTFE) filters (VWR International, Darmstadt, Germany) before direct injection into UHPLC-MS/MS system or solid-phase extraction (SPE) procedure.

2.2. Apparatus

The sunlight irradiation was simulated by means of a solarbox 3000a (CoFoMeGra, Milan, Italy), equipped with a xenon lamp and a soda-lime glass UV filter used to better simulate the outdoor exposure. Uv–vis measurements were performed by UV–vis spectrophotometer V-550 (Jasco International Co., Tokyo, Japan). The LC/MS analyses were performed by Nexera Liquid Chromatography Shimadzu (Kyoto, Japan) system equipped by a DGU-20A3R Degasser, two LC-30AD Pumps, a SIL-30AC Autosampler, a CTO-20AC column compartment and a CMB-20A Lite system controller. The system was interfaced with a 3200 QTrap™ LC-MS/MS system (Sciex, Concord, Canada) by a Turbo V™ interface equipped with an electrospray (ESI) source. The 3200 QTrap™ data were processed by Analyst 1.5.2 software (Toronto, Canada).

2.3. The irradiation procedure

Twenty-eight mL of CPT-11 ultrapure water solution at 10.0 mg L^{-1} was put into a cylindrical quartz cell and undergone to simulated sunlight irradiation in the solarbox (Xe lamp irradiation intensity = 600 W m^{-2}; temperature = 35 °C). The instrumental conditions were chosen on the basis of the sun average irradiation (day-night) and temperature in the period of May–September in Alessandria (Piedmont, Italy) as monitored by the meteorological station of our Department. The irradiation experiments were carried out for 13 days. Sample aliquots (3 mL) were withdrawn after the irradiation at prefixed time intervals (0 h, 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 154 h, 182 h, 296 h, 312 h). After each withdrawing, the quartz cell was emptied and accurately cleaned; a new fresh CPT-11 solution was introduced up to fill the quartz cell and undergone to irradiation for the next prefixed time. To monitor the progress of the photodegradation process, the aliquots were subsequently analyzed by measuring the UV–vis absorbance spectrum (190–490 nm). After UV spectral measurements, the aliquots were preserved in amber vials at −20 °C until further analysis by UHPLC-MS/MS.

2.4. Solid-phase extraction (SPE) procedure

The SPE cartridge was a C18 Isolute (1 mL, 100 mg) from VWR (Merck, Darmstadt, Germany) and it was conditioned with 2.0 mL of methanol and 2.0 mL of ultrapure water. After the loading of the sample (10 mL), the cartridge was washed with 2.0 mL of water and then dried for 5 min under vacuum. The elution was carried out with 1.6 mL of methanol, evaporating to dryness with a gentle stream of nitrogen and reconstituted with 1.0 mL of the mobile phase at the initial gradient conditions (final pre-concentration factor 10x).

2.5. UHPLC-MS/MS conditions

The stationary phase was a Kinetex XB-C18 column (3.0 mm × 100 mm, 1.7 μm) (Phenomenex, Bologna, Italy). The mobile phase was a mixture of water with the addition of 0.1% formic acid (A) and methanol with the addition of 0.1% formic acid (B), eluting at a flow rate 0.45 mL min^{-1}. The final gradient conditions of UHPLC-MS/MS working in selected reaction monitoring (SRM) mode were the following: 0.00–0.50 min 10% B, 8.00 min 90% B, 8.01 min 100% B, 8.01–8.80 min 100% B, and 8.81 min 10% B until to 10.00 min. The injection volume was 5.0 μL and the oven temperature was set at 40 °C.

Turbo V™ interface was used to produce the turbo ion spray (TIS) ionization in positive ion (PI) mode. The instrumental source parameters were set as follows: curtain gas (N_2) at 30 psig, nebuliser gas GS1 (N_2) and GS2 (N_2) at 70 and 65 psig, respectively, desolvation temperature (TEM) at 450 °C, collision activated dissociation gas (CAD) at 6 units of the arbitrary scale of the instrument and ion spray voltage (IS) at + 5300 V. The collision energy (CE) was set at +10 in MS experiment and at +65 V with the addition of ±20 V due to the collision energy spread (CES) in the Enhanced Product Ion (EPI) experiments. The unit mass resolution was established and maintained in each mass-resolving quadrupole by keeping a full width at half maximum (FWHM) of about 0.7 u.

As concerns the Data Dependent Analysis experiments (DDA), the MS worked cyclising an Enhanced MS experiment (EMS) at 1000 Da/s as survey scan, and two dependent experiments, namely an Enhanced Resolution (ER) experiment at 250 Da/s, and an EPI experiment at 1000 Da/s. The EMS works between *m/z* 100 and *m/z* 700 on the most intense ion, using dynamic background subtraction. The total cycle time of the analysis was 1.96 s. The DDA conditions that have to be satisfied in order to trigger the dependent scans were as follows: the ion of survey scan must be greater than *m/z* 100 and smaller than *m/z* 700, and it must exceed the threshold of 10,000 counts per second. If an ion is monitored for more than 3 times (number of occurrences = 3), then it will be excluded for 5 s from the future scans. When the MS worked in DDA, the total run time of the UHPLC-MS run was 20 min, using a flow-rate at 0.30 mL min^{-1} and the following slow gradient profile: 0.00–1.00 min 10% B, 17.00 min 100% B, 17.00–18.00 min 100% B, 18.10 min 10% until to 20.00 min.

3. Results and discussion

3.1. UV–vis spectrophotometric measurements

UV–vis absorbance spectrum measurements were carried out for the aliquots sampled at the prefixed irradiation times. The results (Fig. 1) indicated that about 90% of the initial peak intensities of CPT-11 at $\lambda_1 = 221$ nm was reduced after 7.5 days, corresponding to 182 h. It is important to point out that between 296 h (12.3 days) and 312 h (13 days) of irradiation, no significant reduction of the absorbance values was observed due to the very low amount (<5% of the initial) of the not-degraded parent CPT-11 compound.

3.2. Development and validation of the UHPLC-MS/MS method

In order to develop a sensitive UHPLC-MS/MS method able to identify unknown photodegradation compounds to search for in water samples, it has been necessary to use the hybrid MS detector first in quadrupole-ion trap (Q-LIT) mode and then in triple quadrupole (QQQ) mode. In particular, Q-LIT mode was used to realize the precursor/product ion transitions in order to build a more selective, sensitive and fast SRM method in the QQQ one.

Q-LIT mode allows to carry out non-target analysis and to obtain information about the type of the species that find themselves in the analyzed samples. Information Dependent Acquisition (IDA), a type of DDA, was used for the acquisition of the data. It permits to concatenate a “survey scan” experiment (the first) with others

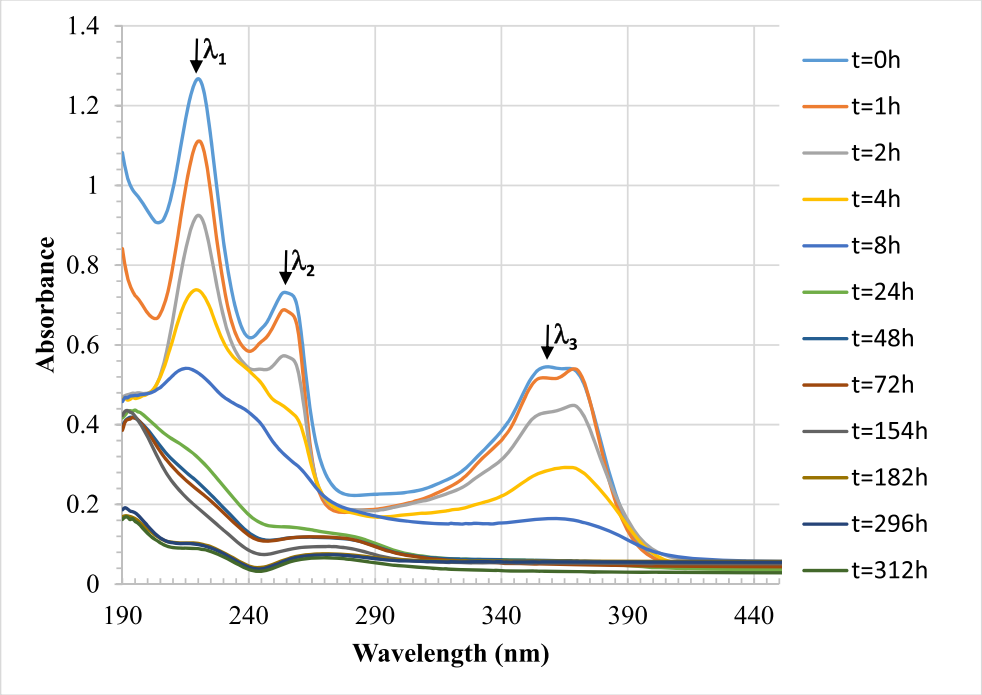


Fig. 1. The photodegradation of CPT-11: UV–vis absorbance spectrum measured as a function of solar light irradiation time ($\lambda_1 = 221$ nm, $\lambda_2 = 255$ nm, $\lambda_3 = 359$ nm).

("dependent scans") if the set IDA criteria are passed. Each cycle (survey-dependent scans) is repeated during the whole time of the chromatographic run.

In order to collect as much information as possible about the characteristic of the species that can form during the irradiation, CPT-11 standard aqueous solutions irradiated at different times were subjected to the above-mentioned IDA approach.

First of all the EMS was selected as survey scan for the non-target screening. In this modality, the Q-TRAP uses the third quadrupole as ion trap for the accumulation of the ions of interest. When the EMS acquires a signal exceeding a determined threshold, then the other two dependent scans experiments (ER and EPI) by default are carried out. The ER mode is useful for the confirmation of the isotope pattern, whereas the EPI mode is an enhanced MS/MS scan, in which the third quadrupole is used as ion trap for providing a higher abundance of product ions.

In this step of the method development the flow-rate is moderate (0.30 mL min^{-1}) and the change of organic phase percentage (B%) in the mobile phase is very slow (starting from 10% B at 1.00 min and arriving at 100% B at 17.00 min). This is useful to elute the largest number of chromatographically resolved peaks from the chromatographic column, whose characteristic *m/z* signals can undergo a DDA procedure.

At last, an SRM method was built using the precursor/product ion transitions assigned to each chromatographic peak. However, the collision energy values were optimized, as the values obtained by EPI experiments were slightly diverse from those that can be obtained from a typical MS/MS performed by a QQQ instrument. For this reason, the PDPs were infused into the MS and all the compound dependent parameters were re-optimized (Declustering Potential, DP, Entrance Potential, EP, Collision Energy, CE, and Collision eXit Potential, CXP). The final SRM transitions are reported in Supplementary Material, Table 1.

At last, the chromatographic run time was reduced to 10 min, changing the gradient profile as reported in the Experimental section.

Table 1
Precision intra- and inter-day and MDL values for each real water samples (river water, groundwater well water and wastewater).

	river water	groundwater	well water	wastewater
Intraday precision (%)	2.0	1.9	2.1	3.4
Interday precision (%)	5.6	5.6	5.7	5.9
MDL (ng mL^{-1})	0.03	0.02	0.03	0.05
MQL (ng mL^{-1})	0.1	0.06	0.09	0.2

3.3. Validation of the method

After proper dilutions with H_2O/CH_3OH 90/10 (v/v) with the addition of 0.1% formic acid, CPT-11 stock solution was used to prepare an external calibration plot. Eleven different standard solutions were prepared (LOQ, 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, 100, 250, 500, and $1.00 \cdot 10^3$ ng mL^{-1}) and injected in randomized order, to prevent a possible experimental error dependence on the analyses execution order. The analyte chromatographic peak area (qualifier transition, *y*) vs the standard concentration (*x*) were correlated (weighting factor $1/x$), obtaining a linear regression fit with $R^2 = 0.9992$. In order to verify that the variance fraction explained by the model was significant, an *F* test was carried out: the F_{calc} value = 150552.77 was definitely greater than the $F_{tab(0.01,1,31)} = 7.53$.

Taking into account the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) regulation, the limit of detection (LOD) and the limit of quantitation (LOQ) were calculated by using the calibration plot as the analyte concentration giving a signal of $3.3s_B/b$ and $10s_B/b$, respectively. s_B is the blank standard deviation, equal to the residual standard deviation ($s_{y/x}$), whereas *b* is the calibration plot slope. The LODs and LOQs were 0.02 ng mL^{-1} and 0.05 ng mL^{-1}, respectively, and confirmed the high sensitivity of this method.

Seven replicates of each investigated blank real water sample (river water, groundwater, well water and wastewater) spiked with

a CPT-11 solution at a concentration giving a S/N ratio between 2.5 and 5 were used for the method detection limit (MDL) determination. It was calculated as $MDL = t_{(n-1,1-\alpha=0.99)} \times S_d$, where $t = 3.14$ corresponds to a *t*-Student's value for 99% confidence level and 6 freedom degrees, and S_d is the standard deviation of the replicate analyses. The method quantification limit (MQL) was defined as 3 times the MDL value. MDLs and MQLs, calculated for river water, groundwater, well water and wastewater were certainly satisfying and were in the range of 0.02–0.05 and 0.06–0.2 ng mL^{-1}, respectively (Table 1).

The intraday precision was determined both for the retention times and the concentrations, analyzing a CPT-11 standard solution at LOQ value for 5 times (n = 5). The interday precision was determined replicating the analyses for 7 days (n = 35). The intraday relative standard deviation (RSD%) of the precision of the retention times and the concentrations is lower than 0.34% and 2.3%, respectively, whereas the interday one is lower than 0.69% and 5.6%, respectively.

The intraday and interday precision were determined also for the real samples, injecting blank real samples previously spiked with a CPT-11 solution at the corresponding MQL value. The intraday and interday relative standard deviation (RSD%) of the precision of the concentration were in the range of 1.9%–3.4% and 5.6%–5.9%, respectively (Table 1).

The four different blank real samples (river water, groundwater, well water and wastewater), were employed also for the investigation of the selectivity: no interfering species at the analyte retention time was observed in any real considered blank samples.

As regarding the presence of the matrix effect *ME*, it was evaluated by calculating the *ME* percentage (*ME%*) as: $ME(\%) = \frac{slope_{add}}{slope_{ext}} * 100 - 100$, where $slope_{ext}$ is the slope of the external calibration plot and $slope_{add}$ that of the standard addition plot built spiking the real water sample with the CPT-11 standard solutions at 50.0, 100 and $1.00 \cdot 10^3$ ng mL^{-1} after the sample pretreatment (analyses were replicated three times). The comparison of the above-mentioned slopes can give as a result of a *t*-test *ME%* = 0, i.e. no matrix effect is present, *ME%*<0 (signal suppression) or *ME%*>0 (signal enhancement). The *t*-test results showed that *ME* was not significant for the CPT-11 in the river, groundwater and well water samples, whereas a signal suppression of 7% was found for the wastewater sample.

The recovery *R* was calculated as C_{obs}/C_{ref}, where C_{obs} was the concentration of CPT-11 determined after the LC-MS/MS analysis, whereas C_{ref} was that of a spiked solution. The CPT-11 solutions were prepared at 0.1, 10.0 and 100.0 ng mL^{-1}, in order to explore as much as possible the whole linearity range, and were used to spike the blank real samples, that were undergone to SPE procedure, replicating the analyses three times. Table 2 reports the *R* values calculated for each spiked concentration level. The *R* values have good reproducibility and were independent of the analyte concentration with respect to the investigated concentration range: in fact, a *t*-test (95% confidence level) showed that there was no difference among the *R* values of the three concentration levels for all the investigated real samples. For this reason, it was possible to calculate an average recovery percentage (*R%*) for each investigated real samples: the values were in the range of 94.8%–105.9%.

3.4. Identification of photodegradation products

First, the MS/MS characterization study of the CPT-11 was performed, in order to provide information about the analyte fragmentation pathway, which can be useful for the identification of unknown PDPs. ESI PI mode was more sensitive and suitable both for the precursor compound and for the PDP ones; therefore, all MS/MS experiments were carried out in this ionization mode. The CPT-11 quasi-molecular ion $[M+H]^+$ was detected with good signal intensity at *m/z* 587.3. The proposed fragmentation pathway of CPT-11 is shown in Fig. 2.

Control experiments conducted in the dark for 13 days revealed no appreciable decay of CPT-11 or the formation of degradation products; whereas eight photodegradation products were identified from the photo-irradiated samples. The total ion chromatogram (TIC) in Fig. 3 shows the disappearance of the CPT-11 peak (*m/z* 587.3, at RT = 5.96) and the increase of the PDPs' peaks, on the left and right of the CPT-11 peak. Eight PDPs of CPT-11 were identified at *m/z* 423.3, *m/z* 437.3, *m/z* 439.3, *m/z* 529.3, *m/z* 557.3, *m/z* 573.3, *m/z* 603.4 and *m/z* 619.4, taking into consideration that PDP5 at m/z 603.4 had two isomers, the first was twice as intense as the latter and eluted at 5.76 min, and the second one coeluted with the peak of the CPT-11.

The evolution profile of the four most abundant PDPs is given in Fig. 4. The trend for most of the PDPs was explained by a sharp increase in the first 4 h, followed by an approximately sharp decline. Then, the PDPs were close to disappearance within 24 h. The only exception was PDP2 with a fast rate of formation in the first 2 h. It was produced steadily for about 1 day and started to decline afterwards. This typical feature of PDP2 could be attributed to the contribution of the other PDPs in its production (Dodds et al., 1997).

The reaction order of the CPT-11 photodegradation was determined by plotting ln *A* (*A* being the chromatographic peak area of CPT-11) against the irradiation time (h) (Supplementary Material, Fig. 2). A linear behavior indicating linear kinetic model of the first order was observed. The corresponding value of the determination coefficient (R^2) was greater than 0.95 and the half-life time ($t_{1/2}$) and the rate constant were 8.24 h and 0.0841 h^{-1}. However, these values do not indicate properly the real decay rate of the drug in natural water, since the initial concentration value of the CPT-11 affects the kinetics of the photodegradation. On the contrary, it was necessary to start from an initial concentration of 10.0 mg L^{-1} of the CPT-11, otherwise we could not have developed the LC-MS method for the PDPs owing to the poor chromatographic peak intensities that we could obtain. In order to give to the reader a more realistic photodegradation half-life time of the CPT-11, experiments carried out at starting concentration values of the drug of 1.0, 0.1 and 0.05 mg L^{-1} gave kinetics half-times of 38.5 h, 57.7 h, and 77.0 h, respectively.

For what regards the solutions preserved in the dark for a month at the same concentration of the solutions undergone to irradiation, no evidence of hydrolysis reactions was observed, i.e. neither appreciable formation of hydrolytic products nor the decreasing of the CPT-11 chromatographic peak area was observed.

In a similar way to CPT-11, MS/MS characterization study of each of the PDPs was conducted to elucidate the chemical structures of PDPs. Initial decarboxylation of CPT-11 (*m/z* 587.3 → *m/z* 543.3) was revealed, a typical fragmentation of camptothecins in the lactone form. Based on the UHPLC-MS/MS data obtained in the present study, the PDPs all appeared to feature modifications to the lactone ring (i.e., the 7-ethyl-camptothecin nucleus). A characteristic loss of the distal piperidine (−85.1 u) was consistent in the fragmentation spectra of the quasi-molecular ions for both CPT-11 and all PDPs. Additionally, the presence of the fragments at *m/z* 110.3, *m/z* 124.3, *m/z* 167.3, and *m/z* 195.3, due to bipiperidine moiety (already found during CPT-11 characterization, see Fig. 2) combined with the absence of decarboxylation of the lactone moiety indicates that this latter moiety was the site of modification for the PDPs. PDP4 and PDP5 were hydroxylated twice and once, respectively, and they dehydrated easily by the initial loss of H_2O. Two isomers of PDP5 were found (namely PDP5′ at RT = 5.76 min and PDP5″ at RT = 6.03 min): the intensity of the PDP5' was twice

Table 2
Recovery values at three different concentration levels (C_{ref}), and average recovery percentage ($\bar{R}\%$) of CPT-11 in real water samples (river water, groundwater well water and wastewater).

Spiked conc. (ng mL^{-1})	C_{ref} (ng mL^{-1})	C_{obs} (ng mL^{-1}) river water	C_{obs} (ng mL^{-1}) groundwater	C_{obs} (ng mL^{-1}) well water	C_{obs} (ng mL^{-1}) wastewater	R (%) river water	R (%) groundwater	R (%) well water	R (%) wastewater
0.1	1.0	1.0	1.0	1.0	0.9				
0.1	1.0	1.0	1.0	1.1	0.9				
0.1	1.0	1.1	1.1	1.0	1.0	104.1 ± 5.8	100.3 ± 5.8	102.9 ± 4.2	95.0 ± 5.5
10.0	100	109	98	102	92				
10.0	100	109	110	111	95				
10.0	100	119	98	110	97	112.5 ± 6.0	102.1 ± 7.1	107.6 ± 5.0	94.7 ± 2.4
100	1000	1075	1019	1116	931				
100	1000	925	1086	1025	910				
100	1000	953	999	1074	997	98.4 ± 8.0	103.5 ± 4.6	107.2 ± 4.6	94.6 ± 4.5
					Average recovery ($\bar{R}\%$)	105.0 ± 7.1	101.9 ± 1.6	105.9 ± 2.6	94.8 ± 0.2

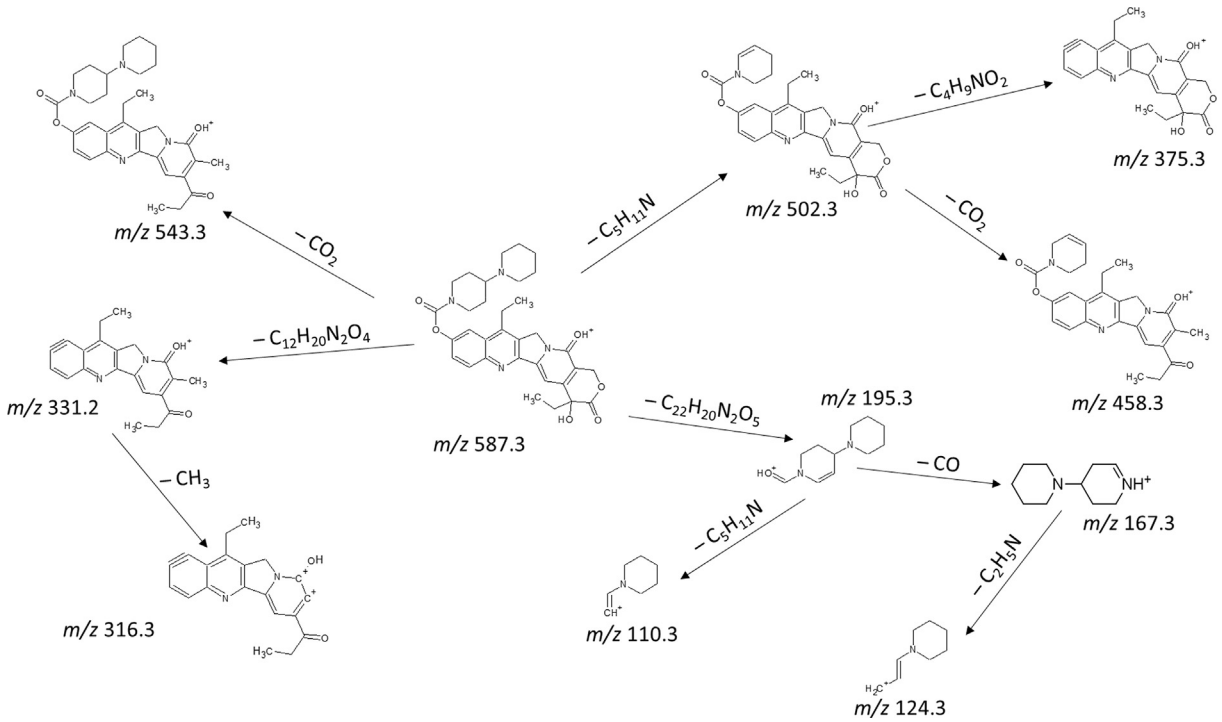


Fig. 2. Proposed fragmentation pathways of CPT-11.

that the second one.

In order to propose the chemical structures of the unknown species formed during the photoirradiation process, we used the following experimental data: (a) the quasi-molecular ion from MS experiment by which the molecular mass is identified; (b) the ER spectrum by which the isotopic pattern is obtained; (c) the number of nitrogen atoms (even or odd) depending on the even or odd molecular mass; (d) the EPI MS/MS spectrum by which it is possible to identify the main fragment ions of the considered species. In this way, the compounds PDP1, PDP2, PDP3, PDP4 and PDP8 were identified for the first time. The MS/MS spectra of the PDPs with assigned structures are reported in Supplementary Materials, Fig. 3.

PDP5' structure confirmed the compound that was isolated from the bile and urine of a patient treated with CPT-11 (Lokiec et al., 1996). The compounds PDP6 and PDP7 were already found, although the experiments were conducted on conditions relevant to those used in pharmacological studies by exposing aqueous solutions to laboratory light (Dodds et al., 1997).

A summary of the data obtained from LC-MS/MS and the proposed structures of the PDPs are presented in Table 3, in which the main product ions of different PDPs are shown.

As can be seen from Table 3, PDP2 has a high degree of transformation and this product persisted longer (Fig. 4), whereas the formation of the other PDPs declined with the disappearance of CPT-11. The longer persistence of PDP2 could be associated with its formation in the later stages through a number of the PDPs as intermediates. PDP3 is also one of the products with a high degree of transformation, but it was further transformed faster than PDP2. Both PDP2 and PDP3 appear to be products of PDP1. Moreover, it seems that PDP5 and PDP6 are possible intermediates for PDP4 and PDP8, respectively.

3.5. Real sample analyses

The whole analytical procedure here developed was applied to different water samples (2 river water, 1 groundwater, 2 well water

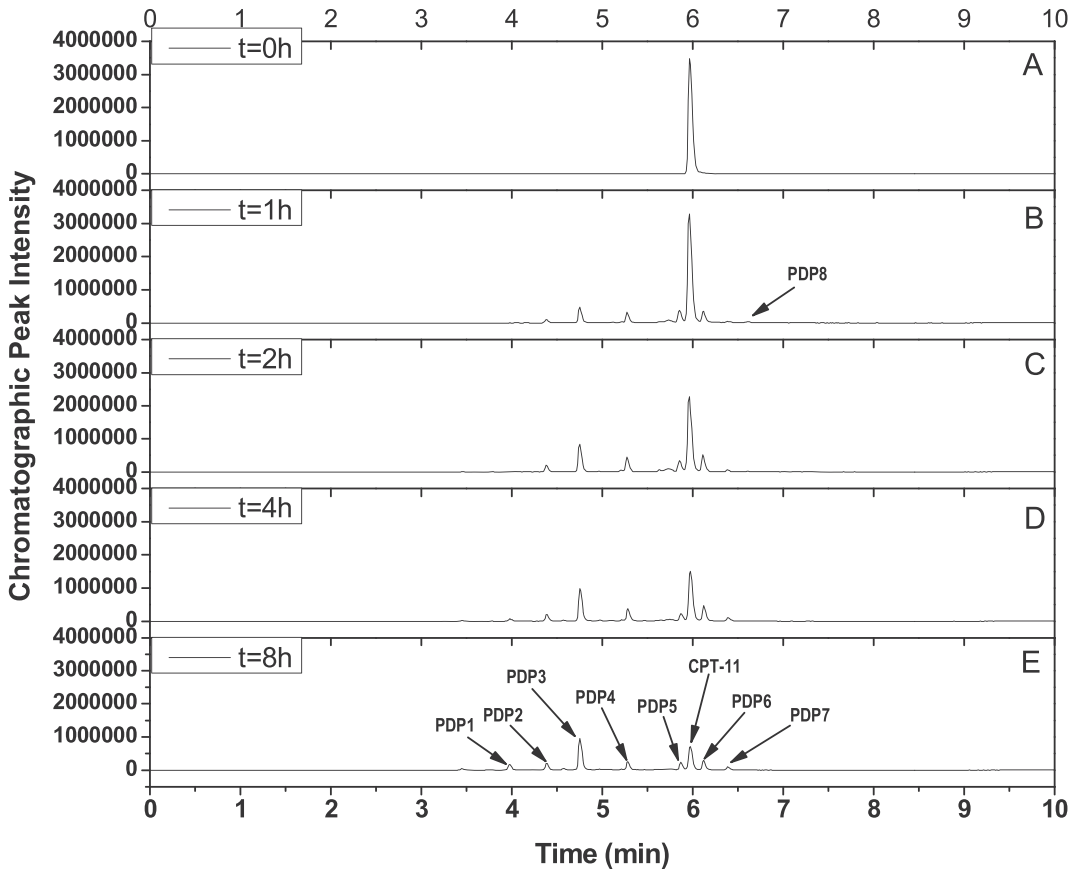


Fig. 3. Total ion chromatogram of CPT-11 and its PDPs for non-irradiated (A), and after irradiation for 1 h (B), 2 h (C), 4 h (D) and 8 h (E) of irradiation. The peaks detected for PDP8 (B) and PDP1-PDP7 (E) are indicated by arrows (an isomer of PDP5″ coelutes with CPT-11).

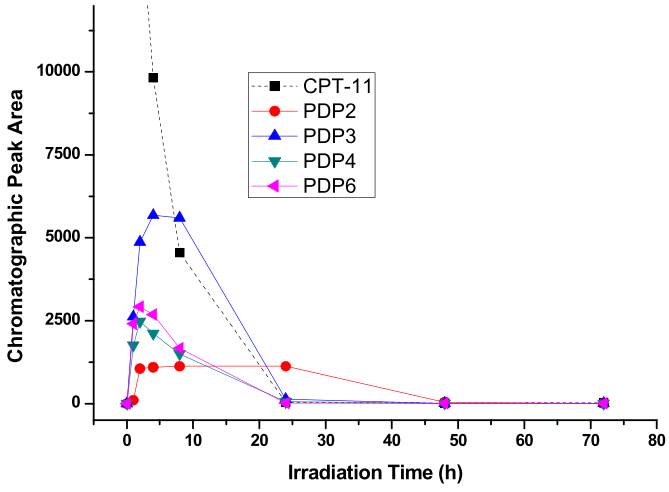


Fig. 4. The evolution profile of the four most abundant PDPs of CPT-11.

(1 before and 1 after chlorination), 3 samples collected close to a depurator outlet), 1 hospital effluent. The analyses were replicated three times for each sample. CPT-11 was detected and quantified only in the hospital effluent sample at the concentration of 0.41 ± 0.2 ng mL^{-1} together with the presence of the PDP3.

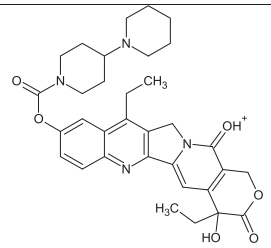
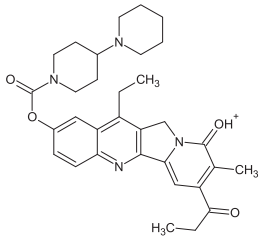
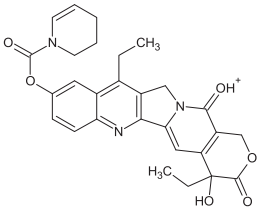
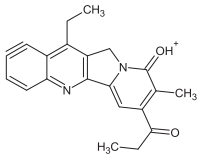
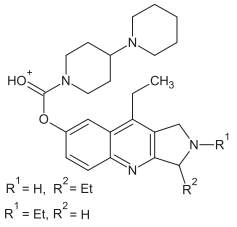
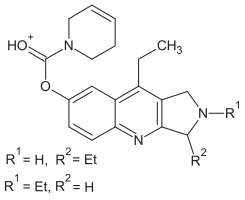
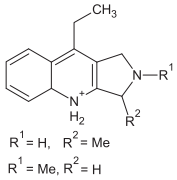
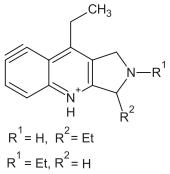
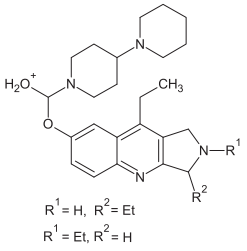
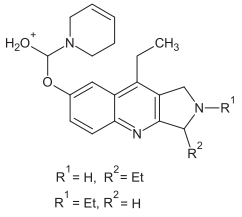
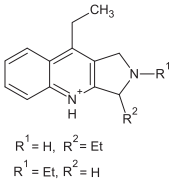
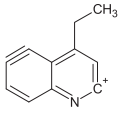
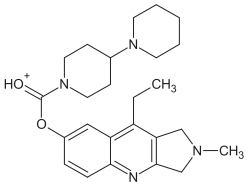
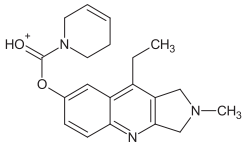
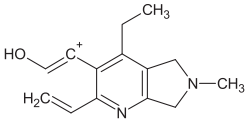
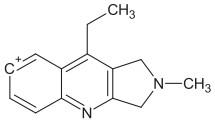
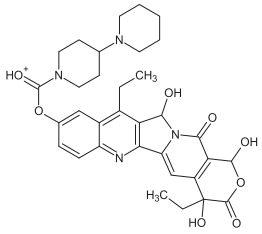
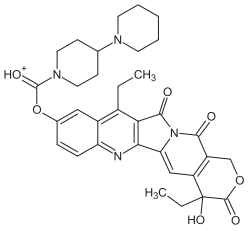
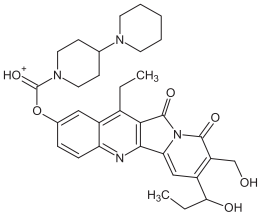
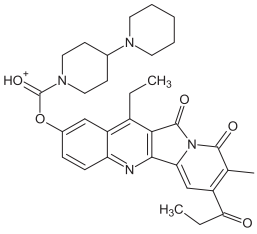
In order to simultaneously identify the maximum number of PDPs, the Po river water was spiked with CPT-11 at the concentration of 10.0 mg mL^{-1} and the experiment was replicated three times. The solutions were photo-irradiated for 8 h (time in which the largest number of degradation product formed), subjected to SPE procedure and successively analyzed by UHPLC-MS/MS.

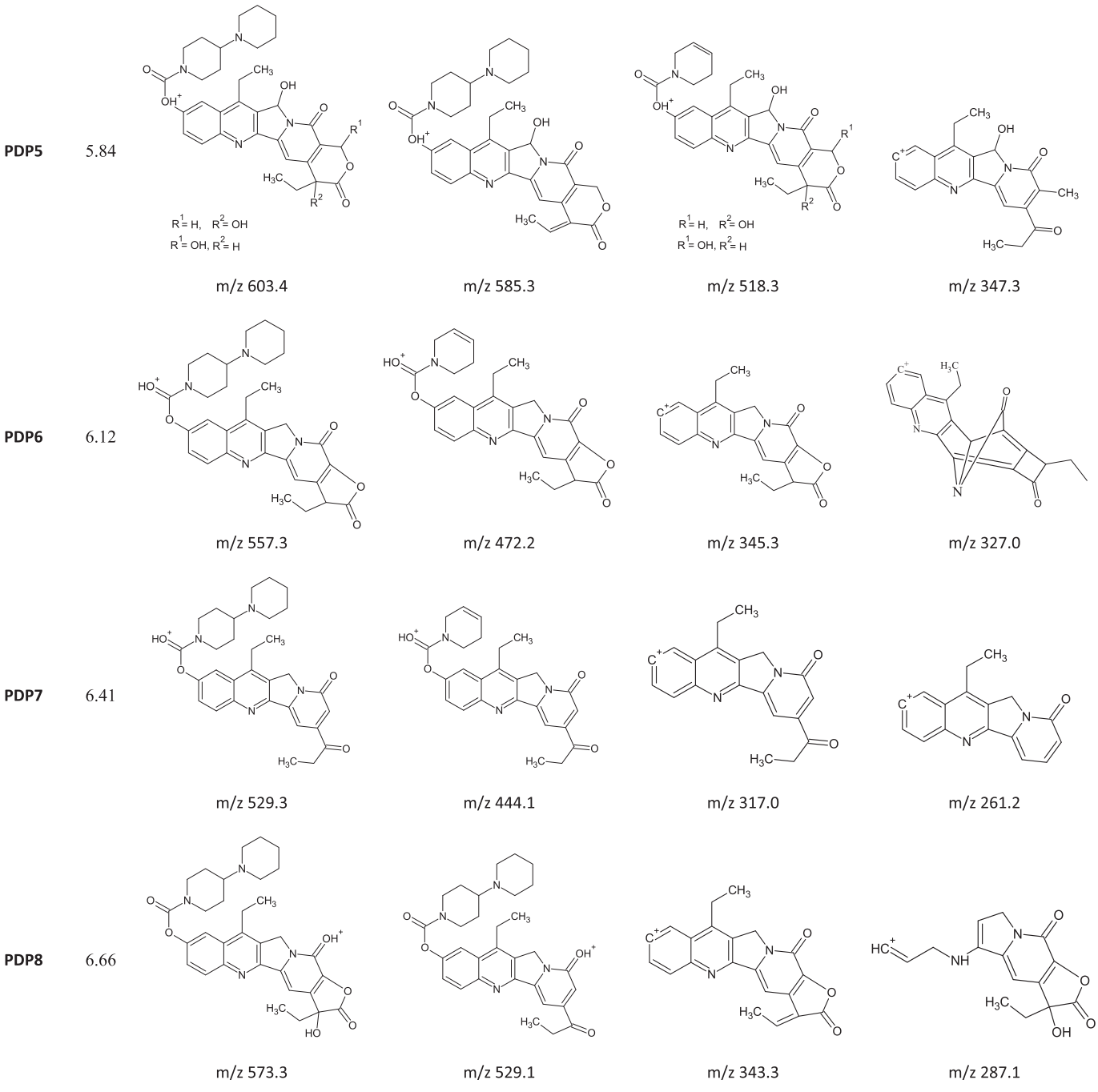
The results showed the formation of the same PDPs of CPT-11 both in river water and in ultrapure one (Supplementary Material, Fig. 4).

4. Conclusions

In this study, we developed and validated a very sensitive UHPLC-MS/MS method for the identification of CPT-11 PDPs in the aqueous system. A total of eight PDPs were identified and their chemical structures proposed. Five of them (PDP1, PDP2, PDP3, PDP4 and PDP8) were identified for the first time and only two of them (i.e. PDP6 and PDP7) were previously detected in aqueous samples. The toxicity of these compounds was not investigated here, but considering that almost all their chemical structures contain the mappicine core, we can suppose that the CPT-11 PDPs could be active against viruses or could have cytotoxic properties (Dodds et al., 1997). The findings of the present study will be significant for updating the current knowledge about the formation and the identification of CPT-11 PDPs and they can be useful to implement the monitoring of micropollutants in water systems.

Table 3
Proposed chemical structures of quasi-molecular ion $[M+H]^+$ and of three corresponding product ions with their retention times (RT). The chemical structures of the most abundant product ions at *m/z* 167.3, *m/z* 124.3 and *m/z* 195.3 are not reported here, as they are already shown in Fig. 2.2

Compound	RT (min)	Structure [M+H]+	Product Ion 1	Product Ion 2	Product Ion 3
CPT-11	5.96	 m/z 587.3	 m/z 543.4	 m/z 502.3	 m/z 331.3
PDP1	3.98	 m/z 437.3	 m/z 352.4	 m/z 229.3	 m/z 225.2
PDP2	4.39	 m/z 439.3	 m/z 354.3	 m/z 227.3	 m/z 154.2
PDP3	4.75	 m/z 423.3	 m/z 338.2	 m/z 229.3	 m/z 211.4
PDP4	5.28	 m/z 619.4	 m/z 601.3	 m/z 575.3	 m/z 557.3



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Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113370.

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