Environmental Pollution 253 (2019) 655–666

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Target quantification of azole antifungals and retrospective screening of other emerging pollutants in wastewater effluent using UHPLC–QTOF-MS☆

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ARTICLE INFO

Article history:
Received 12 April 2019
Received in revised form
19 June 2019
Accepted 15 July 2019
Available online 16 July 2019

Keywords:
Azole antifungal drugs
Suspect screening
Non-target screening
Retrospective screening
Emerging pollutants
Wastewater effluent

ABSTRACT

The information acquired by high resolution quadrupole-time of flight mass spectrometry (QTOF-MS) allows target analysis as well as retrospective screening for the presence of suspect or unknown emerging pollutants which were not included in the target analysis. Targeted quantification of eight azole antifungal drugs in wastewater effluent as well as new and relatively simple retrospective suspect and non-target screening strategy for emerging pollutants using UHPLC-QTOF-MS is described in this work. More than 300 (parent compounds and transformation products) and 150 accurate masses were included in the retrospective suspect and non-target screening, respectively. Tentative identification of suspects and unknowns was based on accurate masses, peak intensity, blank subtraction, isotopic pattern (mSigma value), compound annotation using data bases such as KEGG and CHEBI, and fragmentation pattern interpretation. In the targeted analysis, clotrimazole, fluconazole, itraconazole, ketoconazole and posaconazole were detected in the effluent wastewater sample, fluconazole being with highest average concentration (302.38 ng L^{-1}). The retrospective screening resulted in the detection of 27 compounds that had not been included in the target analysis. The suspect compounds tentatively identified included atazanavir, citalopram, climbazole, bezafibrate estradiol, desmethylvenlafaxine, losartan carboxylic acid and cetirizine, of which citalopram, estradiol and cetirizine were confirmed using a standard. Carbamazepine, atrazine, efavirenz, lopinavir, fexofenadine and 5-methylbenzotriazole were among the compounds detected following the non-targeted screening approach, of which carbamazepine was confirmed using a standard. Given the detection of the target antifungals in the effluent, the findings are a call for a wide assessment of their occurrence in aquatic environments and their role in ecotoxicology as well as in selection of drug resistant fungi. The findings of this work further highlights the practical benefits obtained for the identification of a broader range of emerging pollutants in the environment when retrospective screening is applied to high resolution and high accuracy mass spectrometric data.

1. Introduction

The use of non-conventional water resources such as treated wastewater effluents has been introduced to meet the current and future demands of water (Bellver-Domingo et al., 2017), which is under growing stress (Shevah, 2014; Besha et al., 2017). For example, wastewater reuse for diverse purposes has been implemented by many countries such as France, Italy, Israel, Cyprus, Singapore, Spain, Malta, Jordan, USA, Saudi Arabia, Qatar, Kuwait, Namibia and South Africa (Becerra-Castro et al., 2015; Menge, 2010; Lyu et al., 2016; Ng, 2018; Adewumi et al., 2010).

The presence of tens of thousands of micropollutants in the effluents of wastewater, however, has made the quality of the effluent questionable as most of the conventional wastewater treatment plants are not designed to remove emerging pollutants such as pharmaceuticals, personal care products, disinfection byproducts, industrial chemicals and pesticides (Fatta-Kassinos et al., 2016; Schymanski et al., 2014a; Zuo et al., 2013). The presence of these contaminants in wastewater effluents and their subsequent release to the environment might be followed by

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

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https://doi.org/10.1016/j.envpol.2019.07.075
0269-7491/

detrimental effect to both human health and aquatic ecosystems (Sousa et al., 2017; Zuo et al., 2006; Pahigian and Zuo, 2018). Investigations aimed at studying occurrence of the micropollutants in the effluent wastewater and obtaining better knowledge of chemical status of wastewater is therefore indispensable to determine its quality, predict the potential risks associated with its re-use and development of efficient removal approaches.

Azole antifungal drugs are a group of pharmaceuticals which recently emerged as a new class of environmental pollutants (Castro et al., 2016). Their extensive usage in human as well as in agriculture and personal care products (Liu et al., 2016; García-Valcárcel and Tadeo, 2011) has resulted in significant and widespread presence of their residues in the environment such as wastewater, surface water, groundwater, sludge, sediment, and biosolid amended soils (Gottschall et al., 2012; Chen and Ying, 2015). Their presence in the environment has been associated with negative effect to non-target aquatic organisms (e.g. endocrine disruption) and plants (e.g. retardation of growth) (Zarn et al., 2003; Richter et al., 2016). Moreover, increased use of azoles has resulted in the emergence of less susceptible as well as drug resistant fungal species (Brandão et al., 2010). Due to the concerns both for non-target organisms and a potential health risk for humans, reliable determination of the azoles in different environmental matrices is essential (Barra Caracciolo et al., 2015).

Ultrahigh performance liquid chromatography-Quadrupole Time of flight tandem mass spectrometer (UHPLC-QTOF-MS) is a powerful analytical technique for residue analysis in aquatic environments due to its intrinsic characteristics of accurate mass measurements and high resolution (Zhao et al., 2014). It permits the acquisition of full scan product ion spectra with measurement of accurate mass of product ions thereby securing reliable identification of compounds (Ibáñez et al., 2009; Petrovic and Barceló, 2006). Moreover, full scan acquisition in UHPLC-QTOF-MS has led to the retrospective analysis of data generated for target analysis. Retrospective analysis enables detection of suspect and unknown compounds other than the targets compounds as the full spectrum for accurate mass data can be archived (Kinyua et al., 2015; Hernández et al., 2012a). Retrospective analysis also provides historical record of pollution profile of an aquatic environment by tracking the occurrence in time of the micropollutants (Chiaia-Hernandez et al., 2012). Unlike the discussions which have been going on for some time on retrospective analysis with high resolution mass spectrometry (Geissen et al., 2015; Hernández et al., 2012b), there are few reports in the open literature that have used the approach (Hernández et al., 2011a; Polgár et al., 2012). In this study (i) preliminary investigation on the occurrence of eight commonly used azole antifungal drugs in wastewater effluent was performed using UHPLC-QTOF-MS (ii) the data generated during the target analysis of the azole antifungals was retrospectively analyzed for further identification of other pollutants through suspect and non-target screening. The main objective of this work was to explore the potential of retrospective screening for detection of wide range of emerging pollutants through suspect and non-target screening of high resolution mass spectrometry data.

2. Experimental

2.1. Reagents and materials

All reagents and pharmaceutical standards used were of analytical grade. Clotrimazole, econazole, fluconazole, itraconazole, ketoconazole, miconazole, and voriconazole reference standards, all European Pharmacopoeia standards, were purchased from Sigma Aldrich (Missouri, United States). Analytical standard for posaconazole was also purchased from Sigma Aldrich (Missouri, United States). LC-MS CHROMASOLV® grade methanol and acetonitrile were purchased from Sigma Aldrich (Missouri, United States). Ultrapure water (UPW) was produced by an Integral 10 Elix Milli-Q system with an LC (Bio-pak) polisher (Massachusetts, USA).

Individual stock standard solutions (1000 mg L^{-1}) were prepared in methanol and stored at −20 °C. A mixture of all pharmaceutical standards (100 mg L^{-1}) was prepared in methanol–water (50:50 v/v) by appropriate dilution of individual stock solutions and was used to prepare working solutions. All prepared standard solutions were stored at −20 °C in a freezer.

Atlantic HLB-H disks (47 mm) from Waters Corporation (New Hampshire, USA) were used for the solid phase extraction (SPE) of water samples. Whatman Grade GF/F Glass Microfiber Filters (Missouri, United States) with a diameter of 110 mm was used for filtering water samples before extraction.

2.2. Study area and sample collection

Effluent wastewater sample was collected from one of the wastewater treatment plants in Pretoria, located in the most densely populated Gauteng province, South Africa, namely Daspoort wastewater treatment plant (DWWTP) (25°44′03.8″S 28°10′32.2″E). The wastewater treatment plant receives mostly domestic wastewaters. The existing wastewater treatment works is based on trickling filter and activated sludge technology. DWWTP discharges its effluent to Apies River that connects down to the Pienaars River which is one of the tributaries of Crocodile River. Crocodile River flows into the Hartbeespoort Dam, which is the source of irrigation and drinking water for the local community in Hartbeespoort area, Northwest province in South Africa.

Ambered glass bottles pre-rinsed with ultrapure water and flushed three times with the wastewater were used for sample collection. After collection, samples were kept at 4 °C until arrival to the laboratory and processed within 48 h.

2.3. Sample pre-concentration

The wastewater sample was preconcentrated following the method described by Huang et al. (2010), with slight modification. Briefly, wastewater sample was filtered using 0.7 μm glass microfiber filters, pH adjusted to 2 using formic acid and preconcentrated using Dionex™ AutoTrace™ 280 solid phase extraction (SPE), (Thermo scientific, Massachusetts, United States). SPE disk was sequentially conditioned with 10 mL of methanol and 10 mL of ultrapure water (pH = 2) at flow rate of 10 mL min^{-1}. Then after, 500 mL of wastewater was loaded to the disk at a flow rate of 5 mL min^{-1}. The disk was rinsed with 10 mL of 10% MeOH in water followed by drying with the help of nitrogen for 15 min. Elution was performed with 2 × 5 mL of methanol at 3 mL min^{-1}. The extract was evaporated under a gentle nitrogen stream and reconstituted with 1 mL of methanol–water (50:50, v/v). Finally, reconstituted extracts were filtered using GHP acrodysic syringe filters (25 mm, 0.45 μm) (PALL life sciences, USA) before injection. Extracted sample was analyzed in triplicates.

2.4. Instrumental analysis

Instrumental analysis was performed using a Dionex ultimate 3000 ultrahigh performance liquid chromatography (UPLC) (Thermo scientific, Massachusetts,United States) system equipped with a binary pump, an online degasser, column oven and an autosampler coupled to Impact II Quadrupole time of flight (QTOF) tandem mass spectrometer (Bruker, Germany) with electrospray ionization (ESI). Chromatographic separation was achieved using an Acquity BEH C_{18} column (2.1 × 100 mm x 1.7 μm) supplied by

Waters Corporation (Milford, MA, USA). Ultrapure water (A) and acetonitrile (B), both containing 0.1% formic acid, were used as mobile phases applying gradient elution. The elution gradient started with 40% of eluent B, increasing to 100% in 7 min, holding at 100% B for 2 min and then, back to 40% within 1 min. Mobile phase flow rate of 0.3 mL min^{-1}, injection volume of 5 μL and column temperature at 35 °C were used.

Retention times, mass accuracy of extracted ion chromatogram and the fragmentation pattern of analytes in the samples were matched with those of single standards for reliable identification. For quantification, a 10 point calibration was constructed for each target compounds in the concentration range of 10–50 μg L^{-1} using reference standards prepared in 50:50 (H_2O: MeOH). The MS/MS fragmentation pattern of the target compounds and other LC-MS conditions used for quantification were given in supplementary material 1 (Tables S1 and S2).

MS parameters for the analysis were the following: drying gas (N_2, generated by Peak Scientific Genius nitrogen generator) flow rate was 8 min L^{-1}, drying gas temperature was set at 220 °C, nebulizer gas (N_2) was 1.8 Bar, and the capillary voltage was 4500 V. The mass spectra were recorded across the range of m/z 50–1600 in full scan mode. All compounds were analyzed in positive mode. Nitrogen was kept as a nebulizer and auxiliary gas. Sodium formate cluster was used to calibrate the MS instrument. The calibrant was infused in front of the LC for each sample run and used for mass recalibration as well as calculating the mass accuracy.

Solvent blank, procedural blank, recovery test and determination of method detection limit (MDL) were included in the analysis as a measure of quality assurance (QA) and quality control (QC). MDL and recoveries of the target analytes were 0.1–1 ng L^{-1} and 10–105%, respectively (Supplementary material 1, Table S3). No quantifiable target azoles were detected in the procedural and solvent blanks. The relative standard deviation of triplicate measurements of standards and samples remained within 10%.

2.5. Suspect screening

A suspect list of more than 300 emerging pollutants, both parent and transformation products, was prepared from Norman's list (https://www.norman-network.net/?q=node/81) and literature reports (Ibáñez et al., 2017; Gómez et al., 2010; Hernández et al., 2011b). Compass Data Analysis 4.3 (Bruker, Germany) software package together with the tools found in the software package such as compass isotope pattern and compound crawler were used in the suspect screening workflow. The function edit chromatogram was used to obtain extracted ion chromatogram (EIC) of each compound using their molecular formula. A mass window of 0.005 mDa was used for extracting ion chromatograms. MS/MS spectrum of each compound was then obtained using the find compound spectra function in the Data Analysis. The compound crawler function in the Data Analysis 4.3 software was used to confirm that the peak represents the suspect compounds in online databases of KEGG (Kyoto Encyclopedia of Genes and Genomes), CHEBI (Chemical Entities of Biological Interest), HMBD (Human Metabolome Data Base), and FOR-IDENT. Parameters used in screening were mass accuracy ≤5 ppm, isotopic fit (mSigma) less than or equal to 100, signal to noise ratio of 3, minimum intensity threshold of 500, and presence of minimum of one product ion. The MS/MS spectra of the suspect compounds which were found in either of aforementioned online databases were verified with spectral libraries including MassBank (https://massbank.eu/MassBank/), METLIN (http://METLIN.scripps.edu), Drug Bank (https://www.drugbank.ca/) and In Silico fragmentation platforms (CFM-ID) (cfmid.wishartlab.com/). Available reference standards were then used for unequivocal identification. The work flow used for suspect screening is summarized in supplementary material 1 (Fig. S1, path B).

Decision of confidence of level of identification was based on the criteria developed by Schymanski et al. (2014b). Briefly, Compounds are identified at level 1 when the proposed structure is confirmed via appropriate measurement of a reference standard. Level 2 refers to probable compounds whose MS/MS spectra were verified with spectral libraries or literature.

2.6. Non-target screening

For non-target screening, accurate masses were obtained from base peak chromatogram (BPC) through the average spectrum function of Compass Data Analysis 4.3 software (Bruker Daltonics, Germany). Extracted ion chromatogram (EIC±0.005 mDa) of accurate masses which were not present in procedural blank and the suspect list was added to analysis list using add extracted ion chromatogram function of the data analysis software. Selection of masses was based on its intensity and presence of MS/MS information. Compound crawler function in Compass Data Analysis 4.3 was used to generate the possible molecular formulas based on measured accurate masses. The compound crawler was set to include C, H, O, P, S, Cl, and F elements. Online databases of KEGG, CHEBI, HMBD, and FOR-IDENT were used to annotate the proposed formulas through the Metfrag and Metfusion functions in the compound crawler. Parameters used in screening were similar with parameters used for suspect screening. MS/MS spectra verification and unequivocal identification was also made following the procedure mentioned in the suspect screening. The work flow used for non-target screening is summarized in supplementary material 1 (Fig. S1, path A).

3. Results and discussion

3.1. Determination of azole antifungal drugs

Even though, there is a misconception among researchers that complete chromatographic separation can be minimized or even eliminated in LC-MS/MS methods (Jessome and Volmer, 2006), complete chromatographic resolution can decrease the number of co-eluting compounds thereby improving detectability and minimizing matrix effect (Huang et al., 2010). In this study, a complete separation of eight azole antifungal drugs compounds was achieved using micro column (2.1 mm × 100 mm × 1.7 μm) in a considerably shorter total run time (10 min) (supplementary material 1, Fig. S2). Furthermore, full spectrum of the target azole compounds was acquired in the auto- MS/MS mode using reference standards. Once the full spectrum of each target compound was obtained, the most abundant ion (parent or product ion) in the mass spectrum was selected as a quantifier for each compound (Table S2). Representative fragments of the studied azoles were summarized in supplementary material 1 (Table S1).

Among the target compounds, clotrimazole, fluconazole, itraconazole, ketoconazole and posaconazole were detected in the effluent wastewater sample (Fig. 1). Average concentration of Clotrimazole was 30.03 ng L^{-1}, which falls in the range reported for wastewater effluent in Ireland (Lacey et al., 2012) and is higher than the levels reported in China and UK (Huang et al., 2010; Roberts and Thomas, 2006). Ketoconazole was detected at an average concentration of 12.31 ng L^{-1}, falling in the range reported by Casado et al. (2014) but lower than the reported level in Sweden (Lindberg et al., 2014). Itraconazole and posaconazole were detected for the first time in this study with an average concentration of 2.43 ng L^{-1} and 15.38 ng L^{-1}, respectively. Fluconazole showed the highest concentration with an average concentration of 302.38 ng L^{-1}. The

concentration of fluconazole observed in this study was higher than reports for effluent wastewater in China (6.10 ng L^{-1}) (Chen et al., 2012) and Germany (158.93 ng L^{-1}) (Gurke et al., 2015) and lower than the concentration reported in Sweden (793.00 ng L^{-1}) (Lindberg et al., 2014). Econazole and miconazole were not detected in this study although they were detected in wastewaters of Beijing, China (up to 0.51 ng L^{-1} in effluent) and Ghent, Belgium (in up to 35.70 ng L^{-1} in effluent), respectively (Van De Steene et al., 2010; Huang et al., 2012). The relative higher concentration of fluconazole observed in this study could be due to its ability to be predominantly transported in the aqueous phase, while the other azoles tend to adsorb on solid matrices due to their hydrophobic nature (Peng et al., 2012). Moreover, fluconazole is widely used as a prophylactic drug to control invasive fungal infections (Ericson et al., 2016; Ng et al., 2012). The higher level of the fluconazole is an environmental concern as it is higher than its reported predicted no effect concentration (PNEC, 250 ng L^{-1}), which might lead to selection of drug resistance in the aquatic environments (Bengtsson-Palme and Larsson, 2016). The spatial variation in the observed and reported concentrations of the azoles in the effluent wastewaters of different countries could be attributed to factors such as the size of wastewater treatment plant, removal efficiencies of wastewater treatment technology, consumption pattern of the drugs, and number of population (Reilly et al., 2012). Given the detection of the target antifungals in the effluent, the findings are a call for a wide assessment of their occurrence in aquatic environments and their role ecotoxicology as well as in selection of drug resistant fungi.

3.2. Suspect screening

Effluents from wastewater treatment plants contain tens of thousands of micropollutants and transformation products which cannot be addressed by only target analysis. High accuracy, UHPLC-QTOF-Mass spectrometric data was retrospectively analyzed for suspects and unknown (non-target) compounds manually to complement the target analysis of antifungal azoles.

Molecular formula of suspected compounds was used to extract the exact m/z of the expected compound from the full scan spectrum. Suspect screening was performed using $[M+H]^+$as it is the predominant molecular ion produced in electrospray ionization (ESI) operated in positive mode. A suspect list of more than 300 emerging pollutants both parent and transformation products were included in the retrospective screening for suspect compounds (supplementary material 2, Table S1). Sixteen Compounds were identified in the effluent wastewater all with mass accuracy less than 4 ppm and ion intensities higher than 1000. Pharmaceuticals were the most common group of compounds identified (Table 1) which could be attributed to their wide use. This finding is in agreement with that of Glauner et al. (2016), who reported the dominance of pharmaceuticals in urban wastewater effluents (Glauner et al., 2016). Others such as plasticizer, herbicide, surfactants and transformation products were also detected (Table 1, Fig. 2).

Fig. 3 illustrates the detection and identification of the antihistamine drug cetirizine through suspect screening. A narrow window extraction of ion chromatogram (EIC ± 0.005) using the molecular formula $C_{21}H_{25}ClN_2O_3$ presented a chromatographic peak at a retention time of 1.82 min (Fig. 3A). More than 100 plausible formulas (data not shown) were generated by compound crawler when this function was used at 5 ppm mass tolerance to establish if the peak and spectral information found represents the suspected drug cetirizine. The mass tolerance was lowered to 2 ppm to reduce the number of candidate molecular formulas. The change in mass tolerance from 5 ppm to 2 ppm resulted in 38 molecular formulas ranked by their mSigma, the one with the lowest mSigma (21.6) being the sum formula $C_{21}H_{25}ClN_2O_3$ (Fig. S3). This same formula has also the highest score (100%) among the listed molecular formulas by compound crawler. A Metfrag search using this formula and its exact mass yielded 3 candidates ranked by their score, where cetirizine showed score of 1.0 with highest number of peaks (9/24) explained (supplementary material 1, Fig. S3). The molecular weight of cetirizine (388.155 gm/mol) was confirmed by the presence of peak at m/z 389.1628 in the mass spectrum corresponding to the $[M+H]^+$ adduct ($C_{21}H_{26}ClN_2O_3^+$). Seven main fragments were observed in the MS^2 of the compound. The base peak in the MS^2 corresponded to the m/z 201.0466 ($C_{13}H_{10}Cl^+$) (Fig. 3B) which could be formed by the loss of 2-(2-(piperazin-1-yl) ethoxy)-acetic acid ($C_8H_{16}N_2O_3$) moiety. Other fragments observed in the MS^2 spectrum of the suspect drug

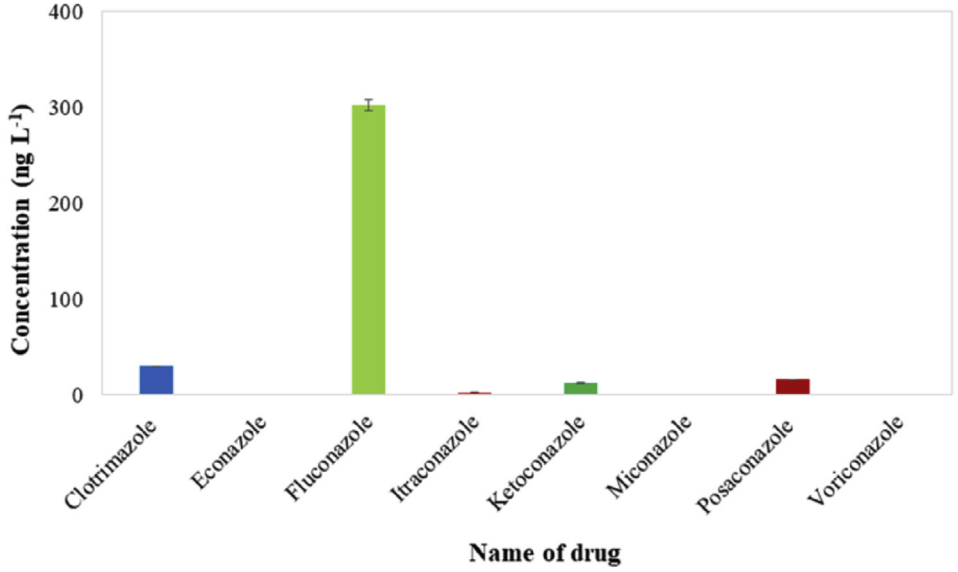

Fig. 1. Average concentration of the target azoles in analyzed wastewater effluent (ng L^{-1}). Error bars represent the standard deviations calculated from triplicate (n = 3) measurement of the effluent using UHPLC-QTOF-MS/MS.

Table 1
List of emerging pollutants tentatively identified through suspect screening (except the compounds used for illustration of suspect screening workflow).

Suspect	Ion Formula (m/z)	RT[a]	ME[b]	mSigma	Selected fragments			Identification level	Class
					Fragment 1	Fragment 2	Fragment 3		
Diethylhexyl phthalate	$C_{24}H_{39}O_4$ (391.2857)	8.65	−3.5	14.0	167.0347	149.0236	71.0857	2	Plasticizer
Atazanavir	$C_{38}H_{53}N_6O_7$ (705.3977)	2.22	−0.9	13.9	534.3082	335.1971	168.0807	2	Pharmaceutical
N,N-Diethyltoluamide	$C_{12}H_{18}NO$ (192.1383)	2.39	−0.2	9.4	119.0493	100.0759	91.0544	2	Insect repellent
Ritonavir	$C_{37}H_{49}N_6O_5S_2$ (721.3232)	3.58	−2.8	17.1	426.1860	296.1433	268.1481	2	Pharmaceutical
Climbazole	$C_{15}H_{18}ClN_2O_2$ (293.1055)	1.90	−1.3	0.8	225.0680	197.0729	166.1091	2	Pharmaceutical
Bezafibrate	$C_{19}H_{21}ClNO_4$ (362.1161)	2.61	−2.0	24.9	316.1109	276.0769	138.9948	2	Pharmaceutical
Citalopram	$C_{20}H_{22}FN_2O$ (325.1705)	1.46	1.8	56.0	307.1559	262.1029	234.0726	1	Pharmaceutical
nonaethylene glycol	$C_{18}H_{39}O_{10}$ (415.2535)	0.99	0.6	48.2	273.11232	177.1124	133.0855	4	Surfactant
Fluorene	$C_{13}H_{11}$ (167.0854)	1.45	0.8	31.9	166.0763	152.0623	121.1017	4	Polyaromatic hydrocarbon
Estradiol	$C_{18}H_{25}O_2$ (273.1855)	5.45	−2.2	2.40	255.1749	225.1275	197.1328	1	Pharmaceutical
Desethylterbuthylazine	$C_7H_{13}ClN_5$ (202.0855)	1.85	−0.5	42.3	146.0231	148.0197	104.0012	2	Transformation product
Desmethylvenlafaxine	$C_{16}H_{26}NO_2$ (264.1958)	1.08	−0.0	16.8	246.1854	119.0852	107.0491	2	pharmaceutical
Losartan carboxylic acid	$C_{22}H_{22}ClN_6O_2$ (437.1490)	2.46	−0.7	8.70	235.0983	207.0919	190.0669	2	Transformation product
Irbesartan	$C_{25}H_{29}N_6O$ (429.2398)	1.80	−0.2	20.4	401.2348	386.2237	235.0981	2	Pharmaceutical

[a] RT = retention time (min).
[b] ME = mass error (ppm).

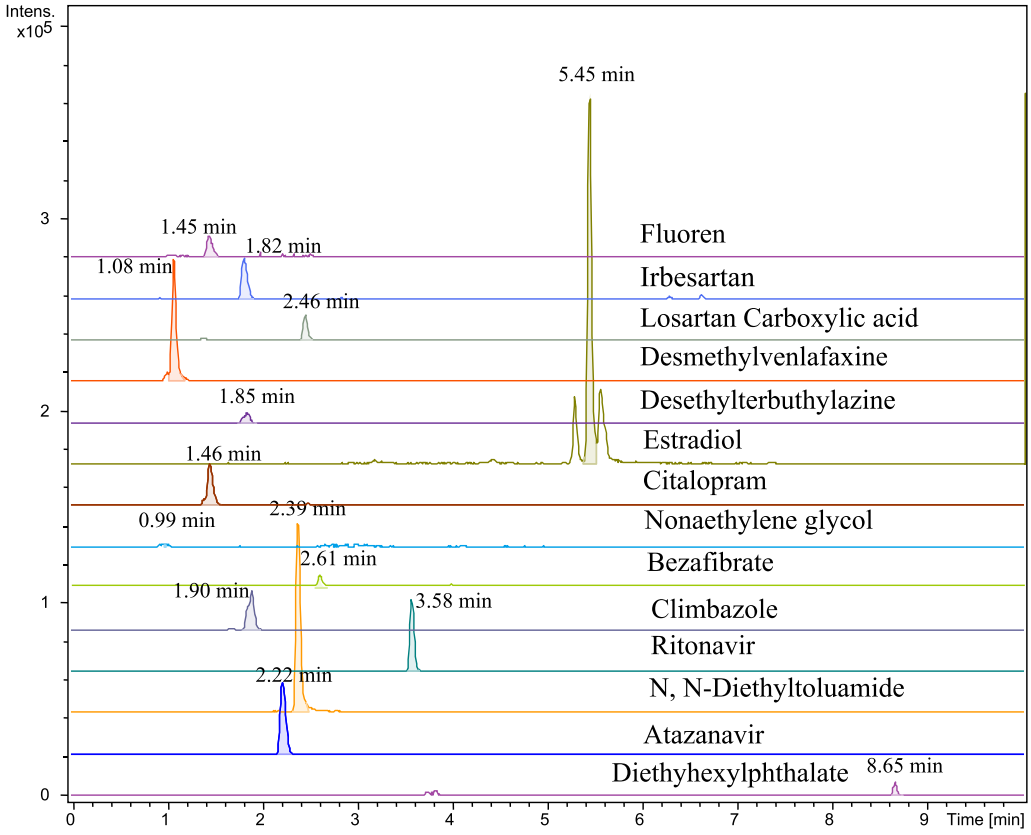

Fig. 2. UHPLC-QTOF-MS/MS chromatogram of tentatively identified compounds in the effluent sample through suspect screening (chromatograms of compounds used for illustration are not included).

include m/z 203.0437 ($C_{13}H_{10}[37]Cl^+$), 193.0758 ($C_{13}H_9N_2+$), 187.1078 ($C_8H_{15}N_2O_3^+$), 166.0774 ($C_{13}H_{10}^+$), and 165.0699 ($C_{13}H_9^+$). Mass errors of all the fragments were below 2.5 ppm. Moreover, the fragment ions with m/z 201.0466, 166.0774 and 165.0699 are in accordance with fragments reported for cetirizine in the literature (Ferrer et al., 2013; Zhou, 2016). The MS^2 spectra of cetirizine in METLIN and MassBank are in agreement with measured spectra of cetirizine (Fig. 3C and D). Cetirizine has been reported up to 510.00 ng L^{-1} in wastewater effluents and up to 720.00 ng L^{-1} in surface waters (Bahlmann et al., 2012; Kosonen and Kronberg, 2009). All these evidences strongly supported the correct identification of the suspect compound as cetirizine at level 2. Cetirizine was then confirmed using a standard.

Majority of the research on pollutants in the environment, both through targeted and non-targeted approach, focuses on the determination of the parent compound. However, many compounds can undergo partial metabolism and/or degradation in the environment resulting in more persistent or toxic than the parent compound and may assume different ecotoxicological profile (Kosma et al., 2017). To this end, more than 40 transformation products were included in our list of suspects.

A detection and identification of one of the transformation

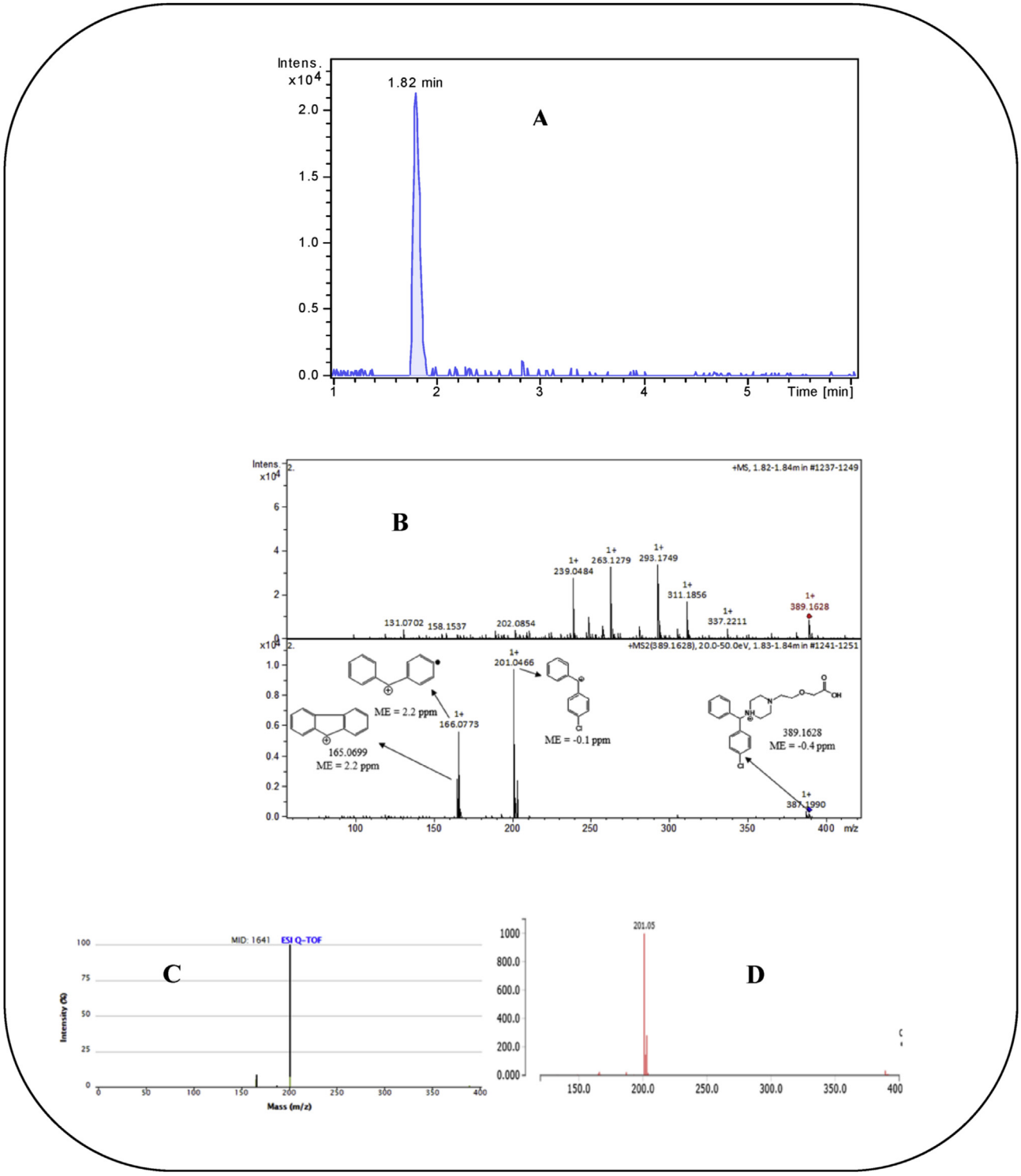

Fig. 3. Extracted ion chromatogram (A), MS and MS^2 Spectra with proposed structure of selected fragments (B) of cetirizine. MS^2 spectra of cetirizine available in METLIN (C) and MassBank (D). ME = mass error.

product of carbamazepine, 10, 11-trans-dihydroxy-10, 11-dihydrocarbamazepine (DiOH-CBZ), ($C_{15}H_{14}N_2O_3$) is shown in Fig. 4. DiOH-CBZ is the result of conversion of the anticonvulsant drug carbamazepine into carbamazepine-10,11-epoxide (CBZ-E) through cytochrome p450 dependent enzymes and subsequent metabolism of the epoxide by epoxy hydroxylase (Aceña et al., 2017). It can also be formed due to metabolism of carbamazepine analogue oxcarbazepine (Leclercq et al., 2009). Furthermore, DiOH-CBZ has been reported as one of the degradation products carbamazepine which is formed during the wastewater treatment processes (Wang et al., 2016). Reported pathways to the formation of DiOH-CBZ are summarized in supplementary material 1 (Fig. S4). Performing a narrow ion chromatogram extraction using the molecular formula of the transformation product presented a

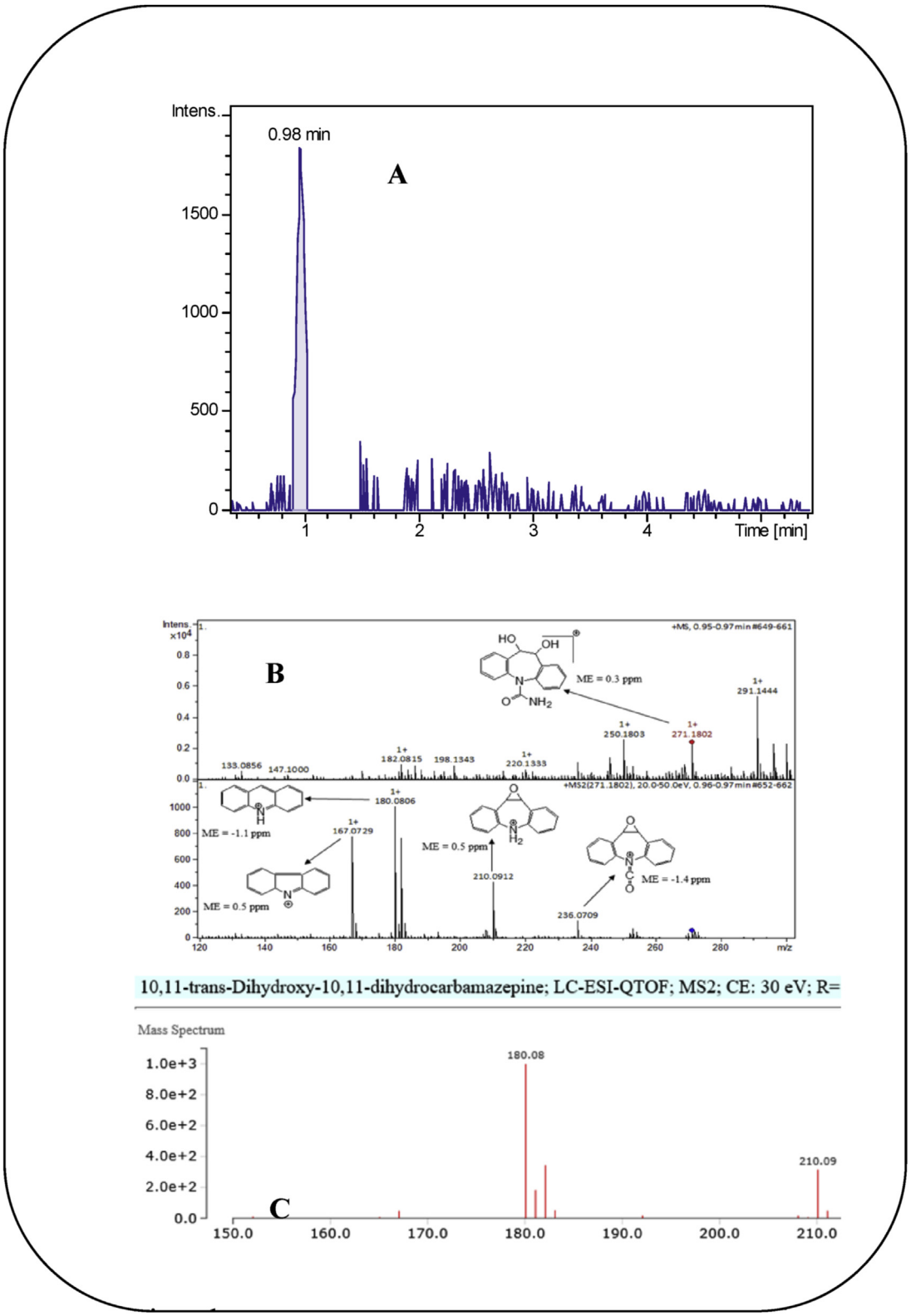

Fig. 4. Extracted ion chromatogram (A), MS and MS^2 Spectra of with proposed structure of selected fragments of 10, 11-trans-dihydroxy-10,11-dihydrocarbamazepine (B). MS^2 spectra of 10, 11-trans-dihydroxy-10,11-dihydrocarbamazepine available in MassBank (C). ME = mass error.

chromatographic peak at 0.98 min. The function compound crawler in Compass Data Analysis 4.3 software (Bruker, Germany) was used to confirm if the peak and spectral information found represents the suspected transformation product 10, 11-trans-dihydroxy-10, 11-dihydrocarbamazepine. Eight plausible formulas ranked by their mSigma were retrieved using the compound crawler (supplementary material 1, Fig. S5). However, none of the formulas suggested by the compound crawler were in agreement with the molecular

formula of the suspect transformation product. This could be attributed to the relatively lower signal intensity of chromatographic peak as well as spectral data. A spectral search for the molecular formula(s) with lower mSigma (<100) and higher score (100%), which is $C_{17}H_{22}N_2O$, in Metfrag, MassBank and METLIN returned zero hits.

The use of the exact mass (270.1729) to search for potential candidates in Metfrag yielded 1 hit named Doxylamine (Supplementary material 1, Fig. S5). The measured MS^2 spectrum was then compared to the MS/MS spectrum of doxylamine retrieved from METLIN data base which was found to be different making doxylamine not very likely (Fig. 4B and supplementary material 1, Fig. S6). The same exact mass was again used to look for potential candidate compounds for the chromatographic peak and spectral information obtained using the molecular formula of the suspect transformation product ($C_{15}H_{14}N_2O_3$) in an online data bases (MassBank and METLIN). 154 and 138 candidate compounds were retrieved from the data bases MassBank and METLIN respectively. The top most candidate of the combined list (MassBank and METLIN) resulted in one prioritized compound which was 10, 11-trans-dihydroxy-10, 11-dihydrocarbamazepine. Although METLIN revealed a slightly different *in silico* predicted spectra, the MS^2 spectra in the MassBank for 10, 11-trans-dihydroxy-10, 11-dihydrocarbamazepine is in agreement with the measured spectrum (Fig. 4C) making this transformation product to be the more likely candidate.

The mass spectrum for 10, 11-trans-dihydroxy-10,11-dihydrocarbamazepine shows a peak at m/z 271.1802 corresponding to the $[M+H]+$ adduct ($C_{15}H_{15}N_2O_3+$) confirming the molecular weight 270.288 gm/mol. A base peak at m/z 180.0806 ($C_{13}H_{10}N+$) is observed in both the MS^2 of the measured spectra and the spectra in MassBank (Fig. 4B and C). Other main fragments revealed in the measured spectrum include 236.0709 ($C_{15}H_{10}NO_2+$), 210.0912($C_{15}H_{10}NO_2+$), 182.0961 ($C_{13}H_{12}N+$) and 167.0729 ($C_{12}H_9N+$) (Fig. 4B). Literature was searched if similar fragments were reported to further increase the confidence of identification of the transformation product. Fragments at m/z 254.0801, 253.0971, 236.0704, 210.0912, 182.0962 and 180.0805 were reported in literature (Miao and Metcalfe, 2003; Kaiser et al., 2014). The differences in some of the fragments could be attributed to the differences in collision energy used for fragmentation.

3.3. Non-target screening

Given accurate masses, which can be obtained from high resolution mass spectrometry full scan spectral data, it is possible to use a formula generator to compute molecular formula of the masses. Data bases can then be used to allocate a molecular structure for the generated molecular formulas and identify unknown compounds (Müller et al., 2011). To this effect, more than 150 accurate masses selected based on their intensities, were included in the manual retrospective non-target screening for the detection of unknown compounds. Eleven relevant compounds, all with very low mass errors (<2 ppm), were detected in the wastewater effluent (Table 2, Fig. 6). Chromatogram of compounds tentatively identified through non-target screening, except for the ones used as illustrative examples, is given in Fig. 5.

As an example, the detection and tentative identification of the antihistamine drug fexofenadine is shown in Fig. S7 (supplementary material 1) and Fig. 6. Performing narrow window extraction of ion chromatogram using the accurate mass 502.2953 revealed a chromatographic peak at 1.76 min (Fig. 6A). The accurate mass together with the measured mass spectrum and MS^2 spectrum, as well as restriction of elements (C, H, O, N, Cl, F, S, P) were used in compound crawler to generate plausible molecular formulas for the targeted accurate mass. 55 molecular formulas were generated, the formula $C_{32}H_{39}NO_4$ being top scoring (100%) and with the lowest mSigma (8.2). The mass error associated with this formula was also considerably low (−0.2 ppm). Metfrag was used to retrieve 2 structures for this formula from KEGG, the antihistamine drug fexofenadine with a score of 1.0 and the mycotoxin aflatrem with a score of 0.89 (Fig. S7). CHEBI and HMBD databases were also used to retrieve structures through Metfrag, where both suggested only fexofenadine (data not shown) making fexofenadine the more likely candidate.

The MS^2 spectrum of the candidate fexofenadine presents a base peak at 502.2953 corresponding to the $[M+H]^+$ of the compound. Other main fragments observed in the MS^2 spectra of the candidate include 484.2859 ($C_{32}H_{38}NO_3+$), 466.2748 ($C_{32}H_{36}NO_2+$), 262.1602($C_{19}H_{20}N+$), 233.1183 ($C_{14}H_{17}O_3+$), 171.1169 ($C_{13}H_{15}+$) and 91.0544 (C_7H_7+) (Fig. 6B). A matching spectrum with measured spectra was found in METLIN database (Fig. 6C). Furthermore, the fragments 171.2000, 233.1000, 262.5000, 466.3000 and 484.2000 have been reported in the scientific literature (Kumar et al., 2009). Fexofenadine occurrence in the aquatic environments such as wastewater effluents is widely reported in the literature (Kosonen and Kronberg, 2009; Loos et al., 2013; Kristofco and Brooks, 2017; Golovko et al., 2014). It has been reported to be one of the pharmaceuticals known to have low removal efficiency during wastewater treatment (Kosonen and Kronberg, 2009; Golovko et al., 2014). These all evidences suggest that the accurate mass 502.2953 corresponds to the antihistamine fexofenadine.

Table 2
List of emerging pollutants tentatively identified through non-target screening (except the compound used for illustration).

Accurate mass	Ion Formula (m/z)	RT[a]	ME[b]	mSigma	Selected fragments			Identified as	Identification level	Class
					Fragment 1	Fragment 2	Fragment 3			
183.0780	$C_6H_{16}O_4P$	1.39	0.2	6.2	127.0156	98.9843	80.9734	triethyl phosphate	2	Flame retardant/intermediate for others/
237.1023	$C_{15}H_{13}N_2O$	1.75	−0.4	9.2	195.1000	194.0963	193.0881	Carbamazepine	l	Pharmaceutical
230.1169	$C_9H_{16}ClN_5$	3.12	−1.0	5.7	176.0514	174.0543	132.0325	Terbuthylazine	2	Herbicide
629.3704	$C_{37}H_{48}N_4O_5$	3.81	−1.0	2.1	447.2655	429.2548	183.1129	Lopinavir	2	Pharmaceutical
316.0348	$C_{14}H_{10}ClF_3NO_2$	4.02	−0.5	2.8	278.0187	244.0142	232.0142	Efavirenz	2	Pharmaceutical
134.0711	$C_7H_8N_3$	1.25	1.5	10.1	106.0653	95.0496	79.0540	5-Methylbenzotriazole	2	Corrosion inhibitor
216.1012	$C_8H_{14}ClN_5$	2.35	−0.6	19.1	174.0543	146.0231	132.0326	Atrazine	1	Herbicide
399.2511	$C_{18}H_{40}O_7P$	5.12	−1.2	4.7	299.1623	199.0732	101.0961	Tris (2-butoxyethyl) phosphate	2	Flame retardant
278.1906	$C_{20}H_{24}N$	1.91	−1.0	72.7	233.1328	191.0859	91.0544	Amitriptyline	2	Pharmaceutical
436.2350	$C_{24}H_{30}N_5O_3$	2.82	−1.5	4.9	418.2259	362.2238	352.1780	Valsartan	2	Pharmaceutical

[a] RT = retention time (min).
[b] ME = mass error (ppm).

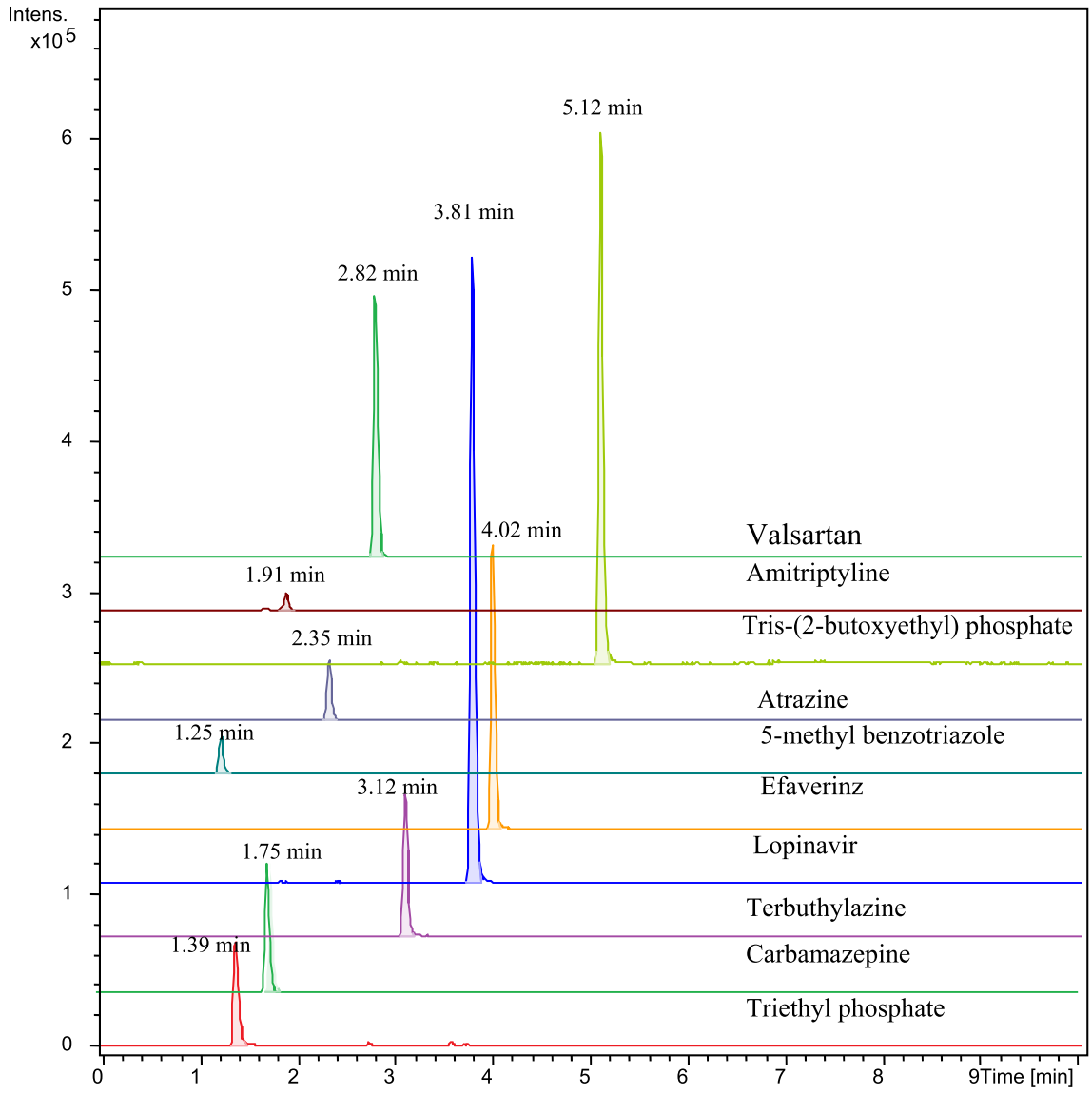

Fig. 5. Chromatogram of compounds tentatively identified through non-target screening.

Obviously, reference standard is required for unambiguous identification of the compound.

Considering these results, the strategy used here provided valuable confirmation for the identification of wide range of emerging pollutants in environmental samples through retrospective suspect and non-target screening. A total of 27 compounds were tentatively identified through retrospective suspect and non-target screening and four compounds were confirmed using standards. This shows that the relatively simple approach used here can still be applied in situations where automatic data management tools are not available or are not applicable. Available automatic mass spectrometric data management tools are often either expensive, developed for data outputs from specific instruments or need high speed computers, which all these might limit fully exploring the potential of suspect and non-target screening.

4. Conclusion

The information acquired by UHPLC-QTOF-MS allows target analysis as well as retrospective look into the presence of other suspect or unknown emerging pollutants which were not included in the target analysis. Targeted quantification of commonly used azole antifungal drugs (clotrimazole, econazole, fluconazole, itraconazole, ketoconazole, miconazole, posaconazole and voriconazole) and retrospective suspect and unknown (non-target) screening of other emerging pollutants in wastewater effluent using UHPLC-QTOF-MS has been carried out in this work. In the target analysis, clotrimazole, fluconazole, itraconazole, ketoconazole and posaconazole were detected in the effluent wastewater sample, fluconazole being with highest average concentration (302.38 ng L^{-1}). The retrospective analysis of the accurate mass data has allowed, in this work, the detection and identification pharmaceuticals such as irbesartan, valsartan, carbamazepine, ritonavir and lopinavir, herbicides such as terbuthylazine, flame retardants such as triethyl phosphate and plasticizers such as diethylhexyl phthalate. Compounds which have been included in the watch list, estradiol, atrazine and carbamazepine, were also detected in the effluent wastewater through the retrospective analysis. Furthermore, transformation products such as 10, 11-dihdro 10, 11-carbamzepine, desethylterbuthylazine and losartan

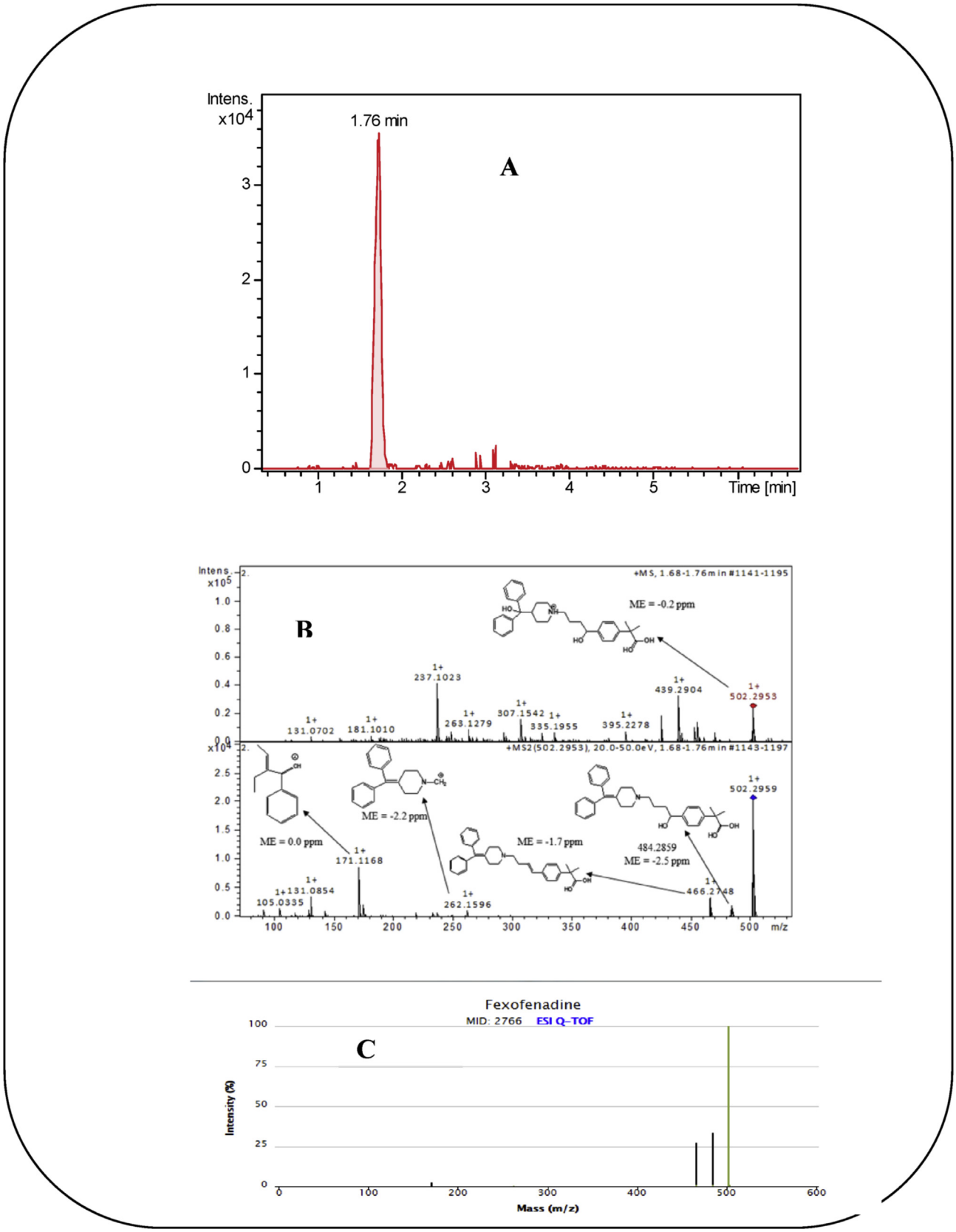

Fig. 6. Extracted ion chromatogram (A), MS and MS^2 Spectra with proposed structure of selected fragments of fexofenadine (B). MS^2 spectra of fexofenadine available in METLIN (C). ME = mass error.

carboxylic acid were detected. Wider assessment of the occurrence of the azole antifungals in different aquatic matrices is recommendable to better understand the risk related to their existence in the environment. The advantage of retrospective data mining from data acquired by resolution QTOF-MS could be used more in the future for wide-ranging emerging pollutants without the need for additional injection of samples.

Declaration of conflict of interest

All authors of the manuscript submitted to Environmental pollution for publication consideration declare no competing interests in whatsoever way.

Acknowledgements

The authors are thankful to Nanotechnology and Water Sustainability Research Unit, University of South Africa (UNISA), South Africa for funding. The first author is also thankful to the Addis Ababa University, Ethiopia, for granting him a study leave.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.07.075.

References

Aceña, J., et al., 2017. Metabolite profiling of carbamazepine and ibuprofen in Solea senegalensis bile using high-resolution mass spectrometry. Anal. Bioanal. Chem. 409, 5441–5450.
Adewumi, J., Ilemobade, A., Van Zyl, J., 2010. Treated wastewater reuse in South Africa: overview, potential and challenges. Resour. Conserv. Recycl. 55, 221–231.
Bahlmann, A., et al., 2012. Immunoassays as high-throughput tools: monitoring spatial and temporal variations of carbamazepine, caffeine and cetirizine in surface and wastewaters. Chemosphere 89, 1278–1286.
Barra Caracciolo, A., Topp, E., Grenni, P., 2015. Pharmaceuticals in the environment: biodegradation and effects on natural microbial communities. A review. J. Pharm. Biomed. Anal. 106, 25–36.
Becerra-Castro, C., et al., 2015. Wastewater reuse in irrigation: a microbiological perspective on implications in soil fertility and human and environmental health. Environ. Int. 75, 117–135.
Bellver-Domingo, A., Fuentes, R., Hernández-Sancho, F., 2017. Shadow prices of emerging pollutants in wastewater treatment plants: quantification of environmental externalities. J. Environ. Manag. 203, 439–447.
Bengtsson-Palme, J., Larsson, D.J., 2016. Concentrations of antibiotics predicted to select for resistant bacteria: proposed limits for environmental regulation. Environ. Int. 86, 140–149.
Besha, A.T., et al., 2017. Removal of emerging micropollutants by activated sludge process and membrane bioreactors and the effects of micropollutants on membrane fouling: a review. J. Environ. Chem. Eng. 5, 2395–2414.
Brandão, L.R., et al., 2010. Diversity and antifungal susceptibility of yeasts isolated by multiple-tube fermentation from three freshwater lakes in Brazil. J. Water Health 8, 279–289.
Casado, J., et al., 2014. Selective determination of antimycotic drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography quadrupole time-of-flight mass spectrometry. J. Chromatogr. A 1339, 42–49.
Castro, G., et al., 2016. Time-of-flight mass spectrometry assessment of fluconazole and climbazole UV and UV/H 2 O 2 degradability: kinetics study and transformation products elucidation. Water Res. 88, 681–690.
Chen, Z.-F., Ying, G.-G., 2015. Occurrence, fate and ecological risk of five typical azole fungicides as therapeutic and personal care products in the environment: a review. Environ. Int. 84, 142–153.
Chen, Z.-F., et al., 2012. Determination of biocides in different environmental matrices by use of ultra-high-performance liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 404, 3175–3188.
Chiaia-Hernandez, A.C., Krauss, M., Hollender, J., 2012. Screening of lake sediments for emerging contaminants by liquid chromatography atmospheric pressure photoionization and electrospray ionization coupled to high resolution mass spectrometry. Environ. Sci. Technol. 47, 976–986.
Ericson, J.E., et al., 2016. Fluconazole prophylaxis for the prevention of candidiasis in premature infants: a meta-analysis using patient-level data. Clin. Infect. Dis. 63, 604–610.
Fatta-Kassinos, D., Dionysiou, D.D., Kümmerer, K., 2016. Advanced Treatment Technologies for Urban Wastewater Reuse, vol. 45. Springer, pp. 5–37.
Ferrer, I., Thurman, E.M., 2013. Chapter 4 - analysis of pharmaceuticals in drinking water, groundwater, surface water, and wastewater. In: Petrovic, M., Barcelo, D., Pérez, S. (Eds.), Comprehensive Analytical Chemistry. Elsevier, pp. 91–128.
García-Valcárcel, A.I., Tadeo, J.L., 2011. Determination of azoles in sewage sludge from Spanish wastewater treatment plants by liquid chromatography-tandem mass spectrometry. J. Sep. Sci. 34, 1228–1235.
Geissen, V., et al., 2015. Emerging pollutants in the environment: a challenge for water resource management. Int. Soil Water Conserv. Res. 3, 57–65.
Glauner, T., Wüst, B., Faye, T., 2016. A comprehensive workflow for target, suspect, and non-target screening by LC/MS demonstrated for the identification of CECs in effluents from waste water treatment plants. In: Assessing Transformation Products of Chemicals by Non-target and Suspect Screening – Strategies and Workflows, vol. 2. American Chemical Society, pp. 113–130.
Golovko, O., et al., 2014. Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. Chemosphere 111, 418–426.
Gómez, M., et al., 2010. Rapid automated screening, identification and quantification of organic micro-contaminants and their main transformation products in wastewater and river waters using liquid chromatography–quadrupole-time-of-flight mass spectrometry with an accurate-mass database. J. Chromatogr. A 1217, 7038–7054.
Gottschall, N., et al., 2012. Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field. Chemosphere 87, 194–203.
Gurke, R., et al., 2015. Occurrence and removal of frequently prescribed pharmaceuticals and corresponding metabolites in wastewater of a sewage treatment plant. Sci. Total Environ. 532, 762–770.
Hernández, F., et al., 2011. Retrospective LC-QTOF-MS analysis searching for pharmaceutical metabolites in urban wastewater. J. Sep. Sci. 34, 3517–3526.
Hernández, F., et al., 2011. Rapid wide-scope screening of drugs of abuse, prescription drugs with potential for abuse and their metabolites in influent and effluent urban wastewater by ultrahigh pressure liquid chromatography–quadrupole-time-of-flight-mass spectrometry. Anal. Chim. Acta 684, 96–106.
Hernández, F., et al., 2012. Use of time-of-flight mass spectrometry for large screening of organic pollutants in surface waters and soils from a rice production area in Colombia. Sci. Total Environ. 439, 249–259.
Hernández, F., et al., 2012. Use of time-of-flight mass spectrometry for large screening of organic pollutants in surface waters and soils from a rice production area in Colombia. Sci. Total Environ. 439, 249–259.
Huang, Q., et al., 2010. Determination of commonly used azole antifungals in various waters and sewage sludge using ultra-high performance liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1217, 3481–3488.
Huang, Q., et al., 2012. Enantiomeric determination of azole antifungals in wastewater and sludge by liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 403, 1751–1760.
Ibáñez, M., et al., 2009. Screening of antibiotics in surface and wastewater samples by ultra-high-pressure liquid chromatography coupled to hybrid quadrupole time-of-flight mass spectrometry. J. Chromatogr. A 1216, 2529–2539.
Ibáñez, M., et al., 2017. UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. J. Hazard. Mater. 323, 26–35.
Jessome, L.L., Volmer, D.A., 2006. Ion suppression: a major concern in mass spectrometry. LC-GC N. Am. 24, p. 498.
Kaiser, E., et al., 2014. Transformation of oxcarbazepine and human metabolites of carbamazepine and oxcarbazepine in wastewater treatment and sand filters. Environ. Sci. Technol. 48, 10208–10216.
Kinyua, J., et al., 2015. A data-independent acquisition workflow for qualitative screening of new psychoactive substances in biological samples. Anal. Bioanal. Chem. 407, 8773–8785.
Kosma, C.I., Lambropoulou, D.A., Albanis, T.A., 2017. Photochemical transformation and wastewater fate and occurrence of omeprazole: HRMS for elucidation of transformation products and target and suspect screening analysis in wastewaters. Sci. Total Environ. 590–591, 592–601.
Kosonen, J., Kronberg, L., 2009. The occurrence of antihistamines in sewage waters and in recipient rivers. Environ. Sci. Pollut. Control Ser. 16, 555–564.
Kristofco, L.A., Brooks, B.W., 2017. Global scanning of antihistamines in the environment: analysis of occurrence and hazards in aquatic systems. Sci. Total Environ. 592, 477–487.
Kumar, L., et al., 2009. Chapter 4 - fexofenadine hydrochloride. In: Brittain, H.G. (Ed.), Profiles of Drug Substances, Excipients and Related Methodology. Academic Press, pp. 153–192.
Lacey, C., et al., 2012. Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland. Environ. Monit. Assess. 184, 1049–1062.
Leclercq, M., et al., 2009. Presence and fate of carbamazepine, oxcarbazepine, and seven of their metabolites at wastewater treatment plants. Arch. Environ. Contam. Toxicol. 56, p. 408.
Lindberg, R.H., et al., 2014. Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. Water Res. 58, 221–229.
Liu, J., et al., 2016. Bioconcentration and metabolism of ketoconazole and effects on multi-biomarkers in crucian carp (Carassius auratus). Chemosphere 150, 145–151.

Loos, R., et al., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Res. 47, 6475–6487.
Lyu, S., et al., 2016. Wastewater reclamation and reuse in China: opportunities and challenges. J. Environ. Sci. 39, 86–96.
Menge, J., 2010. Treatment of Wastewater for Re-use in the Drinking Water System of Windhoek. City of Windhoek, Namibia. http://www.wastewater.co.za/.
Miao, X.-S., Metcalfe, C.D., 2003. Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography– electrospray tandem mass spectrometry. Anal. Chem. 75, 3731–3738.
Müller, A., et al., 2011. A new approach to data evaluation in the non-target screening of organic trace substances in water analysis. Chemosphere 85, 1211–1219.
Ng, P.J.H., 2018. Singapore: transforming water scarcity into a virtue. In: Assessing Global Water Megatrends. Springer, pp. 179–186.
Ng, T.B., et al., 2012. Pharmacotherapy approaches to antifungal prophylaxis. Expert Opin. Pharmacother. 13, 1695–1705.
Pahigian, J.M., Zuo, Y., 2018. Occurrence, endocrine-related bioeffects and fate of bisphenol A chemical degradation intermediates and impurities: a review. Chemosphere 207, 469–480.
Peng, X., et al., 2012. Distribution, behavior and fate of azole antifungals during mechanical, biological, and chemical treatments in sewage treatment plants in China. Sci. Total Environ. 426, 311–317.
Petrovic, M., Barceló, D., 2006. Application of liquid chromatography/quadrupole time-of-flight mass spectrometry (LC-QqTOF-MS) in the environmental analysis. J. Mass Spectrom. 41, 1259–1267.
Polgár, L., et al., 2012. Retrospective screening of relevant pesticide metabolites in food using liquid chromatography high resolution mass spectrometry and accurate-mass databases of parent molecules and diagnostic fragment ions. J. Chromatogr. A 1249, 83–91.
Reilly, T.J., et al., 2012. Occurrence of boscalid and other selected fungicides in surface water and groundwater in three targeted use areas in the United States. Chemosphere 89, 228–234.
Richter, E., et al., 2016. Phytotoxicity of wastewater-born micropollutants–Characterisation of three antimycotics and a cationic surfactant. Environ. Pollut. 208, 512–522.
Roberts, P.H., Thomas, K.V., 2006. The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Sci. Total Environ. 356, 143–153.
Schymanski, E.L., et al., 2014. Strategies to characterize polar organic contamination in wastewater: exploring the capability of high resolution mass spectrometry. Environ. Sci. Technol. 48, 1811–1818.
Schymanski, E.L., et al., 2014. Identifying Small Molecules via High Resolution Mass Spectrometry: Communicating Confidence. ACS Publications.
Shevah, Y., 2014. Water scarcity, water reuse, and environmental safety. Pure Appl. Chem. 86, 1205–1214.
Sousa, J.C., et al., 2017. A review on environmental monitoring of water organic pollutants identified by EU guidelines. J. Hazard. Mater. 344, 146–162.
Van De Steene, J.C., Stove, C.P., Lambert, W.E., 2010. A field study on 8 pharmaceuticals and 1 pesticide in Belgium: removal rates in waste water treatment plants and occurrence in surface water. Sci. Total Environ. 408, 3448–3453.
Wang, W.-L., et al., 2016. Synergistic effect between UV and chlorine (UV/chlorine) on the degradation of carbamazepine: influence factors and radical species. Water Res. 98, 190–198.
Zarn, J.A., Brüschweiler, B.J., Schlatter, J.R., 2003. Azole fungicides affect mammalian steroidogenesis by inhibiting sterol 14 alpha-demethylase and aromatase. Environ. Health Perspect. 111, p. 255.
Zhao, L., et al., 2014. LC-QTOF-MS method for the analysis of residual pharmaceuticals in wastewater: application to a comparative multiresidue trace analysis between spring and winter water. Anal. Methods 6, 6956–6962.
Zhou, Z., 2016. Non-target impurity profiling of marketplace Cetirizine using high-resolution mass spectrometry and multivariate data analysis. Rapid Commun. Mass Spectrom. 30, 1941–1950.
Zuo, Y., Zhang, K., Deng, Y., 2006. Occurrence and photochemical degradation of 17α-ethinylestradiol in acushnet river estuary. Chemosphere 63, 1583–1590.
Zuo, Y., Zhang, K., Zhou, S., 2013. Determination of estrogenic steroids and microbial and photochemical degradation of 17α-ethinylestradiol (EE2) in lake surface water, a case study. Environ. Sci.: Processes Impacts 15, 1529–1535.