LC-QTOF MS screening of more than 1000 licit and illicit drugs and their metabolites in wastewater and surface waters from the area of Bogotá, Colombia

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Abstract

A large screening of around 1000 emerging contaminants, focused on licit and illicit drugs and their metabolites, has been made in urban wastewaters (both influent and effluent) and surface waters from the area of Bogotá, Colombia. After a simple generic solid-phase extraction (SPE) step with Oasis HLB cartridges, analyses were made by ultra high-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UHPLC-QTOF MS) under MS^E mode (sequential acquisition of mass spectra at low energy (LE) and high collision energy (HE)). Accurate-mass measurements and the information provided by MS^E on the presence of the (de)protonated molecule and fragment ions allowed the reliable identification of the compounds detected, even without reference standards being available in some cases (tentative identification). The compounds most frequently found were acetaminophen/paracetamol, carbamazepine and its dihydro-dihydroxylated metabolite, clarithromycin, diclofenac, ibuprofen, gemfibrozil, lincomycin, losartan, valsartan, the two metabolites of metamizole (4-acetamido-antipyrine and 4-formylamino-antipyrine), sucralose, and cocaine and its main metabolite benzoylecgonine. Caffeine, the sweetener saccharin and two hydroxylated metabolites of losartan were tentatively identified in almost all samples analysed. Pharmaceutical lidocaine was tentatively identified and subsequently confirmed with reference standard. For the first time, a general overview of the occurrence of drugs and their metabolites in the aquatic environment of Colombia has been reported. In the near future, target methodologies, typically based on LC-MS/MS, will need to be set up for accurate and sensitive quantification of the contaminants selected on the basis on the information provided in the present paper.

Keywords

Screening, ultra high-performance liquid chromatography, time-of-flight mass spectrometry, drugs, water, Colombia

Introduction

Changes in industry, agriculture and urban development present interlinked challenges to water quality [1, 2]. Due to the increasing use of pharmaceuticals and personal-care products (PCPs) as well as veterinary drugs and illicit drugs, the amount of these newly emerging chemicals detected in waters raise environmental and health concerns. Many of these contaminants survive the passage through conventional wastewater treatment processes resulting in the growing discharge of these compounds into receiving waters where their presence is increasingly common [3, 4]. However, little information is available from Latin America despite the fact that this region undergoes rapid land, economical, and social changes [5] and where, in some areas, the discharge of raw sewage into rivers, lakes and reservoirs are widely practiced [6 - 8]. Concern over the presence of these contaminants is well founded as these rivers not only flow through tropical areas rich in biodiversity, but surface waters are also often used as a source for human consumption.

Monitoring licit and illicit drugs is generally based on multi-residue methodologies using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for target substances considered as harmful for human health or the ecosystem. Analyses are commonly focused on a limited number of priority compounds, and the main objective is the accurate quantification of the target analytes. However, the use of pharmaceuticals and veterinary drugs differs temporally and spatially between regions and countries due to different marketing, regulations, prescription practices, etc. [9]. Thus, drugs used in Latin America may differ from those generally prescribed or consumed in Europe and North America. Accordingly, target approaches applied for routine analysis of water samples in one country might ignore important contaminants from another country, as they are simply not included in the scope of the method. Therefore, a large screening of contaminants is of high interest in order to define priority compounds and to subsequently set up target methodologies for monitoring. To this end, High Resolution Mass Spectrometry (HRMS) plays an important role for screening of emerging contaminants, in relevant fields like environmental pollution [10, 11].

Hybrid HRMS, such as a quadrupole time-of-flight mass spectrometery (QTOF MS) in MS^E mode, has demonstrated its use as an advanced tool that allows the investigation of hundreds of compounds in the same run, making use of home-made databases [12, 13].

The presence of compounds in samples can be investigated once the analysis has been performed and data acquired, without being dependent on the pre-selection of analytes. It allows detection/rapid screening for compounds by means of searching the exact mass of the (de)protonated molecules in the full spectra generated using low collision energy (LE function). Confirmation with reference standards, or tentative identification, could subsequently be performed searching for exact masses of fragment ions in the acquired spectra at high collision energy (HE), where fragmentation of the molecule is promoted.

We performed a previous monitoring in the irrigate district of Usosaldaña, an important agricultural area in Colombia, mainly devoted to the cultivation of rice. Analysis of surface water and soil samples by TOF MS, coupled to both gas and liquid chromatography, revealed the presence of several pesticides and metabolites in most of the samples. Also, some personal care products were identified [14]. However, there is little or no information on the presence of emerging organic contaminants in the area of Bogotá. In the present work, a large screening of pharmaceuticals belonging to different therapeutic groups, veterinary drugs, X-ray agents, PCPs (preservatives and UV filters), sweeteners, illicit drugs and a notable number of metabolites has been performed in urban wastewaters (both influent and effluent) and receiving surface waters from the surrounding area of Bogotá. A customized database with more than 1000 emerging contaminants was developed including pharmaceuticals frequently prescribed in Colombia. As far as we know, this is the first wide-scope screening based on HRMS that has been applied to this aim. Henceforth, future monitoring in this area can be focused on the compounds identified in this initial step.

Material and Methods

Chemicals and standards

868 human and veterinary pharmaceuticals including metabolites (see Supplementary information (SI), **Table SI1**), 29 X-Ray agents (**Table SI2**), 20 UV-filters (**Table SI3**), 5 preservatives (**Table SI4**), 9 sweeteners (**Table SI5**) and 130 illicit drugs including metabolites (**Table SI6**) were studied, of which 216 reference standards were available (* in **Tables SI1** – **SI6**).

Reference standards of these compounds were purchased from Across Organics (Geel, Belgium), Aventis Pharma (Madrid, Spain), Bayer Hispania (Barcelona, Spain), Cerilliant (Round Rock, TX, USA), Fluka (Buchs, Switzerland), Dr. Ehrenstorfer (Augsburg, Germany), Fort Dodge Veterinaria (Gerona, Spain), LGC Promochem (London, UK), National Measurement Institute (Pymble, Australia), Riedel-de Haën (Seelze, Germany), Sigma Aldrich (St Louis, MO, USA), Toronto Research Chemicals (Ontario, Canada), Vetoquinol Industrial (Madrid, Spain), and Witega (Berlin, Germany). All reference materials had purities higher than 98% (w/w), except for marbofloxacin and pefloxacin, which had purities higher than 93%.

HPLC-grade methanol (MeOH), HPLC-grade acetonitrile (ACN), sodium hydroxide (NaOH) (>99% w/w) and formic acid (HCOOH) (>98 % w/w) were purchased from Scharlau (Barcelona, Spain). Leucine enkephalin was purchased from Sigma Aldrich (Madrid, Spain). HPLC-grade water was obtained by purifying demineralised water in a Milli-Q plus system from Millipore (Bedford, MA, USA). Solid-phase extraction (SPE) cartridges used were Oasis HLB 3 cm^3 (60 mg) from Waters (Milford, MA, USA).

Samples

Seven influent wastewater (IWW) samples and seven effluent wastewater (EWW) samples were taken from the Salitre wastewater treatment plant (WWTP), in Northwest Bogotá D.C., Colombia, which serves a population of approximately three million inhabitants. The 24-hour composite samples were collected over seven consecutive days in March 2014 (starting on Wednesday March 12th and ending on Tuesday March 18th) in high density polystyrene bottles, immediately centrifuged and stored in the dark at -20°C until analysis. In addition, ten grab surface water (SW) samples were collected from around Bogota (**Figure 1**) in July 2013, concerning areas of interest along the

Tunjuelo River (samples 1-4) and in La Ramada Irrigation District (samples 5-10). Seven individual samples (250 mL) were taken for each sampling point. These were then combined to form a composite sample to provide a more complete overview of each area. An aliquot (250 mL) was then taken for the sample treatment and subsequent analysis.

Sample Treatment

An SPE step was applied prior to analysis to pre-concentrate the sample. All samples were filtered through 0.45 μm mixed cellulose ester membrane filter (Whatman, Dassel, Germany). SPE was performed using Oasis HLB cartridges (60 mg). The cartridges were conditioned by washing and rinsing with 6 mL MeOH and 6 mL Milli-Q water. The water samples (IWW was four times diluted with MilliQ water, i.e. 25 mL sample in 100 mL; EWW and SW was 100 mL, no dilution) were loaded onto the cartridges, percolated by gravity (flow rate around 3 mL/min) and vacuum dried for approximately 15 min. Analytes were eluted with 5 mL MeOH. The extracts were evaporated to dryness at 35°C under a gentle stream of nitrogen and reconstructed in 1 mL of 10:90 MeOH:H_2O. Analyses were performed by injecting 20 μL of the final extract into the UHPLC-QTOF-MS

Instrumentation

A Waters Acquity UPLC system (Waters, Milford, MA, USA) was interfaced to a hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (XEVO G2 QTOF, Waters Micromass, Manchester, UK), using a Z-Spray ESI interface operating in both positive and negative ion mode. The chromatographic separation was performed using an Acquity UPLC BEH C18 1.7 μm particle size column 100 × 2.1 mm (Waters) at a flow rate of 300 μL/min. The mobile phases used were A = H_2O with 0.01% HCOOH and B = MeOH with 0.01% HCOOH. The initial percentage of B was 10%, which was linearly increased to 90% in 14 min, followed by a 2 min isocratic period and, then, returned to initial conditions during 2 min. The total run time was 18 minutes. Nitrogen was used as drying gas and nebulizing gas. The desolvation gas flow was set at 1000 L/h and the cone gas at 80 L/h. TOF-MS resolution was approximately 20,000 at full width half maximum (FWHM) at *m/z* 556.

MS data were acquired in centroid mode over an *m/z* range of 50–1000 Da. Data were acquired in both positive and negative ionization modes in two separate runs. A capillary voltage of 0.7 kV and 2.5 kV were used in positive and negative ionizations modes, respectively. A cone voltage of 20 V was used. Collision gas was argon 99.995% (Praxair, Valencia, Spain). The desolvation temperature was set to 600°C, and the source temperature to 130 °C. The column temperature was set to 40°C.

For MS^E experiments, two acquisition functions with different collision energies were created. The low energy (LE) function, selecting a collision energy of 4 eV, and the high energy (HE) function, with a collision energy ramp ranging from 15-40 eV in order to obtain a greater range of fragment ions. The LE and HE functions settings were for both a scan time of 0.4 s.

For elucidation of losartan metabolites, MS/MS experiments at different collision energies (10, 20, 30, 40 and 50 eV) were performed to promote higher fragmentation.

Data Processing

Processing of MS data was done using ChromaLynx XS application manager (within MassLynx v 4.1; Waters Corporation). The following parameters were used for screening: mass window 150 ppm (for positive ID $\leq$ 5 ppm), isotope fit as well as retention time (maximum deviation of $\leq$ 2.5%) and fragmentation, when available. Software specific settings were: peak width at 5% height: 6 seconds, peak-to-peak baseline noise: 1000 and threshold absolute area: 200.

Results and discussion

Detection, confirmation and tentative identification

The full-spectrum accurate-mass data, generated by QTOF under MS^E mode, were inspected using a home-made database containing 1061 LC-amenable organic contaminants (**Tables SI1 – SI6**), which is continuously being updated. Analytes were mostly selected based on our own experience and on existing compound lists encountered in the literature on LC–MS methods for determination of organic contaminants. Furthermore, pharmaceuticals frequently prescribed in Colombia were also included. In principle, the only information that needed to be included in the database was the name and elemental composition of the compounds. Empirical data (retention time, adduct information and/or fragment ions) obtained from compounds of which reference standards (up to 216) were available in our laboratory was also included. This information was used for easier detection and unambiguous confirmation of the identity of the compound.

The confidence in HRMS based identification is an important issue as pointed out by Schymanski *et al.* [15]. Discussion on how to best communicate confidence regarding identification for the exchange of results via literature and databases is on-going, but criteria are needed and should be well reported. Criteria used in this study were based on the availability (detection and confirmation of the identity) or non-availability (tentative identification) of reference standards. The information given by QTOF MS spectra, both LE and HE accurate mass spectra, and retention times allowed the compounds to be distinguished using different possibilities:

- *Detection*, based on the presence of 1 accurate-mass ion (mass error ≤ 5 ppm) and retention time agreement (maximum deviation ≤ 2.5%)
- *Confirmation of the identity*, with at least 2 accurate-mass ions (≤ 5 ppm) and retention time (≤ 2.5%)
- *Tentative identification*, with at least 2 accurate-mass ions justified by literature data and /or compatibility with candidate chemical structure

Obviously, for compounds containing chlorine or bromine atoms, the characteristic isotopic profile should be observed. However, we consider that this ion is not as definitive as a fragment

ion, and therefore when only the ions corresponding to the parent compound and the isotopic peak are present, we consider this analyte as detected as not as confirmed.

In the latter case, reference standards and additional MS/MS experiments would be necessary for final unequivocal confirmation of the compounds. However, we do not need expensive reference standards for all 1061 compounds in the database, only for the positive findings. From our previous experience, the subsequent acquisition of reference standards has allowed confirmation of nearly all tentative identifications. This supports the high degree of reliability of tentative identification by using this technique [16].

Screening of Colombian water samples

In order to perform a large screening, a non-selective sample treatment and a generic chromatographic separation was chosen to broaden the system applicability to as many compounds as possible. Due to the large level of dilution in these types of samples, SPE was performed, using a generic Oasis HLB cartridge for pre-concentration and to enable detecting the analytes at the low concentration levels normally present. Using this analytical procedure and from the information obtained by UHPLC-QTOF MS under MS^E mode, several compounds could be detected, confirmed and/or tentatively identified in all type of Colombian water samples analyzed (**Table 1**).

Samples were screened for pharmaceuticals belonging to different therapeutic groups, veterinary drugs, X-ray agents, PCPs (preservatives and UV filters), sweeteners, illicit drugs and several metabolites. The compounds most frequently detected were the analgesics/anti-inflammatories N-acetyl-p-aminophenol (commonly known as acetaminophen or paracetamol), diclofenac, ibuprofen and lidocaine. The antibiotics clarithromycin and lincomycin, the angiotensin II antagonists valsartan and losartan, the anti-epileptic carbamazepine, the beta-blocker metoprolol, the lipid regulator gemfibrozil and the X-ray agent iopromide were also found. It is interesting to remark the detection or tentative identification of several metabolites such as those of metamizole (4-acetylamino-antipyrine and 4-formylamino-antipyrine), carbamazepine-10,11-dihydro-10, 11 dihydroxy and three metabolites of losartan (losartan carboxylic acid, and two hydroxy-losartan isomers). Furthermore, the sweeteners acesulfame, sucralose and saccharin and the psychoactive drugs, cocaine and its main metabolite benzoylecgonine, as well as caffeine were frequently detected or tentatively identified.

UV-filters were not detected, while the preservatives methylparaben and propylparaben were found, but only in wastewater samples.

These data are consistent with studies on over the counter medicines in Colombia, where analgesics are most frequently sold (60%), in Bogotá [17]. In addition, 11% of the population of Bogotá has been diagnosed with hypertension of which 5.5% taking mainly losartan, valsartan and metoprolol for its control [18] and regarding antibiotics, clarithromycin and lincomycin are most often prescribed [19].

Based on general population surveys, cocaine is the second most consumed illicit drug, but the main illicit psychoactive drug consumed in Colombia is cannabis [20]. However, the main metabolite of cannabis (11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH)), which is commonly used as biomarker in wastewater for cannabis consumption was not detected. This is probably due to the low sensitivity of the technique applied for this compound, which is only a part of the difficulties around the determination of THC-COOH at low concentrations in complex matrix samples. The analytical limitations related to this compound have been widely recognized in the literature [21 - 23].

Most compounds were found in both wastewaters (influent and effluent) and surface waters suggesting incomplete removal by WWTPs and possible disposal to receiving surface waters. The pharmaceuticals carbamazepine and its metabolite carbamazepine-10,11-dihydro-10, 11 dihydroxy, acetaminophen, diclofenac, ibuprofen, the highly consumed caffeine, cocaine and its metabolite benzoylecgonine are widely occurring contaminants in the aquatic environment, having been reported to be also present in Brazilian surface waters [5, 7].

In relation to surface waters, the presence of pharmaceuticals was more noticeable in samples collected from Tunjuelo river (samples 1 to 4) -a tributary of the Bogotá river- in the south of Bogotá. In this area, around 2.5 million inhabitants discharge their sewage directly to this river without any treatment. In addition, solid urban waste are located here (Doña Juana landfill, **Figure 1**) and potential leaching surely contributes to the pollution in the area. This can explain that the profile of emerging contaminants found in these surface waters and wastewaters from Salitre WWTP is rather similar, as this plant receives sewage from around three million inhabitants.

In contrast, the irrigation area of Ramada -supplied by the Bogotá river in its northern part- may be affected by industrial and urban waste, albeit with a lower population (around 600.000 inhabitants). Although these surface water samples (5 to 10) had several contaminants in common with those samples from the Tunjuelo river and with wastewater samples, the number of compounds detected was notably lower. These waters are mainly used for irrigation on this agricultural area; therefore, the presence of emerging contaminants may have undesirable consequences for public health.

Below, some examples are given in detail, to illustrate the screening strategy applied in this study, which led to the detection, confirmation or tentative identification of several compounds. The different possibilities considered in this work will be taken into account: presence/absence of reference standards in the laboratory, and searching for known and unknown metabolites on the basis of common fragmentation pathway between parent compound and possible metabolites.

a) Reference standards available

Up to 216 reference standards were available in our laboratory and, therefore, experimental data such as retention time and fragment ions could be included in the home-made database. This information was used for easier detection and unambiguous confirmation of the identity of the compound. **Figure 2** shows the unequivocal confirmation in surface water of 4-acetamido antipyrine, a metabolite of the widely used analgesic metamizole. The LE spectrum (Figure 2a, top) of the peak at 3.78 min showed the *m/z* corresponding to the protonated molecule (*m/z* 246.1237) with a mass error of -2.4 ppm. The HE spectrum (Figure 2a, bottom) also showed the remaining protonated molecule and five fragment ions with mass errors all below 5 ppm. In this figure, narrow-window eXtracted Ion Chromatograms (nw-XICs) for the six *m/z* ions are also depicted, with a chromatographic peak at exactly the same retention time.

In a similar way, most of the detected compounds could be confirmed in the samples (see the compounds marked as √ in **Table 1**). The compounds metoprolol and codeine could not be confirmed despite the reference standards were available, as only the protonated molecule was observed at the corresponding retention time. In these cases the compounds were marked as detected (• in **Table 1**). Additional analysis would be

required to promote the fragmentation, using higher collision energies in new MS/MS experiments.

b) Reference standards not available

When reference standards were unavailable, the only information included in the database was the name and elemental composition of the contaminants. Even in this situation, a tentative identification could be performed on the basis of the relevant information on the accurate mass of the (de)protonated molecule and the fragment ions offered by QTOF MS^E. **Figure 3** illustrates the potential of this screening approach with two case studies: the detection and tentative identification of lidocaine and caffeine in effluent wastewater.

The LE spectrum in ESI positive of an abundant chromatographic peak at 3.97 min, showed an intense signal at *m/z* 235.1808 (**Figure 3a, bottom**). This might correspond to the protonated molecule of lidocaine ($C_{14}H_{23}N_2O$, expressed as protonated molecule), with a mass error of -0.9 ppm in relation with the theoretical exact mass. The LE spectrum also showed an important signal at *m/z* 195.0883 (retention time 3.89 min) which could be attributed to caffeine ($C_8H_{11}N_4O_2$, 0.5 ppm mass error). The HE spectrum showed 3 fragment ions at *m/z* 138.0667 ($C_6H_8N_3O$), 110.0716 ($C_5H_8N_3$) and 86.0971 ($C_5H_{12}N$), all with mass errors below 2 ppm (**Figure 3a, top**). At this point, UHPLC was a valuable tool for selecting almost co-eluting fragment ions that might correspond to different precursor ions. Thus, the fragments at *m/z* 138 and 110 were related to caffeine, whereas the fragment at *m/z* 86 was related to lidocaine. The structure of the fragment ions were justified on the basis of their measured accurate masses, which was moreover in agreement with the information available in scientific literature [24, 25]. Lidocaine could be tentatively identified and subsequently confirmed after acquiring its reference standard (⊕√ in **Table 1**). The confirmation of caffeine would require the injection of the reference standard, which at the time of writing this paper was not available at our laboratory. Similar situations occurred for other compounds tentatively identified, as metformin, pirantel and the sweetener saccharin, due to the lack of reference standard (⊕ in **Table 1**).

c) Searching for metabolites

A detailed discussion is made on the particular case of losartan, an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension), and its metabolites identified making use of the common fragmentation pathway.

Losartan was confirmed to be present in all the samples analyzed. The LE spectrum in ESI+ showed an abundant chromatographic peak at the expected retention time (10.13 min) for this pharmaceutical (*m/z* 423.1695, -1.2 ppm mass error) and also presented the typical isotopic pattern of a chlorine atom ($C_{22}H_{24}N_6OCl$, expressed as protonated molecule). The expected fragment ion at *m/z* 405.1590 ($C_{22}H_{22}N_6Cl$, -1.0 ppm), due to a loss of water, was also observed in the LE spectrum, as well as a minor fragment ion at *m/z* 377.1528 ($C_{22}H_{22}N_4Cl$, -1.3 ppm), corresponding to a N_2 loss from *m/z* 405. The HE spectrum showed a predominant ion at *m/z* 207.0916 ($C_{14}H_{11}N_2$), and minor peaks at *m/z* 235.0979 ($C_{14}H_{11}N_4$), 192.0813 ($C_{14}H_{10}N$), 190.0661 ($C_{14}H_8N$) and 180.0805 ($C_{13}H_{10}N$), all with mass errors below 4 ppm. These fragments correspond to the fragmentation at the benzylic carbon (CH_2 near to the two rings) (*m/z* 235) with losses of N_2 (*m/z* 207), N_3H (*m/z* 192) or N_3H_3 (*m/z* 190). Losartan was also detected in negative mode at *m/z* 421.1552, showing fragment ions at *m/z* 187.0631 ($C_8H_{12}N_2OCl$), 179.0868 ($C_{14}H_{11}$), 157.0529 ($C_7H_{10}N_2Cl$) and 127.0066 ($C_5H_4N_2Cl$). With all this information, the compound detected in the samples was unequivocally confirmed to be losartan.

The presence of three additional chromatographic peaks at 8.25, 9.29 and 10.47 min in all the three XICs at *m/z* 207, 235 and 190 performed at the HE function in ESI+ (for losartan, retention time 10.13 min) suggested that the three compounds were chemically related with this pharmaceutical (**Figure 4**). The common fragmentation pathway strategy has been successfully applied by our group in the elucidation of metabolites of the new psychoactive substance methylenedioxypyrovalerone (MDPV) and of degradation products of cocaine and benzoylecgonine [26, 27]. This encouraged us to investigate the identity of the potential metabolites/transformation products of losartan from the data provided by QTOF MS.

Firstly, the LE spectra (all showing the isotopic distribution corresponding to a chlorine atom) of these 3 possible metabolites were studied in more detail. In relation to the peak at 10.47 min, the protonated molecule corresponded to *m/z* 437.1490 ($C_{22}H_{22}N_6O_2Cl$, -0.7 ppm), which might be attributed to an oxidation of losartan. This compound might

correspond to losartan carboxylic acid, the main metabolite reported in the literature [28].

Regarding the peaks at 8.25 and 9.29 min, accurate masses of *m/z* 439.1643 and 439.1661 were obtained, respectively, both corresponding to the same empirical formulae $C_{22}H_{24}N_6O_2Cl$ with mass errors lower than 3 ppm. This implies the presence of an extra oxygen atom with respect to losartan. Hence, these two compounds might correspond to hydroxylated metabolites of losartan.

The following step consisted on the elucidation of the structure of these three potential metabolites. For this purpose, MS/MS experiments were performed at different collision energies (10-50 eV).

Regarding the peak at 10.47 min (*m/z* 437.1490, $C_{22}H_{22}N_6O_2Cl$, -0.7 ppm), 5 common product ions with losartan were observed at *m/z* 235.0982, 207.0919, 192.0816, 190.0654 and 180.0809. This compound was also detected in negative mode, with *m/z* 435.1327 ($C_{22}H_{22}N_6O_2Cl$, -2.1 ppm). At the lowest collision energy, a product ion was observed at *m/z* 391.1429 ($C_{21}H_{20}N_6Cl$, -2.3 ppm), corresponding to a loss of CO_2. When the collision energy was increased, product ions at *m/z* 363.1375 ($C_{21}H_{20}N_4Cl$, this is a N_2 loss from 391, -0.3 ppm), 157.0530 ($C_7H_{10}N_2Cl$, -1.9 ppm) and 113.9989 ($C_4H_3N_2Cl$, 3.5 ppm) were observed. All these fragments fitted with the chemical structure of losartan carboxylic acid. After this careful evaluation and well-supported tentative identification, the reference standard of losartan carboxylic acid was acquired and injected, allowing the ultimate confirmation of this metabolite in the samples.

Regarding the hydroxylated metabolite 1 (8.25 min, *m/z* 439.1643, $C_{22}H_{24}N_6O_2Cl$, -1.4 ppm), two minor product ions were observed at 10 eV at *m/z* 421.1536 ($C_{22}H_{22}N_6OCl$) and 385.1772 ($C_{22}H_{21}N_6O$), resulting from a loss of water and subsequent loss of hydrochloric acid. At higher collision energies, product ions at *m/z* 235.0978 ($C_{14}H_{11}N_4$), 207.0915 ($C_{14}H_{11}N_2$), 192.0806 ($C_{14}H_{10}N$), 190.0649 ($C_{14}H_8N$) and 180.0803 ($C_{13}H_{10}N$) were obtained (**Figure 5a**). Thus, ESI+ fragmentation advise about what is happening near to the tetrazol group but not about the location of the hydroxyl group. Accordingly, these fragments seem to indicate that the hydroxylation has occurred in the imidazole part. In negative mode, product ions at *m/z* 203.0585 ($C_8H_{12}N_2O_2Cl$) and 173.0488 ($C_7H_{10}N_2OCl$) were observed at 20 eV. When the collision energy was increased up to 50 eV, product ions at *m/z* 155.0384 ($C_7H_8N_2Cl$), 127.0060 ($C_5H_4N_2Cl$), 113.9991 ($C_4H_3N_2Cl$) and 100.9912 ($C_3H_2N_2Cl$) were observed

(**Figure 5b**). Considering all this information, the –OH group could be placed in the benzimidazole part, being an N-oxide the most plausible candidate. Otherwise, if the hydroxyl group was located in the alkylic chain, a second loss of water should in principle be observed in ESI+.

Regarding the hydroxylated metabolite 2 (9.29 min, *m/z* 439.1645, $C_{22}H_{24}N_6O_2Cl$, -0.9 ppm), two important fragment ions were observed at 10 eV (ESI+) at *m/z* 421.1538 ($C_{22}H_{22}N_6OCl$) and 403.1435 ($C_{22}H_{20}N_6Cl$) corresponding to two consecutive losses of water. At higher collision energies, the common fragment ions at *m/z* 235.0983, 207.0920, 192.0811, 190.0659 and 180.0808 were observed. The spectra in negative mode was different from that of losartan and the other hydroxylated metabolite (e.g. respectively *m/z* 187/157 or 203/173 were not seen) and only two product ions at *m/z* 100.9908 ($C_3H_2N_2Cl$) and 131.0008 ($C_4H_4N_2OCl$) were observed. The presence of the –OH group on the other N of the imidazole group would not explain the easy loss of water observed in ESI+. Another possibility would be the hydroxyl group to be located in the benzilic carbon, although this would hamper (but not prevent) the formation of the ions 235/207 in ESI+.

After a literature search [28], three hydroxylated candidates were found (see the hydroxylated reported sites, marked as (•) in **Figure 5**): two metabolites are hydroxylated in the alkylic chain and the other in the benzilic carbon. However, on the basis of the MS/MS fragmentation, it was not possible to unequivocally locate the position of the –OH group.

It is interesting to remark that losartan, its carboxylic acid and the two hydroxylated metabolites were found in all water samples analysed (10 surface, 7 effluent WW, 7 influent WW).

Conclusions

The potential of UHPLC-QTOF MS for large screening of more than 1000 licit and illicit drugs, even without the need of having all reference standards available, has allowed the detection of many of these compounds in water samples from the area of Bogotá. The screening performed in urban wastewater and surface water confirmed the presence of emerging contaminants, mainly pharmaceuticals, in the samples. This work provides new information on the occurrence of these compounds and metabolites in the Colombian water cycle. The availability of full-spectrum acquisition accurate-mass QTOF data will also facilitate in the future, retrospective analysis of other contaminants not considered in this initial screening, if required. The results obtained in this first study may help Colombian institutions to select priority contaminants for future actions. Thus, target methodologies, typically based on LC-MS/MS, will need to be set up in the near future for accurate and sensitive quantification of the contaminants selected on the basis on the information provided in the present paper.

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Supplementary information

In this section, a table is shown of the human and veterinary pharmaceuticals including metabolites (**Table SI1**), X-Ray agents (**Table SI2**), UV-filters (**Table SI3**), preservatives (**Table SI4**), sweeteners (**Table SI5**) and illicit drugs including metabolites (**Table SI6**) studied.

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Table 1. Positive findings by UHPLC-(Q)TOF MS in 10 surface water samples. Results obtained in influent and effluent samples.

COMPOUND	SURFACE WATER (number of the sampling site)										INFLUENT* (n=7)	EFFLUENT* (n=7)
	1	2	3	4	5	6	7	8	9	10		
Pharmaceuticals and veterinary drugs												
4-Acetamido antipyine	√	√	√	√	√	√	√	√	√	√	√	√
4-Formylamino antipyrine		√	√	√	√	√	√	√	√	√	√	√
Acetaminophen/paracetamol	√			√		√	√		√	√	√	√
Carbamazepine	√	√	√	√	√	√	√	√	√	√	√	√
Carbamazepine 10,11-dihydro-10,11-dihydroxy	√	√	√	√	√	√	√	√	√	√	√	√
Clarithromycin		√	√					√	√	√	√	√
Clindamycin						√			√	√		
Codeine				•								•
Diclofenac		√	√	√							√	√
Dimetridazole		√	√									
Gabapentin												•
Gemfibrozil	√	√	√	√		√	√	√	√	√	√	
Ibuprofen		√		√				√			√	√
Iopromide				√								√
Irbesartan											√	√
Ketoprofen				√							√	√
Levamisole			√	√								√
Lidocaine**	⊕√	⊕√	⊕√	⊕√		⊕√	⊕√	⊕√	⊕√	⊕√	⊕√	⊕√
Lincomycin		√	√	√		√	√	√	√	√		√
Losartan	√	√	√	√	√	√	√	√	√	√	√	√
Losartan, carboxylic acid**	⊕√	⊕√	⊕√	⊕√	⊕√	⊕√	⊕√	⊕√	⊕√	⊕√	⊕√	⊕√

Losartan, hydroxy (1)	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Losartan, hydroxy (2)	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Metformin		⊕	⊕	⊕							⊕	⊕
Metoprolol		•	•	•		•		•	•	•	•	•
Metronidazole	√	√	√	√								
Naproxen	√	√	√	√							√	√
Pirantel			⊕	⊕								
Salbutamol				√								
Sulfamethoxazole		√	√	√							√	√
Trimethoprim		√	√	√							√	√
Valsartan		√	√	√		√	√	√	√	√	√	√
Psychoactive drugs												
Benzoylecgonine	√	√	√	√		√	√	√	√	√	√	√
Caffeine	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕
Cocaine	√	√	√	√		√		√	√	√		√
Preservatives												
Methylparaben												√
Propylparaben											√	√
Sweeteners												
Acesulfame		•	•	•	•	•	√	•	•	√	√	√
Saccharin	⊕			⊕	⊕	⊕	⊕	⊕	⊕		⊕	⊕
Sucralose	•	•	•	√	•	•	√	√	√	√	√	√

(•) Detected, not confirmed (1 accurate-mass ion <5 ppm + retention time <2.5%).
(√) Confirmed with at least two accurate-mass ions (<5ppm) and retention time (<2.5%) with reference standard.
(⊕) Tentatively identified (at least two accurate-mass ions justified by literature data and/or compatible with the candidate chemical structure).

*Results given for IWW and EWW correspond to seven samples (one whole week). The compounds indicated as •, ⊕ or √ were found in at least 6 out of 7 samples analysed.
**These compounds were firstly tentatively identified in the samples and afterwards confirmed with reference standards.

Figure captions

Figure 1. Location of sampling sites

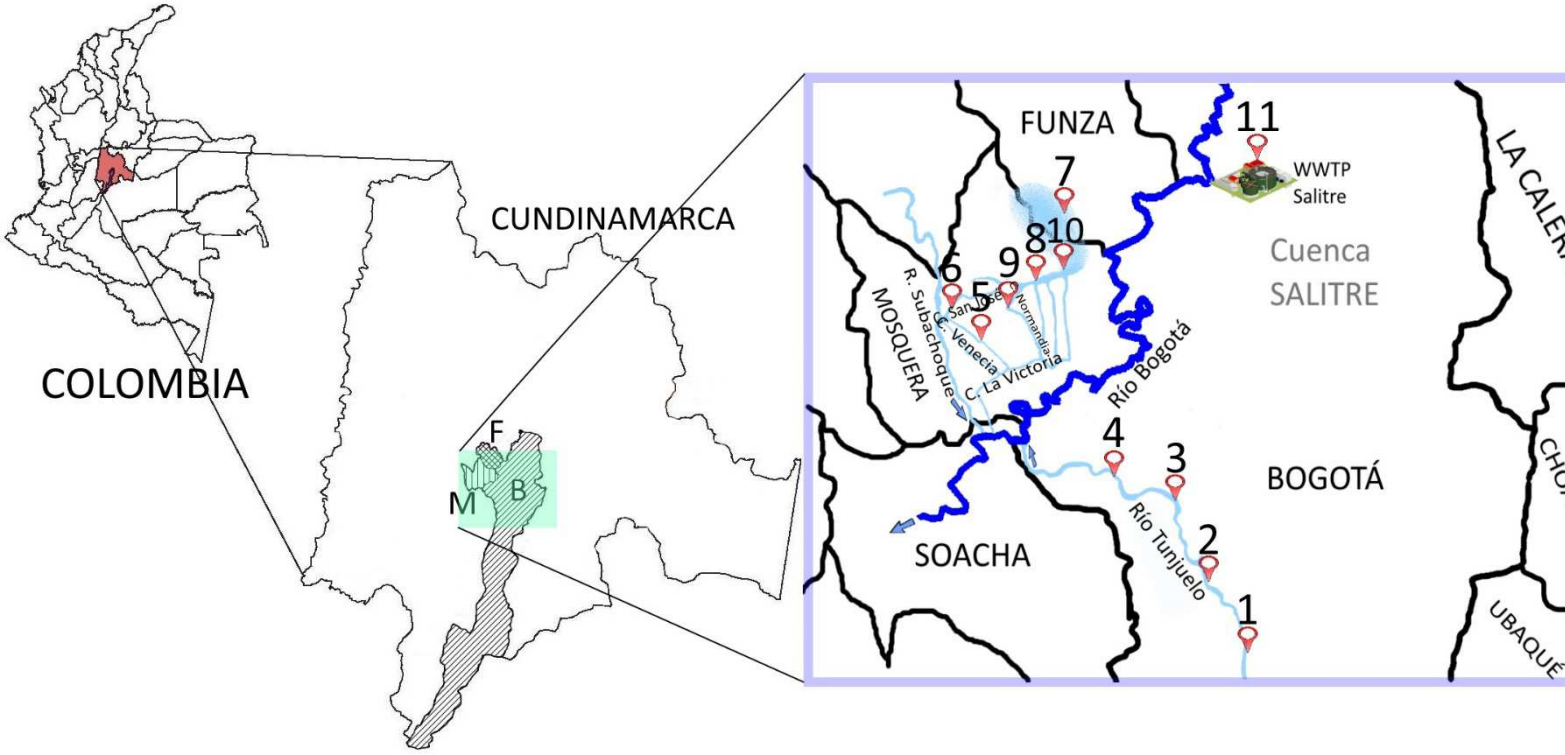
Figure 2. Detection and identification of metabolite 4-acetylamino antipyrine in surface water by UHPLC-QTOF MS. (a) Low energy (LE, bottom) and high energy (HE, top) mass spectra for the peak at 3.78 min; (b) eXtracted Ion Chromatograms (XICs) at 150 ppm mass window for [M+H]+ in LE, and main fragments in HE.

Figure 3. Tentative identification of lidocaine and caffeine in effluent wastewater by UHPLC-QTOF MS. (a) LE and HE mass spectra for the peaks at 3.89 and 3.98 min in positive ionization mode; (b) XICs at 150 ppm mass window for $[M+H]^+$ of lidocaine (3.98 min) and caffeine (3.89 min) in LE, and for the fragment ions (*m/z* 86, 138 and 110) in HE.

Figure 4. Common fragmentation pathway strategy applied for the detection of metabolites of losartan. (a) eXtracted Ion Chromatograms (XICs) at 150 ppm mass window for $[M+H]^+$ of losartan in LE function, and (b) main fragments (*m/z* 207, 235, 190) in HE function; (c) XICs at 150 ppm mass window for $[M+H]^+$ of possible carboxylic acid metabolite in LE function; (d) XICs at 150 ppm mass window for $[M+H]^+$ of possible hydroxylated metabolites of losartan in LE function.

Figure 5. MS/MS spectra at 10 eV (bottom), 20 eV (middle) and 50 eV (top) for hydoxylated metabolite 1 (8.25 min) in (a) ESI+ and (b) ESI-

(•) indicates the possible hydroxylation sites, as reported in the literature.



Sampling points

Number	Name/Description
1	Landfill, Doña Juana (Tunjuelo river)
2	San Benito (tanneries area) (Tunjuelo river)
3	Guadalupe (slaughterhouse) (Tunjuelo river)
4	Bosa (Tunjuelo river)
5	Irrigation channel San Jose- La Victoria (Ramada irrigation area)
6	Irrigation channel San Jose- Los Pinos (Ramada irrigation area)
7	Wetland Güali-Tres Esquinas (Ramada irrigation area)
8	Canal C. Agricultural Center, Marengo (Ramada irrigation area)
9	Canal C. Agricultural Center, Marengo (Ramada irrigation area)
10	Exit of swamp (Ramada irrigation area)
11	Wastewater, Salitre WWTP Seven influent and seven effluent wastewater samples (one whole week)

Figure 1.

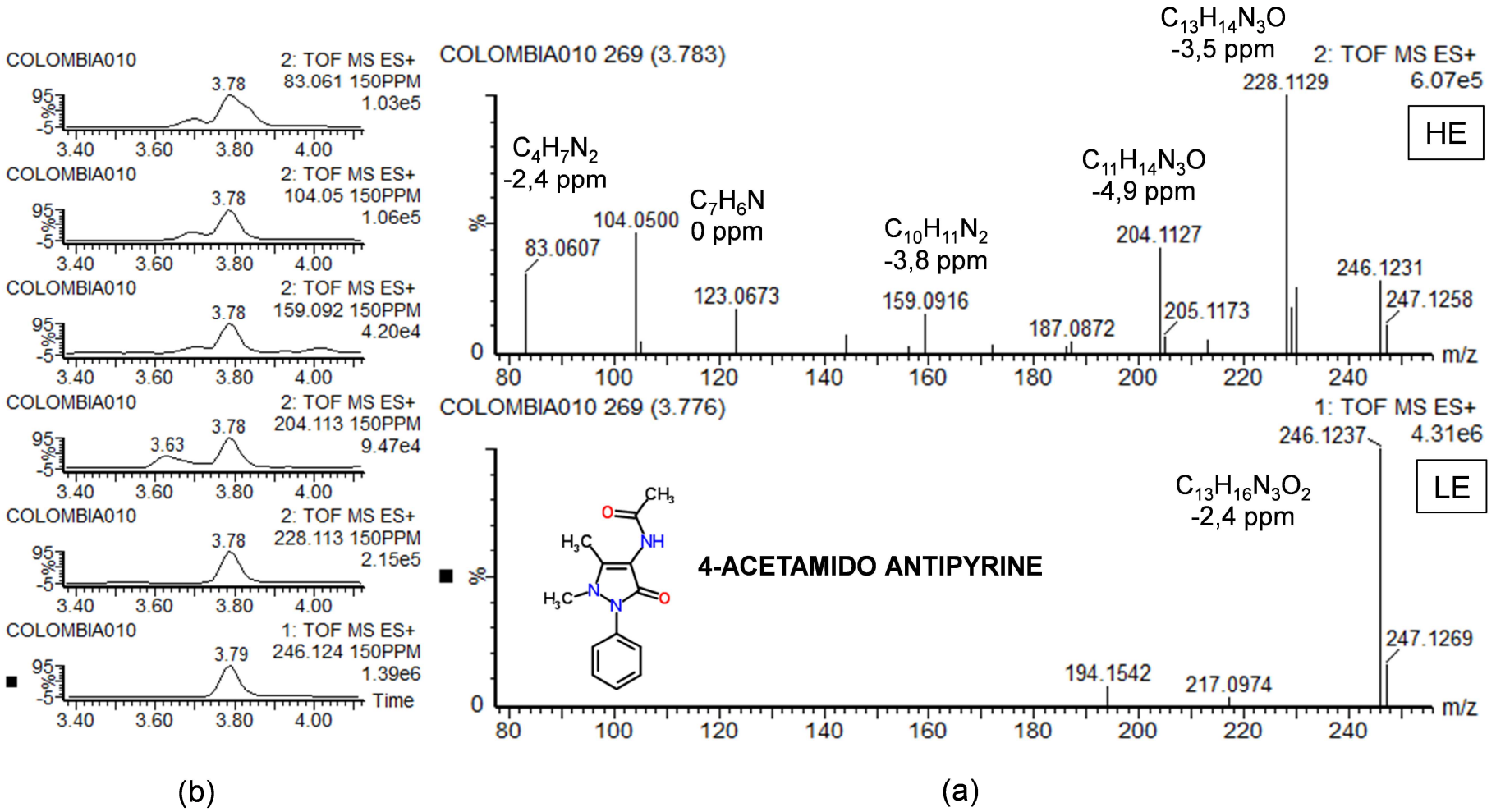
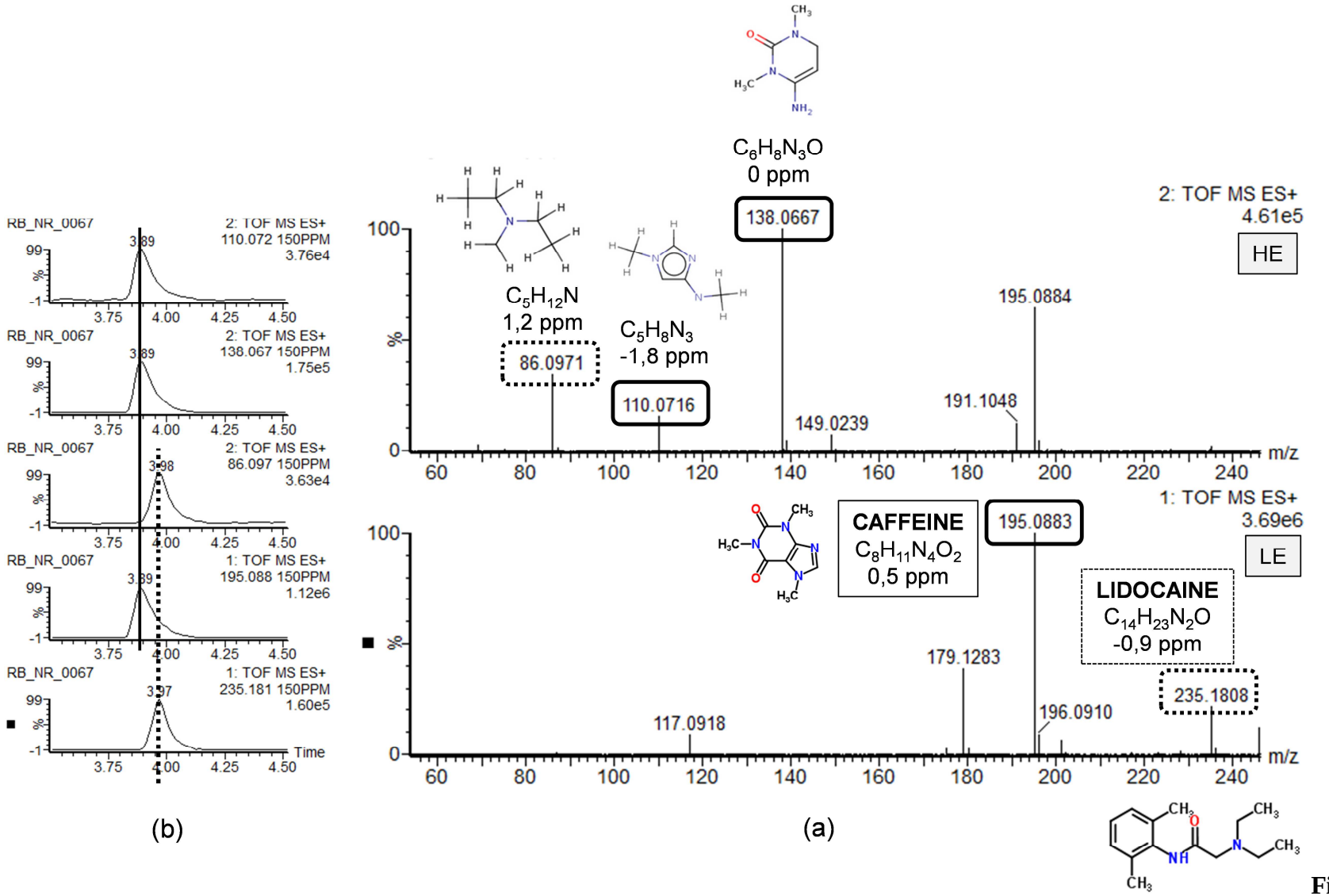

COLOMBIA010
2: TOF MS ES+
83.061 150PPM
1.03e5
3.78
COLOMBIA010
2: TOF MS ES+
104.05 150PPM
1.06e5
3.78
COLOMBIA010
2: TOF MS ES+
159.092 150PPM
4.20e4
3.78
COLOMBIA010
2: TOF MS ES+
204.113 150PPM
9.47e4
3.63
3.78
COLOMBIA010
2: TOF MS ES+
228.113 150PPM
2.15e5
3.78
COLOMBIA010
1: TOF MS ES+
246.124 150PPM
1.39e6
3.79
Time
3.40 3.60 3.80 4.00
(b)
COLOMBIA010 269 (3.783)
C4H7N2
-2,4 ppm
C7H6N
0 ppm
C10H11N2
-3,8 ppm
C11H14N3O
-4,9 ppm
C13H14N3O
-3,5 ppm
2: TOF MS ES+
6.07e5
HE
83.0607
104.0500
123.0673
159.0916
187.0872
204.1127
205.1173
228.1129
246.1231
247.1258
m/z
COLOMBIA010 269 (3.776)
1: TOF MS ES+
4.31e6
LE
C13H16N3O2
-2,4 ppm
246.1237
247.1269
194.1542
217.0974
4-ACETAMIDO ANTIPYRINE
80 100 120 140 160 180 200 220 240
(a)

Figure 2.


RB_NR_0067
2: TOF MS ES+
110.072 150PPM
3.76e4
RB_NR_0067
2: TOF MS ES+
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1.75e5
RB_NR_0067
2: TOF MS ES+
86.097 150PPM
3.63e4
RB_NR_0067
1: TOF MS ES+
195.088 150PPM
1.12e6
RB_NR_0067
1: TOF MS ES+
235.181 150PPM
1.60e5
Time
(b)
C6H8N3O
0 ppm
138.0667
C5H12N
1,2 ppm
86.0971
C5H8N3
-1,8 ppm
110.0716
149.0239
191.1048
195.0884
2: TOF MS ES+
4.61e5
HE
m/z
CAFFEINE
C8H11N4O2
0,5 ppm
195.0883
LIDOCAINE
C14H23N2O
-0,9 ppm
235.1808
179.1283
196.0910
117.0918
1: TOF MS ES+
3.69e6
LE
m/z
(a)

Figure 3

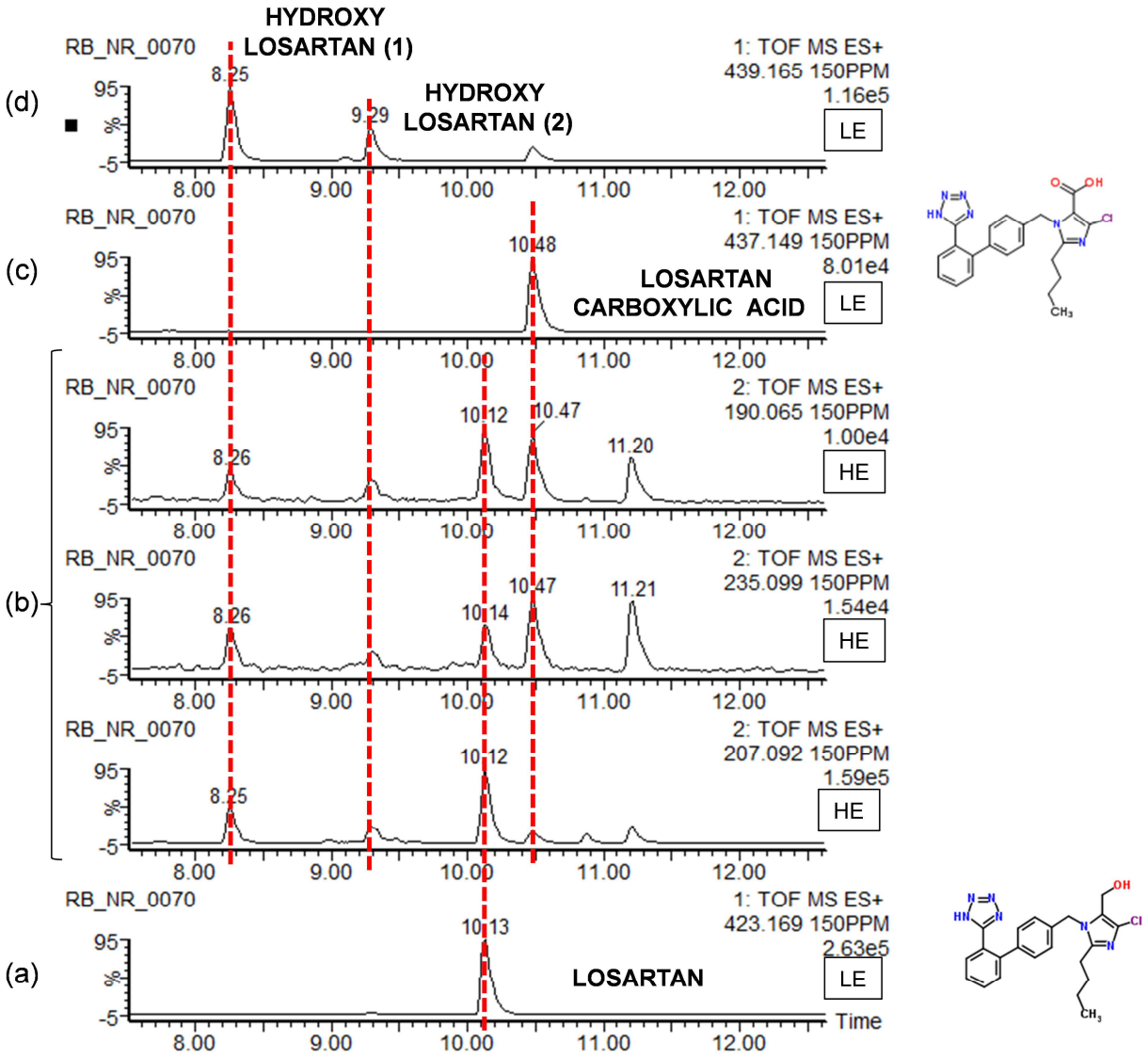
HYDROXY
LOSARTAN (1)
HYDROXY
LOSARTAN (2)
LOSARTAN
CARBOXYLIC ACID
LOSARTAN
(a)
(b)
(c)
(d)
RB_NR_0070
1: TOF MS ES+
439.165 150PPM
1.16e5
LE
8.25
9.29
437.149 150PPM
8.01e4
10.48
2: TOF MS ES+
190.065 150PPM
1.00e4
HE
8.26
10.12
10.47
11.20
235.099 150PPM
1.54e4
10.14
11.21
207.092 150PPM
1.59e5
10.12
423.169 150PPM
2.63e5
10.13
95
%
-5
8.00
9.00
10.00
11.00
12.00
Time

Figure 4

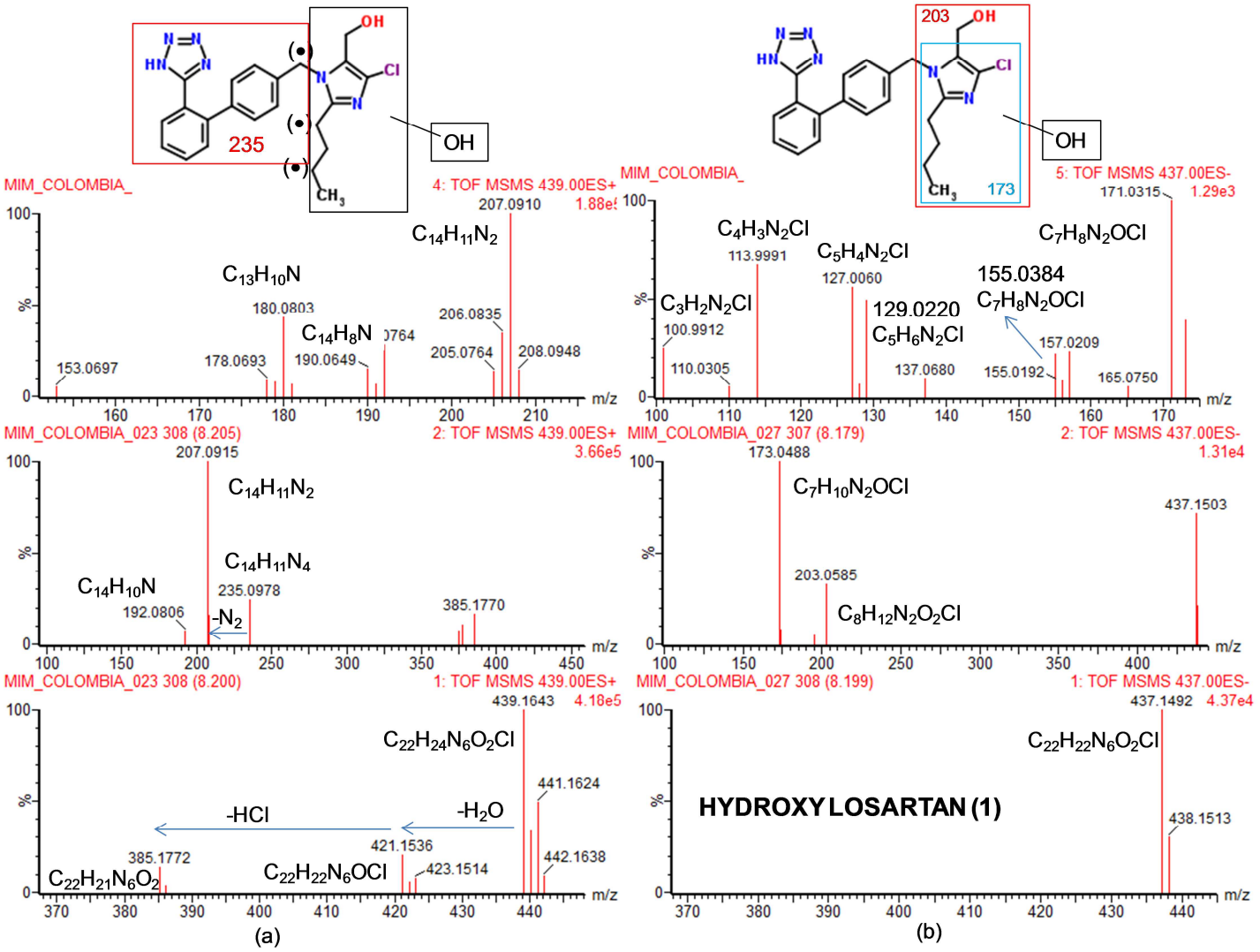
235
OH
Cl
MIM_COLOMBIA_
4: TOF MSMS 439.00ES+
207.0910
C14H11N2
C13H10N
180.0803
C14H8N
206.0835
205.0764
208.0948
153.0697
178.0693
190.0649
MIM_COLOMBIA_023 308 (8.205)
2: TOF MSMS 439.00ES+
207.0915
C14H11N2
C14H11N4
C14H10N
192.0806
235.0978
-N2
385.1770
MIM_COLOMBIA_023 308 (8.200)
1: TOF MSMS 439.00ES+
439.1643
C22H24N6O2Cl
441.1624
-HCl
-H2O
421.1536
385.1772
C22H21N6O2
C22H22N6OCl
423.1514
442.1638
m/z
(a)
203
173
MIM_COLOMBIA_
5: TOF MSMS 437.00ES-
171.0315
C4H3N2Cl
113.9991
C5H4N2Cl
127.0060
C7H8N2OCl
155.0384
C3H2N2Cl
100.9912
129.0220
C5H6N2Cl
157.0209
110.0305
137.0680
155.0192
165.0750
MIM_COLOMBIA_027 307 (8.179)
2: TOF MSMS 437.00ES-
173.0488
C7H10N2OCl
437.1503
203.0585
C8H12N2O2Cl
MIM_COLOMBIA_027 308 (8.199)
1: TOF MSMS 437.00ES-
437.1492
C22H22N6O2Cl
438.1513
HYDROXY LOSARTAN (1)
(b)

Figure 5