



High resolution mass spectrometry-based screening for the comprehensive investigation of organic micropollutants in surface water and wastewater from Pasto city, Colombian Andean highlands

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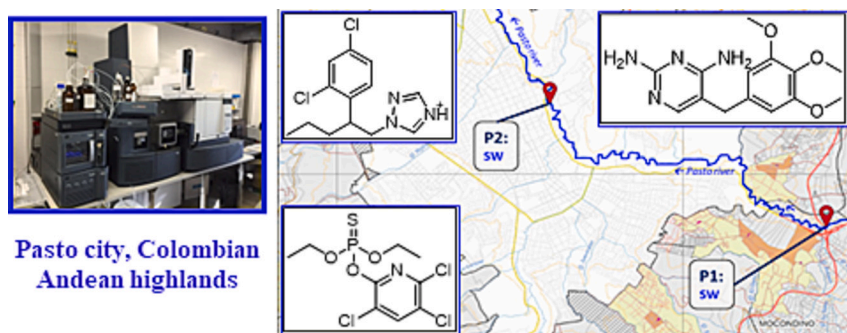
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HIGHLIGHTS

- Combined use of LC- and GC-QTOF MS for screening of surface and wastewater
- Exploratory target and suspect wide-scope screening in Colombian Andean highlands
- Pesticides and pharmaceuticals identified revealed the anthropogenic impact in the study area.
- Preliminary screening allows selecting target analytes for future quantitative analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

The complexity of the aquatic environment scenario, including the impact of urban wastewater, together with the huge number of potential hazardous compounds that may be present in waters, makes the comprehensive characterization of the samples an analytical challenge, particularly in relation to the presence of organic micropollutants (OMPs). Nowadays, the potential of high-resolution mass spectrometry (HRMS) for wide-scope screening in environmental samples is out of question. Considering the physicochemical characteristics of OMPs, the coupling of liquid (LC) and gas chromatography (GC) to HRMS is mandatory. In this work, we have explored the combined use of LC and GC coupled to Quadrupole-Time-of-Flight Mass Spectrometry (QTOF MS) for screening of surface water and wastewater samples from Pasto (Nariño), a town of the Colombian Andean highlands (average altitude 2527 m), located in an important agricultural area. The upper basin of the Pasto

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River is impacted by phytosanitary products used in different crops, whereas the domestic wastewater is directly discharged into the river without any treatment, enhancing the anthropogenic impact on the water quality. The OMP searching was made by target (standards available) and suspect (without standards) approaches, using home-made databases containing >2000 compounds. Up to 15 pesticides (7 insecticides, 6 fungicides and 2 herbicides) were identified in the sampling point of the Pasto River up to the town, while no pharmaceuticals were found at this site, illustrating the impact of agriculture practices. On the contrary, 14 pharmaceuticals (7 antibiotics and 3 analgesics, among others) were found in river samples collected in the middle and down to the town sites, revealing the impact of the urban population. Interestingly, some transformation products, including metabolites, such as carbofuran-3-hydroxy and 4-acetylamino antipyrine were identified in the screening. Based on these data, future monitoring will apply target quantitative LC-MS/MS methods for the most relevant compounds identified.

1. Introduction

Human activities generate wastes and increasing levels of water pollution due to the direct discharge of both organic matter and chemical compounds, mainly composed of detergents, personal care products, medicines and chemicals used in agricultural activities (agrotoxics) (Barceló and López, 2008; Samer, 2015; Rizzo et al., 2019). These discharges, when carried out directly on surface water sources, contribute to considerably reducing water quality and to the growth of pathogenic microorganisms that affect the surrounding ecosystems (Petrović et al., 2003; Bonansea et al., 2013; Cárdenas-Calvachi and Sánchez-Ortiz, 2013; Patel et al., 2019; UNESCO, 2021). The continued presence of organic micropollutants (OMPs) in the aquatic environment represents a danger to human health, the environment and ecosystems, due to their common occurrence in aqueous wastes or sediments (Petrović et al., 2003).

Sanitary discharges and agricultural activities allow OMPs, such as pharmaceuticals or pesticides, to move through sewers to wastewater (Patel et al., 2019); in the best case, these waters enter up in wastewater treatment plants (WWTP) (Diaz and Barkdoll, 2006). However, the processes conventionally applied in WWTPs do not usually ensure the sorption and/or degradation of OMPs, so the effluents discharged into water sources can contain a wide variety of these compounds. It is noteworthy that such water sources are frequently used to irrigate crops, which ultimately poses food safety in this region at risk.

In the rural zones of Pasto city (Colombia) the intensive use of pesticides in agriculture has generated their accumulation in soils, facilitating their mobility through runoff and leaching processes into the surrounding bodies of water (Carrasco et al., 2017). This phenomenon has raised concerns about the deterioration of water quality and its impact on environmental health, without so far having clear data on the presence of OMPs and their impact on the river that crosses the city. The progressive release of this type of compounds could trigger serious effects on the aquatic system, altering biodiversity, biogeochemical cycles, and in some cases, even causing adverse consequences on public health (Barizon et al., 2020). In general, the accumulation of contaminants in the region has increased due to the lack of infrastructure for the adequate wastewater treatment, which also contains an undetermined load of contaminants of emerging concern of pharmaceutical and agronomic origin (CORPONARIÑO, 2011).

Despite recent advances in environmental analytical chemistry, the comprehensive investigation of organic contaminants in waters is still a challenge (Hernández et al., 2023). The main difficulty arises from the elevated number of compounds (in addition to their transformation products) that may be present in the samples and from their different physico-chemical properties (e.g. polarity, volatility), which makes the application of universal analytical methodologies, appropriate for all potential contaminants, unfeasible.

Most analytical methods applied for the determination of OMPs focus on quantitative analysis of a limited number of compounds (commonly <100 compounds), while many relevant contaminants other than the target analytes that might be present in samples remain ignored. The most recent target quantitative methods are usually based

on the use of LC-MS/MS with triple quadrupole and/or GC-MS/(MS) with single or triple quadrupole, depending on the polarity of analytes. However, despite the evident advances in quantitative methods, wide-scope screening methodologies are needed to detect and identify a long list of OMPs, offering, in this way, more realistic and complete information on undesirable compounds present in environmental samples. HRMS offers the possibility for screening a huge number of contaminants in post-targeted approaches (i.e. the selection of compounds to be searched is made once mass data have been acquired) without the need of pre-selecting the analytes for method development. The accurate-mass full-spectrum data provided with reasonable sensitivity allows quite reliable “tentative” identifications, even without the use of reference standards. In addition, searching for compounds not included in the initial list of suspects can be made without the need of additional analysis of the samples (Fabregat-Safont et al., 2021; Hernández et al., 2015; Hogenboom et al., 2009; Ibáñez et al., 2008; Krauss and Hollender, 2008; Krauss et al., 2010; Picó et al., 2007). Time of flight (TOF) and Orbitrap analyzers are the most used in LC-HRMS based methods for screening of different families of medium-highly polar contaminants in the aquatic environment. The list of contaminants investigated using this methodology includes many pesticides, pharmaceuticals/antibiotics, illicit drugs, PFAs and veterinary drugs, among others.

As a complement to LC-MS based methods, less polar contaminants, such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and certain pesticides, perform better with GC-MS methodologies. Although single nominal analyzers (e.g. single quadrupole, ion trap or triple quadrupole) can be used, HRMS is a superior technique for screening purposes, for the same reasons indicated above. GC-HRMS with Orbitrap has irrupted as a powerful technique. The use of electron ionization (EI) facilitates the application of standardized, commercially available, spectra libraries (e.g. NIST) for “tentative” identification of compounds in a non-target approach. The value of accurate mass is of great help in the identification. Commonly, mass error < 5 ppm in relation to the theoretical exact mass is used as criterion for reliable identification (Izquierdo-Sandoval et al., 2022; Tang et al., 2022; Sapozhnikova, 2021; SANTE, 2023).

Although EI source is the preferred ionization technique and the most widely applied in GC-MS due to its robustness, reproducibility, and the availability of standardized commercial spectra libraries, it commonly leads to extensive fragmentation. EI mass spectra are characterized by an abundance of fragment ions, and the absence (or low abundance) of molecular ion in many cases. Oppositely to EI, atmospheric pressure chemical ionization (APCI), implemented in GC-MS instruments in the last years, provides soft and universal ionization. The presence of an abundant molecular ion (and/or the protonated molecule in positive mode) in the mass spectra facilitates the detection and identification of detected compounds by GC-APCI-HRMS (Portolés et al., 2014; Garlito et al., 2019; Aalizadeh et al., 2022). Interestingly, the availability of this source allows the combined use of GC and LC both coupled to a single quadrupole time-of-flight (QTOF MS) instrument, which appears as one of the most potent approaches for wide-scope screening at present. The use of QTOF MS offers interesting

possibilities for identification, such as the acquisition of low (LE) and high collision energy (HE) spectra in one run, or performing additional MS/MS experiments, if necessary (Hernández et al., 2015; Pitarch et al., 2016).

In this work an overview of the occurrence of OMPs in the Pasto River, in the surrounds of the city of Pasto, is reported. For the first time, this type of information is provided in the area of Pasto and its river that suffers discharges of untreated wastewater. GC and LC both coupled to QTOF MS were complementarily used to get as much information as possible on OMPs present in the water. The study was focused mainly on pesticides and pharmaceuticals to assess the impact of agricultural practices and raw urban wastewater on the quality of the river water. Therefore, the home database used included many compounds belonging to these families as well as an important number of transformation products.

2. Experimental

2.1. Reagent and chemicals

Information on reagents and chemicals used in this work is shown in Supplementary Information.

2.2. Study area

The city of Pasto, currently with around 455,000 inhabitants, is located in the Department of Nariño (Colombia). The city stands out for its geography on the Andes mountain, which has exerted a significant influence on its development as a region. It has an average altitude of 2527 m above the sea level (Alcaldía-Municipal-de-Pasto, 2020). Pasto's unique climatic variability causes a wide variety of agricultural activities that change over the course of each year. The convergence of microclimates has provided the region with an environment appropriate for the development of a diverse range of crops, strengthening its position as an agricultural epicenter in Colombia (Corporación-Autónoma-Regional-de-Nariño-CORPONARIÑO, 2011, Quenguan and Eraso,

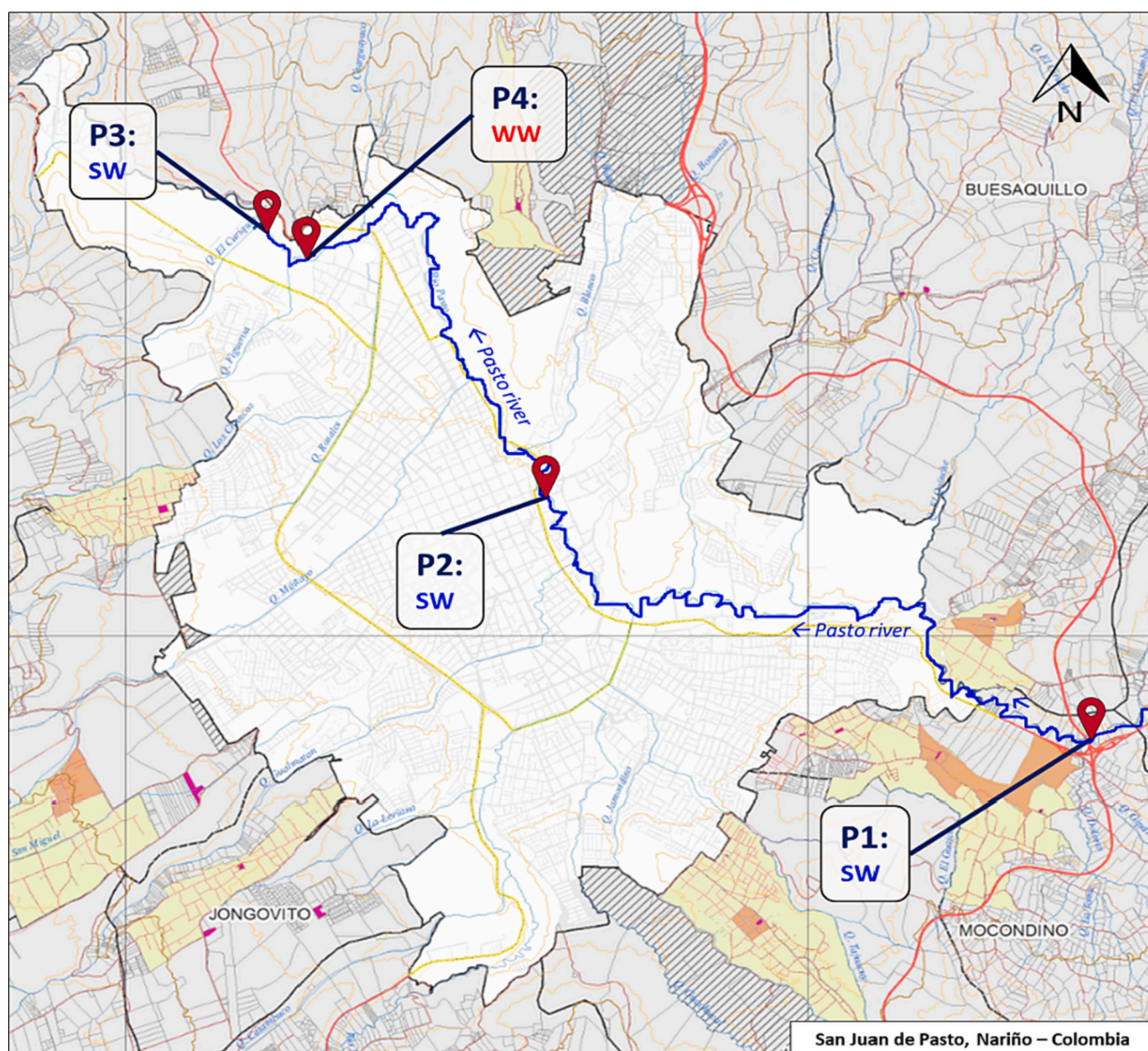


Fig. 1. Pasto's sampling points: P1, P2, and P3 correspond to surface water (SW), and P4 corresponds to wastewater (WW).

2015).

2.3. Sampling

The collection of samples was carried out through August 2022, in 4 points of the city of Pasto (Fig. 1): before entering the urban perimeter that corresponds to an area with a high agricultural vocation (P1: *Intercambiador Vial Dolores*); during its passage through the city (P2: Firefighters Barracks Bridge – geographical half of the city); and after its passage through the urban perimeter (P3: *IDEAM* Hydrometric Station). The sampling at point P4 corresponds to the *Juan XXIII* collector that receives the largest amount of urban wastewater of the city, which is directly discharged (i.e. without prior treatment) onto the river, before reaching sampling point 3. Surface waters were collected as grab samples, while for wastewater it was a composite sample, taking aliquots every hour for 24 consecutive hours, until obtaining a total volume of 10 L of composite sample. Two L of the composite sample were extracted and used for the screening. The samples were transported in plastic containers maintaining temperature at 4.0 °C, and subsequently the process of homogenization and processing of the samples was carried out.

2.4. Sample treatment

In the case of LC, 120 mL of centrifuged water were passed by gravity through Oasis HLB (500 mg, Waters) cartridges, previously conditioned with 10 mL methanol and 10 mL HPLC-grade water. After drying under vacuum, analytes were eluted with 10 mL methanol. The eluate was evaporated under a gentle nitrogen stream at 35 °C down to a volume of 1 mL (final pre-concentration factor x120). In the case of GC, only samples 11S and 13S were analysed. Here, 120 mL of centrifuged water samples were passed by gravity through C18 (500 mg, Supelco) cartridges, previously conditioned with 10 mL DCM, 10 mL MeOH and 10 mL HPLC-grade water. After drying under vacuum, analytes were eluted with 10 mL AcOEt:DCM (1:1). The eluate was evaporated under a gentle nitrogen stream at 35 °C down to a volume of 250 µL (final pre-concentration factor x480). Finally, 20 and 1 µL of the extracts were injected into the UHPLC-(Q)TOF MS and GC-(Q)TOF MS systems, respectively.

2.5. Instrumentation

A hybrid quadrupole-orthogonal acceleration-TOF mass spectrometer (Xevo G2 QTOF, Waters Micromass, Manchester, UK) was interfaced to a Waters Acquity UPLC system (Waters, Milford, MA, USA) or to an Agilent 7890A GC system (Palo Alto, CA, USA) equipped with an Agilent 7683 autosampler, using a single instrument. Xevo G2 QTOF was equipped with an ESI interface working in positive mode, in LC, and with APGC v2.0 working in positive APCI mode, in GC. Instrumental conditions for each technique are detailed below.

2.5.1. UHPLC-QTOF MS

A Cortecs C18 (Waters) analytical column (100 × 2.1 mm, 2.7 µm particle size) was used at a flow rate of 0.3 mL/min. The mobile phases used were A = H₂O and B = MeOH, both with 0.01 % HCOOH, delivered as follows: 10 % of B at 0 min, 90 % of B at 14 min linearly increased, 90 % of B at 16 min, and return to initial conditions at 18 min. Column temperature was set to 40 °C and the injection volume was 20 µL.

Nitrogen (Praxair) was used as desolvation (1000 L/h) and nebulizing gas, while Argon 99.995 % (Praxair) was used as a collision gas. The capillary and cone voltage were set to 0.7 kV and 20 V, respectively. The interface temperature was set to 650 °C and the source temperature to 120 °C. TOF resolution was ~20,000 at FWHM (at *m/z* 556) and a mass range from *m/z* 50 to 1000 was acquired.

Mass-axis was daily calibrated using a mixture of 0.05 M NaOH:5 % HCOOH (50:50), 25-fold diluted with ACN:H₂O (80:20). For accurate

mass measurements, 2 µg/mL leucine enkephalin solution in ACN:H₂O (50:50) with 0.1 % HCOOH was used as lock-mass.

2.5.2. GC-QTOF MS

The GC separation was carried out using a fused silica capillary column with a length of 30 m × 0.25 mm i.d. and a film thickness of 0.25 µm. The oven temperature was set at 90 °C (1 min); 5 °C/min up to 300 °C (2 min). Pulsed splitless (30 psi) injections of 1 µL of sample extracts were carried out with an injector temperature of 250 °C and with a splitless time of 1 min. Helium was used as carrier gas at a constant flow of 1 mL/min.

The interface and ionization source temperatures were set to 340 °C and 150 °C, respectively. N₂ was used as auxiliary gas at 200 L/h, as cone gas at 100 L/h and as make-up gas at 300 mL/min. The APCI corona discharge pin was operated at 1.7 µA and the cone voltage was set to 20 V.

The QTOF was operated at 2.5 spectra/s acquiring the mass range *m/z* 50–1000. The TOF MS resolution was approximately 15,000 (FWHM) at *m/z* 264. Similarly to LC, acquisition was done in MS^E mode, but using a collision energy ramp ranging from 25 to 40 eV in HE.

Heptacose (Sigma-Aldrich, Madrid, Spain) was used for the daily mass calibration. Internal calibration was performed using octa-fluoronaphthalene (Sigma-Aldrich, Madrid, Spain) as lock mass (monitoring the molecular ion, *m/z* 271.9872).

In order to work under proton transfer conditions, an uncapped vial containing water was placed in a designed holder into the APCI source door to enhance protonation.

In both cases, data were acquired under independent acquisition mode (referred to henceforth as MS^E), which allows to obtain information about the protonated molecule and adducts, if present (low energy function, LE), and fragment ions (high energy function, HE) in a single injection. The LE used a collision energy of 4 eV, while the HE applied a collision energy ramp from 15 to 40 eV.

MS data were processed with MassLynx data station operation software version 4.1 (Waters).

2.6. Screening workflow and identification criteria

The analysis of samples was carried out in a post-target way using home-made databases. The identification was possible at different confidence levels, as a function of: 1) standards availability; 2) accurate-mass information and mass errors obtained in the low energy function (LE) for the protonated molecule (or any adduct) in LC or the protonated molecule/molecular ion in GC; 3) fragments ions and mass errors observed, typically in the high energy function (HE). A workflow of the data processing, including the identification criteria, is shown in Fig. 2. These identification confidence levels are aligned with Schymanski's definition (Schymanski et al., 2014).

In the target screening (i.e. standards available, Level 1 according to Schymanski et al., 2014), compound filtering was based on the experimental retention time (RT), mass accuracy and fragmentation. A compound was considered as “detected” when the “precursor” ion was observed at the appropriate retention time (tolerance of ±0.2 min respect to the reference standard), with a mass error lower than 5 ppm (or lower than 1 mDa for *m/z* < 200). For the reliable “identification”, the presence of the precursor ion and, at least, one fragment ion was required, both with mass errors below 5 ppm (1 mDa for *m/z* < 200) at the expected retention time (SANTE, 2023). The list of target compounds (reference standards available) used in the LC-based screening contained pesticides, pharmaceuticals, drugs of abuse as well as metabolites and transformation products (383 compounds). In the case of GC, target database contained pesticides, PCBs, PBDEs, and some emerging contaminants including UV filters, insect repellents, personal care products among others (260 compounds) (Fonseca et al., 2020; Hernández et al., 2015).

In the suspect screening (standards not available), the database

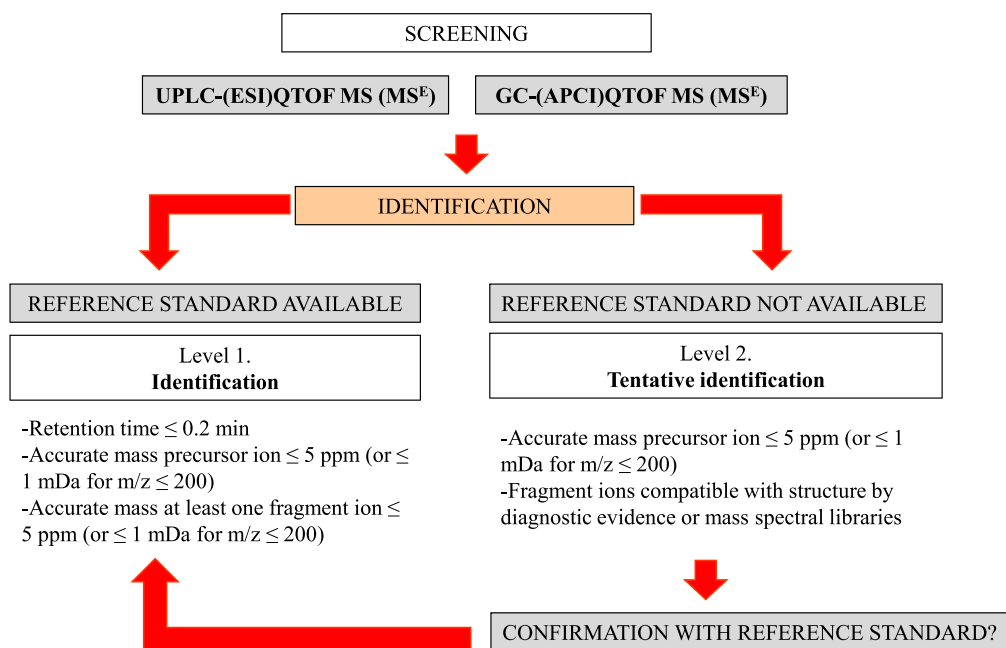


Fig. 2. Overall scheme of the QTOF data processing strategy.

contained information about the elemental composition (and therefore the exact mass) of the compounds. The LC-database contained 631 compounds, including additional pharmaceuticals, illicit drugs, as well as an important number of metabolites and transformation products previously reported in water samples. Information on RT was not included as it could not be tested, but for 85 compounds tentatively identified in previous works, information regarding their fragmentation was accessible. For GC, the suspect database contained around 350 compounds, including mainly pesticides and PAHs derivatives. The first filtering was based on the accurate mass of the ions detected in the LE function, focusing the subsequent tentative identification on those compounds that presented a mass error lower than 5 ppm with respect to the theoretical exact mass. In a second step, the accurate mass fragments were evaluated based on the candidate structure and checked against available MS spectra databases or already published literature (Level 2 according to Schymanski et al., 2014). In the case of TPs, the presence of common fragments shared with the unaltered compounds was also used as an identification parameter (Ibáñez et al., 2017).

3. Results and discussion

A summary of the positive findings in the samples analysed is shown in Table 1. As it can be seen, 90 % of the compounds identified in the river water collected before reaching the city (P1) corresponded to pesticides. Concretely, 7 insecticides (carbaryl, carbofuran, chlorpyrifos, coumaphos, dichlorvos, fipronil, pyriproxyfen) and one transformation product (carbofuran-3-hydroxy), 6 fungicides (azoxystrobin, carbendazim, difenoconazole, diphenylamine, metconazole, penconazole) and 2 herbicides (diuron, terbutylazine) were identified in surface water, illustrating the high impact of agriculture on the river. No pharmaceuticals or illegal drugs were found at this site, and only caffeine and the sweetener saccharin were identified apart from pesticides. The screening detection limit (SDL), defined as the lowest concentration tested in screening validation with at least 95 % of positive detections (SANTE, 2020), was not empirically obtained in this work. Similar screening methodologies have been used in our previous works (Hernández et al., 2015), where most SDLs for compounds included in validation were between 20 and 100 ng/L, which may be used as indicative values in the present study. In any case, considering the strategy used in this work and the value of accurate-mass identifications, positives reported can be

taken as definitive in terms of identification. However, false negatives cannot be ruled out for compounds present at very low concentrations, which could not have been detected in the screening. It cannot be discarded that more sensitive target quantitative methodologies may report some compounds that were not detected in the current screening.

The pesticides found in P1 are insecticides, fungicides, and to a lesser extent herbicides, mainly used to control pests in potato, tomato, onion, and corn crops, in addition to flower crops that are common in the region (CORPONARIÑO, 2011). Considering that P1 corresponds to an area with a high agricultural vocation, the presence of these compounds in surface waters can be associated with fumigation, surface runoff and leaching processes. At points P2 and P3, a reduction in the number of compounds was observed, with a total of 8 pesticides identified at each point, which corresponded to azoxystrobin, carbaryl, carbofuran, chlorpyrifos-ethyl, coumaphos, diuron, fipronil, and dichlorvos (P3), and tentatively penconazole in P2 (Table 1), which essentially display biocidal action as insecticides of medium-high environmental risk. The presence of pesticides in the middle and middle-lower basins of the river (as it passes through the city) may be associated with high mobility of compounds from the upper basin (P1) and to a lesser extent to the direct discharge of wastewater containing pesticides on the river.

In points P2 and P3, the absence of eight compounds previously detected in point P1 was observed. This apparent discrepancy could be attributed to several factors, including degradation processes, molecular adsorption of pesticides by suspended solids or organic matter, but most probably dilution effects; at points P2 and P3, there is an increase in the water volume of the Pasto River due to the arrival of several extra tributary water sources (Fig. 1), alongside domestic wastewater discharges and rainwater (CORPONARIÑO, 2011). These hydrodynamic events could significantly influence the distribution and presence of the analysed compounds. For example, penconazole was tentatively identified in P1 and at the intermediate sampling point of the river (P2), so its presence may be attributed to mobility from point 1; in the case of dichlorvos, found only in points 1 and 3, it may be related with a direct discharge from the sewage networks through the wastewater collector (P4) or their mobility from different nearby agricultural areas that reach the river through streams.

On the other hand, 9 pesticides were identified in wastewater (P4), corresponding to compounds also found P2 and P3, except for carbofuran-3-hydroxy (Table 1), whose concentration probably was not

Table 1

Summary of the results obtained by LC-HRMS and GC-HRMS in the analysis of water samples from Pasto.

| Compound | Family compound | Surface water | | | Wastewater |
|-----------------|------------------------------------|---------------|----|-----|------------|
| | | P1 | P2 | P3 | P4 |
| Pesticides | Azoxystrobin | • ‡ | • | • ‡ | • |
| | Carbaryl | • ‡ | • | • | • |
| | Carbendazim | • | | | |
| | Carbofuran | • ‡ | • | • ‡ | • |
| | Carbofuran-3-hydroxy | • | | | • |
| | Chlorpyrifos ethyl | • ‡ | • | • ‡ | • |
| | Coumaphos | ‡ | • | • ‡ | • |
| | Dichlorvos | • ‡ | | • ‡ | • |
| | Difenoconazole | ‡ | | | |
| | Diphenylamine | ‡ | | | |
| | Diuron | • | • | • | • |
| | Fipronil | • ‡ | • | • ‡ | • |
| | Metconazole | o | | | |
| | Pyriproxyfen | ‡ | | | |
| | Penconazole | o | o | | |
| | Terbuthylazine | • ‡ | | | |
| Pharmaceuticals | 4-aminoantipyrine-N-acetyl (4-AAA) | | • | • | • |
| | 4-aminoantipyrine-N-formyl (4-FAA) | | • | | |
| | Acetaminophen | | • | • | • |
| | Azythromycin | | • | • | |
| | Carbamazepine | | • | • | • |
| | Cephalexin | | • | • | • |
| | Cetirizine | | o | o | o |
| | Clarythromycin | | • | • | |
| | Climbazole | | o | o | o |
| | Diclofenac | | • | • | • |
| | Lincomycin | | • | • | • |
| | Losartan | | • | • | • |
| | Metronidazole | | • | • | |
| | Naproxen | | • | • | • |
| | Sulfamethoxazole | | • | • | • |
| | Trimethoprim | | • | • | • |
| Drugs | Benzoylcegonine | • | • | • | • |
| | Caffeine | | • | • | • |
| | Cocaine | | • | • | • |
| | Ketamine | | • | • | • |
| | Paraxanthine | | • | • | • |
| Others | Sucralose | | • | • | • |
| | Saccharin | • | • | • | • |
| | Iopromide | | | • | • |
| | Ioversol | | | • | • |
| | Iopamidol | | | • | |

o Tentative identification by LC-QTOF MS (reference standard not available).

‡ Identity confirmed by GC-QTOF MS (reference standards).

• Identity confirmed by LC-QTOF MS (reference standards).

Only samples P1 and P3 could be screened out by GC-HRMS.

that significant to be later observed in the most diluted sample collected in P3. The presence of agrotoxic agents in wastewater could be associated with contaminated food washing processes, poor disposal of agricultural wastes, as well as cleaning and fumigation processes in some urban areas. Private use of pesticides cannot be discarded as a source of pollution as well, although there is not reliable information about the contribution of this practice in the area.

As illustrative example of the analytical procedure applied, Fig. 3 shows the identification of the insecticide chlorpyrifos by GC-HRMS. The LE spectrum of the chromatographic peak at the expected retention time (25.98 min, deviation 0.1 min respect to the reference standard) showed the typical isotopic pattern corresponding to the presence of three chlorine atoms in the molecule. Up to 4 fragments were detected in the HE function with high mass accuracy. The identity of this compound (as most of the pesticides found) was confirmed with its reference standard. Among the group of pesticides, only the fungicides metconazole and penconazole were tentatively identified as their reference standards were not available at our lab. Fig. 4 illustrates the detection and tentative identification of penconazole in the surface water sample P1 by UHPLC-QTOF MS. The protonated molecule of penconazole ($C_{13}H_{16}N_3Cl_2^+$) was observed at 11.96 min in the LE function, with a

mass error of -2.1 ppm (Fig. 4a, bottom). The characteristic isotopic profile corresponding to two chlorine atoms was also observed. As the reference standard was not available, chemical structures for the most abundant fragment ions were suggested based on their accurate masses. In the HE function (Fig. 4a, top), 3 fragments were observed at the same retention time and chromatographic peak shape than the protonated molecule, with mass errors lower than 2 mDa in relation to the theoretical predicted exact masses: m/z 172.9907 ($C_8H_7Cl_2^+$), 158.9765 ($C_7H_5Cl_2^+$), both corresponding to dichlorotropylium ions, and 70.0401 ($C_2H_4N_3^+$), corresponding to the triazolium ion. All structures proposed for the fragments were consistent with the chemical structure of penconazole. In addition, the tentative identification of this fungicide was supported by the MS/MS product ions reported in the public mass spectral database Massbank, as the three fragments observed in the HE spectrum had been previously reported for this compound using LC-ESI-QTOF with a collision energy of 45 eV and a resolving power of 35,000 (Massbank).

In relation to pharmaceuticals, a notable number of compounds were detected in wastewater P4, but also in surface waters P2 and P3 where there is influence of urban wastewater discharges (Table 1). The sample P3 was collected around 450 m downstream from the discharge point of

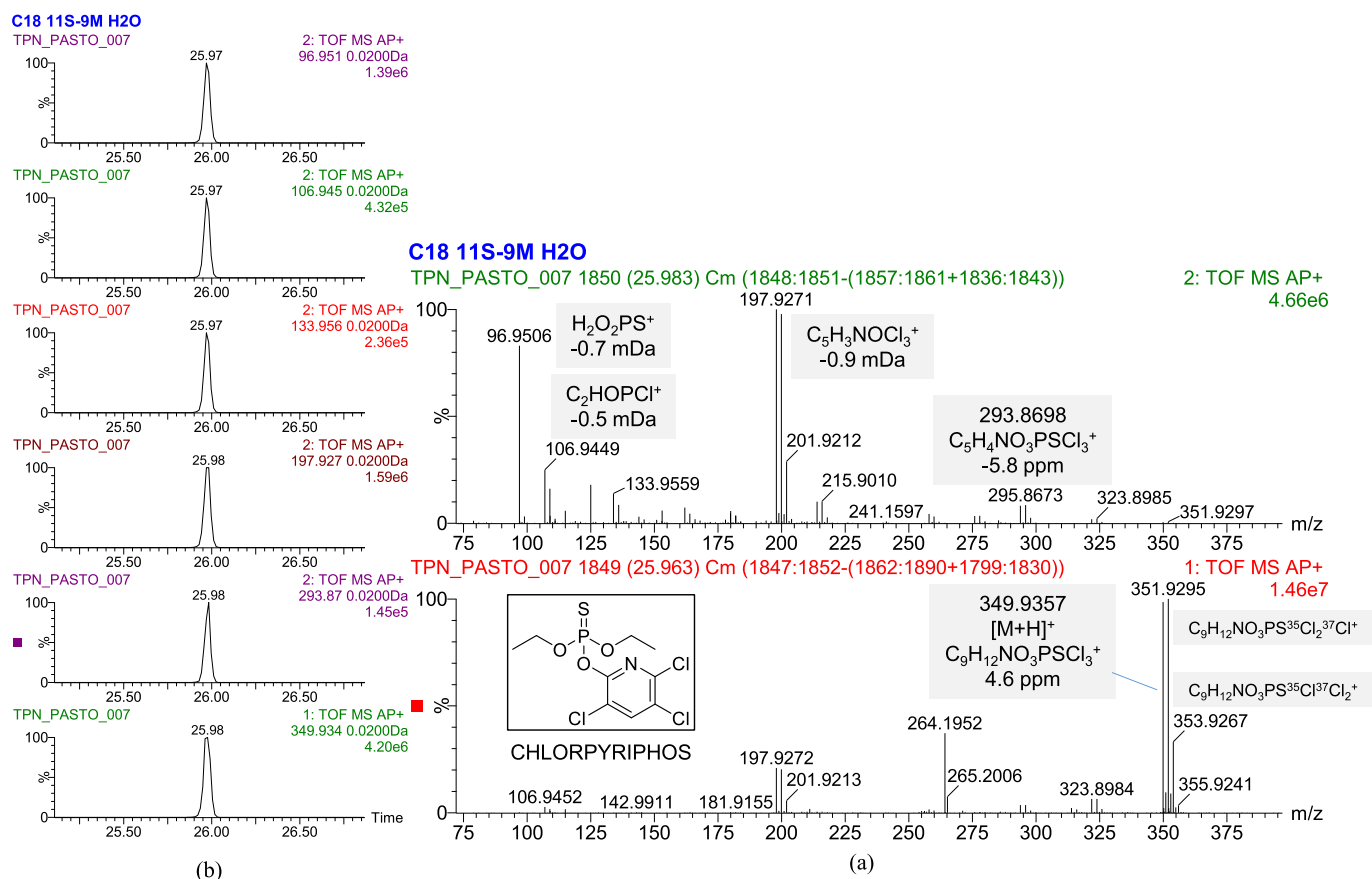


Fig. 3. Target identification of pesticide chlorpyrifos ethyl in surface water P1 by GC-QTOF MS. (a): LE (bottom) and HE (top) mass spectra of chromatographic peak at 25.98 min; (b): nw-XICs (0.02 Da mass window) for protonated molecule in LE (function 1) and different observed ions in HE (function 2).

untreated wastewater (P4). The most detected compounds were by far antibiotics, specifically azithromycin, cefalexin, clarithromycin, lincomycin, metronidazole, sulfamethoxazole and trimethoprim.

As illustrative example, Fig. 5 shows the detection and identification of the antibiotic trimethoprim, confirmed with the reference standard. This compound was found at the expected retention time (3.09 min). In this case, both the protonated molecule (in LE) and four fragment ions (in HE) were observed, all with mass errors lower than 5 ppm (1 mDa for m/z lower than 200).

Several anti-inflammatory/analgesics were also found (namely, acetaminophen, diclofenac, naproxen) as well as the main metabolites of the analgesic metamizole, also known under the commercial trademark Nolotil®: 4-aminoantipyrine-N-formyl (4-FAA) and 4-aminoantipyrine-N-acetyl (4-AAA). The presence of the anti-hypertensive losartan and the anti-epileptic and mood-stabilizing drug carbamazepine was confirmed in three samples. Finally, the anti-fungal climbazole and the anti-histamine cetirizine could be tentatively identified.

In addition to the wide group of pesticides and pharmaceuticals, other compounds were also identified in the samples, including drugs of abuse (cocaine and its main metabolite benzoylecgonine, and ketamine), caffeine and its main human metabolite paraxanthine, and the sweeteners sucralose and saccharin. It is interesting to remark the presence of up to three X-ray contrast agents, concretely iopamidol, iopromide and ioversol in the urban wastewater and in the river sample P3.

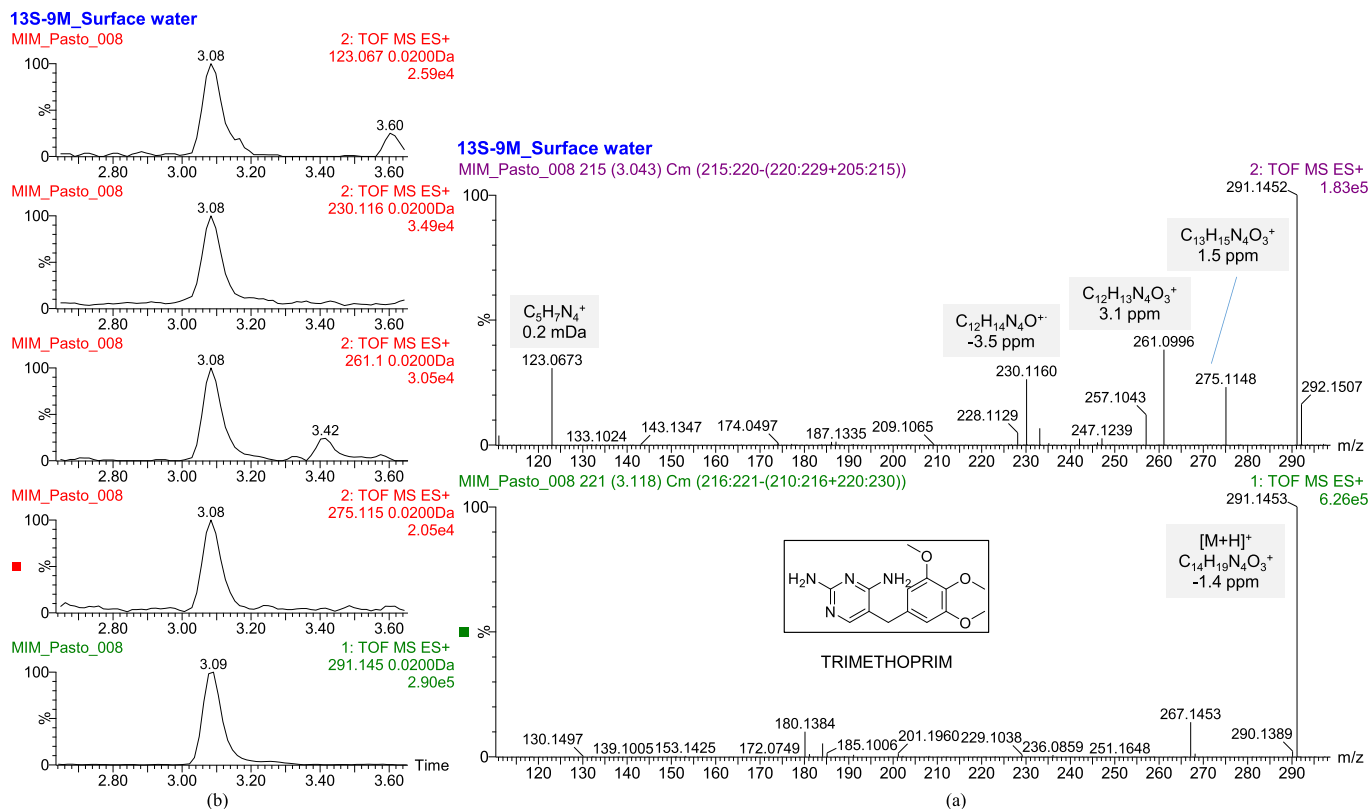
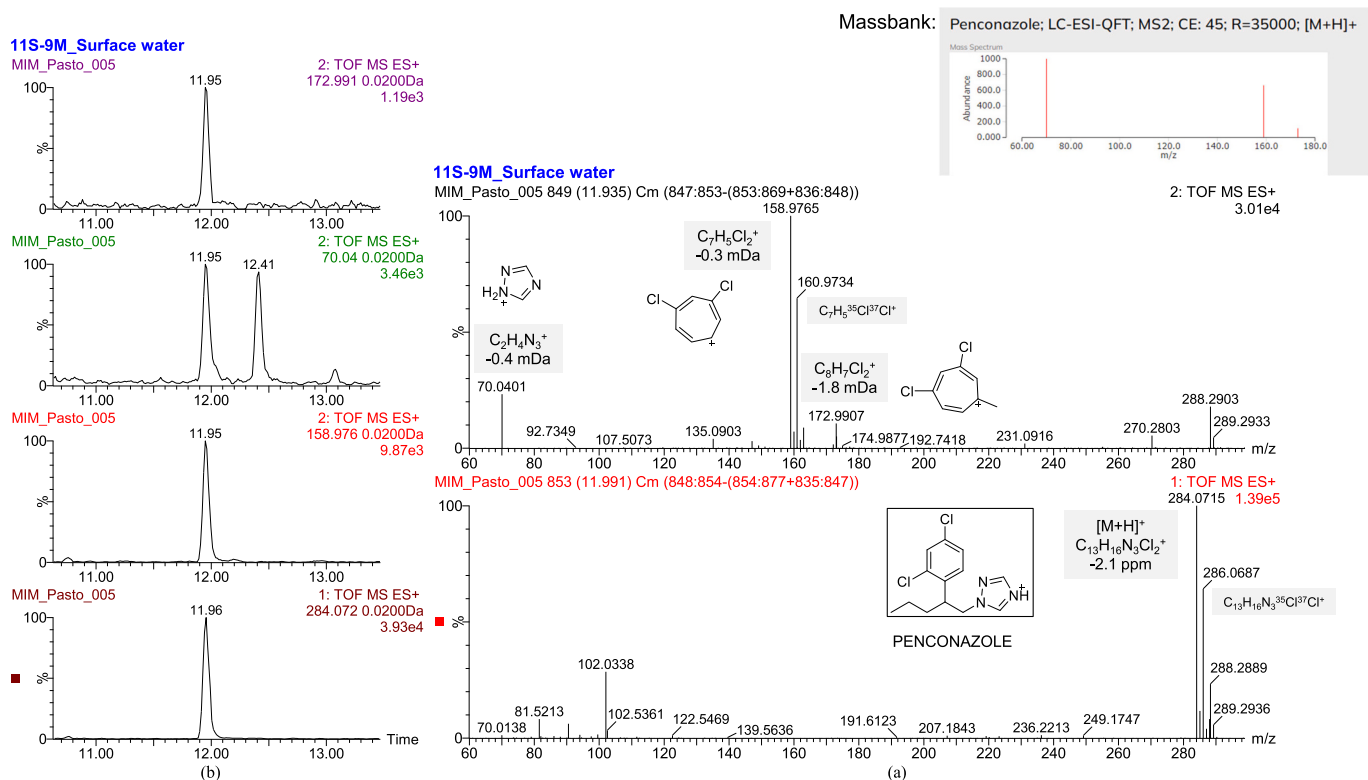
All these findings reveal the impact of the population on the quality of the river water, undoubtedly favoured by the absence of treatments of urban wastewater, which is directly discharged in the river. The impact of agricultural practices in the area is also clearly observed in this work.

In the evaluation of the environmental impact of the identified

compounds, the pesticide risk categorization tool was taken as a reference (Paredes Hincapié et al., 2023). According to this, the highest risk compounds correspond mainly to the insecticides dichlorvos (position 1 in classification), chlorpyrifos, carbofuran, carbaryl and diuron (European-Commission, 2008; European-Parliament-and-of-the-Council, 2020; European-Parliament, 2018; Paredes Hincapié et al., 2023), which presented low limits of acceptability in water for human consumption and high rates of ecotoxicity. These compounds are considered as highly toxic for aquatic organisms, and after continued periods of exposure they can generate adverse effects throughout the whole environment. This is of concern, since the water collected in P1 is also used as a supply source for the city's drinking water treatment plant, and covers the consumption of approximately 85 % of the population (about 385,000 people).

With the preliminary data reported in this work and considering the toxicity/hazard of the compounds identified in the screening, it would be necessary to perform a wide monitoring along the river Pasto in the near future in order to obtain quantitative data (e.g. by application of LC-MS/MS with triple quadrupole methodologies). This would allow an appropriate risk assessment considering not only the toxicity but the concentration levels in the samples as well. Future monitoring could be focused on the compounds identified in this work, or at least on the most relevant based on their toxicity/risk for the aquatic environment.

Despite the "universality" of the approach used in this work, some limitations must be considered. By one side, some potential contaminants can be lost during SPE treatment and the subsequent evaporation of the eluates (e.g. highly polar/ionic compounds, or highly volatile compounds) or some others may be poorly or non-ionized under the conditions applied in this work. By other side, the HRMS screening is commonly less sensitive than quantitative methods (e.g. LC-MS/MS or



GC–MS/MS) specifically developed for a limited group of target analytes. Thus, some compounds might be found at very low concentrations by LC(GC)–MS/MS quantitative methods, but might remain undetected by using screening methodologies. In addition, although the home-made list used in this work contained >1600 compounds, still many other contaminants remain ignored in the analysis. By the reasons indicated above, the compounds identified in the screening can be confirmed to be present in the samples, but the presence of many other contaminants may not be ruled out (false negatives). So, additional efforts are required to widen the screening in future works in order to extend the list of targets for subsequent quantitative monitoring.

4. Conclusions

Nowadays, one of the most attractive approaches to investigate the presence of organic micropollutants in the aquatic environment is the application of wide-scope HRMS-based screening, capable of detecting and identifying a large number of compounds. This allows one to have extensive and realistic information about sample quality without the limitation of most target quantitative methods, normally applied to a limited list of analytes. Thanks to the advances in analytical instrumentation, it is possible to move towards the desired “universal” screening. In this work, the complementary use of GC-QTOF MS and UHPLC-QTOF MS has allowed to investigate a huge number of compounds, with very distinct polarity and volatility, including pesticides, pharmaceuticals, drugs of abuse, PCBs, PBDEs, PAHs, among others, in both surface water and effluent wastewater from the city of Pasto, Colombia.

Up to 16 pesticides were identified in river water collected upstream of the city of Pasto, illustrating the impact of agriculture practices. Data obtained in samples from the river as it passes through the city, suggested that the presence of pesticide contamination may also come from urban origin. In addition, pharmaceutical compounds and some common drugs were also found in both urban wastewater and surface water from the middle and down points of the river, revealing the impact of the urban population on the environmental quality of the river, which water is taken as the main source for the city’s drinking water treatment.

Based on data from this work, monitoring is suggested to be performed by application of target quantitative LC–MS/MS methods for the most relevant compounds identified in the screening.

CRedit authorship contribution statement

Félix Hernández: Writing – review & editing, Supervision, Conceptualization. **María Ibáñez:** Writing – original draft, Supervision, Investigation, Formal analysis. **Tania Portoles:** Writing – review & editing, Investigation, Formal analysis. **Arsenio Hidalgo-Troya:** Writing – review & editing, Supervision, Conceptualization. **Juan David Ramírez:** Writing – review & editing, Methodology. **Manuela Alejandra Paredes:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Andrés Fernando Hidalgo:** Writing – review & editing, Investigation, Conceptualization. **Ana María García:** Writing – review & editing, Methodology, Conceptualization. **Luis Alejandro Galeano:** Writing – review & editing, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.171293>.

References

- Aalizadeh, R., Nikolopoulou, V., Alygizakis, N.A., Thomaidis, N.S., 2022. First novel workflow for semiquantification of emerging contaminants in environmental samples analyzed by gas chromatography–atmospheric pressure chemical ionization–quadrupole time of flight–mass spectrometry. *Anal. Chem.* 94 (27), 9766–9774. <https://doi.org/10.1021/acs.analchem.2c01432>.
- Alcaldía-Municipal-de-Pasto, 2020. Estadísticas - Municipio de Pasto, 24/09/2022. Retrieved 12/10/2022, from <https://www.pasto.gov.co/index.php/nuestro-municipio/estadísticas> (last accessed on October 2023).
- Barceló, D., López, M.J., 2008. Contaminación y calidad química del agua: el problema de los contaminantes emergentes. In: *Jornadas de presentación de resultados: el estado ecológico de las masas de agua. Panel científico-técnico de seguimiento de la política de aguas*, Sevilla, pp. 1–27.
- Barizon, R.R., Figueiredo, R.d.O., de Souza Dutra, D.R.C., Regitano, J.B., Ferracini, V.L., 2020. Pesticides in the surface waters of the Camanducaia River watershed, Brazil. *J. Environ. Sci. Health B* 55 (3), 283–292.
- Bonasea, R.I., Amé, M.V., Wunderlin, D.A., 2013. Determination of priority pesticides in water samples combining SPE and SPME coupled to GC–MS. A case study: Suquia River basin (Argentina). *Chemosphere* 90 (6), 1860–1869.
- Cárdenas-Calvachi, G.L., Sánchez-Ortiz, I.A., 2013. Nitrógeno en aguas residuales: orígenes, efectos y mecanismos de remoción para preservar el ambiente y la salud pública. *Universidad y Salud* 15 (1), 72–88.
- Carrasco, J.d.C.R., Delgado, C.Y.S., Cobos, D.F.O., 2017. Contaminantes emergentes y su impacto en la salud. Emerging contaminants and its impact on the health. *Revista de la Facultad de Ciencias Médicas de la Universidad de Cuenca* 35 (2), 55–59.
- CORPONARIÑO, 2011. Plan de Ordenación del Río Pasto. Pasto, Nariño: 382.
- Corporación-Autónoma-Regional-de-Nariño-CORPONARIÑO (2011). Plan de Ordenamiento del Recurso Hídrico - Río Pasto. C. Nariño. Pasto, Corporariño: 246.
- Diaz, J., Barkdoll, B., 2006. Comparison of wastewater treatment in developed and developing countries. In: *World Environmental and Water Resource Congress 2006: Examining the Confluence of Environmental and Water Concerns*.
- European-Commission, 2008. Directive 2008/105/EC of the European Parliament and the Council on the Environmental Quality Standards in the Field of Water Policy Amending and Subsequently Repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC.
- European-Parliament, 2018. Directive 2008/105/EC of the European-Parliament-and-of-the-Council-and-repealing-Commission-Implementing Decision O. J. o. t. E. Union. Official Journal of the European Union.
- European-Parliament-and-of-the-Council, 2020. Commission Regulation (EU) 2020/1085 of 23 July 2020. Official Journal of the European Union, European-Parliament.
- Fabregat-Safont, D., Ibáñez, M., Bijlsma, L., Hernández, F., Waichman, A.V., Oliveira, R., Rico, A., 2021. Wide-scope screening of pharmaceuticals, illicit drugs and their metabolites in the Amazon River. *Water Res.* 200, 117251 <https://doi.org/10.1016/j.watres.2021.117251>.
- Fonseca, E., Hernández, F., Ibáñez, M., Rico, A., Pitarch, E., Bijlsma, L., 2020. Occurrence and ecological risks of pharmaceuticals in a Mediterranean river in Eastern Spain. *Environ. Int.* 144, 10600. <https://doi.org/10.1016/j.envint.2020.106004>.
- Garlito, B., Portolés, T., Niessen, W.M.A., Navarro, J.C., Hontoria, F., Monroig, O., Varó, I., Serrano, R., 2019. Identification of very long-chain (>C24) fatty acid methyl esters using gas chromatography coupled to quadrupole/time-of-flight mass spectrometry with atmospheric pressure chemical ionization source. *Anal. Chim. Acta* 1051, 103–109. <https://doi.org/10.1016/j.aca.2018.11.001>.
- Hernández, F., Ibáñez, M., Portolés, T., Cervera, M.I., Sancho, J.V., López, J.L., 2015. Advancing towards universal screening for organic pollutants in waters. *J. Hazard Mat.* 282, 56–95. <https://doi.org/10.1016/j.jhazmat.2014.08.006>.
- Hernández, F., Fabregat-Safont, D., Campos-Mañas, M., Quintana, J.B., 2023. Efficient validation strategies in environmental analytical chemistry. Focus on organic micropollutants in water samples. *Ann. Rev. Anal. Chem.* 16, 401–428. <https://doi.org/10.1146/annurev-anchem-091222-112115>.
- Hogenboom, A.C., van Leerdam, J.A., de Voogt, P., 2009. Accurate mass screening and identification of emerging contaminants in environmental samples by liquid chromatography-LTQ FT Orbitrap mass spectrometry. *J. Chromatogr. A* 1216, 510–519. <https://doi.org/10.1016/j.chroma.2008.08.053>.
- Ibáñez, M., Sancho, J.V., McMillan, D., Ramesh, R., Hernández, F., 2008. Rapid non-targetscreening of organic pollutants in water by ultraperformance liquid

- chromatography coupled to time-of light mass spectrometry. *TrAC-Trends Anal. Chem.* 27, 481–489. <https://doi.org/10.1016/j.trac.2008.03.007>.
- Ibáñez, M., Borova, V., Boix, C., Aalizadeh, R., Bade, R., Thomaidis, N.S., Hernández, F., 2017. UHPLC-QTOF MS screening of pharmaceuticals and their metabolites in treated wastewater samples from Athens. *J. Hazard Mat.* 323, 26–35. <https://doi.org/10.1016/j.jhazmat.2016.03.078>.
- Izquierdo-Sandoval, D., Fabregat-Safont, D., Lacalle-Bergeron, L., Sancho, J.V., Hernández, F., Portoles, T., 2022. Benefits of ion mobility separation in GC-APCI-HRMS screening: from the construction of a CCS library to the application to real-world samples. *Anal. Chem.* 94, 9040–9047. <https://doi.org/10.1021/acs.analchem.2c01118>.
- Krauss, M., Hollender, J., 2008. Analysis of nitrosamines in wastewater: exploring the trace level quantification capabilities of a hybrid linear ion trap/Orbitrap mass spectrometer. *Anal. Chem.* 80, 834–842. <https://doi.org/10.1021/ac701804y>.
- Krauss, M., Singer, H., Hollender, J., 2010. LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns. *Anal. Bioanal. Chem.* 397, 943–951. <https://doi.org/10.1007/s00216-010-3608-9>.
- Massbank. <https://massbank.eu/MassBank/>; MS/MS Spectrum Available in MassBank for Penconazole at 45 eV Collision Energy and 35000 FWHM (Massbank Record: MSBNK-Eawag-EQ310703) (accessed 19/10/2023).
- Paredes Hincapié, M., García-Mora, A.M., Hidalgo-Troya, A., Ramírez-González, J.D., Galeano, L.A., 2023. Easy tool for multiparametric categorization of pesticide risk in surface water and wastewater. *Mendeley Data*. <https://doi.org/10.17632/cvvrh456t.1>.
- Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, Jr, C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: chemistry, occurrence, effects, and removal methods. *Chem. Rev.* 119 (6), 3510–3673.
- Petrović, M., Gonzalez, S., Barceló, D., 2003. Analysis and removal of emerging contaminants in wastewater and drinking water. *TrAC Trends Anal. Chem.* 22 (10), 685–696.
- Picó, Y., Farré, M., Soler, C., Barceló, D., 2007. Identification of unknown pesticides in fruits using ultra-performance liquid chromatography–quadrupole time-of-flight mass spectrometry: imazalil as a case study of quantification. *J. Chromatogr. A* 1176, 123–134. <https://doi.org/10.1016/j.chroma.2007.10.071>.
- Pitarch, E., Cervera, M.I., Portolés, T., Ibáñez, M., Barreda, M., Renau-Pruñonosa, A., Morell, I., López, F.J., Albarran, F., Hernández, F., 2016. Comprehensive monitoring of organic micro-pollutants in surface and groundwater in the surrounding of a solid-waste treatment plant of Castellón, Spain. *Sci. Total Environ.* 548–549. <https://doi.org/10.1016/j.scitotenv.2015.12.166>.
- Portolés, T., Mol, J.G.J., Sancho, J.V., Hernández, F., 2014. Use of electron ionization and atmospheric pressure chemical ionization in gas chromatography coupled to time-of-flight mass spectrometry for screening and identification of organic pollutants in waters. *J. Chromatogr. A* 1339, 145–153. <https://doi.org/10.1016/j.chroma.2014.03.001>.
- Quenguan, F., Eraso, E., 2015. Determinación de plaguicidas organofosforados y carbamatos en agua para consumo humano del departamento de Nariño mediante cromatografía líquida de alta eficiencia (HPLC).
- Rizzo, L., Malato, S., Antakyali, D., Beretsou, V.G., Dolic, M.B., Gernjak, W., Heath, E., Ivancev-Tumbas, I., Karaolia, P., Lado, Ribeiro, A.R., Mascolo, G., McArdell, C.S., Schaar, H., Silva, A.M.T., Fatta-Kassinos, D., 2019. Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. *Sci. Total Environ.* 655, 986–1008.
- Samer, M., 2015. Biological and chemical wastewater treatment processes. *Wastewater Treatment Eng.* 1–50.
- SANTE/12682/2019, 2020. Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.
- SANTE/2020/12830, Rev. 2, 2023. Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes.
- Sapozhnikova, Y., 2021. Non-targeted screening of chemicals migrating from paper-based food packaging by GC-Orbitrap mass spectrometry. *Talanta* 226, 122120.
- Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P., Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* 48 (4), 2097–2098. <https://doi.org/10.1021/es5002105>, 18.
- Tang, C., Huang, Q., Zhu, Y., Liang, Y., Zeng, Y., Luo, X., Mai, B., Peng, X., 2022. Nontarget analysis and characterization of alkylamides in electrical product plastics by gas chromatography-positive chemical ionization quadrupole-orbitrap high-resolution mass spectrometry and quasi-molecular ion screening and anchoring algorithm. *J. Chromatogr. A* 1682, 463466. <https://doi.org/10.1016/j.chroma.2022.463466>.
- UNESCO, 2021. Informe Mundial de las Naciones Unidas sobre el Desarrollo de los Recursos Hídricos 2021: El valor del agua (París).