STUDIA UBB CHEMIA, LXVIII, 1, 2023 (p. 19-35)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2023.1.02

HPLC-PDA *VERSUS* GC-MS IN THE ANALYSIS OF PARACETAMOL AND NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN WASTEWATER

Mihaela-Cătălina HERGHELEGIU[a], Aude ERNAULT[b], Mihail Simion BELDEAN-GALEA[a], Maria-Virginia COMAN[c,*]

ABSTRACT. The widespread use of paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) make them increasingly present in environmental factors, especially in water. The aim of this work was to develop an accurate, precise and sensitive analytical procedure for the simultaneous determination of Paracetamol and four NSAIDs (Ketoprofen, Naproxen, Diclofenac, and Ibuprofen). To this aim, the extraction (liquid-liquid extraction, LLE, and solid-phase extraction, SPE) as well as the chromatographic (high-performance liquid chromatography-photodiode array detector, HPLC-PDA, and gas chromatography-mass spectrometry in selected ion monitoring mode, GC-MS-SIM) techniques, in terms of the performances were compared. Different extraction solvents and types of cartridges at pH 3 of samples were tested for the extraction optimization. Low limits of detection and quantification at the μg/L level were achieved. The developed HPLC-PDA and GC-MS-SIM methods were applied to the analysis of selected pharmaceuticals in different wastewater samples.

Keywords: *paracetamol and NSAIDs, solid-phase extraction, liquid-liquid extraction, GC-MS, HPLC-PDA*

[a] *Babeş-Bolyai University, Faculty of Environmental Science and Engineering, 30 Fântânele str., RO-400294, Cluj-Napoca, Romania*
[b] *École Supérieure de Physique et de Chimie Industrielle de la Ville de Paris, Paris Sciences et Lettres Université, 10 Vauquelin Rue, 75231, Paris, France*
[c] *Babeş-Bolyai University, "Raluca Ripan" Institute for Research in Chemistry, 30 Fântânele str., RO-400294, Cluj-Napoca, Romania*
* *Corresponding author: virginia.coman@ubbcluj.ro*

INTRODUCTION

Pharmaceuticals are currently used for many valuable purposes of human and animal health and some of them are classified as “emerging” pollutants. These pharmaceuticals can enter the wastewater system through their excretion after ingestion, in unchanged and/or metabolized (glucuronide conjugates) form or through their incorrect disposal [1–4]. Due to their persistence and continuous input in the environment, pharmaceutical residues are frequently detected in water bodies [5–8] in the range of ng/L to µg/L, which may represent potential threats to living organisms and ecosystem viability [9].

The most commonly prescribed over-the-counter pharmaceuticals, available and popular, are paracetamol (PARA) and non-steroidal anti-inflammatory drugs (NSAIDs) [9, 10]. Paracetamol, also called acetaminophen, is a mild analgesic most widely used to treat fever and headache [11]. NSAIDs are antipyretics and non-narcotic analgesics used for mild to moderate pain, their use does not lead to euphoria and addiction [12]. The most studied NSAID on the Watch List of Substances for European Union-wide Monitoring is Diclofenac together with other NSAIDs (Naproxen, Ketoprofen, Ibuprofen), Paracetamol or hormones [13].

In analytical chemistry, sample preparation for analysis is a very important step with consequences in the identification, confirmation, and quantification of target analytes in various matrices. It involves sampling and extraction procedures [14, 15].

Liquid-liquid extraction (LLE) is one of the first and most well-known sample preparation techniques that use organic solvents to extract the target compounds from different matrices, based on the octanol-water partition coefficient [15]. A widely used extraction method is solid-phase extraction (SPE) which is based on the adsorption of target analytes on the solid-phase (cartridges filled with different adsorbents) that allows their transfer from the liquid sample to the adsorbent, a method that can be applied to analytes with a wide range of polarities [15, 16].

In general, the most popular and easy-to-use extraction procedure for Paracetamol and NSAIDs from different water samples is solid-phase extraction (SPE) [5, 6, 8–10, 17, 18]. Literature data also mention miniaturized extraction (microextraction) methods for Paracetamol and NSAIDs: dispersive liquid–liquid microextraction based on solidification of floating organic droplet [19], ultrasound-assisted emulsification microextraction (with the advantage of no water-miscible organic solvents as dispersers and accelerating the process of target components into the solvent) [7], headspace (migration of volatile target compounds onto the adsorbent material) [20], molecularly imprinted polymers (molecular recognition allowing highly selective retention mechanism) [21].

The continuous increase of high-precision analytical instrumentation in recent years, by high specificity and selectivity, made possible the qualitative and quantitative assessment of Paracetamol and NSAIDs in matrices of different complexity. Liquid chromatography (LC) is able to separate polar compounds from complex mixtures without derivatization [10, 11, 18, 21], while gas chromatography (GC) requires this step [17].

Liquid chromatography-mass spectrometry (LC-MS) technique associated with electrospray ionization has the disadvantage of suppression the analyte signal caused by the matrix effect, fact of which other detectors of higher sensitivity and resolution [11] and with lower matrix effect should be used (QTrap mass spectrometer [10], tandem-mass spectrometry (MS-MS) [11, 18, 21].

Gas chromatography-mass spectrometry (GC-MS) is a convenient, accessible, and versatile technique, commonly used in many laboratories, *versus* LC-MS. GC-MS is applicable to PARA and NSAID analysis, but involves a derivatization step with silylation, acylation or alkylation agents to convert polar compounds into volatile derivatives [9, 11, 20].

The aim of this work is to develop an accurate and sensitive analytical procedure for the simultaneous determination of PARA and four NSAIDs (Ketoprofen, Naproxen, Diclofenac and Ibuprofen) in different wastewater samples. The novelty of this work consists in a pertinent comparison regarding the performance of the most common extraction (LLE and SPE) and chromatographic (high-performance liquid chromatography-photodiode array detector HPLC-PDA and GC-MS) techniques used for the analysis these compounds and their applicability to various wastewater samples.

RESULTS AND DISCUSSION

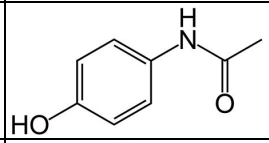
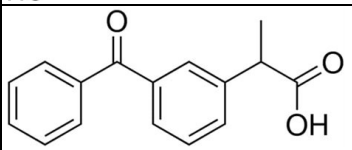
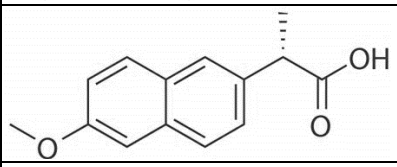
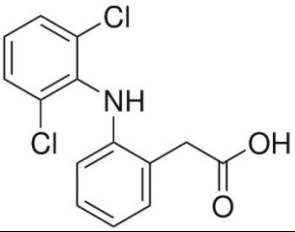
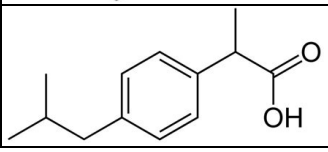
NSAIDs are carboxylic acid derivatives, whereas paracetamol is an N-acetyl-p-aminophenol. The molecular structure, molecular weight (MW), lipophilicity (LogP) and acidity (pKa) constants are presented in Table 1.

Analyzing the physicochemical properties of the compounds in Table 1, it can be seen that, while NSAIDs are weakly lipophilic (log P between 3.12 and 4.51) PARA is rather hydrophilic (log P = 0.91). More NDAIDs have pKa values between 4.15 and 4.91, while the pKa value of PARA is 9.38. These differences could lead to a different behavior of the studied compounds, especially when it is intended to isolate them from aqueous matrices. For these reasons, a study on the influence of pH on the extraction of these compounds from aqueous matrices is necessary.

According to our previously research [19], the pH value of 3 gives the maximum recovery for the studied NSAIDs because due to their pKa values between 4.15 and 4.91. Thus, a low pH value keeps these compounds in neutral form avoiding their dissociation.

Therefore, as a starting point, our experiments were done at pH 3, following which the study of the influence of pH to be done for the extraction method with the highest recovery yield.

Table 1. Molecular structure, molecular weight (MW), lipophilicity (LogP) and acidity (pKa) of the studied pharmaceuticals

Analyte (Abbreviation)	Chemical Structure	MW	LogP	pKa
Paracetamol (PARA)		151.16	0.91	9.38
Ketoprofen (KET)		254.28	3.12	4.45
Naproxen (NAP)		230.26	3.18	4.15
Diclofenac (DIC)		296.14	4.51	4.15
Ibuprofen (IBU)		206.28	3.97	4.91

Physicochemical properties (MW, LogP, pKa) from PubChem databases. https://pubchem.ncbi.nlm.nih.gov/ (Accessed November 2022).

Liquid-liquid extraction. According to the scientific literature [22, 23] the extraction of selected pharmaceuticals from liquid samples are performed using solvent of medium polarity (ethyl acetate, dichloromethane, chloroform) or mixtures of nonpolar to polar solvents such as hexane, acetonitrile, acetone, 1-butanol. For our purpose the efficiency of ethyl acetate and mixture of *n*-hexane:isopropanol (3:2, v/v) were considered. The recovery of each pharmaceutical at the selected pH 3 was calculated for each of the two extraction solvents, ethyl acetate and *n*-hexane:isopropanol (3:2, v/v) respectively, and the results are plotted in Figure 1.

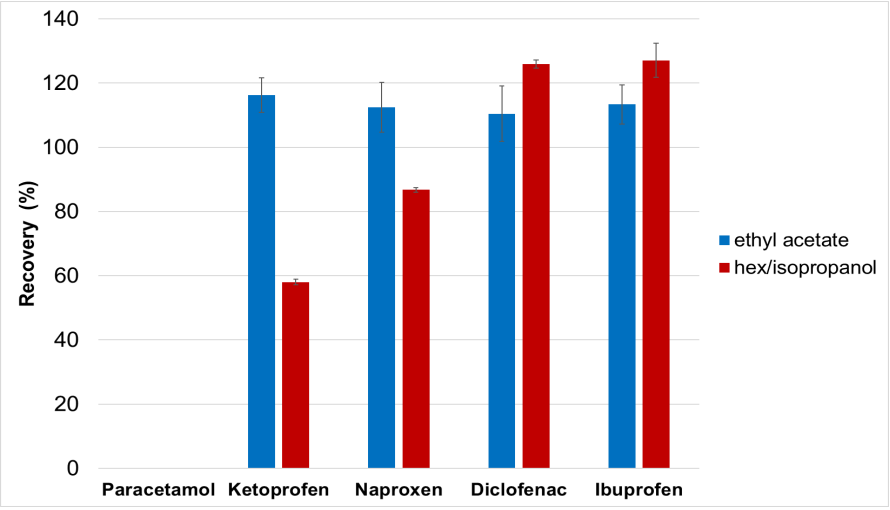


Figure 1. Recovery of studied pharmaceuticals using two extraction solvents

Naproxen, Ketoprofen, Diclofenac and Ibuprofen were detected in the extracts obtained with the both extraction solvents. The recovery over 100% for all the four anti-inflammatories was obtained in the case of ethyl acetate solvent. Paracetamol could not be quantified with either of the two extraction solvents due to the presence of different impurities that co-eluted with it.

These impurities come from the extraction solvent and even if they are present at the trace level, through concentration they reach concentration levels that disturb the analysis. Moreover, the poor retention of PARA in the analysis conditions makes its elution very close to the hold-up volume of the column, which leads to the overlap of the solvent peak and the impurities associated with PARA (see Results and Discussion section, Figure 4).

Since Paracetamol could not be analysed simultaneously with the four NSAIDs, the LLE method was not considered suitable for our aim.

Solid-phase extraction. The three types of SPE cartridges (C18-U, C18-E, Strata X) were tested at pH 3. The best extraction of the studied pharmaceuticals from distilled water samples was obtained on Strata X cartridges that are capable of adsorbing a large group of analytes. Recoveries for all pharmaceuticals were from 41.57 to 89.94% (Figure 2). Therefore, Strata X cartridges were chosen for SPE of the target pharmaceuticals in the wastewater samples.

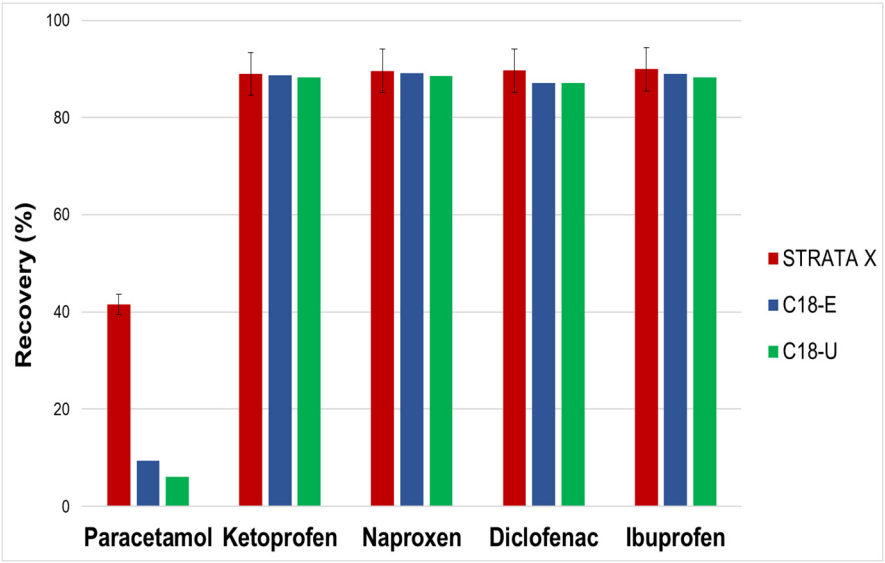


Figure 2. Recovery of studied pharmaceuticals using different SPE cartridges

Influence of pH over the Solid-Phase Extraction. The influence of pH over the SPE recovery was tested at 3 pH levels, such as 3, 4, and 7. The results showed that the most suitable pH is 3, for which both PARA and NSAIDs have the highest extraction recoveries, PARA over 40% and NSAIDs over 80% (Figure 3).

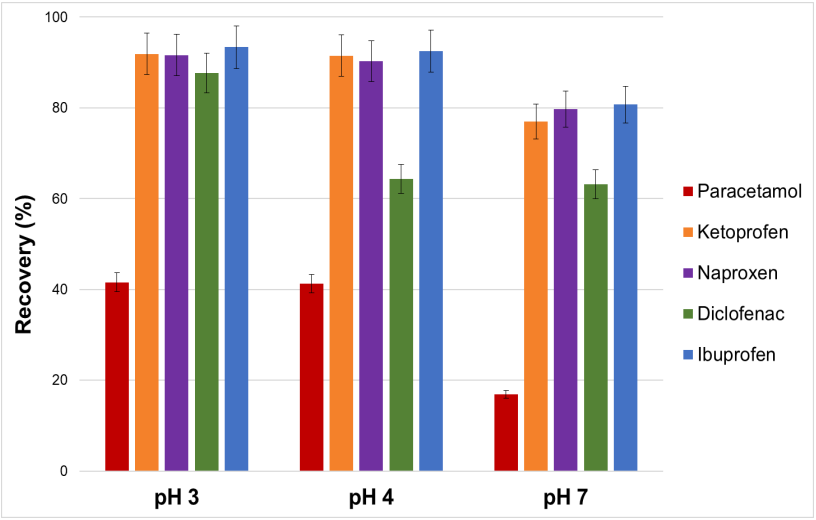


Figure 3. Influence of pH over the recovery of studied pharmaceuticals on Strata X SPE cartridge

Performances of HPLC-PDA and GC-MS-SIM developed methods

For the both chromatographic methods, limit of detection (LOD) and limit of quantification (LOQ) were determined taking into consideration the standard deviation of the response factor (σ) of the detector for each analyte and the slope (S) of each calibration curve. These parameters have been calculated according to equations: LOD = 3.3 σ/S and LOQ = 10 σ/S [24].

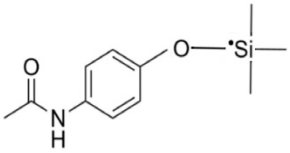
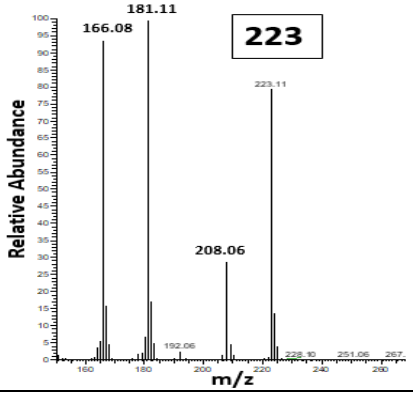
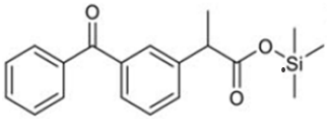
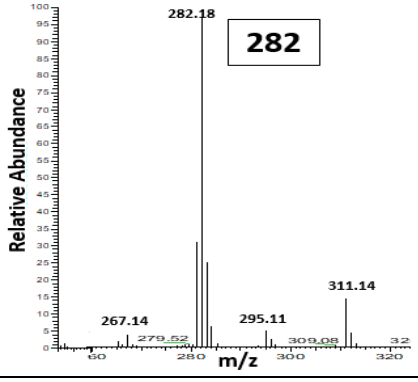
HPLC-PDA method. Linearity (correlation coefficient, R^2) of the method was tested for the concentrations of the studied pharmaceuticals from 10 to 0.25 mg/L. The developed method has good linearity with R^2 over 0.9969, low LODs and LOQs instrument limits in the range of μg/L, and relative standard deviation (RSD) below 3%. If we consider that, the pre-concentration factor of the method is 250, the obtained method detection and quantification limits (MDL, MQL) are in the range of ng/L (Table 2).

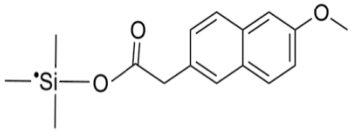
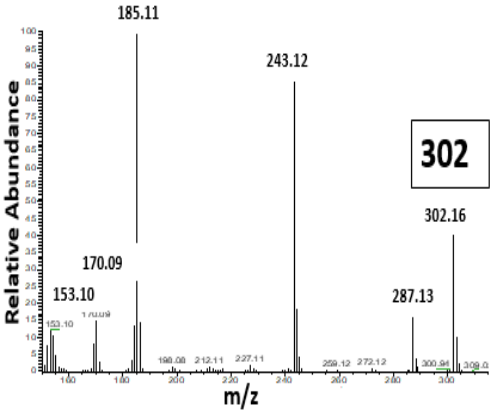
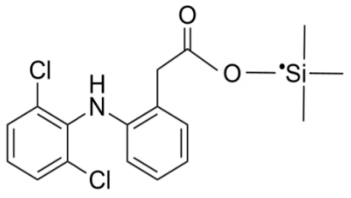
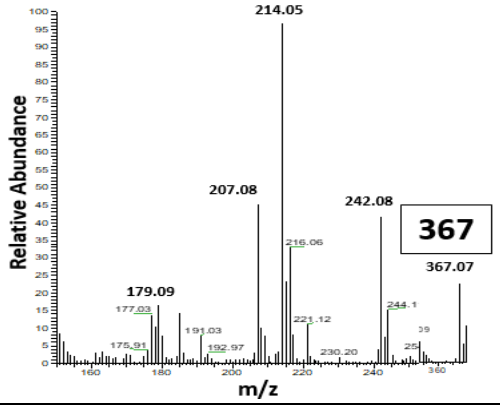
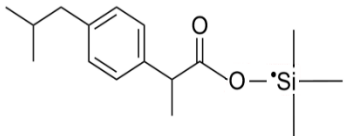
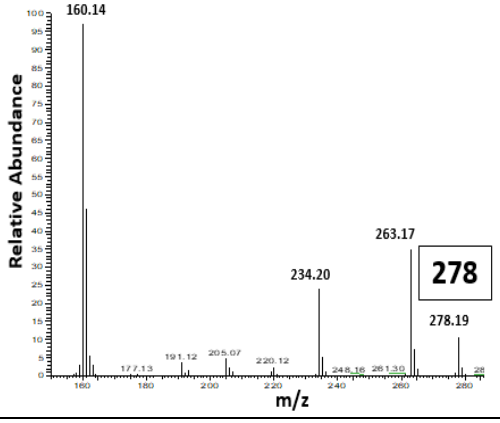
GC-MS-SIM method. Due to their polarity and instability at high temperature NSAIDs and PARA can be usually analysed by GC only as trimethylsilyl (TMS) derivative compounds. The TMS derivatives molecular formula, molecular ion mass and mass spectra are given in Table 3.

Table 2. Performances of HPLC-PDA method

Analyte	Equation	R^2	SD	Instrument limits		Method limits		Intra-day RSD (%)	Inter-day RSD (%)
				LOD (µg/L)	LOQ (µg/L)	MDL (ng/L)	MQL (ng/L)		
PARA	389956x + 24633	0.9980	1774.6	20	50	80	200	0.89	1.90
KET	79873x - 9553	0.9987	429.2	20	50	80	200	1.30	1.57
NAP	323343x - 12731	0.9984	2545.3	30	80	120	320	1.72	1.99
DIC	158932x - 57580	0.9969	602.3	10	40	40	160	1.76	3.19
IBU	219937x - 54428	0.9969	2019.7	30	90	120	360	2.15	2.82

Table 3. Chemical structure and mass spectra of selected pharmaceutical TMS derivatives

Chemical structure of selected pharmaceutical TMS derivative	Mass spectrum and mass of selected molecular ion
1	2
 TMS Paracetamol	
 TMS Ketoprofen	

Chemical structure of selected pharmaceutical TMS derivative	Mass spectrum and mass of selected molecular ion
1	2
 TMS Naproxen	
 TMS Diclofenac	
 TMS Ibuprofen	

Linearity of the method was tested for the concentrations of the studied pharmaceuticals between 10 mg/L and 0.667 mg/L. Method has a good linearity with R over 0.9872, low LODs and LOQs instrument limits in the range of µg/L, but highest relative standard deviations (RSD) (4.91–12.68%) compared with LC-PDA method. If we consider that, the pre-concentration factor of the method is 5000, the obtained method detection and quantification limits (MDL, MQL) are in the range of ng/L (Table 4).

Table 4. Performances of GC-MS-SIM method

Analyte derivative	Equation	R^2	SD	Instrument limits		Method limits		Intra-day RSD (%)	Inter-day RSD (%)
				LOD (µg/L)	LOQ (µg/L)	MDL (ng/L	MQL ng/L		
PARA-TMS	601089x-627380	0.9902	9097.62	50	150	10	30	5.21	4.91
KET-TMS	148494x-161316	0.9872	1399.07	30	90	6	18	8.08	9.09
NAP-TMS	71359x-59322	0.9925	3137.69	150	440	30	88	8.64	10.23
DIC-TMS	16370x-14843	0.9896	375.96	80	230	16	46	8.68	8.90
IBU-TMS	7831.8x-5449.2	0.9969	319.48	130	410	26	82	12.23	12.68

Table 5. Performance of some methods used for analysis of Paracetamol and NSAIDs in wastewater samples

Analyte	Extraction method	Analysis method	Sensitivity		Ref.
			MDLs (ng/L)	MQLs (ng/L)	
PARA, KET, NAP, DIC, IBU.	SPE (Strata-X)	GC-MS-SIM	PARA: 30 KET: 24 NAP: 10 DIC: 18 IBU: 9	PARA: 90 KET: 71 NAP: 25 DIC: 61 IBU: 22	[9]
PARA, KET, NAP, DIC, IBU.	SPE (Oasis HLB)	GC-MS-SIM	PARA: 7.6 KET: 87.4 NAP: 16.9 DIC: 52.9 IBU: 6.6	PARA: 22.4 KET: 257.1 NAP: 56.5 DIC: 158.7 IBU: 19.5	[17]
PARA, KET, NAP, DIC, IBU.	SPE (Strata-X)	GC-MS-SIM	PARA: 10 KET: 6 NAP: 30 DIC:16 IBU: 26	PARA: 30 KET: 18 NAP: 88 DIC: 46 IBU: 82	This work
KET, NAP, DIC, IBU.	DLLME-SFO*	LC-UV	KET: 190 NAP: 75 DIC: 140 IBU: 180	KET: 590 NAP: 220 DIC: 420 IBU: 550	[19]
PARA, KET, NAP, DIC, IBU.	SPE (Strata-X)	LC-PDA	PARA: 80 KET: 80 NAP: 120 DIC:40 IBU: 120	PARA: 200 KET: 200 NAP: 320 DIC: 160 IBU: 360	This work

* Dispersive-liquid-liquid microextraction-solidification floating organic droplet

By comparing our results with the results obtained by other authors worldwide it can be observed that the results are in the same range of sensitivity (Table 5).

Application to real wastewater samples

The two developed methods were applied to the analysis of the selected pharmaceuticals from samples collected in Romania, respectively in wastewater plants (Cluj-Napoca city; Țețchea and Diosig villages in Bihor county), as well as in septic tanks (Turulung and Terebești villages in Satu Mare county).

HPLC-PDA method. The chromatograms obtained were complex, with many different peaks, as it can be seen in Figure 4.

Unexpected compounds co-eluted with pharmaceuticals, making their quantification very difficult. Therefore, SPE-HPLC-PDA method is not suitable for the target pharmaceuticals in wastewater samples.

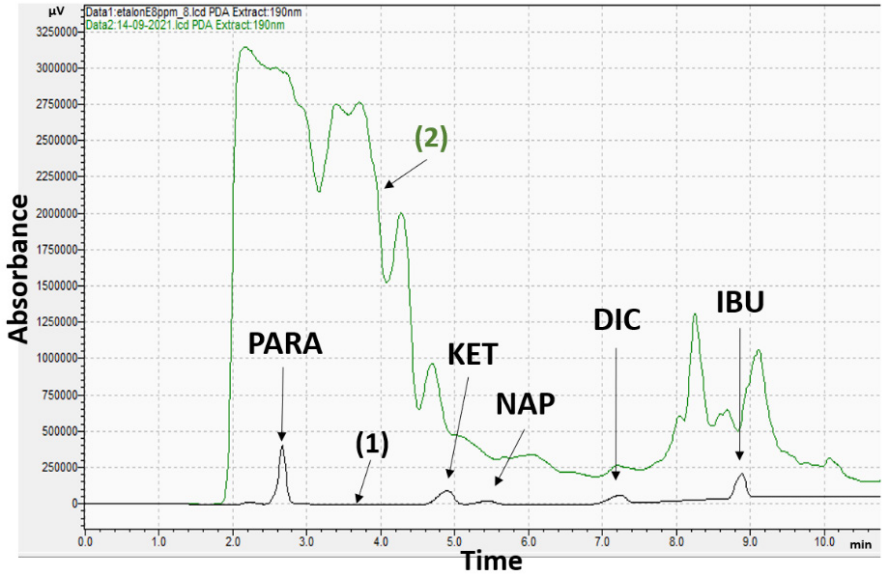


Figure 4. HPLC-PDA chromatograms of studied pharmaceuticals: standards in distilled water (1, black) and wastewater sample (2, green)

GC-MS-SIM method. The selected ion monitoring mode (SIM) allowed the identification of all five pharmaceuticals (Paracetamol, Ketoprofen, Naproxen, Ibuprofen and Diclofenac) as TMS derivatives in wastewater samples (Figure 5).

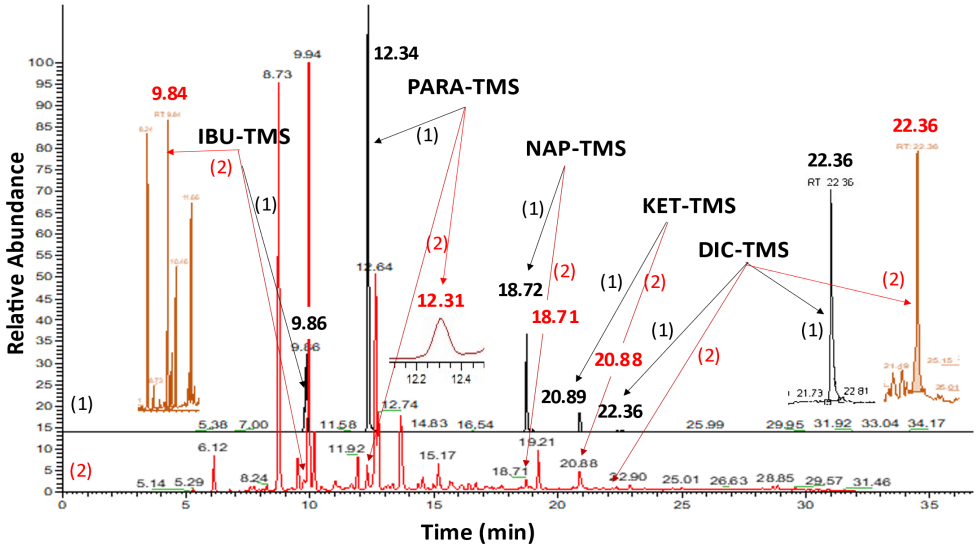


Figure 5. GC-MS-SIM chromatograms of derivatised pharmaceuticals: standard solution (1, black) and wastewater sample (2, red). Details: IBU-TMS (2, red) and PARA-TMS (2, red) in wastewater sample; DIC-TMS in standard solution (1, black) and in wastewater sample (2, red)

The results of analysed wastewater samples showed the presence of the selected compounds in concentration ranged from nd–224.11 ng/L for PARA, from nd–2705.1 ng/L for KET, from nd–1743.4 ng/L for NAP, from nd–16967.8 ng/L for DIC, and from nd–2436.7 ng/L for IBU (Table 6).

Table 6. The concentration of the selected pharmaceuticals in the analysed wastewater samples

Wastewater samples	Found concentration (ng/L)				
	PARA	KET	NAP	DIC	IBU
Cluj-Napoca*	123.19–224.11	567.24–2705.1	440.03–1743.4	351.97–16967.8	1712.26–2436.7
Țețchea*	59.44	318.46	<MQL	68.64	246.82
Diosig*	nd	1986.95	nd	nd	nd
Terebești**	nd	163.61	121.89	89.43	<MQL
Turulung**	nd	nd	nd	nd	nd

*Wastewater treatment plant; **Wastewater from septic tank.

In the samples collected from the wastewater treatment plant, the highest concentrations of the studied pharmaceuticals were found in Cluj-Napoca, while only Ketoprofen was found in Diosig. In the samples collected

from the septic tanks, Ketoprofen, Naproxen and Diclofenac were found in Terebești, while no studied compound was detected in Turulung. Their presence in the septic tank shows their stability during the time and common use of them. Our obtained results are comparable with other studies performed worldwide (Table 7).

Table 7. The concentration of the selected pharmaceuticals analyzed worldwide in wastewater

Analyte (ng/L)					Country	Ref.
PARA	KET	NAP	DIC	IBU		
530.2	nd*	240.0	263.5	280.0	Poland	[6]
7219	4569	7040	2902	6722	Poland	[9]
41.3; 69.1	nd	51.5; 93.8	31.5; 54.6	128.1; 131.5	China	[10]
<MDL	<MDL	240	460	280	Poland	[17]
nd	102–16000	96–35000	47–274	50–150000	Spain	[20]
nd – 224.11	nd – 2705.1	nd – 1743.4	nd – 16967.8	nd – 2436.7	Romania	**

*nd – not detected; **All samples of this work.

CONCLUSIONS

The best recovery for all the pharmaceuticals was obtained by solid-phase extraction on Strata X cartridges.

The GC-MS analysis performed in SIM mode improves the sensitivity and selectivity of the method allowing the simultaneous determination of Paracetamol, Ketoprofen, Naproxen, Ibuprofen and Diclofenac as trimethylsilyl derivatives at ng/L level.

In the analyzed wastewater samples, the concentration of the studied pharmaceuticals range from not detected to hundreds/thousands ng/L depending of the collection point.

The selected pharmaceuticals were found in wastewater samples collected from both sewage systems and septic tanks.

The obtained results show the importance of developing more selective extraction methods as well as the use of detectors of higher selectivity, sensitivity, and specificity for a better quantification of the studied pharmaceuticals in low amounts, even at trace/ultratrace level in wastewaters.

EXPERIMENTAL SECTION

Chemicals

Paracetamol and NSAID standards (>98% purity; Ibuprofen, Ketoprofen, Naproxen, Diclofenac sodium salt) were purchased from Sigma-Aldrich (Steinheim, Germany). The derivatisation agent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was purchased from Cerilliant (Texas, USA). Strata X (200 mg/6 mL), Strata C18-U (200 mg/3 mL) and Strata C18-E (200 mg/3 mL) cartridges were purchased from Phenomenex (Torrance, USA). Chromatographic grade solvents (methanol, acetonitrile, *n*-hexane, ethyl acetate, isopropanol), hydrochloric acid (30%), sodium sulphate, and monopotassium phosphate of analytical grade were supplied from Merck (Darmstadt, Germany).

Stock standard solutions of each individual pharmaceutical at 1000 mg/L concentration were prepared in acetonitrile, except diclofenac prepared in methanol. All solutions were stored at 4ºC in the dark.

Instrumentation and analytical procedures

Resprep 12 Manifold equipped with Rocker 500 Vacuum Pump (Restek, USA) instrument was used for SPE and Hei-VAP Core evaporator (Heidolph, Germany) for solvent evaporation. The derivatisation of the studied pharmaceuticals was performed with BSTFA with 1% TMCS in a lab oven Memmert UFE 400 (Memmert, Germany).

HPLC analyses were carried out with a LC equipment (SLC-40D) with a photodiode detector (SPD-M40) (Shimadzu, Japan). Instrument control and data acquisition was done with LabSolution software (Shimadzu, Japan). The studied pharmaceuticals were separated on a column Luna C18 (250 x 4.6 mm, 5 µm) (Phenomenex, USA) with a flow rate of 1.0 mL/min at 40ºC oven temperature. The injection volume was 20 µL. The gradient elution (acetonitrile and 15 mM aqueous monopotassium phosphate solution) programme was 55% acetonitrile for 4 min, increasing to 83% in 2 min and then held at 83% for 4 min. The specific wavelengths of pharmaceuticals studied using PDA were: Paracetamol – 194 nm, Ketoprofen – 256 nm, Naproxen – 230 nm, Diclofenac – 200 nm, and Ibuprofen – 190 nm.

GC–MS analyses were performed on a Focus GC instrument equipped with DSQ II mass spectrometer (single quadrupole) controlled by a computer running XCalibur software and TriPlus Autosampler (Thermo

Electron Corporation, USA). Column: TR-5MS (30 m x 0.25 mm i.d., 0.25 µm film thickness; Thermo Fisher Scientific, USA); the carrier gas was Helium (purity 99.999%) at a flow rate of 1.2 mL/min. The column temperature was initially set at 120ºC, and then raised with 5ºC/min to 275ºC, with 1 min hold up time. The GC injection port and transfer line temperatures were kept at 280ºC. The mass spectrometer worked in the electron impact mode (70 eV; ion source temperature, 200ºC) by scanning from 150 to 400 *m/z* to obtain full spectra of the studied pharmaceuticals. Their quantification was performed by selected ion monitoring (SIM) mode and comparison of relative retention times.

Our pharmaceuticals were analysed as derivative compounds. Their derivatisation protocol was done with 100 µL BSTFA with 1% TMCS, for 1h at 100ºC, followed by cooling and evaporation under a gentle nitrogen stream and dissolution of the residue extract in 100 µL of *n*-hexane.

Analysis of studied pharmaceuticals from water samples

Liquid-liquid extraction. A volume of 100 mL of distilled water was introduced in a separation funnel, spiked with 100 µg of each pharmaceutical, and acidified at pH 3 with hydrochloric acid 30%, followed by the addition and dissolution of 5 g of NaCl salt. The obtained solution was subjected to LLE with 10 mL of solvent extraction (ethyl acetate or *n*-hexane-isopropanol, 3:2 v/v) for 5 min, followed by the separation of funnel content in two phases. This extraction procedure was repeated three times with fresh solvent and the three organic phases were collected, combined and dried with anhydrous sodium sulphate. Then, the extract was evaporated by a rotary evaporator, and finally the residue was dissolved in 2 mL of acetonitrile and analysed by HPLC-PDA.

Solid-phase extraction. Three types of SPE cartridges (C18-U, C18-E and Strata X) were tested. First, they were successively preconditioned with 6 mL of each: distilled water, acetonitrile, and distilled water again. Then, distilled water samples of 100 mL were each spiked with 100 µg of each pharmaceutical acidified (pH 3), and then passed through the named cartridges at a flow rate of approximately 1 mL/min. Subsequently, the cartridges were dried for 20 minutes under vacuum and then eluted by 4 mL acetonitrile. Each extract was evaporated to near dryness under a gentle stream of nitrogen and then redissolved with 2 mL of acetonitrile for HPLC-PDA analysis.

These two extraction protocols were used to study the extraction recovery of the selected pharmaceuticals from the synthetic water samples.

Analysis of pharmaceuticals from wastewater samples

500 mL of each wastewater sample were acidified at pH 3 with HCl 30%, centrifuged for 5 min at 4000 rot/min to separate the solid particles, and then filtered on 1.6 µm glass fibre filter (Fioroni, France) to remove the smaller particles. Each sample was passed through a Strata X cartridge preconditioned as mentioned before and dried for 20 min under vacuum. The pharmaceuticals of each cartridge were eluted with 4 mL of acetonitrile. Each obtained extract was evaporated under a gentle nitrogen stream.

For HPLC-PDA analysis the residue was dissolved in 2 mL of acetonitrile.

For the GC-MS analysis, the obtained extracts were derivatised as the protocol described previously.

ACKNOWLEDGMENTS

This work was performed in the framework of doctoral studies from the Babeș-Bolyai University, Environmental Science Doctoral School and ESPCI Student Internship at Babeș-Bolyai University. The research leading to these results has received funding from the Norway Grants 2014-2021 under Project contract no. 23 / 2020 (RO-NO-2019-0463). The authors are grateful to Someș Water Company S.A. Cluj-Napoca and also to the public authorities from Terebești, Turulung, Țețchea and Diosig for providing the wastewater samples.

REFERENCES

1. A. Azzouz; E. Ballesteros; *Sci. Total Environ.*, **2012**, *419*, 208-215.
2. H. Montaseri; P. B. C. Forbes; *TrAC.*, **2018**, *108*, 122-134.
3. T. Mackulak; S. Cernanský; M. Fehér; L. Birosová; M. Gál; *Curr. Opin. Environ. Sci.*, **2019**, *9*, 40-48.
4. S. Esteban; Y. Valcárcel; M. Catalá; M. G. Castromil, *Gac. Sanit.*, **2012**, *26(5)*, 457-459.
5. T. Kosjek, E Heath, A Krbavcic, *Environ. Int*., **2005**, *31*, 679-685.
6. J Kumirska; A. Plenis; P. Łukaszewicz; M. Caban, N. Migowska; A. Białk-Bielinska, M. Czerwicka; P. Stepnowski; *J. Chromatogr. A*, **2013**, *1296*, 164-178.

7. C. H. Lee; Y. Shin; M. W. Nam; K. M. Jeong; J. Lee, *Talanta*, **2014**, *129*, 552-559.
8. P. Stepnowski; D. Wolecki; A. Puckowski; M. Paszkiewicz; M. Caban; *Sci. Total Environ.*, **2020**, *745*, 140848.
9. M. Caban; K. Mioduszewska; P. Łukaszewicz; N. Migowska; P. Stepnowski; M. Kwiatkowski; J. Kumirska; *J. Chromatogr. A*, **2014**, *1346*, 107-116.
10. J. Yan; W. Lin; Z. Gao; Y. Ren; *Chemosphere*, **2021**, *279*, 130529.
11. H. Montaseri; P. B. C. Forbes; *TrAC.*, **2018**, *108*, 122-134.
12. K. Wieszczycka; J. Zembrzuska; J. Bornikowska; A. Wojciechowska; I. Wojciechowska; *Chem. Eng. Res. Des.*, **2017**, *117*, 698-705.
13. J. C. G. Sousa; A. R. Ribeiro; M. O. Barbosa; M. F. R. Pereira; A. M. T. Silva, *J. Hazard. Mater.*, **2018**, *344*, 146-162.
14. Y. Chen; Z. Guo; X. Wang; C. Qiu; *J. Chromatogr. A*, **2008**, *1184*, 191-219.
15. L. Nováková; H. Vlcková; *Anal. Chim. Acta*, **2009**, *656*, 8-35.
16. X. Chen; X. Wu; T. Luan; R. Jiang; G. Ouyang; *J. Chromatogr. A*, **2021**, *1640*, 461961.
17. N. Migowska; M. Caban; P. Stepnowski; J. Kumirska; *Sci. Total Environ.*, **2012**, *441*, 77-88.
18. B. Huidobro-López; I. López-Heras; C. Alonso-Alonso; V. Martínez-Hernández; L. Nozal; I. de Bustamante; *J. Chromatogr. A*, **2022**, *1671*, 463006.
19. M. S. Beldean-Galea, R. Klein, M.-V. Coman; *J. AOAC Int.*, 2020, 392-398.
20. T. Martinez-Sena; S. Armenta; M. de la Guardia; F. A. Esteve-Turrillas; *J. Pharm. Biomed. Anal.*, **2016**, *131*, 48-53
21. J. L. P. Pavón; A. M. C. Ferreira; M. E. F. Laespada; B. M. Cordero; *J. Chromatogr. A*, **2009**, *1216*, 6728-6734
22. G. G. Noche; M. E. F. Laespada; J. L. P. Pavón; B. M. Cordero; S. M. Lorenzo; *J. Chromatogr. A*, **2011**, *1218*, 6240-6247.
23. B. Yilmaz; H. Sahin; A. F. Erdem; *J. Sep. Sci.,* **2014**, *37*, 997-1003.
24. International Committee on Harmonization, "Validation of Analytical Procedures: Text and Methodology, Q2(R1), Nov. 2005. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf (Accessed December 2022).