Microchemical Journal 191 (2023) 108869

Contents lists available at ScienceDirect

Microchemical Journal

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

Synthetic cathinones determination by liquid chromatography-mass spectrometry after ultrasound membrane assisted extraction

Cristian Suárez-Oubiña [a], Pamela Cabarcos-Fernández [b], Maria Jesús Tabernero-Duque [b], Pilar Bermejo-Barrera [a], Antonio Moreda-Piñeiro [a], Ana María Bermejo-Barrera [b,*]

[a] Trace Element, Spectroscopy and Speciation Group (GETEE), Institute of Materials iMATUS, Department of Analytical Chemistry, Nutrition and Bromatology, Faculty of Chemistry, University of Santiago de Compostela, Avenida das Ciencias, s/n., 15782 Santiago de Compostela, Spain
[b] Forensic Sciences Institute "Luís Concheiro" (INCIFOR), Faculty of Medicine, University of Santiago de Compostela, Rúa de San Francisco, s/n, 15782 Santiago de Compostela, Spain

ARTICLE INFO

Keywords:
Synthetic cathinones
Urine
LC-MS/MS
Ultrasounds
Membrane assisted solvent extraction

ABSTRACT

The extraction time for membrane-assisted solvent extraction (MASE) when isolating synthetic cathinones from urine has been speeded up to 30 min by using ultrasounds. Separation and determination of eleven synthetic cathinones was further performed liquid chromatography-tandem mass spectrometry (LC-MS/MS). The ultrasound assisted MASE consisted of adjusting 5.0 mL of urine at pH 11.8 and performing the extractive process with 400 μL of n-hexane as an acceptor phase inside the polypropylene membrane under ultrasonication at room temperature. Synthetic cathinones exhibiting LogP values higher than 2.0, such as 3,4-dimethyl methcathinone, methylendioxypyrovalerone, and naphyrone), were efficiently extracted and pre-concentrated. Other synthetic cathinones (LogP between 2.0 and 0.7) were found to exhibit a lower mass transfer. The method was found to be matrix dependent, and a matrix-matched calibration was required for measurements. The achieved limits of detection (LOD) were between 0.03 (flephedrone) to 0.29 (ethylone) μg L-1, with relative standard deviations (RSDs) within the 6–14% and 7–19% ranges for intraday and inter-day assays, and intraday and inter-day analytical recoveries from 84 to 115% and 85 to 118%, respectively. The developed method was finally applied to urine samples from volunteers attending a music festival.

1. Introduction

Data from the World Drug Report 2021 of the United Nations Office on Drugs and Crime (UNODC) confirms an increasing abuse of synthetic stimulants world-wide, being amphetamines and cathinones more popular than cocaine in several countries from South-Eastern and Eastern Europe [1]. The high use of synthetic cathinones is a consequence of a psychoactive effect like that obtained after cocaine and amphetamines consumption [2], but also because of their higher accessibility ('legal' substances) and lower price when comparing with other amphetamine-type stimulants.

In addition to the need to use highly sensitive instrumental techniques [3,4] and highly selective and efficient sample pre-treatments [5], the determination of synthetic cathinones in clinical-forensic samples requires that the sample pre-treatment is not dependent on the chemical structure of the cathinone, allowing therefore the simultaneous extraction of all possible cathinones that could be synthesized in clandestine laboratories. Sample pre-treatments based on conventional liquid–liquid extraction (LLE) [6–12] and solid phase extraction (SPE) [13–23] have been demonstrated as useful methodologies for the simultaneous isolation of synthetic cathinones from urine and blood. In addition, micro-extraction techniques such as dispersive liquid–liquid microextraction (DLLME) [24,25] and solid phase microextraction (SPME) [26] have been successfully applied for synthetic cathinones extraction from biological fluids.

Among the several membrane-based extraction techniques, membrane-assisted solvent extraction (MASE) is based on an equilibrium mechanism between the concentration of the target in a liquid donor phase (sample) and a liquid acceptor phase (organic or aqueous) by diffusion of targets through the pores of a microporous membrane. Therefore, targets transfer through the membrane pores is controlled by the partition coefficients of analytes and chemical equilibrium is required. Mechanical shaking and the use of elevated temperatures increase analyte transfer kinetics [27], and MASE applications have

* Corresponding author.
E-mail address: anamaria.bermejo@usc.es (A.M. Bermejo-Barrera).

https://doi.org/10.1016/j.microc.2023.108869
Received 30 March 2023; Received in revised form 10 May 2023; Accepted 11 May 2023
Available online 16 May 2023

therefore been focused on isolating organic compounds from waters [28–35], as a pre-concentration/clean-up procedure of organic pollutants [36,37] in extracts from solid matrices, and for synthetic cannabinoid receptor agonists in urine [38]. The application of ultrasound for speeding up the MASE process has not been tested, and the aim of this research has been to explore possibilities of ultrasound assistance for enhancing mass transfer in MASE. The study has been focused on synthetic cathinones in urine and on using LC-MS/MS for quantitative purposes.

2. Materials and methods

2.1. Instrumentation

Liquid chromatography–tandem mass spectrometry measurements were performed with an UHPLC Flexar binary pump with integrated vacuum degasser (Perkin Elmer, Waltham, MA, USA) equipped with a 3200 QTRAP ABSciex (Concord, Canada) and a Flexar UHPLC autosampler (Perkin Elmer). Chromatographic column thermostatization was achieved with a GECKO-2000 oven (Amchro GmbH, Hattersheim, Germany). Chromatographic separations were performed with a Zorbax Eclipse Plus C18 column (4.6 × 100 mm, 3.5 μm) from Agilent Technologies (Santa Clara, CA, USA) connected to a C18 pre-column (4.0 mm length × 3.0 mm i.d.) from Phenomenex (Torrance, CA, USA). MultiQuant 2.1 software (ABSciex) was used for data handling. The MASE sample pre-treatment was carried out with 10 mL glass vials equipped with polypropylene (PP) membranes (4.0 cm × 6.0 mm i.d., wall thickness of 0.03 mm), stainless steel funnels with PTFE ring, Viton rings and metallic ring caps, purchased from Gerstel (Mülheim, Germany). Extracts evaporation was performed with a metal block thermostat (VLM EC1) under N_2 stream (VLM, Leopoldshöhe-Greste, Germany). Other laboratory devices were: a Rotabit temperature-controlled mechanical stirring chamber (J.P. Selecta, Barcelona, Spain), a magnetic shaker (Labbox Labware, Barcelona, Spain), a vortex shaker (Heidolph REAX 2000, Schawabach, Germany), thermostatized ultrasound cleaner baths USC60TH (45 kHz, 120 W) from VWR (Leuven, Belgium) and CD-4820 (42 kHz, 70 W) from Jeken (Guangdong, China), and a Basic 20 pH-meter (Crison Instruments, Barcelona, Spain).

2.2. Reagents

Ultrapure water 18 MΩ.cm of resistivity was obtained from a Milli-Q purification system from Millipore Co. (Bedford, MA, USA). The synthetic cathinones methylone hydrochloride, ethylone hydrochloride, flephedrone hydrochloride, penthylone hydrochloride, naphyrone hydrochloride, 3-methylmethcathinone (methcathinone, or 3-MMC), methedrone hydrochloride, and butylone hydrochloride were supplied by Cayman Chemical (Ann Arbor, MI, USA). Other synthetic cathinones, such as cathinone hydrochloride, 3,4-dimethylmethcathinone hydrochloride (3,4-DMMC), and methylenedioxypyrovalerone (MDPV) were supplied by Cerilliant (Round Rock, TX, USA). Deuterated analogues methylone-d_3 hydrochloride and ethylone-d_3 hydrochloride (Cayman Chemical) were used as internal standards. The solid standards were dissolved in adequate volumes of acetonitrile to prepare stock standard solutions at 1.0 mg mL^{-1}. Solid standards, as well as acetonitrile stock solutions, were stored at −20 °C. Organic solvents such as HPLC LiChrosolv grade acetonitrile, LC-MS LiChrosolv hypergrade acetonitrile), 1-butanol, dicloroethane, and dicloromethane were from Merck (Darmstadt, Germany); whereas 99.8% trichloromethane and n-hexane were from PanReac (Barcelona, Spain), and HPLC grade methanol was from Sigma-Aldrich (Steinheim, Germany). Other chemicals were sodium acetate, sodium hydroxide, and monohydrated sodium hydrogencarbonate (Merck); monohydrated sodium dihydrogen phosphate, heptahydrated sodium hydrogen phosphate (J.T. Baker. Deventer, Netherlands); 96% acetic acid, 85% formic acid, potassium chloride, anhydride sodium carbonate (PanReac); and sodium fluoride and 1-methyl-3-octylimidazolium tetrafluoroborate (Sigma-Aldrich).

2.3. Preparation of working standard solutions

Working solutions (0.1 mg mL^{-1} each cathinone), used for calibration and spiking experiments, were prepared from single stock solutions of each cathinone using acetonitrile as a solvent. Internal standard stock solution of methylone-d_3 and ethylone-d_3 at 0.1 mg mL^{-1} each one was also prepared in acetonitrile. All standard solutions were stored at −20 °C.

2.4. Urine samples

Drug-free urine samples were from laboratory staff volunteers. These samples were used for method validation. Other urine samples (used for verifying the applicability of the method) consisted of forensic cases received at the Forensic Sciences Institute "Luís Concheiro" (INCIFOR) at the University of Santiago de Compostela. For all cases, urine samples were collected in clean sealed polyethylene vials, and they were kept at − 20 °C when necessary.

2.5. MASE procedure

2.5.1. Ultrasound assisted – MASE procedure

MASE procedure was performed with 5.0 mL of urine (or drug-free urine spiked with 1.0 μg L^{-1} of each cathinone for validation studies) placed in a 10 mL vial and after pH adjustment at 11.8 (use of 0.1 M sodium hydroxide dropwise). For all cases, deuterated analogues methylone-d_3 and ethylone-d_5 (internal standards) at 0.5 μg L^{-1} were added. The PP membrane was then filled with 400 μL of n-hexane and the device was subjected to ultrasounds (45 kHz y 120 W) for 30 min. The recovered extract was them transferred to a conical glass tube and the empty PP bag was rinsed with 200 μL of n-hexane (rinsing was mixed with the previous n-hexane extract). Finally, the extract was subjected to dryness (N_2 stream), and the residue was re-dissolved in 100 μL of acetonitrile by assisting with vortex shaking. The pre-concentration factor of the MASE procedure is therefore 50.

2.5.2. Polypropylene membrane decontamination/cleaning procedure

The whole MASE device (PP membrane, Teflon rings and conic metallic pieces) were subjected to a cleaning stage to avoid carryover contamination. The procedure consists of soaking the MASE pieces into n-hexane and subjecting them to ultrasound (42 kHz, 170 W, 10 min). Finally, MASE pieces were air-dried at room temperature.

2.6. Liquid chromatography–tandem mass spectrometry measurement

Cathinones separation was performed under gradient conditions (Table 1SM, ESI) using aqueous 0.1 %(v/v) formic acid and 0.1 %(v/v) formic acid in acetonitrile as mobile phases at a fixed flow rate of 0.30 mL min^{-1}. Chromatographic separation was carried out at 40 °C. Data acquisition for MRM [(m/z (precursor ion) → m/z (product ion)] transitions (positive ion mode) are shown in Table 2SM. At least two MRM transitions (quantifier and qualifier transitions) were used for verifying the presence of cathinones in forensic samples. Selected electrospray ionization source parameters (desolvation gas temperature, electrode voltage, and collide, curtain, conical, and auxiliary gas flows) are also listed in Table 2SM.

Standards prepared in acetonitrile and within the 10–200 μg L^{-1} concentration range (plus 25 μg L^{-1} of methylone-d_3 and ethylone-d_5 as internal standards) were used when optimising the ultrasound assisted MASE procedure. Method validation was performed by subjecting drug-free urine samples to the MASE procedure after spiking the urine aliquots at cathinones concentrations between 0.1 and 4.0 μg L^{-1} (concentrations within the 5.0–200 μg L^{-1} range after pre-concentration) and at 0.5 μg L^{-1} for the internal standards (concentration of 25 μg L^{-1} after

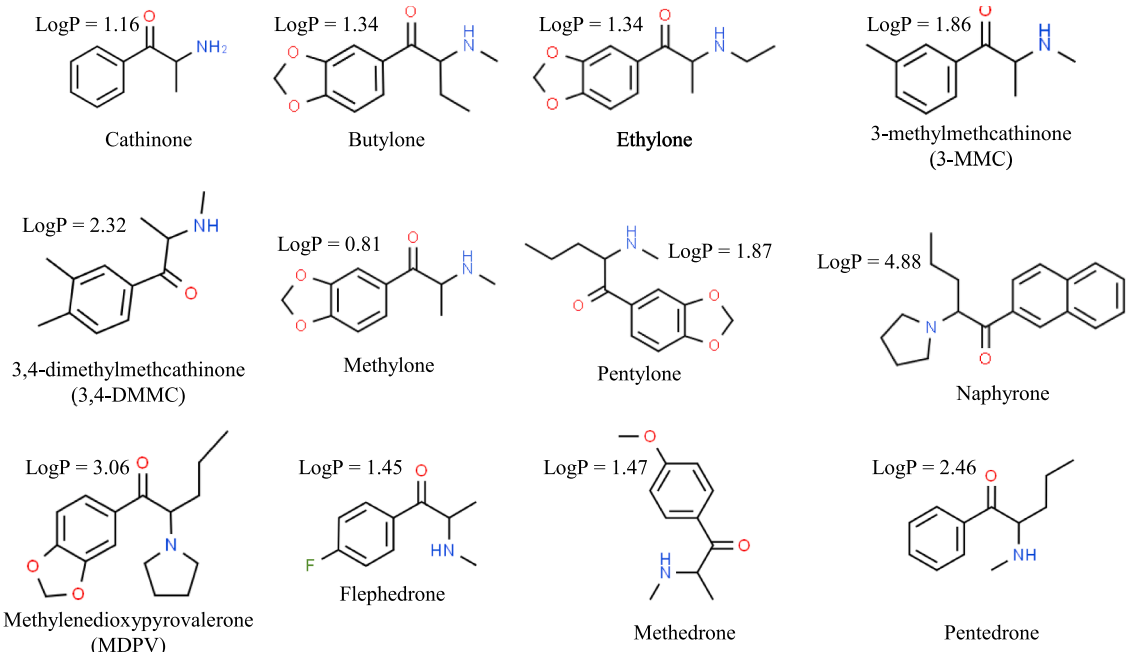

Fig. 1. Chemical structures and LogP of the synthetic cathinones under study.

pre-concentration). Each spiked concentration level (matrix-matched calibration, MMC) was tested in triplicate for performing the analysis of forensic urine samples. Fig. 1 shows the cathinones' structure involved in the current research together with their predicted ACD LogP (ACD/Labs Percepta Platform - PhysChem Module) [39].

3. Results and discussion

3.1. Preliminary experiments for MASE

Organic solvents of low volatility are required as suitable acceptor solvents in MASE since evaporation of the solvent during the extraction process must be minimised. Therefore, n-hexane has been commonly used as acceptor solvent in MASE for the isolation of a great variety of organic compounds which are soluble in polar solvents, or which are easily protonated/deprotonated to reduce polarity [30–36,39]. Based on the octanol–water partition coefficient (LogP values) [42] listed in Fig. 1, cathinones like 3,4-DMMC (LogP = 2.32), pentedrone (LogP = 2.46), MDPV (LogP = 3.06), and naphyrone (LogP = 4.88) would be conveniently extracted, whereas the extraction efficiency of cathinones exhibiting low LogP values, like methylone (LogP = 0.81), would be limited. The pH of the donor phase (urine sample) will play an important role by reducing the polarity of the substances and allowing a better extraction. Preliminary experiments (data not given) have shown that cathinones extraction is enhanced when fixing the pH of the urine sample at alkaline values. On the other hand, MASE is a non-exhaustive technique and has been reported to require long extractive times, even 90 min [39], when using conventional assistance with mechanical or magnetic stirring.

A first set of experiments have been led to establish the convenience of ultrasound assistance to speed up analyte transfer in MASE. Therefore, drug-free urines (5.0 mL) adjusted at pH 10 (0.1 M sodium hydroxide dropwise) and spiked with cathinones and internal standards (2.5 μg L^{-1} each one) were subjected in triplicate to a MASE procedure based on mechanical (orbital – horizontal) stirring at 200 rpm for 10 min, and to ultrasound assisted MASE process at 45 kHz (120 W) also for 10 min. A volume of 400 μL of n-hexane was used as an acceptor phase for all cases, and reagent blanks (5.0 mL of ultrapure water at pH 10 with internal standards at 2.5 μg L^{-1} each one) were also prepared. The amount of extracted cathinones was assessed by using a calibration in acetonitrile covering the 0–200 μg L^{-1} concentration levels. Results in Fig. 2 show an increase on the analytical recovery, mainly for cathinones exhibiting low LogP values (cathinone, methylone, ethylone and butylone), when assisting the extraction with ultrasounds. Analytical recoveries close to 80% have been obtained for cathinones of high LogP values (3,4-DMMC, pentedrone, MDPV, and naphyrone) under ultrasound assistance which suggests further quantitative recoveries after an accurate optimization of the pH and the extraction time. Better extractability with ultrasounds must be attributed to changes on cathinones diffusion through the membrane microporous being less notorious the mass transfer by the concentration gradient mechanism (higher targets concentration in the donor phase than in the acceptor phase into

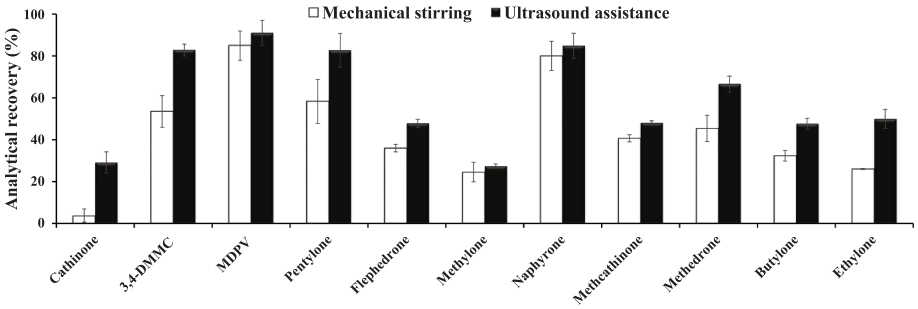

Fig. 2. Effect of mechanical stirring and ultrasounds for MASE assistance on the analytical recovery of synthetic cathinones.

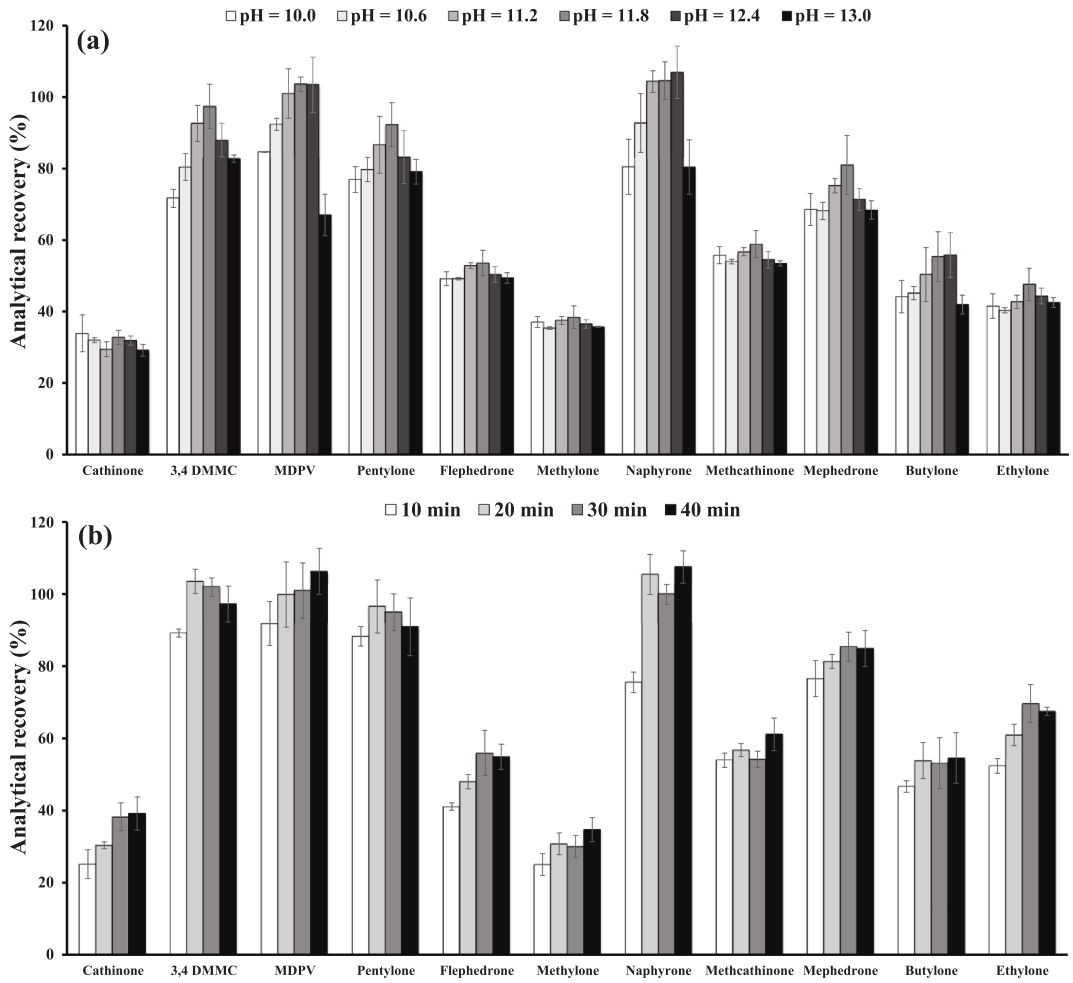

Fig. 3. Effect of urine pH (a) and extraction time (b) on the analytical recovery of synthetic cathinones.

the membrane) in MASE.

3.2. Parameters affecting the ultrasound assisted MASE procedure

As previously commented, sample (urine) pH is quite important since the cathinone extraction efficiency is affected by the ionization degree of targets (high extractability in a non-polar solvent when reducing the polarity of the analytes). Preliminary experiments at neutral and close to neutral pHs (7.0–9.0) were unsuccessful, and the effect of the pH was studied within the 10–13 range. Each pH was tested in triplicate by subjecting 5.0 mL drug-free urine samples (spiking experiments at 2.5 μg L^{-1} for each cathinone and internal standard) to ultrasound (45 kHz, 120 W) assisted MASE for 10 min, using 400 μL of n-hexane as an acceptor phase. Reagent blanks (ultrapure water at the selected pH plus internal standards at 2.5 μg L^{-1}) were also prepared. Analytical recoveries obtained against a 0–200 μg L^{-1} calibration in acetonitrile (Fig. 3(a)) are improved when using pHs within the 10.6–11.8 range, and quantitative recoveries were obtained for some cathinones such as 3,4-DMMC, pentedrone, MDPV, and naphyrone (cathinones exhibiting the highest LogP values). Extraction (non-quantitative) was also enhanced for flephedrone, mephedrone, methcathinone, ethylone and butylone (moderate LogP values) at pHs of 11.2 and 11.8; whereas pH influence was poor for cathinones with the lowest LogP values (cathinone and methylone). A pH of 11.8 was finally selected as a compromise pH value for further

Table 1
Calibration, LOD, LOQ, and matrix effect.

Compound	LOD (μg L^{-1})[a,b]	LOQ (μg L^{-1}) [a,b]	Standard Calibration Slope (A_{ratio} μg^{-1} L)[c]	R^2	Matrix-Matched calibration Slope (A_{ratio} μg^{-1} L)[c]	R^2	Matrix effect (%)[c]
Cathinone	0.20	0.66	0.485 ± 0.011	< 0.998	0.008 ± 0.001	< 0.997	74
3,4-DMMC	0.16	0.53	2.755 ± 0.124	< 0.995	0.317 ± 0.109	< 0.997	84
MDPV	0.21	0.70	0.709 ± 0.097	< 0.997	0.286 ± 0.043	< 0.996	81
Pentylone	0.07	0.23	0.539 ± 0.092	< 0.996	0.072 ± 0.012	< 0.995	79
Flephedrone	0.03	0.10	0.630 ± 0.022	< 0.998	0.030 ± 0.007	< 0.996	80
Methylone	0.12	0.38	0.386 ± 0.022	< 0.998	0.012 ± 0.003	< 0.998	79
Naphyrone	0.28	0.94	0.839 ± 0.094	< 0.999	0.269 ± 0.037	< 0.998	80
Methcathinone	0.07	0.23	0.791 ± 0.065	< 0.995	0.035 ± 0.012	< 0.995	74
Methedrone	0.03	0.10	1.091 ± 0.060	< 0.996	0.057 ± 0.006	< 0.998	76
Butylone	0.19	0.65	2.212 ± 0.241	< 0.996	0.115 ± 0.020	< 0.997	77
Ethylone	0.29	0.96	1.897 ± 0.267	< 0.997	0.085 ± 0.009	< 0.997	76

(a) blank measurements (n = 11); (b) pre-concentration factor opf 50; (c) n = 4

Table 2
Comparison of the LOD (μg/L) and LLOQ (μg/L) of different published methods with the one proposed in this study for 11 cathinones. LLOQs are indicated in bold and LODs in parentheses.

Ref.	Methylone	Ethylone	Flephedrone	Penthylone	Naphyrone	Methcathinone	Methedrone	Buthylone	Cathinone	3,4-DMMC	MDPV
7	–	–	–	–	–	**13** (5)	–	**5** (2)	**8** (4)	–	**4** (2)
8	**50**	–	–	–	–	**25**	–	–	–	–	–
10	–	**100** (30)	**100** (10)	–	**100** (30)	**100** (10)	**100** (10)	**100** (10)	–	–	–
12	–	–	–	–	–	–	–	–	–	–	**0.02**
14	(15)	–	–	(0.7)	(0.7)	(0.7)	(15)	–	(15)	–	(15)
15	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)	**0.5** (0.25)
16	**2.5** (1)	**2.5** (2.5)	–	**2.5** (1)	**2.5** (1)	**2.5** (1)	**2.5** (1)	**2.5** (1)	**2.5** (1)	**5** (5)	**2.5** (1)
17	**1** (0.25)	**5** (1)	–	**5 (1)**	**0.5** (0.5)	**0.25** (0.25)	–	**2** (1)	–	**5** (5)	**2** (1)
18	**1.14** (0.68)	**0.54** (0.32)	**1.58** (0.96)	**0.24** (0.14)	**1.46** (0.88)	–	–	**0.84** (0.5)	**2.52** (1.51)	**1.70** (1.02)	**1.26** (0.76)
19	(0.011)	–	(0.099)	–	–	(0.027)	(0.052)	–	–	–	–
20	–	–	**0.2** (0.16)	–	–	–	–	**0.2** (0.04)	–	**0.2** (0.04)	**0.2** (0.16)
Our method	**0.38** (0.12)	**0.96** (0.29)	**0.10** (0.03)	**0.23** (0.07)	**0.94** (0.28)	**0.23** (0.07)	**0.10** (0.03)	**0.65** (0.19)	**0.66** (0.20)	**0.53** (0.16)	**0.7** (0.21)

experiments.

The extraction (sonication) time was studied within the 10–40 min range by using 5.0 mL drug-free urine aliquots at pH 11.8 and spiked at 2.5 μg L^{-1} for each cathinone and internal standard, 400 μL of n-hexane as an acceptor phase, and ultrasounds (45 kHz, 120 W). Blanks (ultrapure water at a pH of 11.8 and spiked with internal standards at 2.5 μg L^{-1}) were also prepared, and analytical recoveries (use of calibrations in acetonitrile) obtained for each extraction time (experiments in triplicate) are plotted in Fig. 3(b). The highest analytical recoveries have been observed for sonication times of 20, 30 and 40 min, although cathinone, flephedrone, and ethylone recoveries were found to be slightly lower with an extraction time of 20 min. Since similar extraction efficiencies were achieved at sonication times of 30 and 40 min, the sonication (extraction) time was fixed at 30 min which give a fast sample pretreatment.

3.3. Analytical performances

Since the different sensitivity of cathinones, the calibration ranges varied from 5.0 to 200 μg L^{-1} (flephedrone, mephedrone, pentylone, and methcathinone), and from 10 to 250 μg L^{-1} (cathinone, 3,4-DMMC, MDPV, methylone, naphyrone, butylone, and ethylone). For all cases, seven-point acetonitrile calibration curves and seven-point matrix matched calibrations (drug-free urines spiked with the targets at concentrations ranging from 0 to 5.0 μg L^{-1} and subjected to the MASE procedure) have revealed correlation coefficients (R^2) higher than 0.995 (calibration) and 0.9961 (matrix matched calibrations) for all targets (Table 1). Mean slopes and standard deviations for four calibration and matrix matched calibrations are also listed in Table 1, and high differences between slopes of both calibration graphs have been found for all targets. Therefore, a matrix matched calibration must be used for an accurate assessment of cathinones.

Matrix effect (ME), defined as signal suppression or enhancement by matrix constituent which coelutes with the target analytes, was evaluated by comparing the peak areas belong to 200 μg L^{-1} standard solutions in acetonitrile (B) and the peak areas measured for a drug-free urine sample spiked also at 200 μg L^{-1} (A) according to [40]

$$ME(\%) = \frac{B}{A} x100 \quad (1)$$

As listed in Table 1, ME(%) values have found to vary between 74% (cathinone and methcathinone) to 84% (3,4-DMMC) which implies ME (%) values slightly lower than 100% and hence low signal suppression (low ME).

The assessment of the limits of detection (LODs) and the limits of quantification (LOQs) were based on the mean blank + 3 SD criterion (LOD) and mean blank + 10 SD criterion (LOQ) where mean blank and SD are the mean value and the standard deviation of eleven procedural blanks (ultrapure water subjected to the optimised MASE procedure). The peak area values obtained were then divided by the mean slopes of the matrix matched calibration to obtain the LODs/LOQs referred to concentrations. Table 1 lists the LOD and LOQ values considering the pre-concentration factor of 50. LOQ values ranged from 0.10 μg L^{-1} for flephedrone and methedrone to 0.96 μg L^{-1} for ethylone. These LOD/LOQ values are quite lower than those previously reported by other authors for synthetic cathinones in urine (Table 2) allowing quantitative analysis of synthetic cathinones in urine (most of the reported methods in Table 2 are used for screening purposes).

The precision of method was proved by intraday (n = 9) and inter-day (n = 7) assays with extracts prepared from drug-free urine samples spiked at 0.30 μg L^{-1} (low level) and 1.5 μg L^{-1} (high level). The RSD (%) obtained (Table 3) varied from 6% to 14% for intraday assays and from 7% to 19% for inter-day tests.

Table 3 also shows the assessed analytical recovery from intraday (n = 7) and inter-day (n = 7) assays with drug-free urine samples spiked at 0.30 μg L^{-1} (low level) and 1.5 μg L^{-1} (high level). Good analytical

Table 3
Intraday and inter-day precision and analytical recovery of the method.

Compound	Intraday assay[a] Low level (0.30 μg L^{-1}) Analytical recovery (%)	RSD (%)	High level (1.5 μg L^{-1}) Analytical recovery (%)	RSD (%)	Inter-day assay[b] Low level (0.30 μg L^{-1}) Analytical recovery (%)	RSD (%)	High level (1.5 μg L^{-1}) Analytical recovery (%)	RSD (%)
Cathinone	84 ± 8	14	88 ± 3	7	94 ± 8	17	103 ± 9	19
3,4-DMMC	112 ± 6	12	112 ± 7	11	116 ± 9	17	107 ± 6	12
MDPV	104 ± 9	14	89 ± 5	11	85 ± 10	16	93 ± 7	15
Pentylone	87 ± 6	15	106 ± 5	10	95 ± 9	19	105 ± 6	12
Flephedrone	92 ± 6	12	101 ± 3	7	91 ± 8	17	110 ± 6	12
Methylone	91 ± 10	15	85 ± 5	10	93 ± 6	12	118 ± 10	19
Naphyrone	91 ± 5	10	97 ± 3	6	97 ± 9	19	94 ± 5	10
Methcathinone	112 ± 10	14	104 ± 9	18	98 ± 9	18	96 ± 7	14
Methedrone	107 ± 7	13	114 ± 7	12	104 ± 9	19	115 ± 4	7
Butylone	103 ± 7	15	118 ± 9	16	99 ± 7	14	94 ± 8	16
Ethylone	102 ± 6	12	115 ± 13	21	114 ± 6	11	109 ± 7	14

(a) n = 9; (b) n = 7

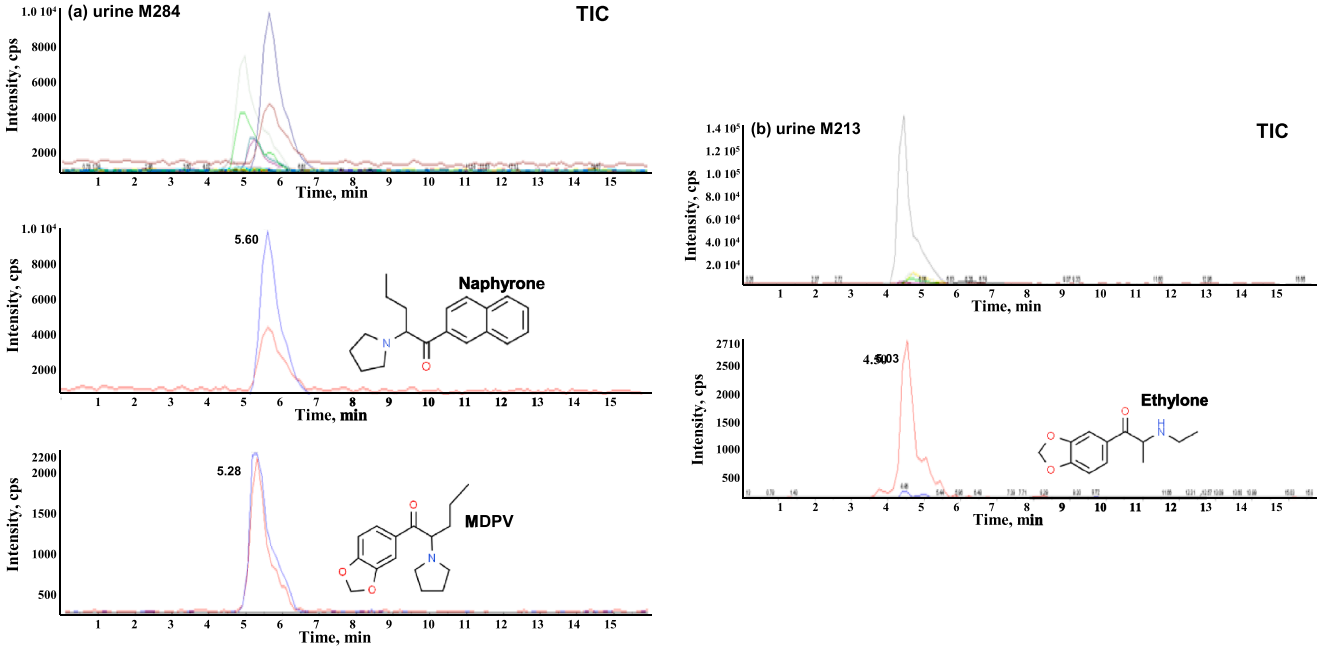

Fig. 4. Total ion and ion extracted chromatograms for urine sample M284 (a) and M213 (b).

recovery was obtained for both intraday (84–115%) and inter-day (85–118%) assays.

Finally, selectivity was studied by analysing eleven drug-free urine samples (blanks) from different volunteers by ultrasound assisted MASE and LC-MS/MS, and there were not found chromatographic signals at the retention times and selected precursor ion → product ion transitions for each target.

3.4. Applications

The MASE LC-MS/MS procedure has been applied to nine urine samples from forensic cases (anonymous individuals attending a music festival). After sample preparation in triplicate and LC-MS/MS analysis in duplicate, only urine samples coded as M284 and M213 were found to contain synthetic cathinones (Fig. 4). Naphyrone (48.3 ± 3.87 μg L^{-1}) and MDPV (1.40 ± 0.191 μg L^{-1}) were quantified in sample M284 (Fig. 4a), whereas ethylone (3.06 ± 0.210 μg L^{-1}) was found in sample M213 (Fig. 4b). MDPV was also detected in urine M213, but the concentration was between the LOQ and the LOD of the method. For all cases the presence of the analytes was verified by monitoring the second $m/z \rightarrow m/z$ transitions as well as the ratio between the peak areas of the second $m/z \rightarrow m/z$ transition and the first $m/z \rightarrow m/z$ transition (transition used for quantification). Additional analysis by COBAS as a screening method gave positive for amphetamines and cannabis in sample M213, and for amphetamines in sample M284.

4. Conclusions

The efficiency of MASE as a sample pre-treatment for target isolation from clinical-forensic samples has been found to be enhanced by assisting the extractive procedures by ultrasound instead of conventional mechanical and magnetic stirring. Naphyrone, 3,4-DMMC, and MDPV (targets with a high Log P) have shown the highest extraction efficiencies, whereas extractability of the remaining synthetic cathinones was found to be moderate. A matrix-matched calibration was therefore required for guaranteeing the accuracy of the extractive process, achieving quantitative analytical recoveries for all targets under study. The proposed method allows the simultaneous identification and quantification of eleven cathinones in urine samples. The application of the developed procedure to urine samples from forensic cases has shown the presence of naphyrone, MDPV and ethylone, the latter at very low concentrations, which means a great applicability scope of the

developed extractive process.

Funding

This work was supported by the *Dirección Xeral de I + D – Xunta de Galicia Grupos de Referencia Competitiva* (project number ED431C 2022/029).

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

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2023.108869.

References

[1] United Nations Office on Drugs and Crime (UNODC), 2021. World Drug Report 2021 Drug market trends: Cocaine amphetamine-type stimulants. United Nations Office on Drugs and Crime, Wien. https://www.unodc.org/unodc/en/data-and-analysis/wdr-2021_booklet-4.html (accessed 16 December 2022).

[2] M.L. Banks, T.J. Worst, D.E. Rusyniak, J.E. Sprague, The synthetic cathinones (the "bath salts"), J. Emerg. Med. 46 (2014) 632–642, https://doi.org/10.1016/j.jemermed.2013.11.104.

[3] M.R. Meyer, H.H. Maurer, Review: LC coupled to low- and high-resolution mass spectrometry for new psychoactive substance screening in biological matrices - Where do we stand today? Anal. Chim. Acta 927 (2016) 13–20, https://doi.org/10.1016/j.aca.2016.04.046.

[4] A. Perez-Alcaraz, F. Borrull, M. Calull, C. Aguilar, Cathinones in urine samples: A review of recent advances for their determination by chromatographic and related techniques, Trends Anal. Chem. 143 (2021), 116347, https://doi.org/10.1016/j.trac.2021.116347.

[5] A.Y. Simao, M. Antunes, H. Marques, T. Rosado, S. Soares, J. Goncalves, M. Barroso, M. Andraus, E. Gallardo, Recent bionalytical methods for the determination of new psychoactive substances in biological specimens, Bioanalysis 12 (2020) 1557–1595, https://doi.org/10.4155/bio-2020-0148.

[6] M. Paul, J. Ippisch, C. Herrmann, S. Guber, W. Schultis, Analysis of new designer drugs and common drugs of abuse in urine by a combined targeted and untargeted LC-HR-QTOFMS approach, Anal. Bioanal. Chem. 406 (2014) 4425–4441, https://doi.org/10.1007/s00216-014-7825-5.

[7] K.S. Hagan, L. Reidy, Detection of synthetic cathinones in victims of sexual assault, Forensic Sci. Int. 257 (2015) 71–75, https://doi.org/10.1016/j.forsciint.2015.07.040.

[8] V. Uralets, S. Rana, S. Morgan, W. Ross, Testing for designer stimulants: metabolic profiles of 16 synthetic cathinones excreted free in human urine, J. Anal. Toxicol. 38 (2014) 233–241, https://doi.org/10.1093/jat/bku021.

[9] E. Gerace, D. Caneparo, F. Borio, A. Salomone, M. Vincenti, Determination of several synthetic cathinones and an amphetamine-like compound in urine by gas chromatography with mass spectrometry. Method validation and application to real cases, J. Sep. Sci. 42 (2019) 1577–1584, https://doi.org/10.1002/jssc.201801249.

[10] M.K. Woźniak, L. Banaszkiewicz, M. Wiergowski, E. Tomczak, M. Kata, B. Szpiech, J. Namieśnik, M. Biziuk, Development and validation of a GC-MS/MS method for the determination of 11 amphetamines and 34 synthetic cathinones in whole blood, Forensic Toxicol. 38 (2020) 42–58, https://doi.org/10.1093/jat/bkw120.

[11] A. Alexandridou, T. Mouskeftara, N. Raikos, H.G. Gika, GC-MS analysis of underivatised new psychoactive substances in whole blood and urine, J. Chromatogr. A 1156 (2020), 122308, https://doi.org/10.1016/j.jchromb.2020.122308.

[12] R. Gottardo, D. Sorio, G. Soldati, M. Ballotari, N.M. Porpiglia, F. Tagliaro, Optimization and validation of a new approach based on CE-HRMS for the screening analysis of novel psychoactive substances (cathinones, phenethylamines, and tryptamines) in urine, Electrophoresis 42 (2021) 450–459, https://doi.org/10.1002/elps.202000304.

[13] M. Sundström, A. Pelander, V. Angerer, M. Hutter, S. Kneisel, I. Ojanperä, A high-sensitivity ultra-high performance liquid chromatography/high-resolution time-of-flight mass spectrometry (UHPLC-HR-TOFMS) method for screening synthetic cannabinoids and other drugs of abuse in urine, Anal. Bioanal. Chem. 405 (2013) 8463–8474, https://doi.org/10.1007/s00216-013-7272-8.

[14] M. Concheiro, S. Anizan, K. Ellefsen, M.A. Huestis, Simultaneous quantification of 28 synthetic cathinones and metabolites in urine by liquid chromatography-high resolution mass spectrometry, Anal. Bioanal. Chem. 405 (2013) 9437–9448, https://doi.org/10.1007/s00216-013-7386-z.

[15] M. Concheiro, M. Castaneto, R. Kronstrand, M.A. Huestis, Simultaneous determination of 40 novel psychoactive stimulants in urine by liquid chromatography–high resolution mass spectrometry and library matching, J. Chromatogr. A 1397 (2015) 32–42, https://doi.org/10.1016/j.chroma.2015.04.002.

[16] L. Glicksberg, K. Bryand, S. Kerrigan, Identification and quantification of synthetic cathinones in blood and urine using liquid chromatography-quadrupole/time of flight (LC-Q/TOF) mass spectrometry, J. Chromatogr. B 1035 (2016) 91–103, https://doi.org/10.1016/j.jchromb.2016.09.027.

[17] J. Sánchez-González, S. Odoardi, A.M. Bermejo, P. Bermejo-Barrera, F.S. Romolo, A. Moreda-Piñeiro, S. Strano-Rossi, HPLC-MS/MS combined with membrane-protected molecularly imprinted polymer micro-extraction for synthetic cathinones monitoring in urine, Drug Test. Anal. 11 (2019) 33–44, https://doi.org/10.1002/dta.2448.

[18] T. Murakami, Y. Iwamuro, R. Ishimaru, S. Chinaka, H. Hasegawa, Molecularly imprinted polymer solid-phase extraction of synthetic cathinones from urine and whole blood samples, J. Sep. Sci. 41 (2018) 4506–4514, https://doi.org/10.1002/jssc.201800874.

[19] S. Pascual-Caro, N. Fontanals, F. Borrull, C. Aguilar, M. Calull, Solid-phase extraction based on cation-exchange sorbents followed by liquid chromatography high-resolution mass spectrometry to determine synthetic cathinones in urine, Forensic Toxicol. 38 (2020) 185–194, https://doi.org/10.1007/s11419-019-00508-8.

[20] T. Lau, M. Concheiro, G. Cooper, Determination of 30 synthetic cathinones in postmortem blood using LC-MS-MS, J. Anal. Toxicol. 44 (2020) 679–687, https://doi.org/10.1093/jat/bkaa071.

[21] M. Antunes, M. Sequeira, M.D. Pereira, M.J. Caldeira, S. Santos, J. Franco, M. Barroso, H. Gaspar, Determination of selected cathinones in blood by solid-phase extraction and GC-MS, J. Anal. Toxicol. 45 (2021) 233–242, https://doi.org/10.1093/jat/bkaa074.

[22] S. Karampela, J. Smith, I. Panderi, Determination of 19 psychoactive substances in premortem and postmortem whole blood samples using ultra-high-performance liquid chromatography-tandem mass spectrometry, Separations 8 (2021) 78, https://doi.org/10.3390/separations8060078.

[23] Y.W. Li, R. Lian, F.Y. Yang, Z.R. Xu, F.Q. Cao, R. Wang, C. Liang, Y.R. Zhang, Rapid quantitation of three synthetic cathinones in urine by magnetic dispersive solid-phase extraction combined with DART-HRMS, Anal. Methods 13 (2021) 5048–5055, https://doi.org/10.1039/d1ay01280k.

[24] S. Odoardi, M. Fisichella, F.S. Romolo, S. Strano-Rossi, High-throughput screening for new psychoactive substances (NPS) in whole blood by DLLME extraction and UHPLC–MS/MS analysis, J. Chromatogr. B 1000 (2015) 57–68, https://doi.org/10.1016/j.jchromb.2015.07.007.

[25] V. Gallo, P. Tomai, V. Di Lisio, C. Dal Bosco, P. D'Angelo, C. Fanali, G. D'Orazio, I. Silvestro, Y. Picó, A. Gentili, Application of a low transition temperature mixture for the dispersive liquid-liquid microextraction of illicit drugs from urine samples, Molecules 26 (2021) 5222, https://doi.org/10.3390/molecules26175222.

[26] J. LaPointe, B. Musselman, T. O'Neill, J.R.E. Shepard, Detection of "bath salt" synthetic cathinones and metabolites in urine via DART-MS and solid phase microextraction, J. Am. Soc. Mass Spectrom. 26 (2015) 159–165, https://doi.org/10.1007/s13361-014-1006-9.

[27] N. Jakubowska, Ż. Polkowska, J. Namieśnik, A. Przyjazny, Analytical applications of membrane extraction for biomedical and environmental liquid sample preparation, Crit. Rev. Anal. Chem. 35 (2005) 217–235, https://doi.org/10.1080/10408340500304032.

[28] X. Shi, Z. Tang, A. Sun, L. Zhou, J. Zhao, D. Li, J. Chen, D. Pan, Simultaneous analysis of polychlorinated biphenyls and organochlorine pesticides in seawater samples by membrane-assisted solvent extraction combined with gas chromatography–electron capture detector and gas chromatography–tandem mass spectrometry, J. Chromatogr. B 72 (2014) 58–64, https://doi.org/10.1016/j.jchromb.2014.09.036.

[29] A. Iparraguirre, P. Navarro, R. Rodil, A. Prieto, M. Olivares, N. Etxebarria, O. Zuloaga, Matrix effect during the membrane-assisted solvent extraction coupled to liquid chromatography tandem mass spectrometry for the determination of a variety of endocrine disrupting compounds in wastewater, J. Chromatogr. A 1356 (2014) 163–170.

[30] N. Salgueiro-González, I. Turnes-Carou, S. Muniategui-Lorenzo, P. López-Mahía, D. Prada-Rodríguez, Pressurized hot water extraction followed by miniaturized membrane assisted solvent extraction for the green analysis of alkylphenols in sediments, J. Chromatogr. A 1383 (2015) 8–17, https://doi.org/10.1016/j.chroma.2015.01.022.

[31] S. Ncube, G. Lekoto, E. Cukrowska, L. Chimuka, Development and optimisation of a novel three-way extraction technique based on a combination of Soxhlet extraction, membrane assisted solvent extraction and a molecularly imprinted polymer using sludge polycyclic aromatic hydrocarbons as model compounds, J. Sep. Sci. 41 (2018) 918–928, https://doi.org/10.1002/jssc.201701216.

[32] S. Ncube, N. Tavengwa, A. Soqaka, E. Cukrowska, L. Chimuka, Development of a single format membrane assisted solvent extraction-molecularly imprinted polymer technique for extraction of polycyclic aromatic hydrocarbons in wastewater followed by gas chromatography mass spectrometry determination, J. Chromatogr. A 1569 (2018) 36–43, https://doi.org/10.1016/j.chroma.2018.07.061.

[33] E. Poonguzhali, A. Kapoor, P.S. Kumar, S. Prabhakar, Effective separation of toxic phenol from aquatic system using membrane assisted solvent extraction system, Desalin. Water Treat. 221 (2021) 316–327, https://doi.org/10.5004/dwt.2021.27037.

[34] A. Mañana-López, J. Sánchez-Piñero, J. Moreda-Piñeiro, I. Turnes-Carou, S. Muniategui-Lorenzo, P. López-Mahía, Polycyclic aromatic hydrocarbons analysis in tea infusions and tea beverages using membrane assisted solvent extraction, Microchem. J. 167 (2021), 106278, https://doi.org/10.1016/j.microc.2021.106278.

[35] S. Khulu, S. Ncube, Y. Nuapia, L.M. Madikizela, H. Tutu, H. Richards, K. Ndungu, E. Mavhunga, L. Chimuka, Multivariate optimization of a two-way technique for extraction of pharmaceuticals in surface water using a combination of membrane assisted solvent extraction and a molecularly imprinted polymer, Chemosphere 286 (2022), 131973, https://doi.org/10.1016/j.chemosphere.2021.131973.

[36] N. Carro, I. Garcia, M. Ignacio, A. Mouteira, Microwave-assisted extraction, membrane-assisted solvent extraction combined with gas chromatography/electron-capture detection applied to the analysis of polychlorinated biphenyls in bivalve molluscs, Int. J. Environ. Anal. Chem. 89 (2009) 759–774, https://doi.org/10.1080/03067310802610250.

[37] C. Ramos-Contreras, E. Concha-Graña, P. López-Mahía, F. Molina-Pérez, S. Muniategui-Lorenzo, Determination of atmospheric particle-bound polycyclic aromatic hydrocarbons using subcritical water extraction coupled with membrane microextraction, J. Chromatogr. A 1606 (2019), 460381, https://doi.org/10.1016/j.chroma.2019.460381.

[38] C. Suárez-Oubiña, I. Álvarez-Freire, P. Cabarcos, A.M. Bermejo, P. Bermejo-Barrera, A. Moreda-Piñeiro, Isolation and quantification of synthetic cannabinoid receptor agonists in human urine using membrane-assisted solvent extraction followed by liquid chromatography – tandem mass spectrometry, Anal. Methods 15 (2023) 333–342, https://doi.org/10.1039/d2ay01491B.

[39] ChemSpider Search and Share Chemistry, http://www.chemspider.com/ (accessed 16 December 2022).