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Análisis y Control de Calidad Químicos.

Determination of contaminants in urine: bisphenols, phthalates, and other chemical substances and their relationship with human health

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ABSTRACT

The so-called emerging contaminants, which have gained prominence in recent years, are linked to personal care products, pharmaceuticals, and plasticizers, among others. These substances represent potential risks to human health due to their widespread presence in the environment and their ability to enter the human body through various exposure routes. Bisphenols (BPs) and phthalates have received significant attention due to their toxicity and ubiquity in consumer products. Even at low concentrations, they can have adverse effects on health, making essential their comprehensive analysis and regulation.

Human biomonitoring studies play a fundamental role in assessing contamination by contaminants of emerging concern (CECs) in populations, particularly through the analysis of urine samples. Urine is preferred for its large sample volume, non-invasiveness, and ease of collection. In this work, we examined the presence and concentration of 36 chemical substances in urine samples from 40 pregnant women from Barcelona, obtained following ethical guidelines and respecting data protection.

LC-MS/MS analysis was performed using a UHPLC Sciex Exion system connected to a Sciex 6500+ triple-quadrupole mass spectrometer from Sciex (Washington, DC, USA). The mass spectrometer was equipped with an electrospray ionization (ESI) source and operated in both positive and negative modes within the same run. An exhaustive method validation process was conducted, including extraction recoveries, precision, limits of quantification (LOQs), limits of detection (LODs), and matrix effects, for 3 compounds. Seventy-six per cent of the target analytes successfully passed the validation standards demanded. Semi-quantification was used for some compounds due to matrix complexity, setting the minimum amount of these compounds expected to be in the samples. Strategies to mitigate ionization suppression were discussed, highlighting the need for optimization in sample preparation and analytical protocols to ensure result accuracy. This study provides insights into emerging contamination in humans, shedding light on potential health risks associated with these pollutants.

1. INTRODUCTION

Contaminants of emerging concern (CECs) are chemicals that raise concerns within the scientific community due to their toxicity and widespread presence. CECs encompass a diverse range of pollutants, including endocrine disrupting chemicals (EDCs), personal care products (PCPs), pharmaceutically active chemicals (PhACs), or plasticizers, among others. These chemicals can enter the human body through various exposure pathways such as inhalation, ingestion, or dermal contact according to the Environmental Protection Agency (EPA). Among these pollutants, bisphenols (BPs) and phthalates have attracted significant attention due to their widespread use in the production of various consumer products, such as plastics, food packaging, and personal care products [1]. Even at low concentrations, they can have adverse effects with long-term exposure, potentially leading to severe health issues. Hence, it is crucial to comprehensively understand their presence and levels to identify which chemicals may pose threats to human health and establish regulatory measures for their control.

Human biomonitoring (HBM) studies focus on this and include the development and application of analytical methods to accurately determine the presence of these contaminants and their concentration. Among the various biofluids, the application of these analytical methodologies to human urine is justified by the access to high sample volume, ease of collection, and non-invasive nature, in comparison to other biofluids. In this context, intense sampling campaigns are allowed to assess the contamination in large populations.

Bisphenols (BPs) are one of the most common phenols in the environment, characterized by the presence of two phenols connected by an alkyl group.

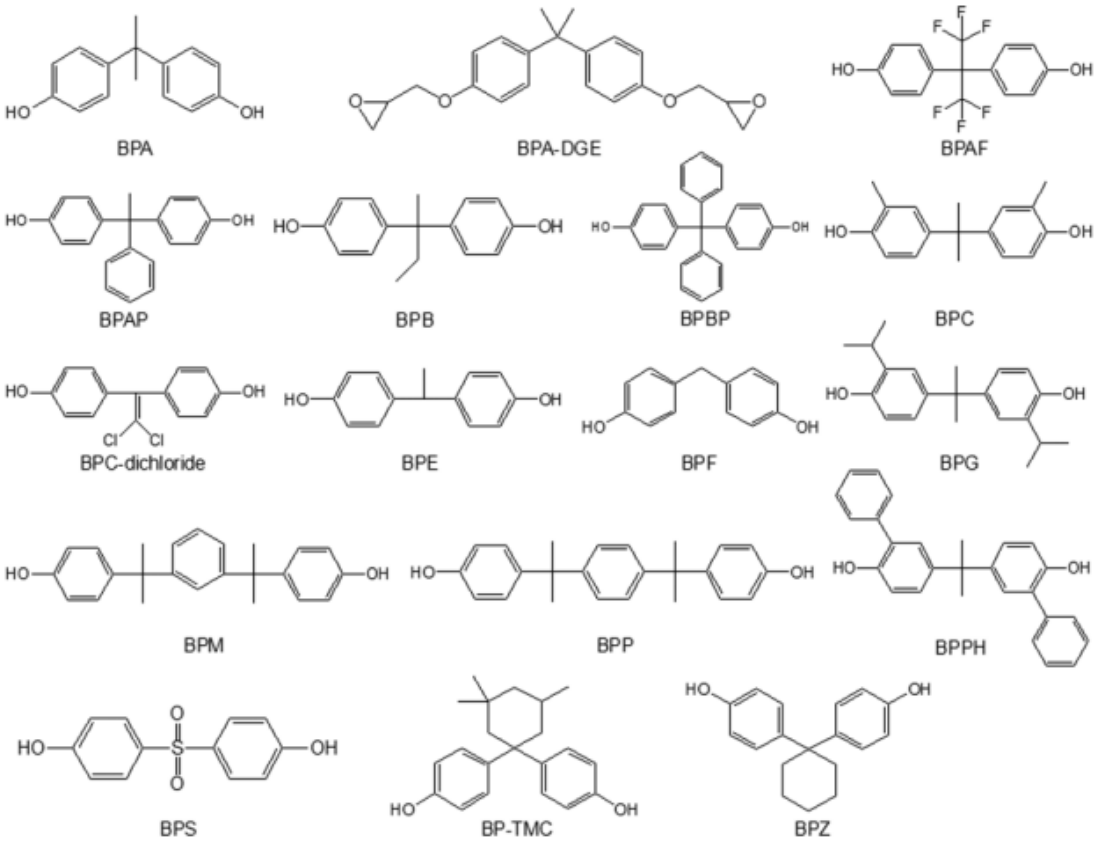


Figure 1. Chemical structures of bisphenol analogues [2].

BPs are essential components of polycarbonate plastics, widely used in consumer goods and packaging that store food and beverages. They are also employed in the production of epoxy resin coatings in metal-based cans for food and beverages, as well as in other consumer products such as thermal paper, medical equipment, toys, electronics, and water pipes [3]. Bisphenol A is commonly measured in urine to monitor human exposure to this compound. Recent studies have started to evaluate urinary concentrations of other bisphenol analogues, but available data is still limited (see Table 1).

Region	Year	n	Units	BPA	BPAF	BPAP	BPB	BPE	BPF	BPP	BPS	BPZ	Reference
China	2013	94	ng/mL	0.886	0.018	-	-	-	0.228	-	0.029	-	[4]
Saudi Arabia	2014	130	ng/mL	4.92	0.05	0.3	0.05	-	0.19	0.093	13.3	0.06	[5]
India	2012-2013	76	ng/mL	5.08	-	-	-	-	-	-	0.04	-	[6]
USA	2016	380	ng/mL	1.32	-	-	-	-	-	-	-	-	[7]
USA	2000	79	ng/mL	1340	-	-	-	-	340	-	-	-	[8]
USA	2001	67	ng/mL	1290	-	-	-	-	300	-	-	-	[8]
USA	2007	27	ng/mL	740	-	-	-	-	160	-	-	-	[8]
USA	2009	122	ng/mL	1340	-	-	-	-	540	-	-	-	[8]
USA	2010	43	ng/mL	2070	-	-	-	-	170	-	-	-	[8]
USA	2011	95	ng/mL	960	-	-	-	-	150	-	-	-	[8]
USA	2013	141	ng/mL	670	-	-	-	-	180	-	-	-	[8]
USA	2014	42	ng/mL	360	-	-	-	-	410	-	-	-	[8]

Table 1. Concentrations of bisphenols in urine across different regions and years.

Exposure to BPs can occur through the ingestion of food and liquids that have encountered containers or coatings which contain these compounds. Additionally, humans can be exposed through inhalation of contaminated dust particles and direct dermal contact with products containing bisphenols. This exposure derived in the presence of BPs in human serum, urine, placental tissue, umbilical cord blood, and breast milk, revealing global distribution [1], [9], [10].

Thus, a growing concern exists regarding the potential negative impact of bisphenols on human health and the environment. Bisphenol A (BPA), a widely produced chemical, and other bisphenols have been found to disrupt the endocrine system, affecting human development and function [1]. Research indicated adverse effects of BPA on reproduction, development, neural networks, cardiovascular health, metabolism, and the immune system. The risk of over widespread human exposure and associated adverse effects has led to regulations on BPA production and use in North America and the European Union [11]. Additionally, analogues such as BPF, BPS, BPAF, BPB, and BPC demonstrated similar or greater toxicity estrogenic and antiandrogenic potency compared to BPA, thus requiring its assessment as well [12][13].

Phthalates, derived from phthalic acid, have different chemical structures: some are di-phthalates, replacing two hydrogen atoms, while others are mono-phthalates, replacing one hydrogen atom.

They have low water solubility, long-lasting properties, and various toxicity levels depending on their side chains. Common phthalates include DMP (dimethyl phthalate), mono-2-ethylhexyl phthalate (MEHP), DEP (diethyl phthalate), BBzP (butyl benzyl phthalate), DnBP (dibutyl phthalate), and DiBP (diisobutyl phthalate), used in solvents, lubricants, textiles, personal care products, paints, and adhesives [14]. Phthalates have been found in human urine samples, indicating widespread exposure (see Table 2).

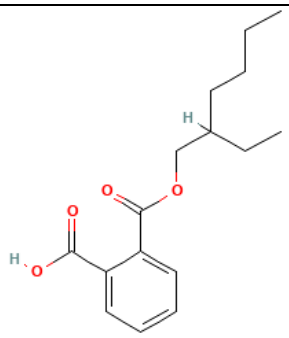
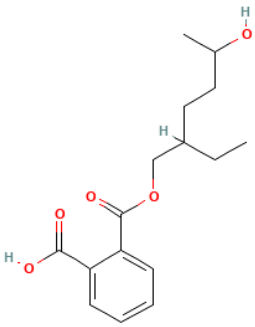
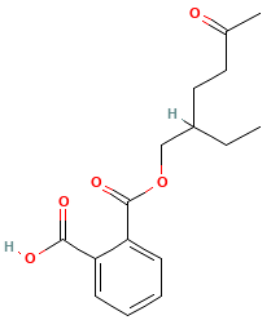
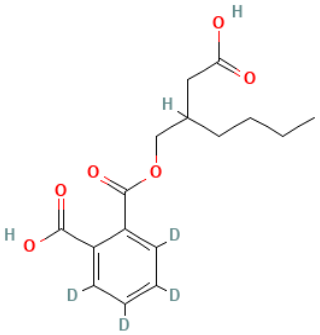
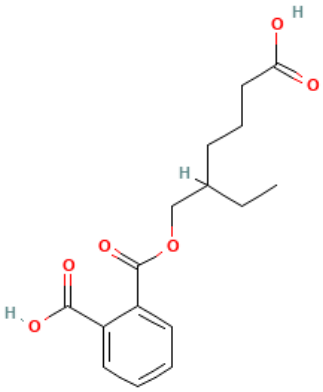
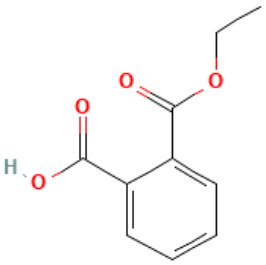
MEHP	MEHHP	MEOHP
		
MCMHP	**MECPP**	**MEP**
		

Figure 2. Chemical structures of phthalates.

Region	Year	n	Units	MEHP	MEHHP	MEOHP	MECPP	MCMHP	MEP	MnBP	MiBP	MBzP	Citation
Germany	-	19	ng/mL	9.8	47.5	39.7	85.5	36.6	-	-	-	-	[15]
USA	-	129	ng/mL	3.3	15.1	7.8	16.2	5.2	-	-	-	-	[16]
USA	1999-2000	328	ng/mL	4.9	-	-	-	-	50	40	-	29	[16]
USA	2001-2002	393	ng/mL	4.4	33	23	-	-	48	32	4.4	27	[16]
USA	2003.2	342	ng/mL	2.7	37	26	52	-	54	37	7	25	[16]
USA	2005-2006	356	ng/mL	3	36	25	54	-	48	32	9	24	[16]
USA	2007-2008	389	ng/mL	2.2	27	17	44	-	45	29	11	18	[16]
Germany	2007	111	ng/mL	4.7	17	15	28	-	-	37	43	7.2	[17]
South Korea	2008	621	ng/mL	25	-	2	-	-	-	51	-	-	[18]
Spain	2005-2006	30	ng/mL	6.2	57	45	115	-	755	30	42	33	[19]
Denmark	2007	129	ng/mL	-	-	137	-	-	46	188		32	[15]
Egypt (urban)	2009	28	ng/mL ng/mL	4.7	29	19	2	-	99	54	25	2.2	[20]

Egypt (rural)	2009	29	ng/mL	3.5	23	16	1	-	43	48	18	0.4	[20]
USA	2016	380	ng/mL	9.9	34.8	20.1	48.7	-	147.4	-	9	7.9	[7]

Table 2. Bisphenol Concentrations in Urine: Regional and Temporal Variations.

Exposure to phthalates can occur through ingestion, inhalation, and dermal absorption. Foods with high fat content, packaging, and processing conditions contribute to phthalate levels. Medical materials, pharmaceuticals, and nutritional products can also be sources. Long-chain phthalates are primarily ingested, while short-chain phthalates are mainly inhaled. Enclosed environments with decorative materials, PVC flooring, and air fresheners can cause inhalation exposure. Dermal absorption primarily occurs through low-molecular-weight phthalates found in cosmetics and personal care products [21], [22].

Phthalates and their metabolites have been linked to various adverse health effects, including reduced semen quality, neurodevelopmental problems, childhood asthma, anogenital distance in boys, low birthweight, endometriosis, decreased testosterone, ADHD, type 2 diabetes, and breast/uterine cancer. Further research is recommended in these areas, particularly reproductive effects in women, which are underrepresented [23].

On the other hand, different pharmaceutical contaminants are detectable in human urine. The pathways through which these contaminants enter the environment are significantly shaped by human activities, including oral consumption, injections, and metabolic processes within the body. Eventually, these substances are released into the sewer system in diverse forms. Previous research has demonstrated that the body absorbs only a small fraction of pharmaceuticals, with the majority being excreted in urine as either unchanged drugs or metabolites [24].

Over the past few years, advances in detection and analysis technology have led to the widespread identification of pharmaceutical contaminants in sewage, surface water, soil, and human urine. These contaminants exhibit characteristics such as biological toxicity, environmental persistence, bioaccumulation, and other factors, which could potentially pose risks and hazards to both water ecosystems and human health [25].

In order to organize and better understand analyte properties and uses, Table 3 shows the classification of the compounds of interest into eight different categories.

Antibiotics	Levofloxacin Sulfadiazine Sulfathiazole Sulfapyridine Tylosin Apramycin Trimethoprim Metronidazole Ofloxacin Nalidixic acid Norfloxacin
Hormones	Progesterone Estrone B-estradiol Dexamethasone
Antiepileptics	Carbamazepine
Analgesic and anti-inflammatory drugs	Ibuprofen Naproxen Codeine
Blood lipid regulators	Atorvastatin
β-blockers	Atenolol Propranolol
Other pharmaceutical drugs	Crotamiton Gemfibrozil
Veterinary drugs	Tiamulin Florfenicol
Stimulants	Caffeine
Plastic additives	Bisphenols Phthalates
Insect repellents	Diethyltoluamide (DEET)
Other chemical compounds	4-nonylphenol

Table 3. Classification of chemical compounds.

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly utilized for pain and inflammation management in various diseases. Ibuprofen (2-[4-(2-methylpropyl) phenyl] propanoic acid) is a significant drug with anti-inflammatory, analgesic, and antipyretic properties. It is known to have a lower incidence of gastrointestinal adverse effects compared to other NSAIDs. The presence of ibuprofen in urine can indicate its therapeutic use or the ingestion of products containing this compound [26] , in the literature we can find a value of 411 µg/mL for this compound [24]. The presence of enrofloxacin, a broad-spectrum antibiotic used in veterinary medicine, in human urine may be due to accidental exposure to this compound. Sulfathiazole, sulfadiazine, and levofloxacin are antibacterial compounds that can be found in urine as a result of their therapeutic administration for the treatment of bacterial infections or their consumption through contaminated food, we find a value of 380 µg/mL for sulfadiazine in the literature [24]. Trimethoprim and nalidixic acid are antimicrobial compounds used in the treatment of urinary tract infections, so their presence in urine may be indicative of their therapeutic use. 4-Nonylphenol is a chemical compound used in the manufacture of plastics and can be detected in urine as a result of environmental exposure or consumption of contaminated food. The presence of progesterone in urine can be indicative of its endogenous production in the body or its administration as part of hormonal therapies. Propranolol, atorvastatin, atenolol, apramycin, tylosin, and metronidazole are pharmaceutical compounds that can be detected in urine as a result of their therapeutic administration for the treatment of various conditions. Carbamazepine (CBZ) is a commonly prescribed anti-epileptic drug that is widely utilized in the treatment of various types of seizures, including psychomotor seizures, generalized tonic-colonic seizures, and complex partial seizures, in the literature we find a value of 22,7 µg/mL for carbamazepine [24]. Its effectiveness in managing these seizure disorders has made it one of the most widely used medications in this therapeutic area. It can be detected in urine as a result of its pharmacological administration [27].

The analysis of bisphenols, phthalates and pharmaceutical contaminants in urine samples from pregnant women remains an underexplored field. It is crucial to assess the current contamination status and generate high-quality knowledge that can be useful for future research linking these endocrine-disrupting

contaminants (EDCs) with the health of pregnant women and their offspring. Understanding the impact of these contaminants on the well-being of pregnant women and their descendants is of utmost importance and can provide valuable insights for better health management.

In this study, urine samples from 40 pregnant women were analyzed to determine the presence and quantity of emerging contaminants, specifically BPs, phthalates, as well as other emerging pollutants such as PhACs in the human body. The main objective is to understand the exposure of pregnant women to these contaminants, thus contributing to the current knowledge about their presence in the human body for future research in environmental health and toxicology.

2. MATERIALS AND METHODS

2.1. Chemicals and materials

The following analytical standards: bisphenol A (BPA) (≥99.0%), bisphenol AF (99.9%), tylosin (99%), florfenicol (99.82%), bisphenol M (99.7%), bisphenol P (97.4%), bisphenol Z (100%) and mono-2-ethylhexyl phthalate (99.3%), were provided by LGCStandards (Barcelona, Spain). Ibuprofen (98%), carbamazepine (100%), sulfathiazole (98%), 4-nonylphenol (100%), progesterone (99%) in powder form, and levofloxacin (98%), nalidixic acid (98%), trimethoprim (99%), dexamethasone (98%), norfloxacin (98%), apramycin (95%), sulfadiazine (99%), aprazolam (100%), codeine phosphate (100%), propranolol hydrochloride (100%), naproxen (100%), and tiamulin (98%) were provided by Sigma-Aldrich (Steinheim, Germany). Sulfapyridine (98%) was provided by Acros Organics. Atorvastatin Calcium (2 molecules of Atorvastatin) (100%) and atenolol were supplied by Pharmaceutical Toronto Research Chemicals. 17-b-estradiol (E2) (98%), DEET (98%), gemfibrozil (98%), crotamiton (97%), metronidazole (99%), estrone (99%), ofloxacin (98%) and caffeine (98.5%) were obtained from Fisher Scientific.

Further information about the chemicals is presented in Table S.1.

Additionally, the following internal standards (IS) were employed: methylparaben-d4, clofibricacid-d4, ibuprofen-d3, naproxen-d3, bisphenol A-d8, ethylparaben-d5, propylparaben-d7, salicylic acid-d4, triclosan-d3, diclofenac-d4, sulfadiazine-d4, sulfadimidine-d4, ciprofloxacin-d8, sulfamethoxazole-d4, danofloxacin-d3 and enrofloxacin-d5. All these internal standards, obtained from Pharmaceutical and Toronto Research Chemicals. They are high purity. Analytical standard quality.

LC-MS grade methanol (MeOH) and acetonitrile (ACN), as well as formic acid (FA, 98%) and ammonium acetate (96%), were provided by Scharlau (Barcelona, Spain). β-glucuronidase enzyme (G07151-100KU, ≥300,000 units/g solid) was acquired from Sigma Aldrich. Ultrapure deionized water was obtained in-house using a Milli-Q Advantage A10 water purification system from Merck Millipore. The filters used for the samples were Captiva filters 3 mL Non-Drip, 100/pk, from Agilent.

For the buffer preparation, ammonium acetate (20.9 g), glacial acetic acid (8,97 mL), milli-Q water (250 mL), and the enzyme β-glucuronidase (4.3 mg) were mixed following the recommendations of the commercial company.

2.2. Sample collection and preparation

Urine samples (n = 40) have been provided from the INSULIN cohort (TECSPR19-1-0022) by researchers from Rovira i Virgili University and the Joan XXIII Hospital. This project has been approved by the CEIm (Comitè Ètic d'Investigació amb medicaments) of the Institut d'Investigació Sanitària Pere Virgili. Data protection has been ensured through the coding of the samples, and all information collected within the framework of this study has been strictly kept confidential. Appropriate security measures have been implemented to guarantee not only confidentiality but also integrity, availability, authenticity, and traceability. All samples were kept frozen at -80°C until analysis. The age of the donors ranged from 19 to 36 years, with an average age of 31 years.

The sample preparation protocol was based on a previous study by Gutiérrez-Martín et al. [28]. Briefly, 1 mL of urine sample was firstly centrifuged (3,500 rpm, 5 min) and the supernatant was transferred trough a Captiva cartridge. Next, 500

µL of the filtered solution was mixed with 1 mL of buffer preparation, and deconjugation was performed at 48°C for 3 hours. Then, 950 µL of the liquid was transferred to a chromatographic vial and stored at -80°C. Prior to instrumental analysis, 50 µL of the IS mix at 1 ppm were added to every sample.

2.3. Instrumental analysis

LC-MS/MS was carried out using a UHPLC Sciex Exion system connected to a Sciex 6500+ triple-quadrupole mass spectrometer from Sciex (Washington, DC, USA). The mass spectrometer was equipped with an electrospray ionization (ESI) source and operated in both positive and negative mode within the same run. Chromatographic separation was accomplished using a Phenomenex (Washington, DC, USA) reversed-phase column Kinetex EVO C18 (2.1 mm × 50 mm, particle size 1.7 µm), which was temperature-controlled at 40 °C throughout the entire chromatogram.

The gradient method employed water (mobile phase A) and MeOH (mobile phase B) as described in Table S2. A 10 µL injection volume was utilized.

For mass spectrometry acquisition, the selected-reaction monitoring (SRM) mode was employed. This mode recorded the transitions between the precursor ion and the two most abundant product ions for each target analyte, resulting in four identification points per compound (2002/657/EC) [29]. The specific UHPLC-MS/MS conditions can be found in the Supplementary data (Table S3). Additionally, the ESI operational settings were as follows: capillary voltage, 4500 V; capillary temperature, 400 °C; gas 1 and 2 pressure, 45 psi. SciexOS software was employed for data acquisition and evaluation.

2.4. Method quanrification and validation

The methodology employed in this study builds upon the previous validated methodology by Gutiérrez-Martín et al. [28], which focused on CECs, specifically pharmaceuticals, plastic additives, food related chemicals, personal care products, insect repellents and UV-filters. The focus of the current investigation was to test the methodology on a more comprehensive list of analytes including

36 CECs, in particular, plastic additives (BPs and phthalates) and PhACs (antibiotics, analgesic and anti-inflammatory drugs) and biocides (see Table S.1.). The validation process relied on several parameters: extraction recoveries, precision, limits of quantification (LOQs), limits of detection (LODs), and the matrix effect.

In this context, a urine pool (n=5) was made. To establish a calibration curve, the urine pool underwent the same pretreatment as the samples and was spiked with the target analytes right before executing UHPLC-MS/MS analysis. To adjust for chemicals already present in the urine, peak areas identified in a non-spiked urine pool sample were deducted from the calibration curve's peak areas. A parallel calibration curve in a solvent composed of 95% water and 5% MeOH was established following the same protocol. The concentration levels built in both calibration curves were 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, 52, 100 ng/mL. LOQs were estimated as the minimum concentration at which a peak was noticeable on the matrix-matched calibration curve. LODs were derived by taking three-tenths of the LOQ values. A linear range was established between the LOQ and the uppermost concentration on the calibration curve, ensuring linearity.

To account for potential losses during the sample treatment, extraction recoveries (R%) were computed. Fourteen pooled samples underwent processing. Six of these samples were pre-spiked with target analytes at an in-vial concentration of 10 ng/mL (n=3) and 50 ng/mL (n=3), respectively, prior to the sample treatment. An additional six-sample batch were spiked with target analytes at an in-vial concentration of 10 ng/mL (n=3) and 50 ng/mL (n=3), respectively, right before the instrumental analysis (post-spikes). Two samples remained non-spiked to track for chemicals already existing in the urine pool. Subsequently, R% was calculated as outlined in Equation 1, for both concentration levels.

$$\text{Eq. 1:} \quad R\% = \frac{\text{Area for each analyte in pre-spiked sample}}{\text{Area for each analyte in post-spiked sample}} \times 100$$

The matrix effect (ME) was assessed by comparing the average peak area for the post-spiked sample (n=3, 10 ng/mL concentration level) with the peak area obtained from spikes in the solvent for each analyte at the same concentration level, as shown in Equation 2.

$$\text{Eq.2:} \quad ME\% = \frac{\text{Area for each analyte in post-spiked sample}}{\text{Area for each analyte in solvent}} \times 100$$

Precision was calculated by computing the coefficient of variation (CV%) of the peak area from a quality control sample (10 ng/mL), injected nine times within a single day.

To account for potential contamination during sample treatment or instrumental analysis, eight procedural blanks were performed (2 blanks per 10 samples), following the same treatment procedure using Milli-Q water instead of urine.

Quantification was executed for chemicals that satisfactorily passed the validation process. It was achieved by interpolating the peak area obtained for the chemicals in each sample, corrected by the peak area for the procedural blanks, to the matrix-matched calibration curve. In cases where a matrix-matched calibration curve was unavailable (NA), as a result of unsatisfactory outcomes during the experimental phase, semi-quantification was performed using the calibration curve in the solvent. Given that suppression is generally observed in urine, semi-quantification based on the calibration curve in the solvent may result in an underestimation of the chemical concentration in urine samples.

2.5. Quality assurance and quality control

To ensure the prevention of any contamination during sample treatments or instrumental analysis, rigorous quality assurance and quality control (QA/QC) measures were implemented. Glass materials were thoroughly cleaned with water and rinsed with distilled water, ethanol and acetone, prior to their utilization. Standards and internal standards were carefully stored in amber glass vials,

shielded from light, and maintained at a temperature of -80 °C. This storage condition was adopted to prevent degradation. Procedural blanks were carried out using the same protocol steps to account for any potential contamination that may arise during the process. To assess the repeatability of the signal, a calibration curve of the pooled urine was established, and a spiked pooled solution with a concentration of 10 µg L^{-1} was injected every 20 injections. Methanol injections were performed every 10 injections to monitor and control any possible carry-over issues. Clothianidin-d3, serving as surrogate, was employed to monitor the sample treatment performance. The rest of the IS were added just before the LC-MSMS analysis to monitor the instrument performance and correct any potential matrix effects. The signal of the IS was checked to see potential losses during sample treatment (surrogate signal) or during the LC-MSMS analysis.

3. RESULTS AND DISCUSSION

3.1. Method validation

The method used has been previously validated by Gutiérrez-Martín et al. [28], providing additional reassurance about its suitability and effectiveness in analyzing the target substances. Building upon this validation, we can be confident that our research is built on a validated method, ensuring the reliability and integrity of our findings. The objective of this validation is to assess the reliability, precision, and accuracy of the analytical procedure when applied for the analysis of the target analytes of this study before its application to real samples.

Thus, out of the 36 initial compounds, 27 yielded successful results during the validation process, which corresponded to a 75% of the total compounds evaluated. Within this subset of 27 compounds, a distinction was made between those undergoing quantification, for which calibration curves in matrix and recovery were employed, and those for which semi-quantification was performed making use of a solvent-based calibration curve, assuming a 100% recovery rate.

It is crucial to recognize that the semi-quantification approach potentially introduced a bias of underestimation in concentration determination. This is due to the inherent complexity of real matrices, in this case, urine, where ionization suppression phenomena usually occur [31]. Ionization suppression affects the efficiency of analyte ionization in the analytical process, thereby influencing the accuracy of the obtained results. This phenomenon can lead to a significant reduction in signal intensity or even the absence of analyte detection.

Specifically, the results of the validation were:

- Calibration Curves

Matrix-matched calibration curves were satisfactory, with coefficient of determinations (R^2) higher than 0.96 for 87% of the chemicals. This indicated a good correlation between the concentration of the substances and the analytical responses in such a complex matrix as urine.

However, as expected, a comparison of slopes between the calibration curves in solvent and urine revealed noticeable differences, as shown in Figure 3. As indicated above, matrix effects often lead to a reduced ionization in urine samples, resulting in calibration curves with lower slopes compared to those observed in solvent. In our study, the presence of a suppression matrix effect was clearly observed in several compounds, including progesterone, carbamazepine, propranolol, metronidazole, ofloxacin, and levofloxacin. For these compounds, the matrix effect varied significantly, ranging from 11% to 92%.

As a consequence of this phenomenon, the accuracy and reliability of measurements can be affected. This means that the calculated concentrations of these compounds in urine samples may be biased by the characteristics of the urine matrix, potentially leading to inaccurate results.

Therefore, it becomes fundamental to quantify using a specific matrix-matched calibration curve in urine to account for this matrix effect.

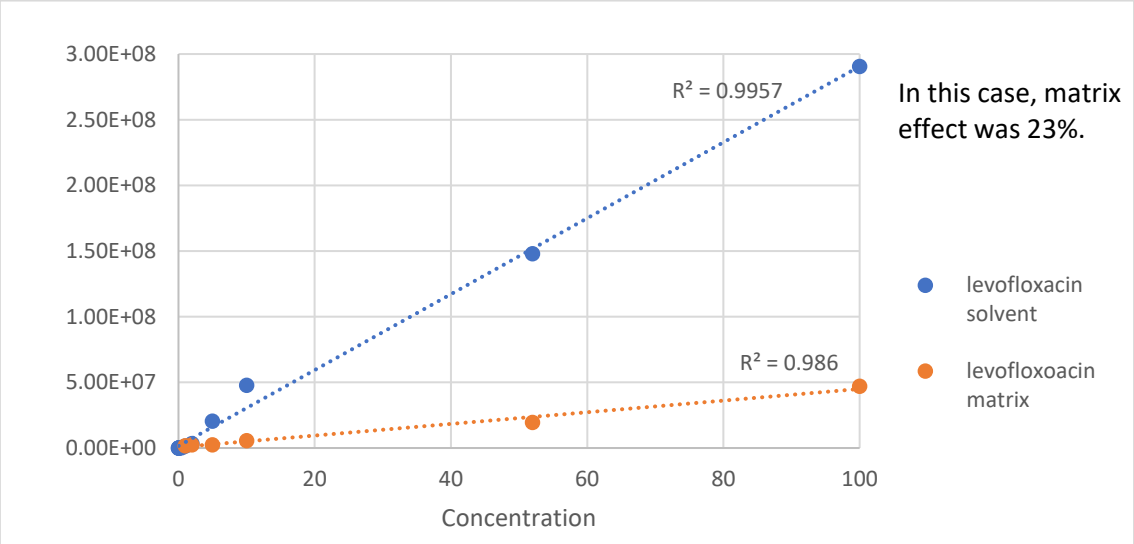


Figure 3. Comparison of levofloxacin slopes in solvent vs. Matrix.

However, in certain cases, specifically in 14, where calibration curves were not available for specific compounds in urine (Table 4) their concentrations can be estimated using the calibration curves built in the solvent. It should be noted that this estimation may lead to actual concentration underestimation due to the observed suppression effect in urine [28].

Chemical	LOQ (ng/mL)	iLOQ (ng/mL)	LODs (ng/mL)	iLODs (ng/mL)	Precision (CV%)
Levofloxacin	1	0.2	0.33	0.07	NA
Sulfathiazole	-	10	-	3.33	NA
Tylosin	-	2	-	0.67	NA
Apramycin	-	1	-	0.33	NA
Trimethoprim	52	0.2	17.33	0.07	42%
Progesterone	5	0.5	1.67	0.17	58%
Carbamazepine	1	0.01	0.33	0.00	22%
Propranolol	2	0.2	0.67	0.07	24%
Metronidazole	0.05	0.05	0.02	0.02	NA
Ofloxacion	1	2	0.33	0.67	71%
Nalidixic acid	NA	0.5	-	0.17	NA
Atorvastatin	5	0.5	1.67	0.17	39%
Atenolol	-	2	-	0.67	NA
Caffeine	NA	0.2	-	0.07	NA
DEET	1	0.01	0.33	0.00	47%
Crotamiton	5	0.2	1.67	0.07	38%
Estrone	-	0.1	-	0.03	90%
Alprazolam	10	0.01	3.33	0.00	48%
Ibuprofen	5	1	1.67	0.33	57%
Bisphenol A	-	2	-	0.67	49%
Nonylphenol	-	5	-	1.67	NA
Gembfibrozil	1	1	0.33	0.33	12%
MEHP	0.01	0.01	0.00	0.00	19%
BP AF	-	5	-	1.67	NA

BP M	-	5	-	1.67	NA
BP Z	-	5	-	1.67	NA
BP P	-	5	-	1.67	NA
Tiamulin	52	0.2	17.33	0.07	93%
B-Estradiol	-	5	-	1.67	40%
Codeine phosphate	-	1	-	0.33	37%
Dexamethasone	-	5	-	1.67	NA
Sulfapyridine	52	0.5	17.33	0.17	NA
Norfloxacin	100	5	33.33	1.67	200%
Sulfadiazine	-	0.02	-	0.01	NA
Florfenicol	-	0.1	-	0.03	173%
Naproxen	-	0.2	-	0.07	NA

Chemical	R2 solvent	R2 matrix	Matrix effect	Recovery 10 ppb	Recovery 50 ppb
Levofloxacin	0.995	0.986	92%	130%	86%
Sulfathiazole	0.990	NA	NA	NA	NA
Tylosin	0.994	NA	NA	NA	NA
Apramycin	0.981	NA	NA	NA	NA
Trimethoprim	0.991	0.983	13%	NA	19%
Progesterone	0.981	1.000	23%	98%	19%
Carbamazepine	0.990	0.995	11%	117%	94%
Propranolol	0.994	0.983	51%	149%	13%
Metronidazole	1.000	1.000	26%	NA	90%

Ofloxacion	1.000	0.990	76%	96%	64%
Nalidixic acid	1.000	NA	26%	NA	78%
Atorvastatin	0.998	0.935	6%	59%	163%
Atenolol	1.000	NA	NA	NA	NA
Caffeine	0.998	NA	NA	NA	NA
DEET	0.992	0.999	20%	186%	85%
Crotamiton	0.994	0.996	21%	133%	102%
Estrone	0.989	NA	NA	NA	NA
Alprazolam	1.000	0.828	7%	75%	99%
Ibuprofen	0.996	0.971	32%	131%	168%
Bisphenol A	0.968	NA	NA	NA	NA
Nonylphenol	1.000	NA	NA	NA	NA
Gembfibrozil	0.999	0.984	49%	99%	72%
MEHP	0.990	0.963	37%	100%	62%
BP AF	0.979	NA	NA	NA	NA
BP M	0.991	NA	NA	NA	NA
BP Z	0.935	NA	NA	NA	NA
BP P	0.989	NA	NA	NA	NA
Tiamulin	0.994	NA	84%	NA	326%
B-Estradiol	0.987	NA	NA	188%	93%

Codeine phosphate	0.984	NA	NA	127%	56%
Dexamethasone	0.962	NA	NA	NA	NA
Sulfapyridine	1.000	NA	NA	NA	38%
Norfloxacin	1.000	NA	NA	NA	NA
Sulfadiazine	0.991	NA	0%	NA	NA
Florfenicol	0.997	NA	NA	NA	NA
Naproxen	0.998	NA	0%	NA	NA

Table 4. Validation Table. (The compounds highlighted in dark blue are those that were quantified, the ones in light blue are those that were semi-quantified, and those left in white are those for which validation was not possible. NA, not available).

- Accuracy of the Method:

Accuracy was expressed as the percentage of recovery, which ranged from 58% to 133% for all substances analyzed in both matrices. The outcome was considered satisfactory, especially given the sample handling simplicity and the method versatility. These results were compared with data published in the literature, such as in the study conducted by Ye et al. [8], exclusively focused on BPs. In this study, urinary concentrations of BPA and three analogs, bisphenol S (BPS), bisphenol F (BPF), and bisphenol AF (BPAF), were measured in a total of 616 archived samples collected from convenience samplings of U.S. adults at eight different time points between 2000 and 2014. It was observed that BPA was the most frequently detected compound, being present in 74-99% of the samples, with average concentrations ranging from 360-2070 ng/L. Next was BPF, detected in 42-88% of the samples, with average concentrations of 150-540 ng/L, followed by BPS, which was present in 19-74% of the samples, with average concentrations below 100-250 ng/L. Lastly, BPAF was rarely detected, being present in less than 3% of all samples. In this study the recovery percentages ranged from 91% to 107%. This allows us to assess the effectiveness of the proposed analytical method in quantifying the same substances in our samples. Additionally, the limits of quantification (LOQs) were found to be satisfactory, with 13 compounds showing LOQs below 5 ng/mL, which accounts for 34% of the compounds under analysis.

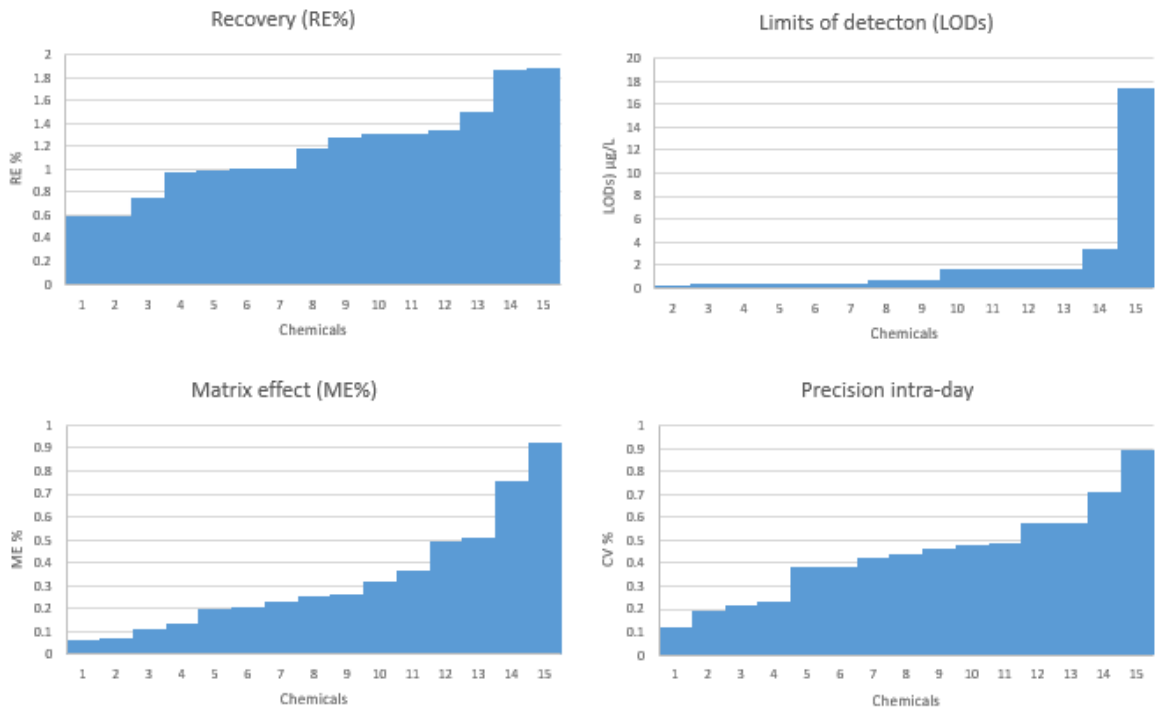


Figure 4. Validation data overview.

3.2. Occurrence of xenobiotics

In our research, a total of 15 out of the 27 validated compounds were detected in the urine samples. These compounds encompass a wide variety of uses and categories, including antibiotics such as levofloxacin, trimethoprim, metronidazole and ofloxacin; lipid-lowering medications like gemfibrozil and atorvastatin; cardiovascular drugs such as propranolol; analgesics and anti-inflammatories like ibuprofen; antiepileptic drugs and mood stabilizers like carbamazepine and alprazolam; steroids like progesterone; insect repellents like DEET; antipruritic agents like crotamiton; plastic additives like BPA; and plasticizers like mono-2-ethylhexyl phthalate.

If we observe the results presented in Table 5, we can see that β-estradiol has been found in all samples except one. β-estradiol is a steroid hormone that plays a crucial role in pregnancy, as it is produced in large quantities by the ovaries and the placenta during this period. Therefore, the detection of estradiol in the urine of pregnant women is a normal result. Furthermore, estrone and progesterone have also been detected in many of the samples. Estrone is a metabolite of estradiol, and its detection is indicative of normal hormonal activity during

pregnancy. Progesterone, on the other hand, is essential for the maintenance of pregnancy and is produced in significant quantities during gestation [30]. Considering all of this, it doesn't seem surprising that these hormones are among the compounds that show the highest frequency and concentration in our study.

The compounds listed in Table 5 have diverse sources of exposure, including medical use, industrial manufacturing, environmental exposure, and other processes. The presence of these compounds in the urine matrix highlights the importance of assessing their impact on human health and the environment.

Chemical	Urine-169	Urine-170	Urine-171	Urine-172	Urine-173	Urine-174	Urine-175	Urine-176	Urine-177	Urine-178
Levofloxacin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfathiazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tylosin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Apramycin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Progesterone	35.3	ND	572.2	ND	1668.1	ND	ND	ND	ND	ND
Carbamazepine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Propranolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metronidazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ofloxacion	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nalidixic acid	20.8	ND	ND	ND	ND	ND	ND	ND	3.5	ND
Atorvastatin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Atenolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Caffeine	ND	ND	ND	2.0	ND	ND	ND	ND	2.3	ND
DEET	ND	<LOQ	ND	ND	ND	<LOQ	ND	ND	ND	ND
Crotamiton	ND	ND	ND	ND	ND	ND	ND	7.0	ND	ND
Estrone	22.5	ND	9.0	ND	ND	ND	15.9	54.3	ND	ND
Alprazolam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ibuprofen	ND	ND	31.9	ND	178.5	ND	ND	ND	71.2	ND
Bisphenol A	ND	ND	ND	ND	<LOQ	ND	ND	ND	ND	ND
Nonylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gembfibrozil	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MEHP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP AF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

BP M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP Z	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tiamulin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B-Estradiol	338300	1062000	4565000	2790000	2234000	5259000	2411000	2838000	3129000	1356000
Codeine phosphate	ND	ND	ND	ND	159300	ND	ND	ND	478900	337000
Dexamethasone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfapyridine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Norfloxacin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfadiazine	ND	ND	ND	ND	ND	345600	ND	379400	ND	ND
Florfenicol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Naproxen	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Chemical	Urine-179	Urine-180	Urine-181	Urine-182	Urine-183	Urine-184	Urine-185	Urine-186	Urine-187	Urine-188
Levofloxacin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfathiazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tylosin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Apramycin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Progesterone	6728	ND	27.0	22882	ND	52.9	20.1	ND	7.6	ND
Carbamazepine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Propranolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metronidazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ofloxacion	ND	ND	ND	ND	ND	ND	ND	ND	7.2	7.4
Nalidixic acid	ND	ND	ND	ND	ND	ND	4.3	ND	ND	ND

Atorvastatin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Atenolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Caffeine	ND	57.7	ND	14.7	11.1	3.1	50.2	9.4	35.3	ND
DEET	ND	ND	ND	ND	ND	ND	<LOQ	ND	<LOQ	ND
Crotamiton	11.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
Estrone	ND	11.0	27.5	35.8	ND	ND	41.0	36.6	ND	21.2
Alprazolam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ibuprofen	ND	41.3	ND	ND	80.9	25.2	20.8	70.1	ND	32.9
Bisphenol A	ND	ND	ND	ND	ND	89.6	<LOQ	ND	ND	ND
Nonylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gembfibrozil	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MEHP	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP AF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP Z	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tiamulin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B-Estradiol	6120000	1889000	9132000	5884000	5441000	7144000	14380000	5042000	1258000	2060000
Codeine phosphate	ND	345000	248100	ND	349800	ND	305100	1077000	141300	ND
Dexamethasone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfapyridine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Norfloxacin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfadiazine	ND	ND	ND	ND	ND	852900	1199000	ND	ND	ND
Florfenicol	656200	475500	ND	ND	ND	ND	ND	ND	ND	ND
Naproxen	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Chemical	Urine-189	Urine-190	Urine-141	Urine-142	Urine-143	Urine-144	Urine-145	Urine-146	Urine-147	Urine-148
Levofloxacin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfathiazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tylosin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Apramycin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Progesterone	ND	46.3	ND	ND	19.3	ND	ND	ND	ND	ND
Carbamazepine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Propranolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metronidazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ofloxacion	ND	ND	ND	ND	ND	5.9	ND	ND	ND	ND
Nalidixic acid	ND	ND	ND	9.6	10.1	ND	ND	ND	ND	ND
Atorvastatin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Atenolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Caffeine	ND	ND	ND	7.9	18.7	ND	53.7	2.4	ND	ND
DEET	<LOQ	ND	ND	ND	ND	<LOQ	1.1	ND	ND	ND
Crotamiton	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.7
Estrone	ND	ND	73.8	ND	ND	ND	ND	27.3	ND	ND
Alprazolam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ibuprofen	14.8	ND	61.1	ND	ND	ND	24.9	ND	ND	ND
Bisphenol A	ND	ND	ND	18.0	ND	ND	ND	ND	ND	ND
Nonylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gembfibrozil	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MEHP	ND	ND	ND	ND	ND	0.0	<LOQ	ND	0.1	ND
BP AF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP Z	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

BP P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tiamulin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B-Estradiol	772300	6097000	6016000	5933000	5367000	1261000	909900	2177000	1473000	1945000
Codeine phosphate	318700	139700	ND	351100	ND	ND	80270.0	ND	ND	ND
Dexamethasone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfapyridine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Norfloxacin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfadiazine	ND	590100	1464000	1148000	ND	ND	ND	ND	469400	343800
Florfenicol	ND	684100	1333000	ND	1167000	ND	ND	ND	ND	457800
Naproxen	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Chemical	Urine-149	Urine-150	Urine-151	Urine-152	Urine-153	Urine-154	Urine-155	Urine-156	Urine-157	Urine-158
Levofloxacin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfathiazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tylosin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Apramycin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Trimethoprim	ND	ND	ND	ND	ND	ND	ND	ND	6.3	ND
Progesterone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carbamazepine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Propranolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Metronidazole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ofloxacion	ND	ND	ND	ND	ND	5.4	ND	ND	ND	ND
Nalidixic acid	ND	ND	ND	ND	ND	ND	ND	5.4	5.3	ND
Atorvastatin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Atenolol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Caffeine	ND	ND	ND	34.8	ND	41.9	81.8	ND	ND	ND
DEET	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Crotamiton	6.7	ND	ND	ND	5.9	ND	ND	ND	ND	ND
Estrone	22.2	ND	ND	ND	22.6	ND	ND	25.5	21.6	ND
Alprazolam	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Ibuprofen	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bisphenol A	ND	ND	ND	ND	ND	3.3	ND	ND	ND	ND
Nonylphenol	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Gembfibrozil	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MEHP	ND	ND	0.1	ND	ND	ND	0.1	0.1	ND	0.1
BP AF	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP M	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
BP Z	ND	ND	ND	ND	ND	33.6	ND	ND	ND	ND
BP P	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Tiamulin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B-Estradiol	ND	2216000	1116000	1847000	5064000	626400	3326000	2492000	3452000	1859000
Codeine phosphate	ND	125900	ND	198300	ND	ND	151800	ND	185100	ND
Dexamethasone	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfapyridine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Norfloxacin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Sulfadiazine	ND	ND	ND	ND	418300	ND	ND	ND	646700	ND
Florfenicol	785400	572300	ND	ND	ND	ND	ND	ND	ND	762100
Naproxen	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 5. Concentrations of different chemical compounds in urine samples (the compounds highlighted in dark blue are those that were quantified, the ones in light blue are those that were semi-quantified, and those left in white are those for which validation was not possible. ND, non-detectable).

Chemical	DF	Average (ng/mL)	Median (ng/mL)
Levofloxacin	0.03	0.03	0
Sulfathiazole	0	0	0
Tylosin	0	0	0
Apramycin	0	0	0
Trimethoprim	0	0	0
Progesterone	0.30	1336	3.13
Carbamazepine	0	0	0
Propranolol	0	0	0
Metronidazole	0.03	1.4E-05	0
Ofloxacion	0.10	1.2E+00	0
Nalidixic acid	0.18	1.5E+00	0
Atorvastatin	0	0	0
Atenolol	0	0	0
Caffeine	0.40	0	10.67
DEET	0.18	0	0.41
Crotamiton	0.13	0	0.96
Estrone	0.4	0	11.70
Alprazolam	0	0	0
Ibuprofen	0.3	0	16.34
Bisphenol A	0.13	0	2.86
Nonylphenol	0	0	0
Gembfibrozil	0	0	0
MEHP	0.28	0	0.015
BP AF	0	0	0
BP M	0	0	0
BP Z	0.03	0	0.84
BP P	0	0	0

Tabla 6. Summary table of concentrations.

BP levels in urine ranged from non-detected (ND) to 284 ng/mL, with nearly 5% of the women having detectable concentrations of BPs (Table 5). In the present work, concentrations of BPA in urine samples ranged between 3.3 and 89.6 ng/mL, we can compare it with the data previously published in the literature, it was observed that the concentrations of BPA detected in urine samples range from 0.886 ng/mL in some studies in China [4] to 2070 ng/mL in a study in the United States [8], we can observe that the lower limit in the bibliography is similar to that in our study, while the upper limit we obtain is significantly lower than the bibliographic one. As for BP Z, it has been identified in only one sample with a value of 33.6 ng/mL, whereas in previous publications from the literature, it was

found to have a value of 0.06 ng/mL in a sample from Saudi Arabia [5]. No other bisphenols have been detected in the analyzed samples.

Regarding phthalates, only the presence of mono-2-ethylhexyl phthalate has been detected in the analyzed samples. The levels ranged from non-detectable to 0.09 ng/mL, with nearly 9.5% of the women showing concentrations of phthalates above LOD (Table 5). The levels of concentration were quite low when compared to the bibliographic data. Nonetheless they varied from levels as low as 9.8 ng/mL in Germany [15], 6.2 ng/mL in Spain [19], 4.7 ng/mL in urban Egypt [20], 2.2 ng/mL in the United States [16] up to concentrations as high as 25 ng/mL in South Korea [18].

Regarding progesterone, it ranged from 6.3 ng/mL to 22881.46 ng/mL in the samples analyzed in the study, with detectable concentrations observed in 12 of the participants, we can compare these values with those found in the literature, where concentrations of progesterone in serum range from 0.08 to 1.57 ng/mL, with an average value of 0.24 ng/mL [31]

Caffeine levels were found in a range from 2.0 ng/mL to 81.8 ng/mL, with 16 participants showing detectable concentrations [32]. We can compare with the values from the bibliography, caffeine and its metabolites were detectable in the urine of most individuals. Median concentrations ranged from 560 ng/mL to 58600 ng/mL [32]. We can observe that our experimental values are below the reference values. This could be attributed to the fact that caffeine concentration levels tend to be lower in the urine of pregnant women, as they are advised to limit their caffeine consumption during pregnancy.

Similarly, nalidixic acid exhibited concentrations spanning from 3.5 ng/mL to 20.8 ng/L, detectable in 7 individuals. Again, this study entails the first time concentrations of this antibiotic is reported for urine. Then, as no previous data is available for urines, concentrations in urban wastewater were used as a reference. Hence, Ghosh et al. [30] reported an average concentration of 40 ng/mL for nalidixic acid in influent wastewater to urban wastewater treatment plants in Singapore.

DEET, on the other hand, displayed concentrations ranging from <LOQ to 1.1 ng/L, detected in 7 participants. We can compare with the values from the bibliography, where we found concentrations spanning from 0.0475 ng/mL to 2.57 ng/mL, with an average concentration of approximately 0.3439 ng/mL [33].

The analysis of crotamiton revealed concentrations varying from 5.9 ng/mL to 11.0 ng/mL, detectable in 5 participants out of the total 40 participants. The analysis of crotamiton in urine in this study was novel and no previous data is available for comparison. Crotamiton was reported to be present in wastewater samples collected from residential areas at concentrations extending from less than 0.0005 ng/mL to 0.387 ng/mL, with an average concentration of 0.0346 ng/mL [33].

Estrone levels spanned from 9.0 ng/mL to 73.8 ng/L, detectable in 16 individuals, we can compare these values with the literature, where urinary estrone levels, measured in nanograms per milligram of creatinine (ng/mg-Cr), range from 2.7 to 13.4 ng/mg-Cr, with an average value of 7.5 ng/mg-Cr [31], since we're studying urine from pregnant women, it's normal for our values to be higher.

Finally, ibuprofen concentrations ranged from 14.8 ng/mL to 178.5 ng/L, with detectable levels observed in 12 participants. Ibuprofen has been found in urine before showing concentrations averaging 411000 ng/L [24]. Thus, levels of ibuprofen observed in the present study were clearly lower. However, this discrepancy can be explained as the bibliographic values were based on data from the general population, while our data was extracted from a specific group of pregnant women. In this context, it is understandable that the concentration of ibuprofen is lower, as this medication is generally not recommended during pregnancy due to safety concerns.

Regarding levofloxacin, it was not detected in any of the examined samples. Similarly, sulfathiazole, tylosin, apramycin, trimethoprim, carbamazepine, propranolol, atorvastatin, atenolol, alprazolam, nonylphenol, gemfibrozil, tiamulin, B-Estradiol, codeine phosphate, dexamethasone, sulfapyridine, norfloxacin, sulfadiazine, florfenicol and naproxen were not detected either. The lack of detection of these compounds in the urine samples of pregnant women could be attributed to the fact that these substances are generally avoided by

expectant mothers on medical advice due to the potential adverse effects they could have on embryonic and fetal development. The chemical compounds bisphenol AF, bisphenol P, bisphenol B, bisphenol M and bisphenol Z have also not been detected, which is in accordance with what it was observed in the study conducted by Ye et al [8].

4. CONCLUSIONS

This study has provided an enriching insight into the presence of emerging contaminants in human urine samples, emphasizing the importance of understanding their relevance and the potential health risks they pose. Emerging contaminants have become a subject of growing concern due to their ubiquity in consumer products and their ability to negatively impact human health, even at very low concentrations.

It is worth noting that, although 36 compounds were analyzed, satisfactory results were obtained for only 27 of them. This highlights the complexity of analyzing urine samples due to the matrix and the need to use semi-quantification in some cases. Despite the inherent challenges posed by the urine matrix, a 76% success rate was achieved in method validation, demonstrating the robustness of the applied methodology.

The significance of this study lies in its impact on public health. The findings provide valuable information about the population's exposure to these emerging contaminants through urine biomonitoring. Such studies can influence future regulations aimed at mitigating the risks associated with these contaminants and protecting public health and the environment.

Ultimately, this work underscores the need for ongoing research and the optimization of analytical methods to improve the accuracy of emerging contaminant detection and to assess potential health risks more precisely. This study significantly contributes to the understanding of emerging contaminants and their impact on the human environment.

5. SUPLEMENTARY INFORMATION

Supplementary information is available at the end of this document.

6. ACKNOWLEDGMENTS

Authors acknowledge the Regional Government of Castilla y León and the EU-FEDER (CL-EI-2021-07, 587 UIC 320, UIC 338). IDAEA-CSIC author acknowledge the Spanish Ministry of Science and Innovation through the support received as "Centro de Excelencia Severo Ochoa 2019–2023". We thank the effort put in the collection and distribution of samples from the INSULIN cohort (TECSPR19-1-0022) to researchers from Rovira i Virgili University and the Joan XXIII Hospital.

7. BIBLIOGRAPHY

[1] J. R. Rochester, "Bisphenol A and human health: A review of the literature," *Reproductive Toxicology*, vol. 42. 2013. doi: 10.1016/j.reprotox.2013.08.008.

[2] D. Chen *et al.*, "Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity - A Review," *Environmental Science and Technology*, vol. 50, no. 11. 2016. doi: 10.1021/acs.est.5b05387.

[3] C. J. Catenza, A. Farooq, N. S. Shubear, and K. K. Donkor, "A targeted review on fate, occurrence, risk and health implications of bisphenol analogues," *Chemosphere*, vol. 268. 2021. doi: 10.1016/j.chemosphere.2020.129273.

[4] Y. Yang, J. Guan, J. Yin, B. Shao, and H. Li, "Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China," *Chemosphere*, vol. 112, 2014, doi: 10.1016/j.chemosphere.2014.05.004.

[5] A. G. Asimakopoulos *et al.*, "Urinary biomarkers of exposure to 57 xenobiotics and its association with oxidative stress in a population in Jeddah, Saudi Arabia," *Environ Res*, vol. 150, 2016, doi: 10.1016/j.envres.2015.11.029.

[6] J. Xue, Q. Wu, S. Sakthivel, P. V. Pavithran, J. R. Vasukutty, and K. Kannan, "Urinary levels of endocrine-disrupting chemicals, including bisphenols, bisphenol A diglycidyl ethers, benzophenones, parabens, and triclosan in obese and non-obese Indian children," *Environ Res*, vol. 137, 2015, doi: 10.1016/j.envres.2014.12.007.

[7] D. E. Cantonwine, J. D. Meeker, K. K. Ferguson, B. Mukherjee, R. Hauser, and T. F. McElrath, "Urinary concentrations of bisphenol A and phthalate metabolites measured during pregnancy and risk of preeclampsia," *Environ Health Perspect*, vol. 124, no. 10, 2016, doi: 10.1289/EHP188.

[8] X. Ye, L. Y. Wong, J. Kramer, X. Zhou, T. Jia, and A. M. Calafat, "Urinary Concentrations of Bisphenol A and Three Other Bisphenols in Convenience Samples of U.S. Adults during 2000-2014," *Environ Sci Technol*, vol. 49, no. 19, 2015, doi: 10.1021/acs.est.5b02135.

[9] T. Geens *et al.*, "A review of dietary and non-dietary exposure to bisphenol-A," *Food and Chemical Toxicology*, vol. 50, no. 10. 2012. doi: 10.1016/j.fct.2012.07.059.

[10] L. N. Vandenberg, R. Hauser, M. Marcus, N. Olea, and W. V. Welshons, "Human exposure to bisphenol A (BPA)," *Reproductive Toxicology*, vol. 24, no. 2. 2007. doi: 10.1016/j.reprotox.2007.07.010.

[11] D. Chen *et al.*, "Bisphenol Analogues Other Than BPA: Environmental Occurrence, Human Exposure, and Toxicity - A Review," *Environmental Science and Technology*, vol. 50, no. 11. 2016. doi: 10.1021/acs.est.5b05387.

[12] J. R. Rochester and A. L. Bolden, "Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol a substitutes," *Environmental Health Perspectives*, vol. 123, no. 7. 2015. doi: 10.1289/ehp.1408989.

[13] M. Audebert, L. Dolo, E. Perdu, J. P. Cravedi, and D. Zalko, "Use of the γh2AX assay for assessing the genotoxicity of bisphenol A and bisphenol F in human cell lines," *Arch Toxicol*, vol. 85, no. 11, 2011, doi: 10.1007/s00204-011-0721-2.

[14] R. U. Halden, "Plastics and health risks," *Annual Review of Public Health*, vol. 31. 2010. doi: 10.1146/annurev.publhealth.012809.103714.

[15] H. Frederiksen, N. E. Skakkebæk, and A. M. Andersson, "Metabolism of phthalates in humans," *Mol Nutr Food Res*, vol. 51, no. 7, 2007, doi: 10.1002/mnfr.200600243.

[16] S. Langer *et al.*, "Phthalate metabolites in urine samples from Danish children and correlations with phthalates in dust samples from their homes and daycare centers," *Int J Hyg Environ Health*, vol. 217, no. 1, 2014, doi: 10.1016/j.ijheh.2013.03.014.

[17] H. M. Koch and A. M. Calafat, "Human body burdens of chemicals used in plastic manufacture," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 364, no. 1526. 2009. doi: 10.1098/rstb.2008.0208.

[18] S. C. Cho *et al.*, "Relationship between environmental phthalate exposure and the intelligence of school-age children," *Environ Health Perspect*, vol. 118, no. 7, 2010, doi: 10.1289/ehp.0901376.

[19] L. Casas *et al.*, "Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children," *Environ Int*, vol. 37, no. 5, 2011, doi: 10.1016/j.envint.2011.02.012.

[20] J. A. Colacino *et al.*, "Exposure to phthalates among premenstrual girls from rural and urban Gharbiah, Egypt: A pilot exposure assessment study," *Environ Health*, vol. 10, no. 1, 2011, doi: 10.1186/1476-069X-10-40.

[21] H. M. Koch and A. M. Calafat, “Human body burdens of chemicals used in plastic manufacture,” *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 364, no. 1526. 2009. doi: 10.1098/rstb.2008.0208.

[22] F. Carlstedt, B. A. G. Jönsson, and C. G. Bornehag, “PVC flooring is related to human uptake of phthalates in infants,” *Indoor Air*, vol. 23, no. 1, 2013, doi: 10.1111/j.1600-0668.2012.00788.x.

[23] J. Eales *et al.*, “Human health impacts of exposure to phthalate plasticizers: An overview of reviews,” *Environment International*, vol. 158. 2022. doi: 10.1016/j.envint.2021.106903.

[24] X. Li, B. Wang, F. Liu, and G. Yu, “Occurrence and Removal of Pharmaceutical Contaminants in Urine: A Review,” *Water (Switzerland)*, vol. 15, no. 8. 2023. doi: 10.3390/w15081517.

[25] Q. Sun, M. Lv, A. Hu, X. Yang, and C. P. Yu, “Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in a wastewater treatment plant in Xiamen, China,” *J Hazard Mater*, vol. 277, 2014, doi: 10.1016/j.jhazmat.2013.11.056.

[26] A. R. Khaskheli *et al.*, “Estimation of ibuprofen in urine and tablet formulations by transmission Fourier Transform Infrared spectroscopy by partial least square,” *Spectrochim Acta A Mol Biomol Spectrosc*, vol. 102, 2013, doi: 10.1016/j.saa.2012.10.021.

[27] H. A. Mashayekhi, P. Abroomand-Azar, M. Saber-Tehrani, and S. W. Husain, “Rapid determination of carbamazepine in human urine, plasma samples and water using DLLME followed by RP-LC,” *Chromatographia*, vol. 71, no. 5–6, 2010, doi: 10.1365/s10337-009-1456-6.

[28] D. S.-R. E. R.-M. E. G. O. L.-S. R. A. R. T. N. M. M. G.-F. P. G.-S. R. Gutiérrez-Martín, “Comprehensive profiling and semi-quantification of exogenous chemicals in human urine using HRMS-based strategies,” *Under review in Analytical and Bioanalytical Chemistry*, 2023.

[29] M. Boonsaner and D. W. Hawker, “Evaluation of food chain transfer of the antibiotic oxytetracycline and human risk assessment,” *Chemosphere*, vol. 93, no. 6, 2013, doi: 10.1016/j.chemosphere.2013.05.070.

[30] N. Magon and P. Kumar, “Hormones in pregnancy,” *Nigerian Medical Journal*, vol. 53, no. 4, 2012, doi: 10.4103/0300-1652.107549.

[31] M. Newman, S. M. Pratt, D. A. Curran, and F. Z. Stanczyk, “Evaluating urinary estrogen and progesterone metabolites using dried filter paper samples and gas chromatography with tandem mass spectrometry (GC-MS/MS),” *BMC Chem*, vol. 13, no. 3, 2019, doi: 10.1186/s13065-019-0539-1.

[32] M. E. Rybak, M. R. Sternberg, C. I. Pao, N. Ahluwalia, and C. M. Pfeiffer, “Urine excretion of caffeine and select caffeine metabolites is common in the US population and associated with caffeine intake,” *Journal of Nutrition*, vol. 145, no. 4, 2015, doi: 10.3945/jn.114.205476.

[33] N. H. Tran, J. Li, J. Hu, and S. L. Ong, “Occurrence and suitability of pharmaceuticals and personal care products as molecular markers for raw wastewater contamination in surface water and groundwater,” *Environmental Science and Pollution Research*, vol. 21, no. 6, 2014, doi: 10.1007/s11356-013-2428-9.

Supplementary information

Determination of contaminants in urine: bisphenols, phthalates, and other chemical substances and their relationship with human health

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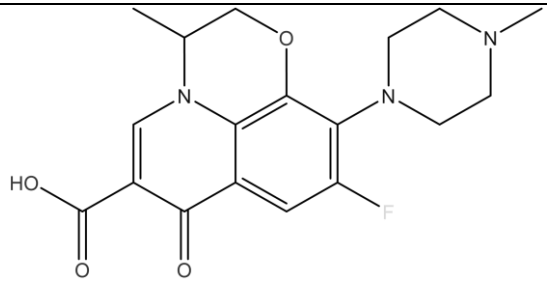
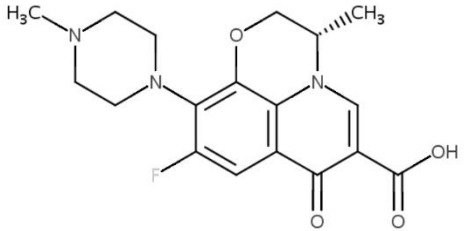
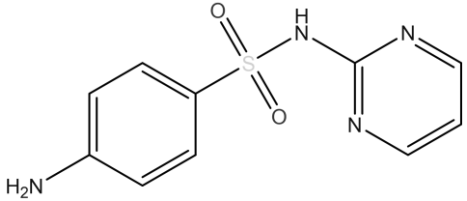
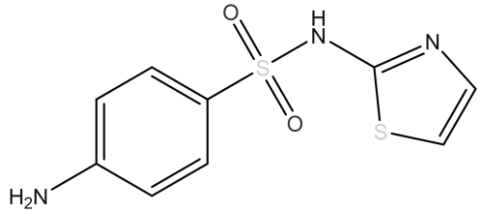
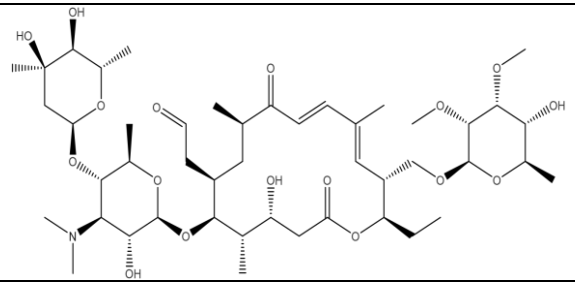
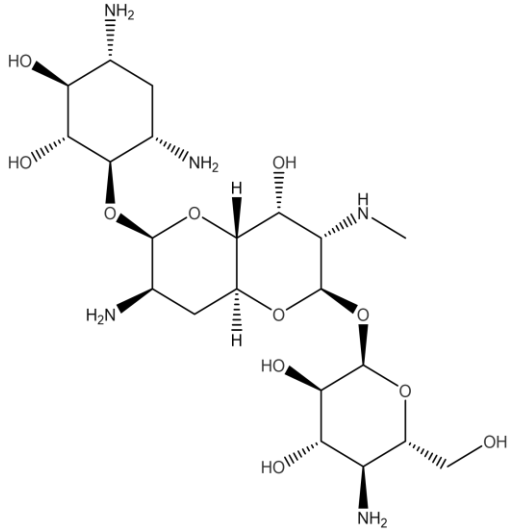
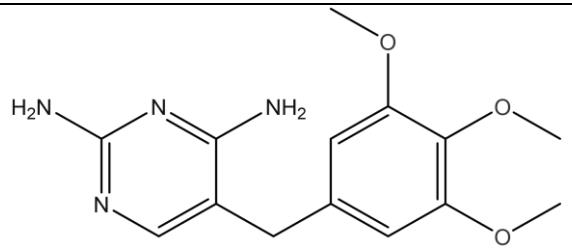
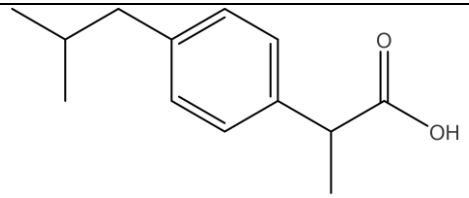
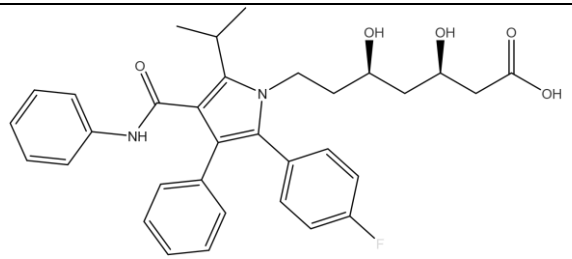
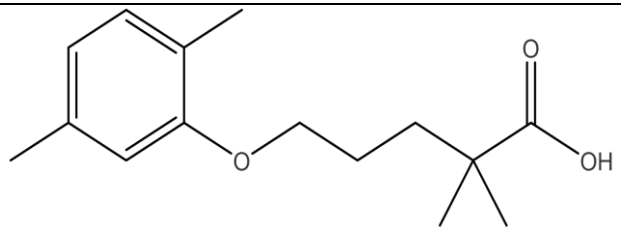
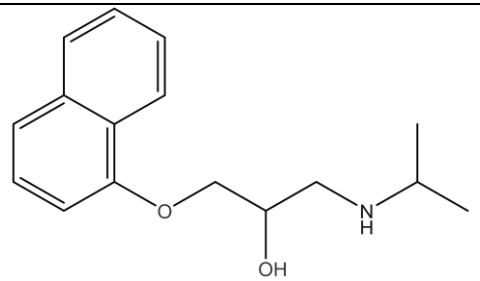
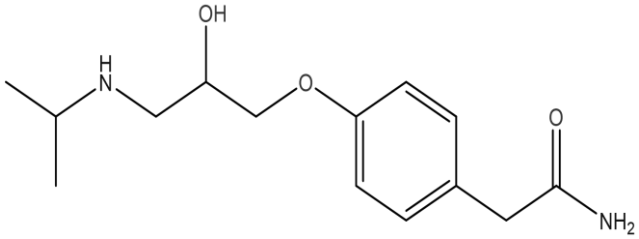
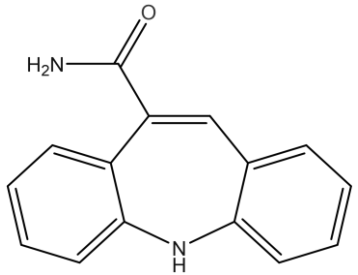
2022/2023

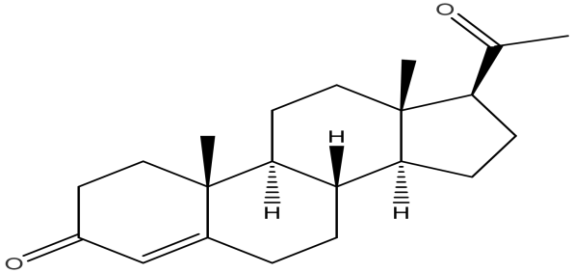
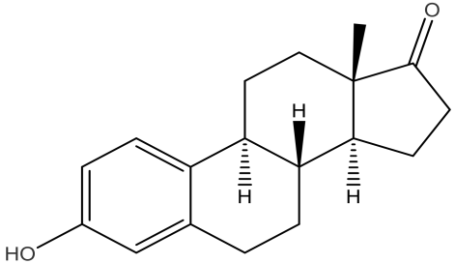
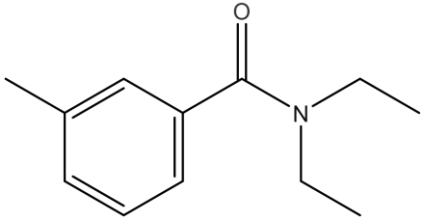
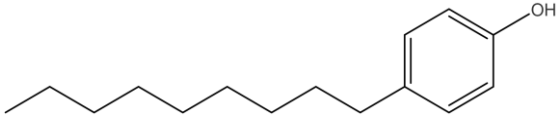
Table S3. List of chemical compounds.

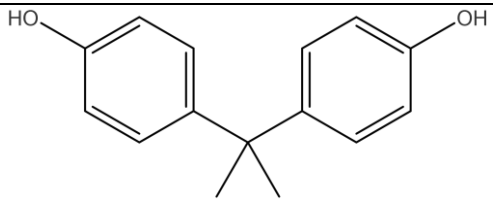
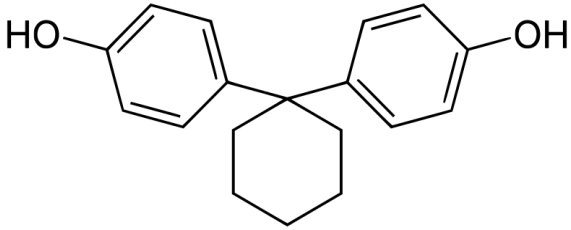
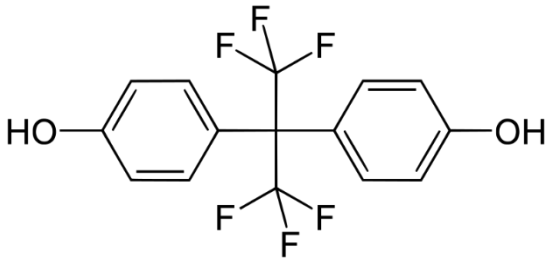
Chemical	Class	Subclass	Molecular formula	CAS	log P at 25 ºC	pKa at 25 ºC	Chemical structure
Ofloxacin	Antibiotic	Quinolone	$C_{18}H_{20}FN_3O_4$	82419-36-1	1.855	5.2 / 7.3	
Levofloxacin	Antibiotic	Quinolone	$C_{18}H_{20}FN_3O_4$	100986-85-4	2.401	6 / 8.6	
Sulfadiazine	Antibiotic	Sulfonamide	$C_{10}H_{10}N_4O_2S$	68-35-9	-0.074	6,81 / 1	

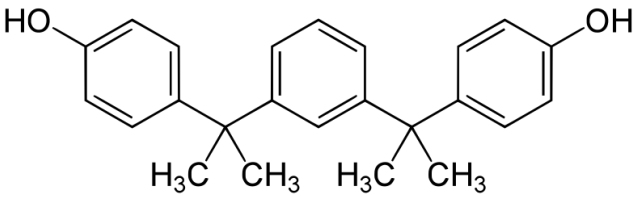
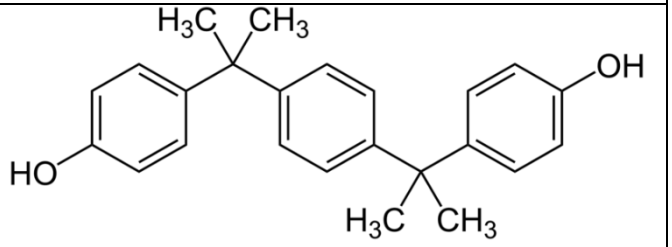
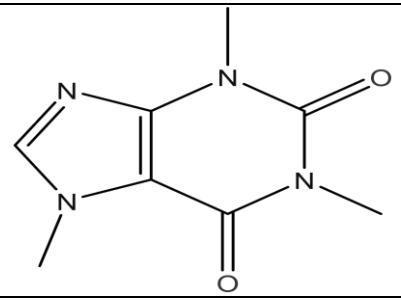
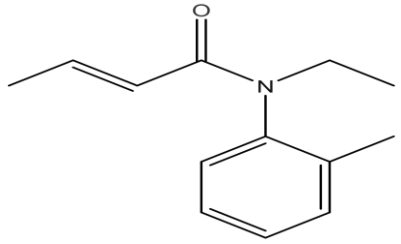
Sulfathiazole	Antibiotic	Sulfonamide	$C_9 H_9 N_3 O_2 S_2$	72-14-0	0.050	7,24 / 2,19	
Tylosin	Antibiotic	Macrolide	$C_{46} H_{77} N O_{17}$	74610-55-2	0.628	13,06 / 7,39	
Apramycin	Antibiotic	Aminoglycoside	$C_{21} H_{41} N_5 O_{11}$	37321-09-8	-3.427	9,48 / 12,91	

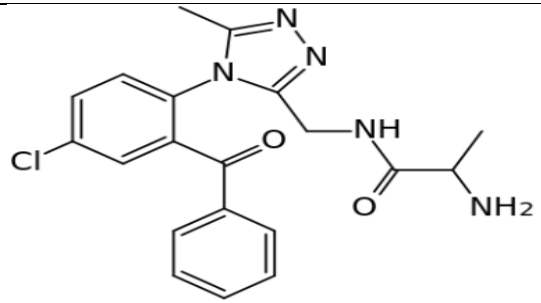
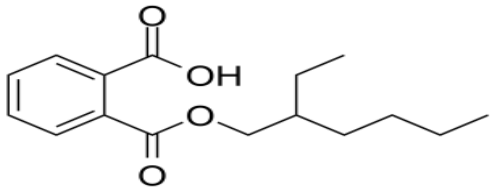
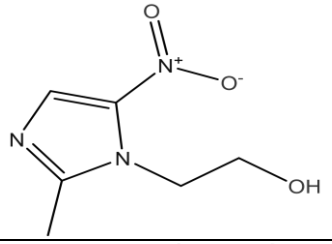
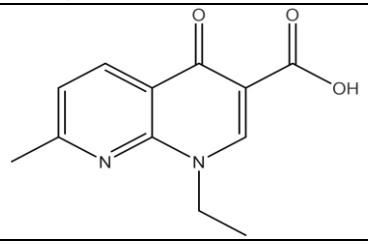
Trimethoprim	Antibiotic	Others	$C_{14}H_{18}N_4O_3$	738-70-5	0.594	7.04	
Ibuprofen	Analgesic/Anti-inflammatory	-	$C_{13}H_{18}O_2$	15687-27-1	3.502	4.41	
Atorvastatin	Lipid regulator	-	$C_{33}H_{35}FN_2O_5$	134523-03-8	3.846	0.38 / 4.29	
Gemfibrozil	Lipid regulator	Fibrate	$C_{15}H_{22}O_3$	25812-30-0	4.302	4.75 (MA)	

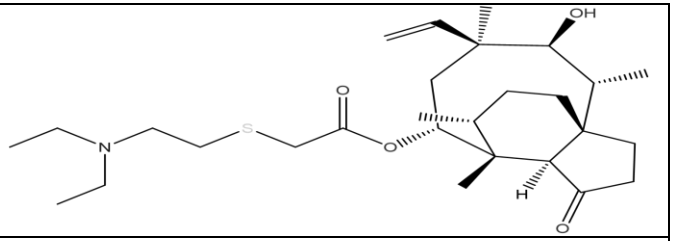
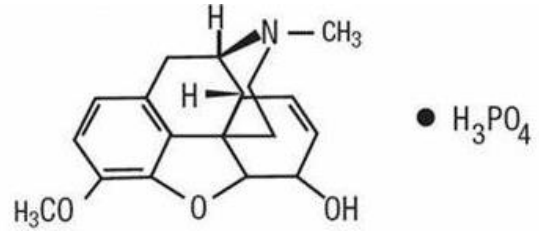
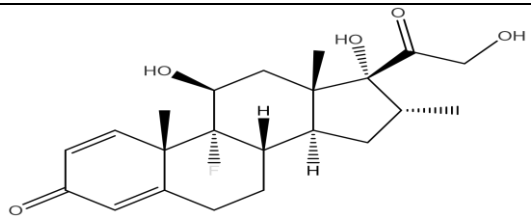
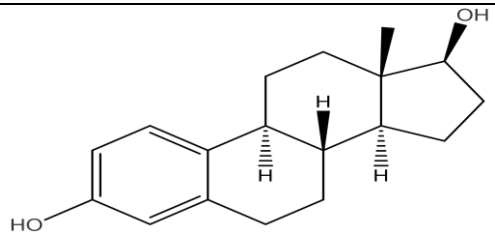
Propranolol	Cardiovascular drug	-	$C_{16}H_{21}NO_2$	525-66-6	2.900	9.50 / 13.84	
Atenolol	Cardiovascular drug	-	$C_{14}H_{22}N_2O_3$	29122-68-7	0.335	9.43 / 13.88	
Carbamazepine	Psychiatric drug	-	$C_{15}H_{12}N_2O$	298-46-4	1.895	-0.49 / 13.94	

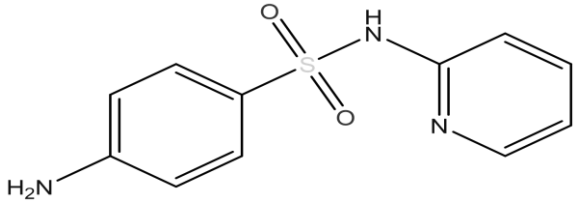
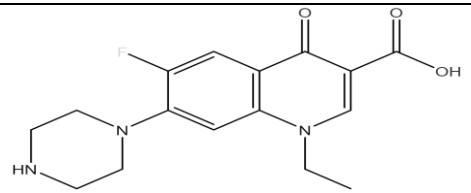
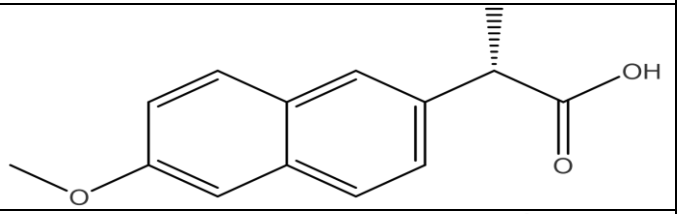
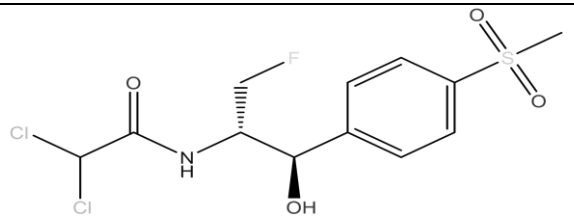
Progesterone	Hormone	-	$C_{21}H_{30}O_2$	57-83-0	3.827	-	
Estrone (E1)	Hormone	-	$C_{18}H_{22}O_2$	53-16-7	3.624	10.25	
DEET	Insect repellents	-	$C_{12}H_{17}NO$	134-62-3	2.419	-1.37 (MB)	
4-Nonylphenol	Surfactants	-	$C_{15}H_{24}O$	104-40-5	6.142	10.15 (MA)	

Bisphenol A	Industrial chemicals (plastic additives…)	-	$C_{15}H_{16}O_2$	80-05-7	3.641	10.29 (MA)	
Bisphenol Z	Industrial chemicals (plastic additives…)	Bisphenol	$C_{18}H_{20}O_2$	91174-67-3	-	-	
Bisphenol AF	Industrial chemicals (plastic additives…)	Bisphenol	$C_{16}H_{14}F_4O_2$	1478-61-1	9.5	-	

Bisphenol P	Industrial chemicals (plastic additives…)	Bisphenol	$C_{24}H_{26}O_2$	13595-25-0	-	-	
Bisphenol M	Industrial chemicals (plastic additives…)	Bisphenol	$C_{24}H_{26}O_2$	2167-51-3	-	-	
Caffeine	Stimulant	xanthines	$C_8H_{10}N_4O_2$	58-08-2.	-0.628	0.52 (MB)	
Crotamiton	Anti-itching drugs	-	$C_{13}H_{17}NO$	483-63-3	2.464	1.14	

Alprazolam	Anxiolytic	Benzodiazepines.	$C_{17}H_{13}ClN_4$	28981-97-7	2.12	-	
MEHP	Phthalat	-	$C_{16}H_{22}O_4$	103-09-3	5.3310.	-	
Metronidazole	Antimicrobial	-	$C_6H_9N_3O_3$	443-48-1	-0.135	2.58 / 14.44	
Nalidixic acid	Antibiotic	Quinolone	$C_{12}H_{22}N_2O_3$	389-08-2	0.025	3.45 / 6.12	

Tiamulin	Antibiotic	Pleuromutilin	$C_{28}\ H_{47}\ N\ O_4\ S$	55297-96-6	4,38 E +00	14,65 / 9,74	
Codeine Phosphate	Opioids	Narcotic analgesic	$C_{18}\ H_{21}\ N\ O_3 \bullet H_3PO_4$	52-28-8	-	8.22	
Dexametasone	Analgesic/Anti-inflammatory	Corticosteroid	$C_{22}\ H_{29}\ F\ O_5$	50-02-2	2.033	12.13	
17-beta-estradiol (E2)	Hormone	-	$C_{20}\ H_{24}\ O_2$	50-28-2	4.106	10.24	

Sulfapyridine	Precusor of compounds	-	$C_{11}H_{11}N_3O_2S$	144-83-2	0.469	2.13 / 8.54	
Norfloxacin	Antibiotic	Quinolone	$C_{16}H_{18}FN_3O_3$	70458-96-7	1.744	0.16 / 8.68	
Naproxen	Analgesic/Anti-inflammatory	-	$C_{14}H_{14}O_3$	22204-53-1	2.867	4.84	
Florfenicol	Antibiotic	-	$C_{12}H_{14}Cl_2FNO_4S$	73231-34-2	1.175	10,73 / -1,79	

Data for log P at 25°C and pKa at 25°C were sourced from pubchem.

Table S2. Chromatographic parameters

Time (min)	Flow (mL min^{-1})	Mobile pase A (%)
0	o.5	95
2.00	o.5	5
5.00	o.5	5
5.10	o.5	95
12.00	o.5	95

Table S3. List of SRMs and mass spectrometry instrumental conditions for A) the target analytes and B) the internal standards

A)

RT (min)	Analyte	Q_1 (m/z)	Q_3 (m/z)	DP (V)	CE (V)	CXP (V)
0.64	Atenolol 1	267.1	145.1	11	33	24
	Atenolol 2		190.3	11	29	4
0.80	Metronidazole 1	172.0	128.4	41	21	6
	Metronidazole 2		82.1	41	35	14
1.15	Sulfadiazine 1	251.0	155.9	71	23	20
	Sulfadiazine 2		108.2	71	31	12
1.50	Sulfathiazole 1	255.9	155.9	96	21	8
	Sulfathiazole 2		108.1	96	33	12

1.84	Sulfapyridine 1	250.1	156.1	61	23	10
	Sulfapyridine 2		108.1	61	35	12
1.93	Trimethoprim 1	291.0	230.1	51	33	18
	Trimethoprim 2		261.1	51	35	16
3.30	Apramycin 1	271.0	156.1	50	20	19
	Apramycin 2		180.0	50	40	19
3.64	Caffeine 1	195.0	137.9	71	27	18
	Caffeine 2		110.6	71	31	16
3.76	Ofloxacin 1	362.0	318.3	86	29	26
	Ofloxacin 2		261.1	86	37	14
3.82	Norfloxacin 1	320.1	276.2	96	27	18
	Norfloxacin 2		233.1	96	37	16
4.18	Florfenicol 1	357.8	339.9	66	13	24
	Florfenicol 2		241.3	66	25	14
4.42	Bisphenol A 1	227.0	227.1	-60	-14	-15
	Bisphenol A 2		211.1	-60	-26	-13
4.46	Propranolol 1	260.1	183.1	66	25	12
	Propranolol 2		116.1	66	25	8

4.52	Tiamulin 1	494.1	192.2	51	29	10
	Tiamulin 2		119.7	51	59	12
4.72	Tylosin 1	916.2	772.3	156	43	36
	Tylosin 2		174.1	156	51	10
4.79	Nalidixic acid 1	233.1	187.1	21	37	18
	Nalidixic acid 2		159.9	21	45	18
4.86	Carbomazepine 1	237.0	194.2	66	29	12
	Carbomazepine 2		193.3	66	47	6
4.98	DEET 1	192.0	119.3	56	23	10
	DEET 2		90.3	56	41	10
5.03	Dexamethasone 1	392.9	355.1	41	19	20
	Dexamethasone 2		147.3	41	39	10
5.06	4-nonylphenol 1	219.1	132.9	-65	-42	-7
	4-nonylphenol 2		117.0	-65	-80	-13
5.13	Naproxen 1	231.1	185.1	56	21	12
	Naproxen 2		170.5	56	37	10
5.14	Atorvastatin 1	559.2	440.2	26	33	38
	Atorvastatin 2		250.1	26	59	18

5.32	Ibuprofen 1	205.0	159.1	-35	-10	-15
	Ibuprofen 2		160.9	-35	-12	-21
5.37	Progesterone 1	315.1	109.2	141	31	10
	Progesterone 2		297.2	141	23	28
5.39	Estrone (E1) 1	271.1	253.3	101	19	10
	Estrone (E1) 2		133.1	101	35	12
5.50	β-Estradiol (E2) 1	273.0	255.0	46	17	14
	β-Estradiol (E2) 2		107.8	61	41	14
5.55	Crotamiton 1	204.1	69.4	61	35	12
	Crotamiton 2		136.1	61	27	14
5.56	Gemfibrozil 1	248.9	121.0	-5	-30	-7
	Gemfibrozil 2		127.5	-85	-14	-5
5,28	Bisphenol P 1	345	329	-15	-36	-23
	Bisphenol P 2		315	-15	-48	-17
5.26	Bisphenol M 1	345	329	-115	-40	-17
	Bisphenol M 2		250	-115	-38	-15
5.01	Bisphenol Z 1	267	173	-65	-36	-9
	Bisphenol Z 2		145	-65	-48	-9

4.99	Bisphenol AF 1	335	266	-40	-32	-15
	Bisphenol AF 2		198	-40	-52	-11
4.54	Levofloxacin 1	362	318	6	27	20
	Levofloxacin 2		261	6	37	24
4.78	Bisphenol A 1	226	133	-5	-32	-19
	Bisphenol A 2		211	-60	-26	-13
5.43	MEHP 1	277	233	-20	-20	-13
	MEHP 2		133	-20	-22	-17
4.76	Alprazolam 1	309	280	81	37	16
	Alprazolam 2		204	81	57	24

Q_n: Mass of pseudion ``n´´; DP: entry orifice potencial; CE: collision energy; CXP: collision cell exit potencial

B)

RT (min)	Internal standard	Q_1 (m/z)	Q_3 (m/z)	DP (V)	CE (V)	CXP (V)
1.5	Sulfadiazine-d4	255.075	160	51	23	16
3.76	Sulfadimidine-d4	283.053	186	61	25	20
4.04	Ciprofloxacin-d8	340.107	322.1	46	31	20
4.15	Sulfamethoxazole-d4	258.064	160.1	41	23	10
4.16	Danofloxacin-d3	361.059	343.1	66	35	20

4.18	Enrofloxacin-d5	364.923	321	91	29	20
4.42	Salicylic acid-d4	140.930	97	-30	-22	-5
4.46	Bisphenol A-d8	235.064	137	-80	-38	-7
4.48	Methylparaben-d4	156.971	125.1	81	23	12
4.84	Ethylparaben-d5	169.898	137.9	-45	-20	-7
5.03	Propylparaben-d7	185.991	136.1	-45	-24	-9
5.14	Clofibric acid-d4	216.924	130.8	-5	-28	-13
5.14	Naproxen-d3	234.107	188.1	41	21	10
5.37	Diclofenac-d4	297.961	254	-5	-18	-31
5.37	Ibuprofen-d3	208.034	161.1	-15	-12	-17
5.5	Triclosan-d3	289.897	289.9	-5	-6	-21

Q_n: Mass of pseudion ``n´´; DP: entry orifice potencial; CE: collision energy; CXP: collision cell exit potencial