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AN ULTRAHIGH PERFORMANCE LIQUID CHROMATOGRAPHY - MASS SPECTROMETRY METHOD FOR THE ANALYSES OF PHENOL DERIVATIVES FROM WATERS

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ABSTRACT. In this study, a new procedure, based on solid-phase extraction (SPE) and analysis by Ultrahigh Performance Liquid Chromatography coupled with Mass Spectrometry method (UPLC-MS/MS), has been developed for the simultaneous, multianalyte determination of 15 selected phenols in water. SPE was carried out on LiChrolut RP-18 and Oasis HLB cartridges by percolating 500 mL water samples. The analytical methods allowed the separation of the 15 phenols in less than 8 minutes, with a recovery higher than 70%, and a quantification limit between 3 – 5 ng/L. The developed UPLC-MS/MS method showed high precision, as it was confirmed by the low values of relative standard deviation (RSD) for water samples spiked with 10 and 100 ng/L analytes. In the optimized method, LOQ higher than 5 ng/L, satisfactory precision (relative standard deviations < 20%) and accuracies (recovery percentages between 70 and 95%) were obtained for most investigated compounds.

Keywords: *Phenol derivatives, UPLC-MS/MS, SPE*

INTRODUCTION

The development of sensitive methods for the determination of organic contaminants in wastewater has become a major issue, because of both the presence of many different toxic compounds in this type of samples and of the strict European Union legal requirements for surface water quality [1, 2]. General reviews relating to surface water analysis and emerging environmental pollutants [3-6] have drawn the attention of the scientific community. For the

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analysis of organic compounds from rivers and lakes, the most used techniques are gas chromatography (GC) and liquid chromatography (LC), both coupled to mass spectrometry (MS). Enrichment by solid-phase extraction (SPE) using relatively low sample volumes, followed by the above mentioned analytical techniques may resolve complex samples, containing more than a dozen compounds in less than 10 minutes [7-10]. LC–MS, at high pressure, is the most appropriate analytical technique [11-14] for polar contaminants analysis and for monitoring plasma samples [15].

Identification and determination of phenol derivatives is a challenging task because of the extremely low levels at which they are present in the environment. Compared to other European countries, only limited research on distribution, occurrence and fate of phenol derivatives has been done in Romania.

In order to detect the pollution of rivers caused by industrial activities, there were successfully developed methods that use UPLC with UV detection and SPE procedure, as an alternative solution for the activity of a laboratory that does not possess expensive LC–MS/MS equipment [16].

Phenol derivatives, and especially chlorophenols, are toxic and they can affect both the odour and the taste of drinking water even at concentrations as low as a few μg/L [17]. Chlorophenols and nitrophenols are included in the list of priority pollutants of both the US Environmental Protection Agency (EPA) and the European Union (EU) [18, 19]. In fact, the maximum concentration of these compounds for drinking water is set up at 0.5μg/L by EU Directive 2455/2001/EC.

In this context, the main objective of this work was to develop a LC-MS/MS method for the simultaneous multianalyte determination of phenol derivatives from water samples. As target analytes (see Table 3), different compounds representative of diverse classes of phenols (chlorinated and alkyl-derivatives), were selected based on the extent of their use, ubiquity and consideration as priority pollutants.

RESULTS AND DISCUSSION

Sample Extraction

In order to concentrate the sample and eliminate as much as possible the interferences of other compounds with the target analytes, an off-line SPE pre-concentration step was applied. In this step there were used two types of SPE cartridges: LiChrolut RP-18 and Oasis HLB (divinylbenzene-N-vinylpyrrolidine copolymer). Slightly better results were obtained with Oasis HLB, because some of the target compounds are relatively polar (with logP smaller than 2).

The influence of the volume of the sample was studied by passing through the SPE cartridge 100, 200 and 500 mL water sample. The best results were obtained with 500 mL sample volume. The weak step of the sample preparation procedure was the evaporation to dryness of the extract. The loss of analytes was somehow reduced by adding protecting solvents, like diethyl ether, acetone and acetonitrile. The best results were obtained when the SPE cartridge was eluted with a mixture of diethyl ether–methanol (9:1; v/v). For the studied analytes, having the logP between 1.4 and 5.8, the optimized procedure for sample preparation by solid phase extraction consisted in adding 500 µL of 20% aqueous methanol solution (v/v) containing 1% acetic acid (v/v) to the residuum obtained after the evaporation of the organic solvents from the SPE eluate. In this way, a 1000-fold pre-concentration was obtained in the off-line SPE procedure.

Sample Analysis

Previous to the coupling with the mass spectrometer, an optimization of the liquid chromatographic separation was carried out using a PDA [20]. In order to optimize the separation and the peak shapes of all the target analytes, different mobile phases were studied. The two organic solvents, acetonitrile and methanol, commonly used in reversed phase liquid chromatography were tested. Taking into consideration the resolution of the separated peaks, the methanol gave slightly better results than acetonitrile. It was noticed that, to suppress the ionic mobility of the analytes, acidification of the LC mobile phases was necessary. For this, there were tested formic acid, acetic acid and trifluoroacetic acid. The best ionization and separation of target analytes was obtained using 1% acetic acid as additive in the mobile phases. To obtain a good resolution for the 15 compounds, taken under study, an elution gradient program was used, that allowed their separation in 7 minutes (8 min with column re-equilibration).

The main parameters for ESI interface in negative ionization modes were: desolvation temperature 350°C, source temperature 150°C, cone gas flow rate 50 L/h, desolvation gas flow rate 650 L/h, vaporizer gas (nitrogen) pressure 7.0 bar, and capillary voltage 4 kV. Full-scan data were acquired in negative mode by scanning from m/z 50 to 250, using a MS inter-scan 0.003 s, and inter-channel delays 0.003 s. For optimization of MS-ESI parameters, the analytes were injected directly in MS interface (direct infusion) at 15 µL flow (concentration around 1 mg/mL). In this case, the scan time was set to 0.1 s. For each analyte, the most abundant and characteristic ion was chosen for quantification as shown in the Table 1.

Table 1. Retention times and optimized MRM parameters of the target compounds

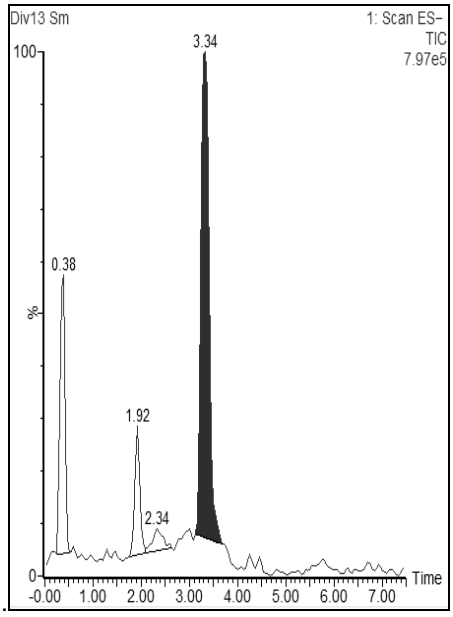
Analyte	Rt range (min)	Con voltage (V)	Collision energy (kV)	Quantification transition	Collision energy (kV)	Confirmation transition
Phenol	0.34-0.45	15	17	93>65	22	93>35
2,4-Dinitrophenol	0.51-0.84	20	15	183>137	20	183>91
4-Nitrophenol	0.91-1.32	15	15	138>93	25	138>65
4-Mehtylphenol	1.78-1.93	20	20	107>79	25	107>51
2-Methyl-4,6-dinitrophenol	2.25-2.43	25	25	197>105	30	197>51
3.5-Dimethylphenol	2.5-2.88	25	25	121>107	30	121>79
4-Clorophenol	3.05-3.34	20	20	127>100	25	127>91
3-Methyl-4-nitrophenol	3.38-3.6	20	18	152>106	25	152>78
2,4,6-Trimethylphenol	3.96-4.05	22	23	134>119	35	134>79
4-Chloro-3,5-dimethylphenol	4.3-4.6	28	22	217>182	30	217>90
Bisphenol A	5.2-5.59	20	15	227>212	20	227>133
3,5-Dichlorophenol	5.71-5.98	20	20	162>127	25	162>91
2,4,6-Trichlorophenol	6.25-6.5	15	25	196>160	33	196>90
4-Tert-octylphenol	6.5-6.8	25	27	205>147	35	205>106
4-Nonylphenol	6.7-7.1	25	27	220>133	35	220>106

Method validation

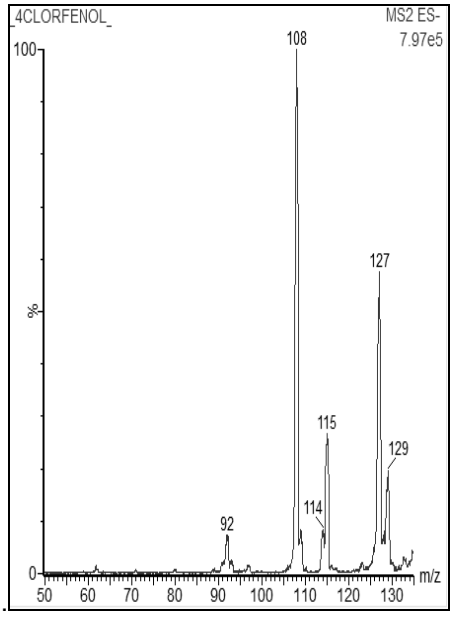
Linearity of the method was good up to 250 µg/L (equivalent to 250 ng/L in samples, taking into account the pre-concentration factor) for all target compounds. The correlation coefficients were higher than 0.99. Recovery of the overall analytical procedure was evaluated by spiking tap water samples (n = 3) at two levels (10 and 100 ng/L). Recovery, expressed in percentage, represents the amount of analyte obtained in the last quantification step (after sample extraction procedure) in relation to the amount of compound added to the initial sample. These results can be seen in Table 2. The method was found to have good precision (with RSD < 20%), with recovery values ranged between 70 and 95% for all analytes. The limit of quantification (LOQ) was established at 5 ng/L for the majority of the analytes, and at 3 ng/L for those compounds having a recovery better than 80%. The procedure was found to be highly specific as no relevant signals were observed in the blanks at the retention times of the analytes.

Table 2. Average recoveries and relative standard deviations for three replicates of tap water spiked at two levels of analytes

Compound	10 ng/L		100 ng/L	
	Rec.(%)	RSD (%)	Rec.(%)	RSD (%)
Phenol	77.33	12.99	84.47	10.45
2,4-Dinitrophenol	78.33	11.27	83.57	9.80
4-Nitrophenol	77.67	12.97	85.43	10.33
4-Mehtylphenol	80.67	9.81	84.87	9.00
2-Methyl-4,6-dinitrophenol	81.33	9.52	88.33	8.46
3.5-Dimethylphenol	86.33	7.63	87.33	7.33
4-Clorophenol	84.67	8.50	95.03	5.21
3-Methyl-4-nitrophenol	83.00	9.22	96.00	6.22
2,4,6-Trimethylphenol	78.33	13.10	82.67	9.83
4-Chloro-3,5-dimethylphenol	76.33	13.28	81.33	9.49
Bisphenol A	83.67	8.96	87.67	6.80
3,5-Dichlorophenol	81.67	9.60	86.00	7.94
2,4,6-Trichlorophenol	81.67	12.84	82.67	8.98
4-Tert-octylphenol	74.33	13.65	78.00	14.48
4-Nonylphenol	71.00	16.32	76.00	13.04



a.



b.

Figure 1. a. The chromatogram of a sample of water collected from Bega watershed in the vicinity of Margina village (Timiş county); **b.** the MS spectrum which prove that the eluted peak at 3.34 minute is 4-chlorophenol (daughters of m/z = 127)

The UPLC-MS/MS method was used to determine the target analytes in water samples collected from Bega watershed. An example of such analysis is presented in Figure 1. An assessment of the pollution with some phenol derivatives from Bega superior watershed is presented elsewhere [21].

CONCLUSIONS

In this work, a method based on UPLC-MS/MS analysis, has been developed for the simultaneous, multianalyte determination of some selected phenol derivatives. With this method, most of the selected compounds can be determined with acceptable precision and accuracy, according to the method performance evaluation carried out with spiked tap water, at concentrations lower than 100 ng/L.

EXPERIMENTAL SECTION

Chemicals and Reagents

The working standards were all purchased from Sigma – Aldrich and some of their characteristics are listed in Table 3. For the stock solution there was used 1 µg/mL of each phenol derivative prepared in HPLC-grade methanol (14262 Fluka).

Table 3. List of target compounds included in the analyses

Compound	Abbreviation	CAS registry number	Elemental composition	Molecular mass
Phenol	Ph	108-95-2	C_6H_6O	94.11
2,4-Dinitrophenol	DNP	51-28-5	$C_6H_4N_2O_5$	184.11
4-Nitrophenol	NP	100-02-7	$C_6H_5NO_3$	139.11
4-Mehtylphenol	MP	95-48-7	C_7H_8O	108.14
2-Methyl-4,6-dinitrophenol	MDNP	534-52-1	$C_7H_6N_2O_5$	198.14
3.5-Dimethylphenol	DMP	108-68-9	$C_8H_{10}O$	122.17
4-Clorophenol	CP	106-48-9	C_6H_5ClO	128.56
3-Methyl-4-nitrophenol	MNP	59-50-7	$C_7H_7NO_3$	153.14
2,4,6-Trimethylphenol	TMP	527-60-6	$C_9H_{12}O$	136.2
4-Chloro-3,5-dimethylphenol	CDMP	88-04-0	C_8H_9ClO	156.61
Bisphenol A	BS	80-05-7	$C_{15}H_{16}O_2$	228.29
3,5-Dichlorophenol	DCP	591-35-5	$C_6H_4Cl_2O$	163
2,4,6-Trichlorophenol	TCP	88-06-2	$C_6H_3Cl_3O$	197.45
4-Tert-octylphenol	TOP	140-66-9	$C_{14}H_{22}O$	206.33
4-Nonylphenol	NOP	25154-52-3	$C_{15}H_{24}O$	220.36

The other solvents and reagents were of chromatography quality, purchased from Sigma – Aldrich: mobile phase additive acetic acid 99% p.a. (A6283-ReagentPlus), diethyl ether (CHROMASOLV, 309966 Sigma), methanol LC-MS Ultra CHROMASOLV, tested for UHPLC-MS (14262 Fluka). SG Ultra Clear 2001-B Water Deionization System (Cole-Parmer) was used for the preparation of HPLC grade water and then filtered through syringe filters PTFE 0,22 µm (Teknokroma, Barcelona, Spain) right before use.

Sample Extraction

The sample preparation procedure was optimized using tap water samples that do not contain any trace of target analytes, fortified with a mixture of analytes of interest as a surrogate, at a concentration of 30 ng/L each. The solid-phase extraction cartridges (LiChrolut RP-18 and Oasis HLB) were conditioned with 5 mL of diethyl ether, 5 mL methanol and 5 mL of deionized water on a SPE manifold (Merck, Darmstadt, Germany) at a rate of 1–2 mL/min. A volume of 500 mL of water sample (neat tap water, tap water fortified with the analytes or real sample from Bega watershed), acidified with acetic acid to pH 5, was passed through the SPE cartridges at a flow rate of 2–3 mL/min. During the subsequent washing step, basic interferences were reduced by washing the cartridge with 5 mL of 5% methanol aqueous solution (v/v) containing 2% acetic acid (v/v) and 5 mL of deionized water; thereafter, the acidic interferences were removed by washing the cartridge with 5 mL of 5% methanol aqueous solution (v/v) containing 2% ammonium hydroxide (v/v) and 5 mL of deionized water. The compounds of interest were eluted with 6 mL of a mixture of diethyl ether–methanol (9:1; v/v). After elution, the solutions were evaporated to dryness at 40°C under a gentle stream of nitrogen. A volume of 500 µL of initial mobile phase was added in order to re-dissolve the residues and the resulting extracts were injected into the LC system after filtration through PTFE syringe disk filter.

Sample Analysis

Chromatographic analyses were performed using an AcquityUPLC™ system (Waters, Milford, MA, USA) and separations were carried out using an AcquityUPLC™ BEH C18 column (100×2.1 mm, 1.7 µm particle size) from Waters. The C18 column was equilibrated at 30°C. The analytes were separated with a gradient elution profile realized with a mobile phase consisting of methanol with acetic acid 1% (v/v) (eluent A) and an aqueous solution of acetic acid 1% (v/v) (eluent B). The analysis started with 20% of eluent A at a flow rate of 0.35 mL/min, for 0.3 minute. Then, the percentage of mobile phase A was increased linearly up to 75% in 3.0 minutes and further to 100% in 2.2 minutes; this composition was held for 2.0 minutes before being returned to 20% of eluent A, in 0.1 min, followed by a re-equilibration time of 0.4 minutes (total run time, 8 minutes). The injection volume was always 5 µL (full sample loop).

The UPLC system was coupled to a XevoTQD (T-wave quadrupole) mass spectrometer with an orthogonal Z-spray–electrospray interface (Micromass, Manchester, UK). For the purpose of optimizing the MS parameters, the selected analytes were dissolved in methanol: water mixture (50:50, v/v) with acetic acid 0.1%, at a concentration of 0.1 µg/mL and infused at 15 µL/min. The MS was operated in the negative electrospray (ESI–) mode with a capillary voltage 4 kV [22]. The source and desolvation temperatures used were 150 and 350ºC, respectively. Nitrogen was used as the desolvation and cone gas at the corresponding flow rates of 650 and 50 L/h, respectively. Collision-induced dissociation was performed using argon (99.995%, Linde, Timisoara, Romania) at a pressure of 2×10^{-3} mbar in the T-wave cell. The selected precursor ions of the analytes were fragmented to their product ions in the collision cell and the two most intensive product ions per analyte were chosen for quantitative and confirmation purposes. The ions were monitored for a dwell time of 0.022 or 0.036 s. Data acquisition was performed using MassLynx 4.0 software with QuanLynx program (Waters, Milford, MA, USA).

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