Selective determination of sartan drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography tandem mass spectrometry

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Abstract

Herein, a method for the simultaneous determination of the currently prescribed sartan drugs (eprosartan, EPR; olmesartan, OLM; losartan, LOS; candesartan, CAN; telmisartan, TEL; irbesartan, IRB; and valsartan, VAL), and the biodegradation product valsartan acid (VALA), in water samples (raw and treated wastewater, river and tap water) was developed. Solid-phase extraction (SPE) and ultra-performance liquid chromatography (UPLC) tandem mass spectrometry (MS/MS) were employed as concentration and determination techniques, respectively. Different sorbents and elution solvents were tested for sample preparation. Under optimized conditions, samples at neutral pH (6 to 8 units) were concentrated using mixed-mode (reversed-phase and anionic exchange) cartridges. Thereafter, the sorbent was washed with 5 mL of a methanol: water (1:1) solution, dried under a nitrogen stream and compounds were eluted with 2 mL of methanol: NH_3 (98:2). The accuracy of the method (accounting for SPE efficiency and matrix effects during electrospray ionization) was investigated using solvent-based calibration standards. Global recoveries, obtained for different water matrices (tap, river, treated and raw wastewater), ranged from 82% to 134%, with standard deviations between 2 and 18%. LOQs varied from 2 to 50 ng L^{-1}. Analysis of un-spiked samples confirmed: (1) the incomplete removal of sartans at sewage treatment plants (STPs), (2) the formation of VALA during municipal water treatment, and (3) the presence of VALA in the processed tap water samples. Additional findings of the current study are the detection of hydroxylated derivatives of the sartan drugs IRB and LOS in wastewater, and the *E-Z* isomerization of EPR in environmental water samples.

Keywords: sartans; valsartan acid; mixed-mode solid-phase extraction; liquid chromatography tandem mass spectrometry; water analysis.

1. Introduction

Sartans are a class of pharmaceuticals employed in the treatment of hypertension (Giebułtowicz et al., 2016; Oosterhuis et al., 2013). Currently, seven drugs from this family are marketed in Europe. After administration, they are excreted through faeces and urine (Bayer et al., 2014; Stankiewicz et al., 2015) entering municipal sewers. The octanol-water partition coefficients (log Kow) of sartans point out to medium, or to very low polarities; however, the existence of acidic moieties in their structures (carboxylic acids and/or a tetrazolic ring) converts them in negatively charged species at the pH of environmental water samples (Bayer et al., 2014). This fact increases significantly their mobility in the aquatic media.

Some members of the sartan group are often included in multianalyte methodologies applied to the screening of emerging pollutants in the aquatic environment (Cantwell et al., 2018; Hermes et al., 2018; Nödler et al., 2016), in methods dealing with the analysis of cardiovascular drugs (Giebułtowicz et al., 2016; Stankiewicz et al., 2015), and, less often, in more specific studies restricted to this family of pharmaceuticals (Bayer et al., 2014). From these previous works, it can be concluded that sartans are ubiquitous in raw wastewater, with average concentrations usually above 1000 ng L^{-1} (Bayer et al., 2014; Stankiewicz et al., 2015). Their removal percentages at municipal sewage treatment plants (STPs) are variable. For example, valsartan (VAL) is an easy to degrade compound (with removal percentages above 90%) (Bayer et al., 2014; Oosterhuis et al., 2013). However, irbesartan (IRB) presents low removal efficiencies (Bayer et al., 2014; Oosterhuis et al., 2013). Given that: (1) the available removal data for different sartans have not always been obtained under identical conditions (for the same STP) ; (2) sartans have been recently reported in sludge from STPs (Castro et al., 2018; Ivanová et al., 2018) ; and (3) sartan drugs with a biphenyl tetrazolic sub-structure (5 out of the 7 prescribed drugs in this family) might be biodegraded to valsartan acid (VALA) (Bayer et

al., 2014; 2009; Helbling et al., 2010) the reported removal efficiencies at municipal STPs must be considered with caution.

From municipal wastewaters, these compounds reach surface water reservoirs, including fresh and coastal waters (Cantwell et al., 2018; Giebułtowicz et al., 2016; Moreno-González et al., 2015; Nödler et al., 2016). Some sartans are stable during oxidative water treatments (photolysis, free chlorine and ozone addition), either tested at laboratory scale (Blum et al., 2017), or applied in tap water production plants (Huerta-Fontela et al., 2011). In other cases, such as losartan (LOS), transformation products arising from chemical oxidative treatments are deemed to be more toxic than the precursor drug (Carpinteiro et al., 2019). In addition to their stability during oxidative treatments, olmesartan (OLM) and candesartan (CAN) are not removed during soil infiltration of treated wastewaters (Hellauer et al., 2018), which is usually employed to recharge groundwater aquifers. Thus, the presence of sartan residues in tap water has been predicted and confirmed in samples obtained from different European countries, such as Germany and Poland (Giebułtowicz et al., 2016; Stankiewicz et al., 2015).

In addition to the prescribed drugs, the transformation product VALA is particularly concerning as regards its stability and mobility. Previous studies have proved that it is resistant to further biodegradation, ozonation, sand and soil filtration (Nödler et al., 2013; Schaffer et al., 2015). VALA shows also a low sorption on granular activated carbon, although it is susceptible to biodegradation on the surface of this sorbent (Sperlich et al., 2017). VALA has been found in bank filtrated samples (Hermes et al., 2018) and in tap water (Nödler et al., 2013), produced from bank filtrates, in the range of concentrations from 57 to 72 ng L^{-1}.

Sample preparation procedures employed for the extraction and concentration of these species are normally based on solid-phase extraction (SPE), using low selectivity reversed-phase sorbents (such as the OASIS HLB polymer). Compounds are eluted with

a relatively large volume of organic solvent (up to 10 mL of methanol or acetone), followed by dryness evaporation and re-constitution of the extract before liquid chromatography (LC) tandem mass spectrometry (MS/MS) determination (Giebułtowicz et al., 2016; Oosterhuis et al., 2013). Such strategy is coherent with the inclusion of some members of the sartan family in screening methods dealing with a large number of analytes with different properties. On the other side, a finely tuned method covering the simultaneous extraction and concentration of all the currently commercialized sartan drugs, and VALA, has not been reported yet.

Herein, an effective analytical procedure for the determination of 7 sartan drugs, and VALA, in water samples with different complexities, from municipal wastewater to surface and tap water, is developed. Efforts were focused on combining high extraction yields, with the lowest possible effect of the sample matrix in the efficiency of ESI ionization, minimum consumption of organic solvents and low manipulation of the primary sample extract before analysis. The existence of acidic functionalities in the structure of sartans was considered to test the viability of selective extraction-concentration approaches based on the use of mixed-mode (reversed-phase and anionic exchange) sorbents. The optimized method was applied to investigate the occurrence of these compounds in wastewater from a medium size city in the Northwest of Spain, as well as in several rivers and tap water samples collected in the same geographic area. In addition, the study investigates the existence of sartan transformation products, arising from metabolization and/or biodegradation processes, in the extracts obtained from waste and river water. To this end, accurate MS and product ion scan data were obtained using an LC quadrupole time-of-flight (TOF) mass spectrometer.

2. Materials and methods

2.1. Chemicals

Standards of eprosartan (EPR, as mesylate), OLM, LOS (as potassium salt), telmisartan (TEL), IRB and VAL were acquired from Sigma-Aldrich (Milwaukee, WI, USA); VALA and the internal surrogates (ISs): valsartan acid-d4 (VALA-d4) and irbesartan-d4 (IRB-d4), were supplied by Toronto Research Chemicals (North York, ON, Canada). CAN was obtained from Alfa Aesar (by Thermo Fisher Scientifics). Individual solutions of each compound (600-1000 mg L^{-1}) were prepared in methanol (MeOH). The ISs were supplied as methanolic solutions (100 mg L^{-1}). Chemical structures of these compounds and some properties of interest to predict their behaviour during sample preparation are provided as supplementary information, Table S1.

MeOH, HPLC-grade purity, glacial formic acid (FA) (98%), and ammonia (NH_3, 25% solution in methanol) were supplied by Merck (Darmstadt, Germany). Ultra-pure deionized water (18.2 MΩ cm^{-1}) was obtained from a Milli-Q Gradient A-10 system (Millipore, Billerica, MA, USA).

2.2. Samples and sample preparation

Different water matrices have been considered in this research. Wastewater samples were obtained from a municipal STP, serving a population of 100.000 inhabitants, equipped with primary and biological (activated sludge) treatment units. A detailed description of the units in the STP can be found elsewhere (Carballa et al., 2004). River water was obtained from different streams known to be affected, or not, by STPs discharges in Galicia (Northwest Spain). Some samples of river water were taken in the vicinity of drinking water production plants. Tap water samples were obtained from five different villages in the same geographic area (Galicia). The geographic coordinates corresponding to the different sampling points are provided as supplementary information, Table S2. Samples were collected in glass bottles, transported to the laboratory at 4ºC, and submitted to the extraction process within the next 48 hours. Filtered extracts were stored at -20 ºC and analysed within the next 7 days. Sampling

dates were comprised between the 2nd half of July and the middle October 2018. During this period, rivers contain the highest proportion of wastewater.

The extraction and concentration of sartan drugs was carried out by SPE. Several sorbents: reversed-phase OASIS HLB (200 and 60 mg) and mixed-mode (reversed-phase and anionic exchangers) OASIS WAX (150 mg) and OASIS MAX (60 and 150 mg), all of them provided by Waters (Milford, MA, USA), were investigated for the effective and selective concentration of sartans from water samples. Unless otherwise stated, SPE conditions were optimized using filtered samples of treated wastewater (400 mL aliquots) spiked with target compounds at 5000 ng L^{-1}. Breakthrough studies were investigated by passing the spiked water samples through each of the above sorbents online connected to an OASIS HLB 60 mg cartridge. After the concentration step, cartridges were eluted separately. The minimum volume of solvent required to recover compounds from SPE cartridges was determined by collecting consecutive fractions of 2 mL from each of the evaluated solvents.

Under optimized conditions, water samples, at neutral pH (6 to 8 units) and spiked with the ISs, were concentrated using the mixed-mode (reversed-phase and weak anionic exchange) 150 mg WAX cartridges. Thereafter, the sorbent was washed with 5 mL of a mixture of MeOH: water (1:1), dried with a stream of nitrogen, and eluted with MeOH: NH_3 (98:2). The volume of extract was limited to 2 mL. This extract was neutralized by addition of 0.06 mL of FA (acidification is required to improve the retention of the compounds in the UPLC column), passed through a 0.45 µm syringe filter, and injected in the UPLC-ESI-MS/MS system.

2.3. LC-MS/MS determination conditions

Determination of sartan drugs was performed in a LC-MS/MS system (Xevo TQD) supplied by Waters. The mass spectrometer was a triple quadrupole (QqQ) instrument

furnished with a Z-ESI source. Compounds were separated in a UPLC column, Zorbax Eclipse Plus C_{18} rapid resolution (50 mm x 2.1 mm, 1.8 µm), acquired from Agilent Technologies (Wilmington, DE, USA). The column was connected to a C_{18} 2.1 mm i.d. Security Guard^{TM} cartridge supplied from Phenomenex (Torrance, CA, USA). Column and pre-column were maintained at 40 ºC. Mobile phases were ultrapure water (A) and methanol (B), both containing 0.1% FA. The composition of the mobile phase was programmed as follows: 20% B (0-0.5 min), linear rated to 100% B (6-7 min), 20% B (7.1-10 min) (Castro et al., 2018). The injection volume was 0.5 µL and the column flow 0.4 mL min^{-1}.

Compounds were ionized operating the ESI source in positive mode. Nitrogen (99.999%) was employed as nebulization (50 L h^{-1}) and drying gas (450 °C at 1000 L h^{-1}) in the ionization source, which was set at 150 °C. Argon (99.99%) was used as collision gas in the MRM detection mode. The nebulizer needle was maintained at 1.5 kV. MS/MS transitions were recorded within a window of 60 s around the retention time of each compound. The dwell-time per transition was automatically adjusted by the MassLynx software (also used to control every UPLC-MS/MS parameter) to obtain 12 points per peak, considering an average baseline peak width of 5 s.

A QTOF-MS instrument (Agilent 6550), connected to an Agilent 1260 UPLC system, was employed to investigate the identity of an extra peak noticed in the chromatograms corresponding to MRM transitions of EPR, and to evaluate the presence of potential transformation products of sartan drugs in wastewater. Chromatographic conditions were the same as those employed in the QqQ instrument. Accurate MS and product ion scan spectra (MS/MS) were acquired operating the ESI source in positive (+) and negative (-) ionization modes (in two different injections), whilst the QTOF MS instrument was used in the 4 GHz high resolution mode (FWHM mass resolution 30000 at *m/z* 322).

Accurate product ion spectra were acquired at four different collision energies: 10, 20, 30 and 40 eV.

2.4. SPE recoveries, matrix effects and overall extraction efficiencies

The recoveries of the optimized SPE process, see section 2.2, were assessed for spiked (addition level 2000 ng L^{-1}) aliquots of wastewater (400 mL and 200 mL for raw and treated water, respectively). Recoveries were calculated as the ratio between responses obtained for samples spiked before (addition to water) and after the extraction step (addition to the SPE extract). The obtained values were multiplied by 100.

Potential matrix effects (MEs,%) during ESI(+) ionization were defined as the ratios between the slopes of calibration curves prepared with spiked extracts from wastewater samples and with solvent-based standards. The obtained values were multiplied by 100. Slope ratios below and above 100% point out to a reduction and to an enhancement in the efficiency of ESI(+) ionization for sample extracts versus solvent standards, respectively. Ratios around 100% suggest similar ionization efficiencies in both cases, which is normally associated with a low level of co-extracted organic species in the SPE extract.

Potential compound losses during the filtration step were estimated with the ratios between responses obtained for each compound in the extracts from 400 mL aliquots of the same treated wastewater sample fortified, at 5000 ng L^{-1}, before and after filtration, multiplied by 100.

Overall extraction efficiencies (accounting for the yield of the SPE step and MEs) were calculated as the difference in concentrations measured for spiked and non-spiked aliquots of different water matrices divided by the added value. Concentrations existing in sample extracts were measured against solvent-based calibration standards.

2.5. Eprosartan photoisomerization experiments

The potential conversion of *E-EPR* in the *Z* isomer was investigated with ultrapure water solutions, spiked with the former compound (added concentration 500 µg L^{-1}) and exposed to sunlight in a quartz tube. Control (zero time) and sample aliquots after 4 h of irradiation were injected in the LC-QTOF-MS system. The accurate MS and product ion scan spectra of the observed transformation product were compared to those corresponding to the EPR analogue detected in river water extracts.

3. Results and discussion

3.1. UPLC-ESI-MS/MS parameters

Table 1 summarizes the most relevant features of the UPLC-MS/MS determination procedure, without considering the sample preparation step. Linearity was investigated in the range of concentrations from 2.5 to 500 µg L^{-1} (from 5 to 1000 µg L^{-1} for VAL), considering an injection volume of 0.5 µL. Within this interval, the plots of corrected responses (peak area divided by the peak area of the corresponding IS) versus concentration fitted a linear model, with determination coefficients (R^2) above 0.999. The instrumental limits of quantification (LOQs) were estimated from responses obtained for the lowest level calibration standard, assuming a signal to noise (S/N) ratio of 10 for the quantification transition (Q1), and a ratio between qualification (Q2) and Q1 transitions within a 30% of the average value provided in Table 1. LOQs varied from 0.5 µg L^{-1} for IRB up to 5 µg L^{-1} for VAL.

3.2. Optimization of SPE conditions

SPE conditions were optimized regarding the extraction sorbent, the washing and the elution solvents. Three different sorbents: OASIS HLB (200 mg), OASIS MAX (60 and 150 mg) and OASIS WAX (150 mg), were tested for the retention of sartan compounds from spiked (5000 ng L^{-1}), filtered aliquots (400 mL) of wastewater. At the natural pH of

this sample (7.2 units), the reversed-phase HLB cartridges (200 mg sorbent) failed to retain VALA. Around 30% of the response obtained for this compound was noticed in the extract from the back on-line connected cartridge (60 mg OASIS HLB). Acidification of the water sample (pH 3) overcame breakthrough problems; however, this option is recognized to increase the retention of acidic natural organic matter, such as humic and fulvic acids, in reversed-phase materials (Fang et al., 2017). Breakthrough problems were not noticed for any of the two mixed-mode (OASIS MAX and OASIS WAX, 150 mg) cartridges for the same water matrix.

MeOH and MeOH containing different percentages of FA or NH_3 (up to 2%) were tested as elution solvents in combination with reversed and mixed-mode sorbents. MeOH (2 mL) was effective to recover all compounds from the HLB cartridge; however, it failed during elution of mixed-mode sorbents (none of the eight target compounds was detected in the first 10 mL obtained from mixed-mode cartridges). As regards the MAX sorbent (reversed-phase and strong anionic exchanger), VALA (the most acidic of the compounds) showed a slow elution profile using MeOH: FA (98:2) as eluent. More than 10 mL of solvent were required to recover this compound. Even using the 60 mg cartridges, VALA displayed a slow elution profile. Elution of target compounds from the WAX cartridges (reversed-phase and weak anionic exchangers) was achieved in the first fraction (2 mL) of MeOH:NH_3 (98:2). Thus, this polymer was selected to follow with the optimization of the extraction process.

Ultrapure water and MeOH (up to 5 mL) were tested as washing solvents, before elution of WAX cartridges. Neither sartans, nor VALA were detected in the corresponding washing fractions. On the other hand, the use of acidified water, or MeOH with 1% of FA, led to partial desorption of the less acidic sartans (particularly TEL, EPR and IRB). As compiled in Table S1, these compounds exhibit a partial positive charge at acidic pH

values. Thus, their electrostatic interactions with the positively charged sites in the WAX sorbent are broken in presence of FA.

Under optimized conditions, SPE recoveries ranged from 93 to 107%, with standard deviations between 1 and 3%, Table 2. MEs (%) were also investigated with both matrices, following the procedure described in section 2.4. The ratios between slopes of spiked wastewater extracts and solvent-based standards ranged from a minimum of 76% to a maximum of 119% (Table 2), pointing out to moderate variations in the efficiency of the ESI ionization for sample extracts versus solvent-based standards.

Filtration losses varied from 82% for TEL (the most lipophilic species) to 93% for VAL, Table S3. Thus, compounds remained associated to the water phase, which is in agreement with their existence as negatively charged species at pH 7, Table S1.

3.3. Performance of the analytical method

Taking into account recoveries and MEs compiled in Table 2, the accuracy of the developed procedure was evaluated using solvent-based calibration standards. Sample aliquots (from 200 to 500 mL depending on the matrix) were spiked with the ISs, fortified with target compounds, filtered and concentrated as described above. Non-spiked fractions of each matrix (fortified only with ISs) were also processed. Overall extraction recoveries (accounting for filtration losses, efficiency of SPE and MEs) were calculated as defined in section 2.4. Table 3 summarizes data obtained for different water matrices and addition levels. River water was spiked at three different concentration levels (attempting to mimic from heavy polluted to pristine surface water samples), and tap water at 100 ng L^{-1}, the maximum allowable concentration of most organic pollutants (pesticides) in this matrix (Council Directive 98/83/EC, 1998). In both cases, the concentrated sample volume was 500 mL. The procedural LOQs of the method for both matrices are also included in Table 3. Recoveries ranged from 88 to 134%, with

associated standard deviations in the range from 3 to 18%. The LOQs obtained for both water types varied from 2 to 20 ng L^{-1}. The pre-concentration factor provided by the developed procedure was of 250 times, without the need of a solvent exchange step, and/or concentration of the primary SPE extract before injection in the UPLC-ESI-MS/MS system.

In case of raw and treated wastewater, the obtained recoveries and the LOQs, only referred to the first and the most complex matrix, are also compiled in Table 3. For these matrices, recoveries ranged from 82 to 115%, with standard deviations lower than 16%. The linear response range of the method for raw wastewater extended up to 5000 ng L^{-1} (10000 ng L^{-1} for VAL) and the achieved LOQs varied from 5 to 50 ng L^{-1}. For treated wastewater, LOQs and the upper limit of the linear response range are twice lower. As further comment, all the processed wastewater samples contained relevant concentrations of target compounds; so, for this matrix it is practically impossible to verify the goodness of LOQs. LOQs reflected in Table 3 for raw wastewater are estimated values, considering the enrichment factor provided by the SPE step (100 and 200 times for raw and treated wastewater, respectively) and the instrumental LOQs of the UPLC-ESI-MS/MS system.

The existence of potential contamination problems was evaluated by processing aliquots of ultrapure water (500 mL), spiked with the mixture of ISs, and submitted to the developed sample preparation process. None of the target compounds was detected in the obtained extracts.

3.4. Concentrations of sartans in water samples

The presence of target analytes was assessed in grab samples obtained from four different environments: municipal wastewater, rivers affected, or not, by known discharges of municipal STPs, rivers used as sources of tap water and tap water samples. Positive identifications require retention times differences lower than 0.1 min

with calibration standards, and Q2/Q1 ratios within a ± 30% interval of values compiled in Table 1. A procedural blank was processed every 5 samples to detect possible contamination problems. Table 4 summarizes the obtained data with their standard deviations (n=3 replicates). Dates and coordinates of sampling points are provided as supplementary information, Table S2.

Codes 1 to 6 correspond to three pairs of wastewater samples. Each pair was simultaneously obtained from the outlet (treated water) and inlet (raw water) flows of the same STP. The seven sartans and the degradation product VALA were found in these six samples at relevant levels. The highest average concentrations in the raw wastewater samples corresponded to VAL and EPR, respectively, Fig. 1A. In some samples, the concentrated water volume was reduced to 100 mL in order to maintain compounds within the linear response range of the method. From data available from the Spanish Drug Agency (*Agencia Española de Medicamentos y Productos Sanitarios, AEMPS*) (AEMPS and Service, n.d.), VAL is the most often prescribed drug with 13.7 daily doses per 1,000 inhabitants (DHD), and an average of 80 mg of active compound per dose and day. In contrast, the lowest prescription rate corresponded to EPR (DHD 0.85); however, the daily dose of this drug (average value 600 mg) is the highest within the group of sartans; so, it is in the 3rd place as regards the total consumed amount, Table S4. Attending to the total consumed amounts, average concentrations of IRB and LOS in the processed raw wastewater are lower than expected.

Fig. 1B displays the average apparent removal efficiencies for the seven sartan drugs in the considered STP. Obtained values varied from 80% for EPR to 35% for TEL. Globally, these percentages agree with those reported by Bayer and co-workers (Bayer et al., 2014) for 5 out of the 7 sartan prescription drugs considered in their work. Formation of VALA during wastewater treatment is also confirmed since the values of this compound

were always higher in the effluent than in the influent samples obtained from the same STP, Table 4.

Codes 7 to 11 correspond to samples obtained from 3 different rivers affected by treated wastewater discharges. They were taken 2-4 km downstream the discharge point. In case of samples 7 and 8, a fraction of the municipal wastewater flows directly to the river, without entering the STP, which explains the relatively high levels of VAL, Table 4. VAL was also found at high concentrations in river sample code 9. On the other hand, sample codes 10 and 11 (obtained from a small stream affected by discharges from a modern STP furnished with primary, biological and UV treatments) contained higher levels of the biodegradation species VALA than residues of any of the prescribed sartans. For these two samples, the ratio VAL/VALA stayed well below 1, as it is expected for wastewaters after the activated sludge treatment (Nödler et al., 2016).

Sample codes 12 and 13 were obtained in river areas not affected by controlled discharges of municipal STPs. Despite this assumption, low concentrations (below 100 ng L^{-1}) were measured for some of the investigated species, Table 4. Codes 14 to 21 correspond to pairs of grab samples obtained the catchment areas of drinking water production plants, and in the tap water received by consumers. In the four catchments points (codes 14, 16, 18 and 20), river water contained low, but measurable, concentrations of several sartans and VALA. In the corresponding tap water samples (codes 15, 17, 19 and 21) VALA was again ubiquitous, with concentrations in the range from 21 to 64 ng L^{-1}. Code 22 corresponds to tap water from a different small village. Measured concentrations remain in the same range of values as those found in the previous processed samples of tap water (codes 15, 17, 19 and 21). The UPLC chromatograms for Q1 and Q2 transitions of VALA for a non-spiked tap water sample (code 15, Table 4), and for a procedural blank are shown in the supplementary information section, Fig. S1.

3.5. Isomerization of eprosartan in the aquatic environment

Fig. 2 shows the UPLC traces for the Q1 and Q2 transitions of EPR in the extracts from two different water types: raw wastewater (Fig. 2A) and river water (Fig. 2B). The peak observed for the first matrix shows the same retention time as that obtained for the commercial standard of the *E* isomer of EPR. The UPLC traces for river water (code 8, Table 4) (Fig. 2B) present a second peak with a slightly longer retention time (0.11 min) than that of *E*-EPR, and a similar Q2/Q1 ratio. The same peak was noticed in all the river water samples polluted with *E*-EPR (codes 7, 8, 9 and 14, Table 4). The accurate ESI(+)-MS spectra for both peaks (obtained using a UPLC-ESI-QTOF-MS system) confirmed that they correspond to species with the same empirical formulae of EPR ($C_{23}H_{24}N_2O_4S$). In addition, their product ion (MS/MS) spectra contain several common product ions (in some cases they present different relative intensities when recorded with the same collision energy), Fig. 2C and 2D. Other product ions (i.e. those at *m/z* values of 163.1226 and 207.1122) are specific, or display a much higher intensity, for *E-EPR*, Fig 2C. On the contrary, ions at m/z 295.1457, 273.1052 and 162.0368 are more intense in the spectrum of the species displaying the longer retention time, Fig. 2D. Differences between both spectra are compatible with intramolecular re-arrangement in the structures of fragment ions obtained for geometric (*E/Z*) isomers of EPR. The possibility to discriminate between *E* and *Z* forms for EPR from their ESI(+) MS/MS spectra has been previously recognized in the literature (Brum and Hannah, 1997). The main product ions in the low resolution APCI(+)-MS/MS spectra obtained in their previous publication matched those shown in Fig. 2C and Fig. 2D.

To the best of our knowledge, *E/Z* isomerization of EPR in environmental water samples has not been previously reported; however, it is a common process for other emerging pollutants, such as certain UV filters, when exposed to solar light (Balmer et al., 2005).

Photodegradation experiments described in section 2.5 revealed that above 90% of *E*-EPR was transformed into a second species, with the same empirical formula, after 4 h of solar irradiation. The new compound shows the same retention time as that appearing in river water samples polluted with *E*-EPR. Moreover, the ESI(-) product ion scan spectra of both compounds (parent and transformation product) in model photodegradation experiments, performed at higher concentrations than those existing in environmental samples, are identical, Fig. S2.

3.6. Transformation products of sartan drugs in wastewater

Chando and co-workers (Chando et al., 1998) have described several metabolites of IRB in human faeces and urine after oral administration of the parent drug. Hydroxylation and oxidation of non-aromatic carbons in the structure of the compound, together to formation of a carboxylic acid derivative were recognized as the main metabolization paths of this drug. Considering the possible formation of similar transformation products for other sartans, extracts from wastewater samples (codes 1 to 6, Table 4) were injected in the UPLC-QTOF-MS system. Thereafter, the pseudo-molecular ions ($[M+H]^+$ and $[M-H]^-$, depending on the ionization mode) for the empirical formulae of above described transformation products were searched within a mass window of 10 ppm. Chromatographic peaks with a significant intensity were noticed only for hydroxylated forms of IRB, under positive ionization, and LOS in ESI (+/-). As expected, they showed a lower retention in the C_{18} UPLC column than the parent drugs, Fig. S3. The number of double bound equivalents, mass errors (ppm) and normalized score (accounting for mass accuracy, isotopic profile and space between ions) obtained from the accurate MS spectra of these compounds are given in Table S5. MS data are coherent with hydroxylation of precursor drugs, without modifying the number of rings and double bounds. The ESI(+)-MS/MS spectra of parent drugs and these potential hydroxylated

forms are shown in Fig. S4. The most intense product ion in the spectra of IRB and IRB-OH appeared 207.0924 Da (calculated mass); thus, the biphenyl tetrazolic substructure of the drug is not involved in the hydroxylation reaction. Moreover, the spectrum of IRB-OH depicted in Fig. S4 matched that previously published for a derivative observed during reaction of IRB with free chlorine and assigned to IRB hydroxylated in the terminal carbon of the alkyl chain attached to the imidazole ring (Carpinteiro et al., 2019). The presence of a hydroxylated form of IRB in treated wastewater and surface water samples was also described by Letzel and co-workers (Letzel et al., 2015). However, these authors suggested that this species was formed due to biodegradation of IRB in STPs.

The ESI (+)-MS/MS spectra for both hydroxylated forms of LOS share several common ions with those observed in the spectrum of LOS, Fig. S4. These ions confirm again that the biphenyl moiety is not involved in the hydroxylation process. Their ESI(-)-MS/MS spectra are shown in Fig. 3. The product ions at *m/z* 187.0640 and 157.0531 Da observed in the spectrum of LOS (Fig. 3A) are shifted 15.9949 Da in that of LOS-OH1, Fig. 3B. Taking into account the structures assigned to both ions, the hydroxyl group is bounded to the butyl group of LOS, likely to the terminal carbon. Above ions are not observed for LOS-OH2, whose spectra (Fig. 3C) contained a relevant number of fragments. Taking into account the structures assigned to these fragments, formation of LOS-OH2 is believed to be the result of hydroxylation of carbon number 5 in the imidazole ring, followed by ring opening and oxidation of the hydroxyl group to a carbonyl moiety.

The average ratio between the responses of IRB-OH and IRB in raw wastewater was 0.71 ± 0.05 (n=9 samples). That for the sum of LOS-OH1 and LOS-OH2 divided by the response of the parent drug was 0.52 ± 0.05 (n=9 samples). Assuming that both, hydroxylated transformation products and parent drugs, behave similarly during sample

preparation and determination steps, then, the hydroxylated forms of IRB and LOS reach significant concentrations in raw wastewater.

The comparison of the corrected responses for hydroxylated species (compound peak area/VALA-d4 peak area) between treated and raw wastewater, normalized to the same volume of sample passed through the SPE cartridge, provides a draft estimation of their mass balance in the STP. Values above 1 point out to significant hydroxylation of parent drugs during the treatment of sewage water. On the other side, ratios below the unit mean neat removal of hydroxylated metabolites. The obtained values (0.57 ± 0.14 and 0.39 ± 0.10 for IRB-OH and the sum of LOS-OH species, respectively) supported the 2nd hypothesis. These data do not serve to discriminate whether these hydroxylated species are human excretion metabolites, or biodegradation species generated in sewage; however, in the latter case, their formation would take place in an earlier stage, at sewers, given that their highest responses were found in the untreated wastewater.

4. Conclusions

For the first time, the efficiency of a mixed-mode SPE protocol for the selective extraction of the seven currently prescribed sartan drugs, and the biodegradation product VALA, in environmental water samples has been demonstrated. In combination with UPLC-ESI-MS/MS determination, compounds are accurately quantified using solvent-based calibration standards, with a low consumption of organic solvents and without a solvent exchange step. Data obtained for municipal sewage water confirm the incomplete removal of this family of drugs in STPs, as well as the formation of VALA during the wastewater treatment process. The existence of hydroxylated forms of IRB and LOS in raw wastewater, and the *E-Z* isomerization of EPR in the aquatic environment are also reported for the first time in this study. The systematic occurrence of VALA in tap water samples confirms the usefulness of this compound to detect contamination of drinking water sources with treated municipal wastewater, and the need to implement new water

treatments aiming to remove non-biodegradable, polar compounds from water samples intended for human consumption.

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Conflict of interest: none.

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Table 1. Summary of chromatographic and MS/MS determination parameters, linearity (determination coefficient, R^2) and limits of quantification (LOQs) of the UPLC-MS/MS system, without considering the sample preparation step.

Compound	Retention time (min)	Precursor ion (m/z)	Cone Voltage (V)	Q1 (CE)	Q2 (CE)	Q2/Q1 ratio	Linearity (2.5–500 µg L^{-1})	LOQ (µg L^{-1})
[a]EPR	3.43	425.1	55	135.1 (36)	97.1 (30)	0.33	0.9995	2
[a]OLM	3.49	447.2	32	207.0 (23)	429.2 (12)	0.69	0.9998	2
[a]VALA	3.68	267.1	32	206.1 (18)	178.1 (28)	0.38	0.9998	2.5
[b]LOS	4.59	423.0	30	207.0 (20)	405.0 (10)	0.66	0.9997	2
[b]CAN	4.63	441.1	30	192.1 (28)	235.1 (22)	0.58	0.9995	3
[b]TEL	4.71	515.1	68	276.0 (46)	497.0 (35)	0.57	0.9997	1
[b]IRB	4.76	429.0	40	207.0 (26)	195.0 (20)	0.23	0.9998	0.5
[b]VAL	5.11	436.0	22	235.0 (18)	207.0 (25)	0.77	0.9997	5
[a]VALA-d_4	3.66	271.0	32	210.0 (20)	182.0 (30)	0.37	-	-
[b]IRB-d_4	4.75	433.0	40	211.0 (26)	195.0 (20)	0.28	-	-

[a], [b], denote the IS assigned to each compound .

CE, collision energy, eV.

Table 2. Recoveries of the SPE procedure and matrix effects (MEs, %) for wastewater samples with their standard deviations (SD). Sample volume 400 and 200 mL for treated and raw wastewater. Addition level 2000 ng L^{-1}, n=3 replicates.

	Treated wastewater		Raw wastewater	
Compound	Recoveries (%) ± SD	ME (%) ± SD	Recoveries (%) ± SD	ME (%) ± SD
EPR	98 ± 1	99 ± 3	104 ± 2	83 ± 3
OLM	102 ± 2	113 ± 3	105 ± 2	99 ± 6
VALA	101 ± 2	101 ± 3	102 ± 3	80 ± 1
LOS	107 ± 2	104 ± 4	103 ± 2	97 ± 2
CAN	105 ± 2	105 ± 2	100 ± 2	94 ± 2
TEL	99 ± 2	104 ± 3	103 ± 1	102 ± 3
IRB	102 ± 3	99 ± 2	104 ± 2	76 ± 4
VAL	93 ± 2	119 ± 3	102 ± 2	105 ± 15

Table 3. Overall recoveries (%) of the analytical procedure for spiked samples of different water types. Mean values, with standard deviations within parenthesis, obtained against solvent-based standards, n=3 replicates.

	River Water			Tap water	Treated wastewater		Raw wastewater		LOQs (ng L^{-1})	
Compound	[a]50 ng L^{-1}	[a]200 ng L^{-1}	[a]500 ng L^{-1}	[a]100 ng L^{-1}	[a]1000 ng L^{-1}	[a]2000 ng L^{-1}	[a]3000 ng L^{-1}	[a]5000 ng L^{-1}	River, Tap water	Raw wastewater
EPR	104 (14)	102 (6)	93 (7)	101 (8)	84 (12)	109 (5)	91 (11)	99 (9)	8	20
OLM	134 (6)	111 (7)	96 (9)	125 (14)	96 (10)	104 (4)	108 (3)	109 (7)	8	20
VALA	108 (12)	114 (10)	93 (6)	101 (4)	97 (16)	99 (7)	103 (2)	100 (12)	10	25
LOS	111 (18)	100 (7)	112 (4)	96 (11)	96 (11)	106 (4)	110 (5)	102 (7)	8	20
CAN	115 (14)	102 (8)	92 (6)	90 (11)	91 (6)	103 (8)	107 (2)	91 (9)	12	30
TEL	98 (8)	102 (13)	112 (5)	91 (6)	98 (9)	103 (5)	107 (5)	105 (13)	4	10
IRB	105 (12)	98 (8)	111 (4)	88 (4)	82 (9)	93 (7)	93 (2)	95 (11)	2	5
VAL	132 (9)	104 (9)	106 (5)	99 (3)	88 (7)	115 (4)	115 (7)	107 (9)	20	50

[a]Added concentration

Table 4. Summary of concentrations (mean values ± SD, ng L^{-1}) measured in different water samples, n=3 replicates.

Code	Water type (River, village)	EPR	OLM	VALA	LOS	CAN	TEL	IRB	VAL
1	T.W.	230 ± 8	972 ± 13	224 ± 9	260 ± 12	219 ± 8	590 ± 27	768 ± 49	1616 ± 60
2	R.W.	4405 ± 100	1308 ± 44	42 ± 5	605 ± 30	362 ± 40	1037 ± 16	1096± 49	3781 ± 96
3	T.W.	2284 ± 25	1368 ± 2	1884 ± 36	367 ± 3	257 ± 5	957 ± 3	813 ± 10	1682 ± 16
4	R.W.	6001 ± 56	2109 ± 43	118 ± 4	627 ± 7	407 ± 17	1340 ± 36	1414 ± 15	9986 ± 87
5	T.W.	554 ± 10	985 ± 52	1344 ± 17	311 ± 12	289 ± 22	903 ± 20	950 ± 33	927 ± 2
6	R.W.	5458 ± 124	1932 ± 216	86 ± 36	853 ± 6	497 ± 34	1324 ± 102	1833 ± 38	7242 ± 149
7	River (Sar)	65 ± 2	429 ± 23	67 ± 4	197 ± 4	136 ± 12	455 ± 13	543 ± 11	1208 ± 45
8	River (Sar)	2128 ± 26	1294 ± 9	915 ± 9	377 ± 11	258 ± 13	788 ± 2	738 ± 8	3432 ± 2
9	River (Anllóns)	326 ± 17	264 ± 19	124 ± 16	190 ± 10	61 ± 5	196 ± 19	158 ± 12	956 ± 48
10	River (Tinto)	n.d.	296 ± 7	1439 ± 14	13 ± 2	97 ± 8	219 ± 16	334 ± 7	49 ± 3
11	River (Tinto)	n.d.	702 ± 16	3111 ± 13	19 ± 2	180 ± 20	433 ± 16	543 ± 7	111 ± 3
12	River (Barbadás)	n.d.	26 ± 2	22 ± 2	n.d.	n.d.	8 ± 1	30 ± 1	n.d.
13	River (Tinto)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	12 ± 1	n.d.
14	River (Arnoia)	59 ± 5	48 ± 4	55 ± 0	n.d.	13 ± 1	20 ± 1	27 ± 0	66 ± 2
15	Tap (Cartelle)	n.d.	n.d.	64 ± 4	n.d.	7 ± 2	13 ± 3	n.d.	41 ± 3
16	River (Tambre)	n.d.	n.d.	29 ± 4	n.d.	n.d.	4 ± 2	13 ± 1	12 ± 3
17	Tap (Santiago)	n.d.	n.d.	24 ± 7	n.d.	n.d.	n.d.	n.d.	n.d.
18	River (Ulla)	n.d.	n.d.	33 ± 5	n.d.	n.d.	n.d.	15 ± 1	n.d.
19	Tap (Teo)	n.d.	n.d.	29 ± 2	n.d.	n.d.	n.d.	n.d.	n.d.
20	River (Anllóns)	n.d.	48 ± 4	108 ± 7	29 ± 1	n.d.	23 ± 1	22 ± 2	107 ± 9
21	Tap (Carballo)	n.d.	8 ± 1	21 ± 7	n.d.	n.d.	n.d.	n.d.	26 ± 8
22	Tap (Sober)	n.d.	63 ± 3	65 ± 6	n.d.	12 ± 3	23 ± 1	4 ± 0	46 ± 2

T.W., treated wastewater; R.W., raw wastewater.

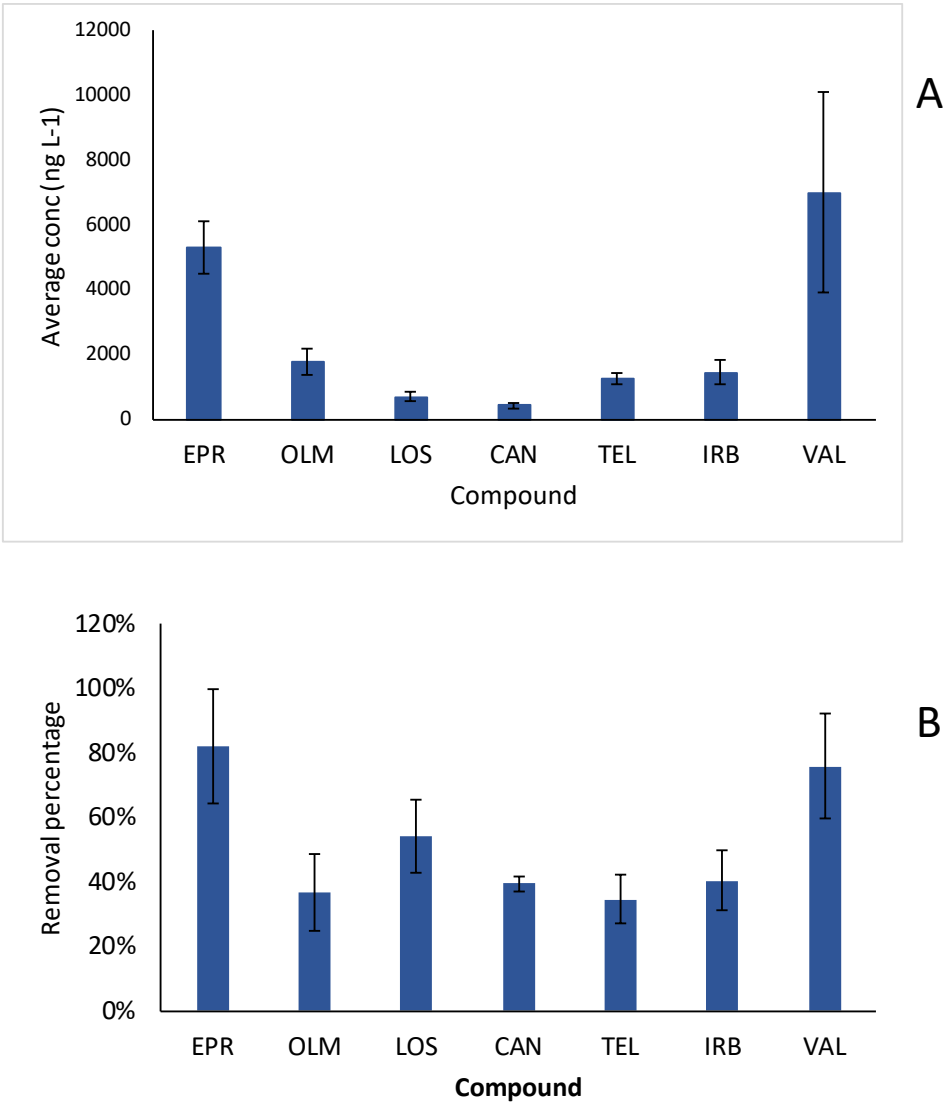

Figure 1. Average concentrations of sartan drugs in raw wastewater from a municipal STP (A), and apparent removal rates in the same plant (B). Data obtained from 3 pairs of grab samples processed in triplicate.

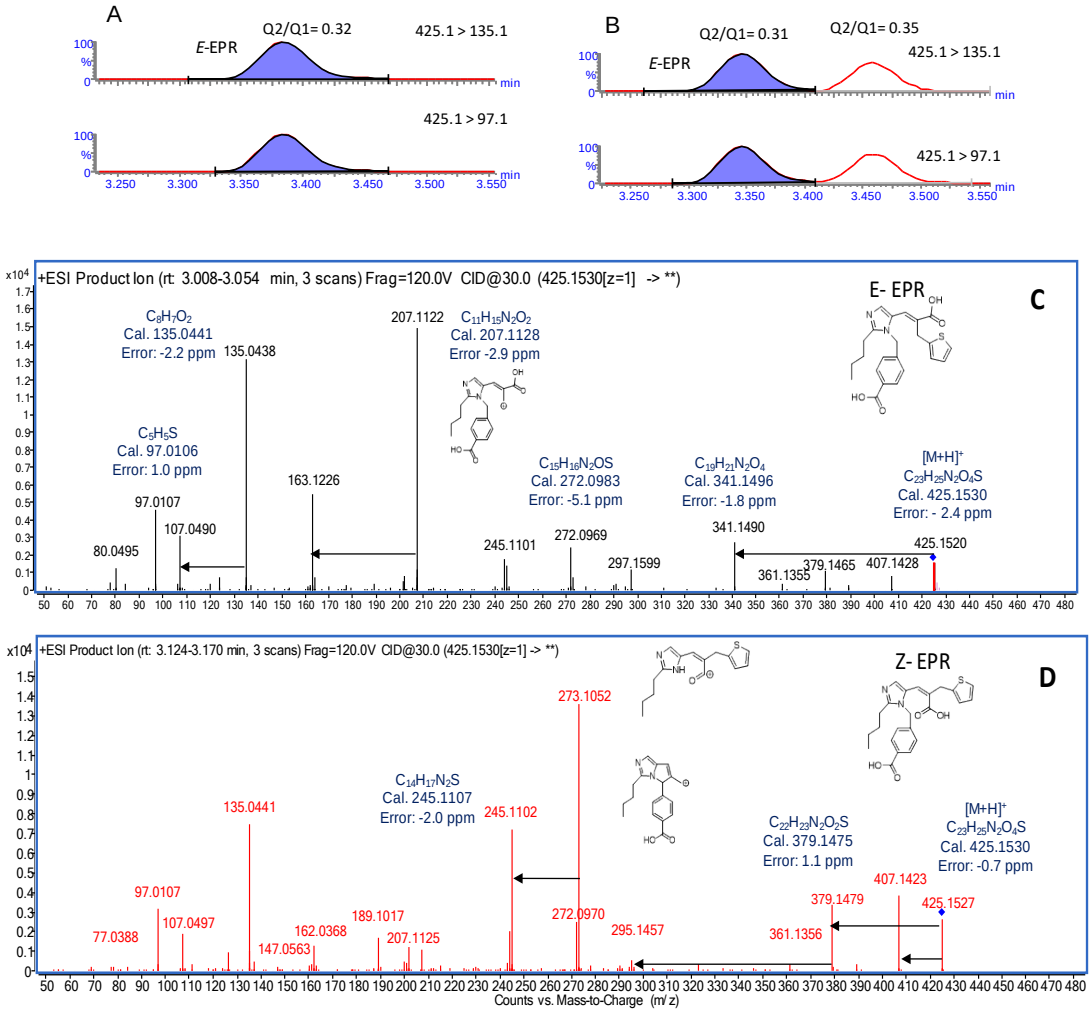

Figure 2. MRM chromatograms corresponding to Q1 and Q2 transitions of EPR in non-spiked samples of raw wastewater (A) and river water (B).

ESI(+) product ion scan spectra obtained for *E*-EPR (C) and the possible Z isomer (D).

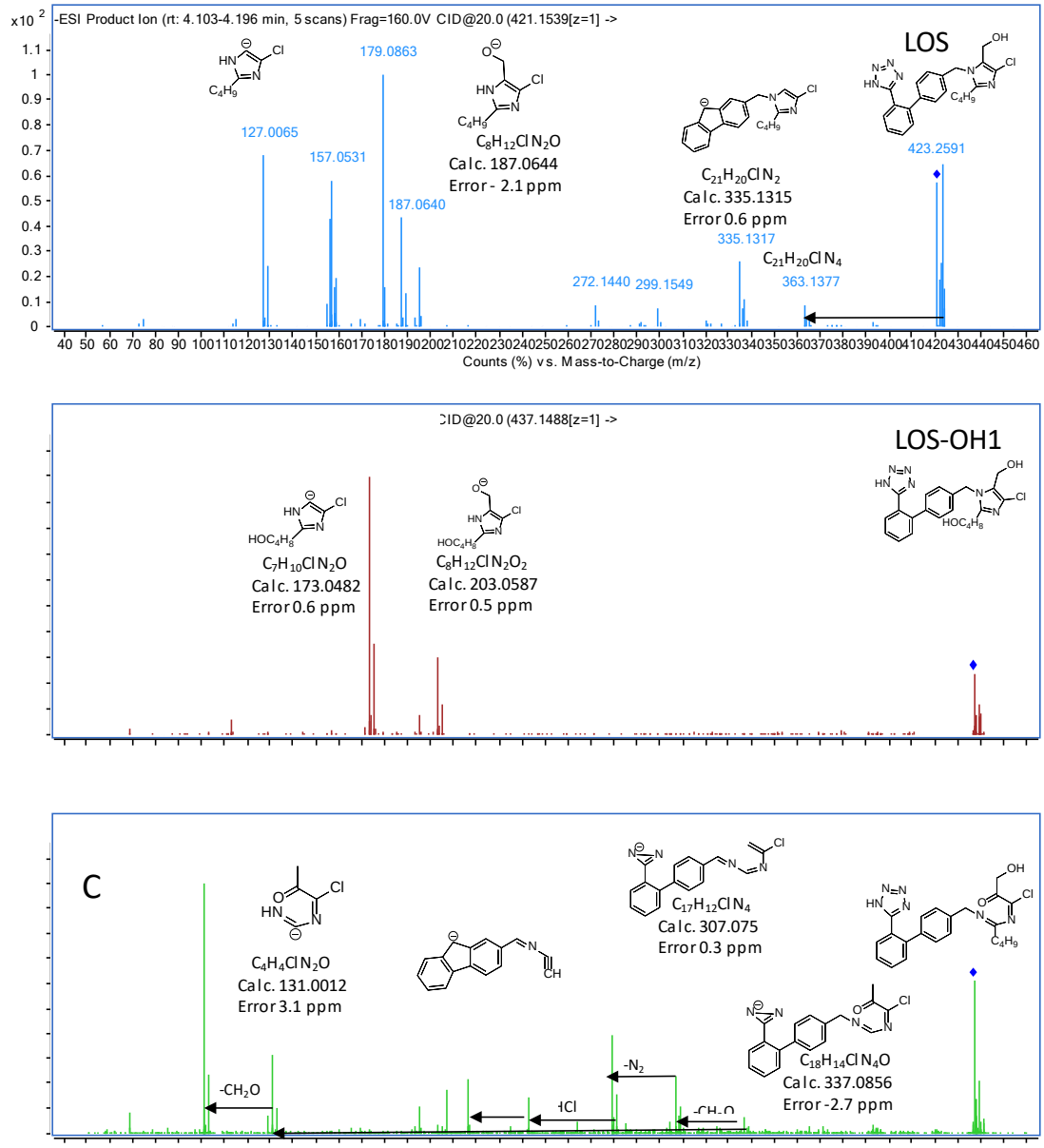

Figure 3. ESI(-) product ion scan spectra for LOS (A), LOS-OH1 (B) and LOS-OH2 (C).

Supplementary information to manuscript:

Selective determination of sartan drugs in environmental water samples by mixed-mode solid-phase extraction and liquid chromatography tandem mass spectrometry

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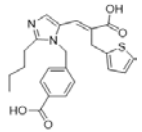
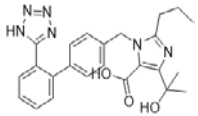
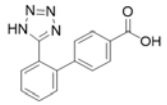
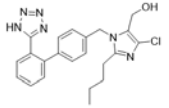
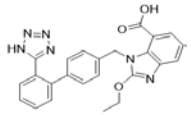
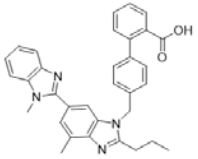
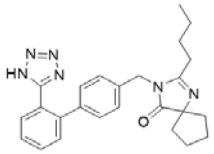
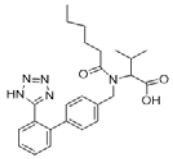
Table S1. Chemical structures, CAS numbers and relevant properties of studied compounds.

Compound	Abbreviation	Structure	CAS	[a]log P	[a]pKa	[a]Charge (pH 7)	[a]Log D (pH 7)
Eprosartan	EPR		133040-01-4	3.75	3.47 (acidic), 6.67(basic)	-1.68	-0.18
Olmesartan	OLM		144689-24-7	2.16	0.89 (acidic), 5.33 (basic)	-1.90	0.05
Valsartan acid	VALA		164265-78-5	3.17	4.03, 5.86 (both acidic)	-1.90	-1.79
Losartan	LOS		114798-26-4	4.06	4.26 (acidic), 3.82 (basic)	-1.00	3.88
Candesartan	CAN		139481-59-7	4.68	3.44 (acidic), 1.5 (basic)	-1.93	0.44
Telmisartan	TEL		144701-48-4	6.13	3.62 (acidic), 5.86 (basic)	-0.93	5.07

Irbesartan	IRB		138402-11-6	5.39	5.85 (acidic), 4.12 (basic)	-1.00	4.20
Valsartan	VAL		137862-53-4	4.59	4.00 (acidic), -0.52 (basic)	-2.00	0.97

[a] Predicted values provided by ChemAxon software.

Table S2. Data corresponding to type of water samples, sampling point and dates.

Code	Water type	City or River	Sampling date	Sampling place coordinates
1	T.W.	STP Santiago	23/07/18	42.870313, -8.598076
2	R.W.	STP Santiago	23/07/18	42.870313, -8.598076
3	T.W.	STP Santiago	06/09/18	42.870313, -8.598076
4	R.W.	STP Santiago	06/09/18	42.870313, -8.598076
5	T.W.	STP Santiago	26/09/18	42.870313, -8.598076
6	R.W.	STP Santiago	26/09/18	42.870313, -8.598076
7	River	Sar	23/07/18	42.857623, -8.631209
8	River	Sar	06/09/18	42.857623, -8.631209
9	River	Anllóns	31/09/18	43.202444,- 8.713944
10	River	Tinto	24/07/18	42.809618, -8.632687
11	River	Tinto	06/09/18	42.809618, -8.632687
12	River	Barbadas	29/07/18	42.321127, -7.879475
13	River	Tinto	24/07/18	42.825999, -8.611045
14	River	Arnoia	29/07/18	42.201042, -7.969702
15	Tap	Cartelle	29/07/18	-
16	River	Tambre	06/09/18	42.946338, -8.528260
17	Tap	Santiago	06/09/18	-
18	River	Ulla	09/09/18	42.755205, -8.559375
19	Tap	Teo	09/09/18	-
20	River	Anllóns	31/09/18	43.211888, -8.680268
21	Tap	Carballo	31/09/18	-
22	Tap	Sober	14/10/18	-

Table S3. Normalized values with their standard deviations (SD) of responses obtained for aliquots of the same treated wastewater sample, spiked before and after the filtration step. Data for n=3 replicates without internal standard correction.

Compound	Normalized response (%)	SD
EPR	84	4
OLM	84	5
VALA	83	4
LOS	86	2
CAN	83	2
TEL	82	2
IRB	87	6
VAL	93	5

Table S4. Data corresponding to the consumption of sartan pharmaceuticals in Spain during year 2017.

Compound	[a]DHD (Daily doses per 1.000 inhabitants)	[b]DDD (Average daily dose, mg)	[c]Total amount (Kg x 10^3)
EPR	0.845	600	8.633
OLM	7.053	20	2.402
LOS	10.061	50	8.566
CAN	9.519	8	1.297
TEL	5.553	40	3.782
IRB	4.599	150	11.746
VAL	13.701	80	18.663

[a] Values obtained from the AEMPS without considering the combination of sartans with other drugs.
[b] Daily dose recommended by the World Health Organization.
[c] Estimated prescribed amount (Kg) for a population of 46.65 million.

Table S5. Proposed formula, rings and double bond equivalents (RDBs) and normalized scores corresponding to UPLC-ESI-TOF-MS data of hydroxylated forms of IRB and LOS.

Ionization mode	Compound	Formula	RDBs	Calculated Mass (Da)	Experimental Mass (Da)	Mass error (ppm)	Normalized Score (0-100)
ESI (+)	IRB-OH	$C_{25}H_{28}N_6O_2$	15	445.2347	445.2341	-1.3	90.4
ESI (-)	IRB-OH	$C_{25}H_{28}N_6O_2$	15	443.2198	443.2201	0.7	98.3
ESI (+)	LOS-OH1	$C_{22}H_{23}ClN_6O_2$	14	439.1644	439.1649	1.1	91.2
	LOS-OH2	$C_{22}H_{23}ClN_6O_2$	14	439.1644	439.1637	-1.6	89.3
ESI (-)	LOS-OH1	$C_{22}H_{23}ClN_6O_2$	14	437.1498	437.1491	-1.6	90.2
	LOS-OH2	$C_{22}H_{23}ClN_6O_2$	14	437.1498	437.1489	-2.1	86.6

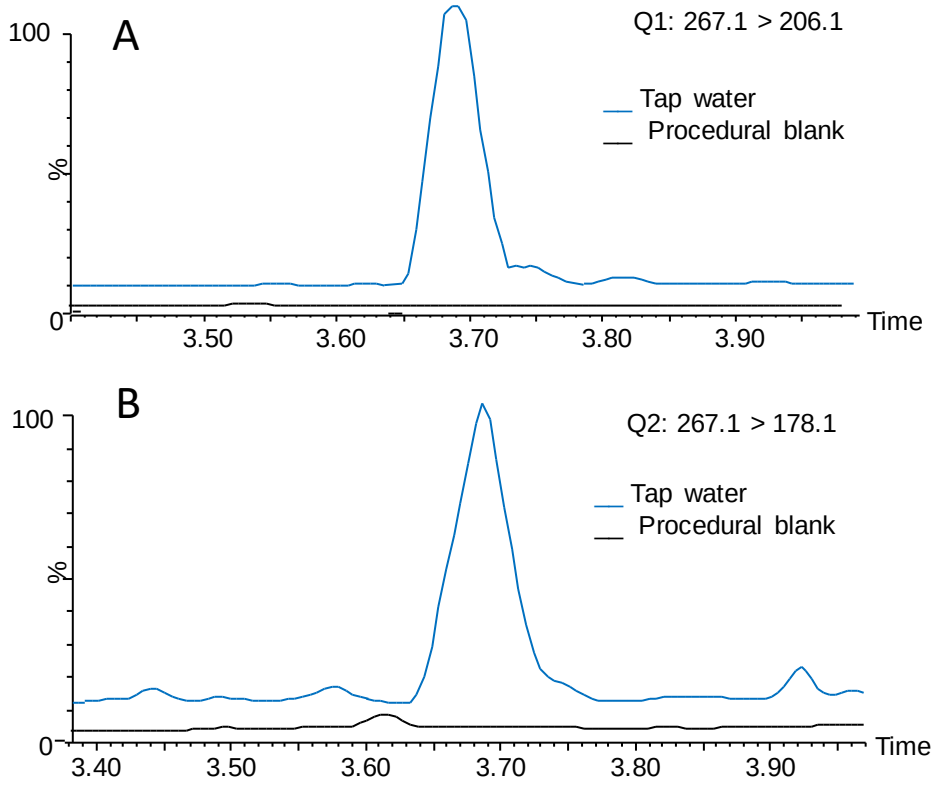


Fig. S1. Overlay of UPLC-ESI-MS/MS chromatograms of VALA in a tap water sample (sample code 15, Table 4, measured concentration 64 ng L^{-1}) and in a procedural blank. A, Q1 transition. B, Q2 transition.

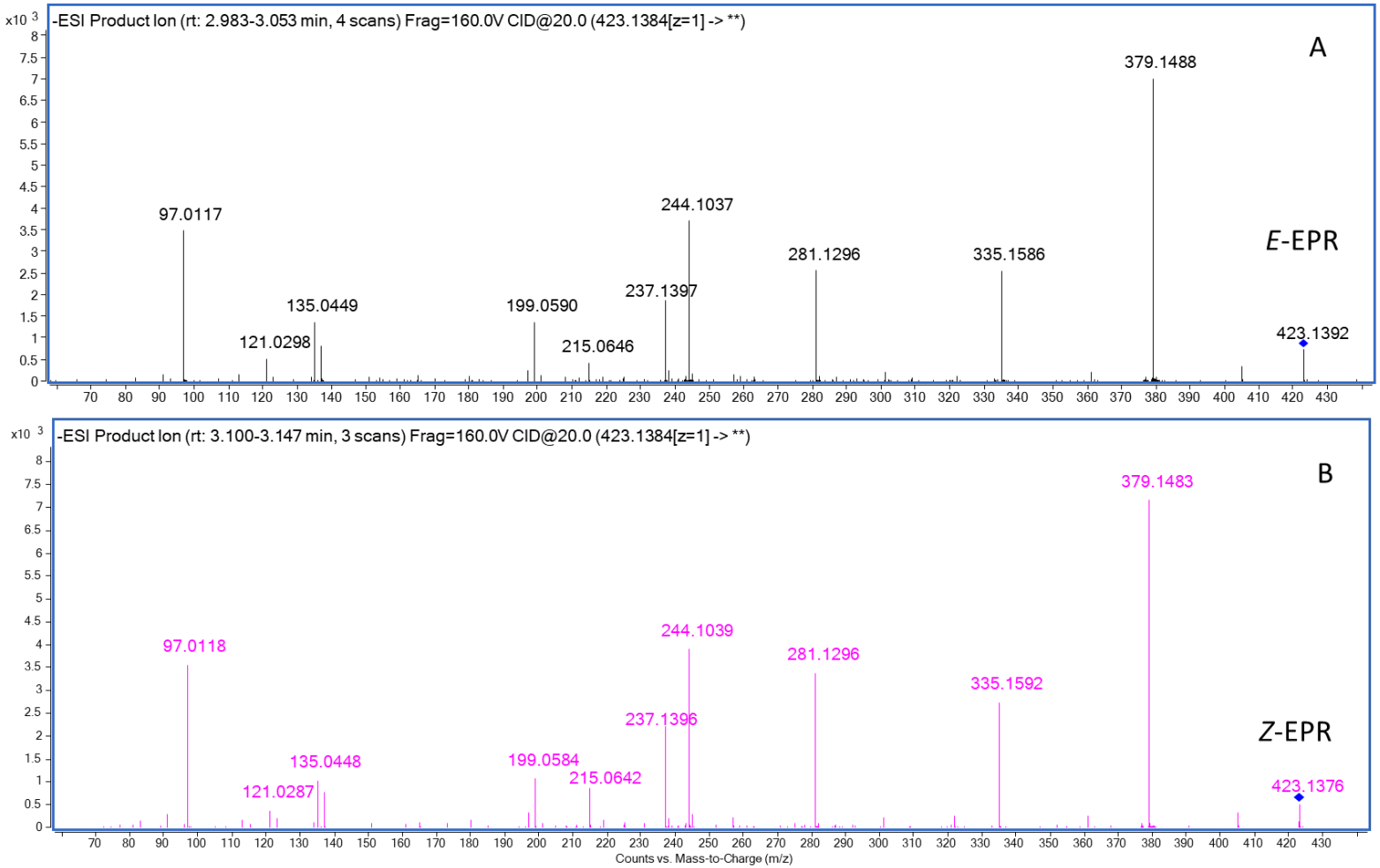


Fig. S2. ESI(-) Accurate product ion spectra obtained for *E*-EPR (A) and the isomer (identified as *Z*-EPR) obtained after sunlight photolysis of an aqueous solution of *E*-EPR (B).

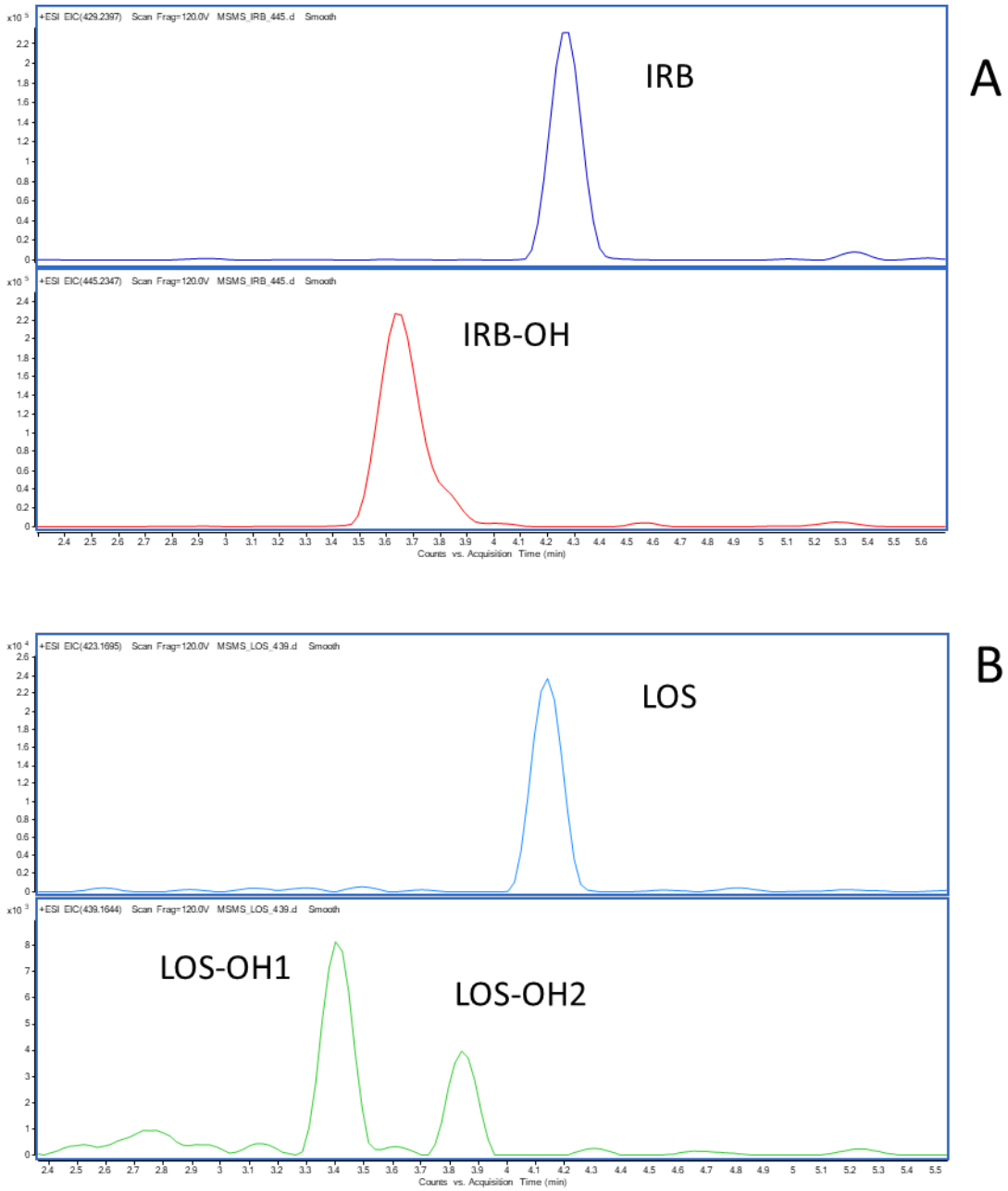


Fig. S3. Extracted ion chromatograms corresponding to the [M+H]+ ions of IRB and IRB-OH (A); LOS and LOS-OH1 and LOS-OH2 (B) in the SPE extract from a non-spiked sample of raw wastewater. Mass extraction window 10 ppm.

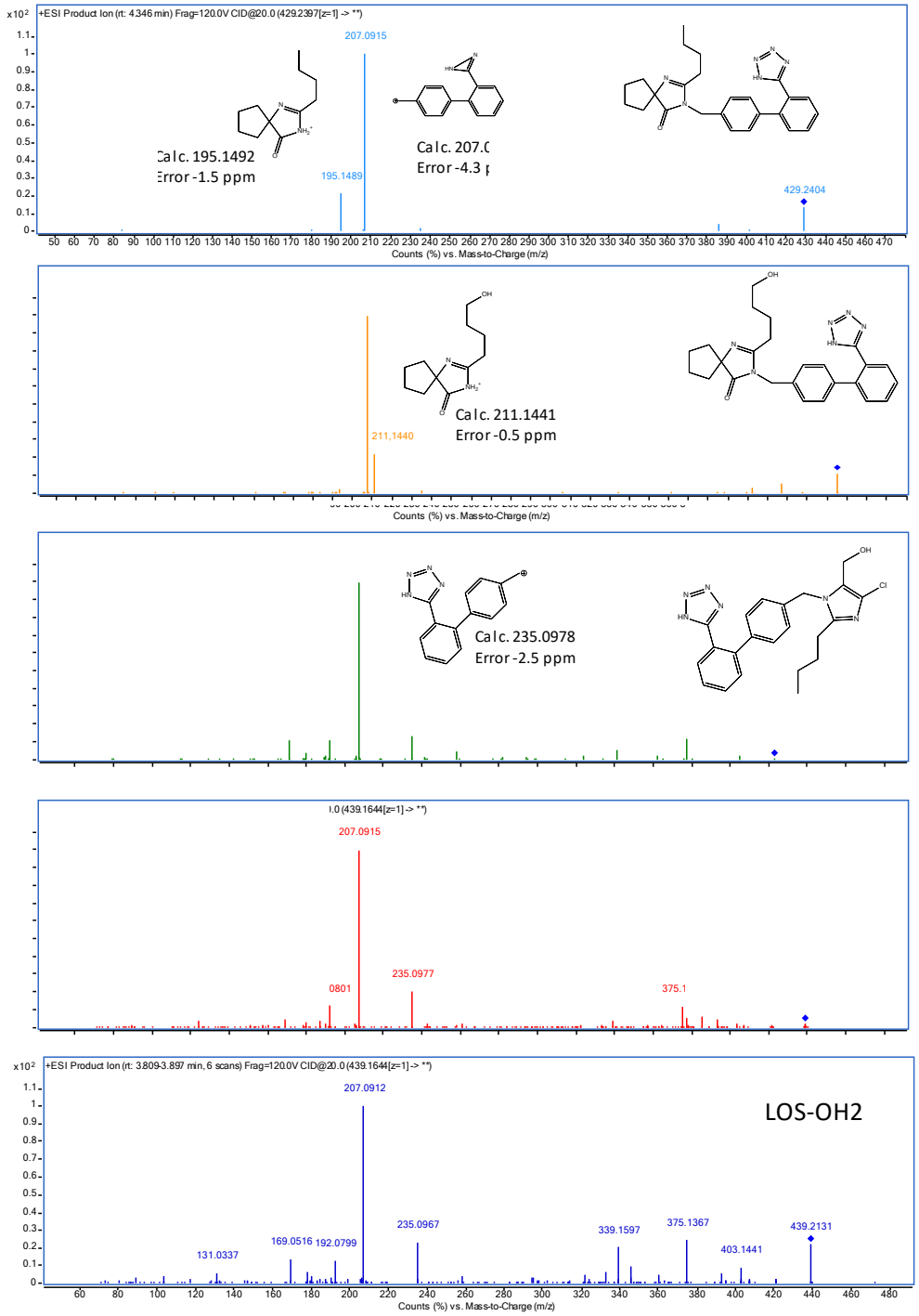


Fig. S4. ESI(+) accurate product ion spectra for IRB, LOS and the entities assigned to their hydroxylated metabolites in raw wastewater.