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Gas chromatographic analysis of cresols in aquatic solution by solid phase microextraction

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A simple and sensitive method for analysis of a mixture of cresol isomers in aquatic solution was developed using headspace solid-phase microextraction (HS-SPME) and gas chromatography with flame ionization detection (GC-FID). Separation of o-, m- and p-cresols in mixture was performed on an Elite Wax column. The effect of temperature, time and sodium chloride on cresol extraction as well as the effect of temperature and time on the desorption of the compounds was established. The following conditions were revealed to be optimal: headspace extraction at 40 °C for 60 min in the presence of 0.3 g/mL of sodium chloride and desorption at 210 °C for 60 s. Detection limits for o-cresol, m-cresol and p-cresol were established to be 0.68, 0.96 and 1.31 μg/L, respectively, when PDMS-DVB fibre was used. The relative standard deviation (RSD) of the measurements was ≤ 5.9%. The dose–response relationship was linear within the range 1.5 to 10.0 mg/L ($R^2 = 0.997$).

Key words: phenolics, headspace, GC-FID, SPME, optimisation

INTRODUCTION

Phenolic compounds, including cresol isomers, are widely spread in the environment. For detection of these compounds various methods were used: photometric, ultraviolet (UV) or electrochemical detection [1–4], gas chromatography and high-performance liquid chromatography with either mass spectrometric (MS) or flame ionization detection (FID) [5–9]. To analyse phenolics, gas chromatography (GC) is widely used. However, GC is usually carried out after compound extraction from various matrixes and preconcentration. Liquid–liquid extraction (LLE), solid-phase extraction (SPE) or solid-phase microextraction (SPME) techniques [5–9], vapour steam distillation [10], microwave-assisted extraction, ultrasonication and supercritical fluid extraction [11, 12] were used. SPME is one of the extraction techniques that combines both sampling and sample preparation. This method is fast, simple, inexpensive and solvent-free [13–15]. Therefore, SPME has been intensively used for the analysis of phenol derivatives in biological, pharmaceutical, environmental, food, flavour and fragrance samples [5–9, 13–15].

Several kinds of SPME coatings are commercially available from Supelco, the only producer of the fibres [16]. For extraction of cresol isomers and some other alkylphenols from oil-contaminated groundwater as well as for extraction and analysis of phenols and nitrophenols from rainwater, polyacrylate (PA) coated fibre was used [6, 8]. Phenol and some of its volatile derivatives present in water samples were analysed using polyaniline (PANI) coated fibre [7]. Carbowax / Divinylbenzene (CW / DVB) was proposed for phenol, o- and p-cresol sampling indoors and outdoors of animal farms and also in farm soil [9]. The same fibre coating was found to be the best for analyses of o-cresol in urinary samples of humans exposed to solvents at their working place [5]. For analysis of phenols present in wastewater samples, laboratory-made calixarene fibre was used [15].

Several modes of SPME have been introduced by various researchers, among those headspace-solid phase microex-

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traction (HS-SPME) and direct immersion (DI-SPME) [13]. However, in case of DI-SPME application some problems may arise, including irreversible adsorption onto the coating of major components of the matrix, leading to the earlier fibre deterioration [17].

The important parameters affecting SPME results include the type of fibre coating employed, the extraction temperature and duration, salt concentration in the sample, desorption time and temperature, agitation, etc. [13–15, 17].

The purpose of the present study was to optimize the parameters influencing the efficiency of headspace extraction and gas chromatographic analysis of cresols' mixture in aquatic solutions. Validation of the analytical method based on HS-SPME-GC-FID for analysis of the compounds was carried out.

EXPERIMENTAL

Methanol (≥99.5%, Lachema), NaCl (analytical grade, Reachim, Ukraine), o-cresol (≥99.7%, Fluka), m-cresol (≥99.7%, Fluka), p-cresol (≥99.7%, Fluka) were used with no extra purification. Standard stock solutions of o-cresol (0.612 mg/mL), m-cresol (0.668 mg/mL) and p-cresol (0.770 mg/mL) were prepared in methanol by weighing. The solutions were stored at +4 °C. Working standard solutions were prepared daily by diluting the stock standard solution with methanol. Methanol volume was kept low with the aim to minimize its sorption on the fibre, thus water was used for the final dilution when 1 mL of water was added to 10 µl of the methanolic working solution. One mL of the finally diluted solution was placed in a 10 mL vial for investigation. The vial was covered with aluminium foil and placed in a water bath at a temperature of 40 °C. After 15 min, when the thermal balance was reached, an SPME needle was inserted into the headspace of the vial. Polydimethylsiloxane-divinylbenzene (PDMS-DVB, 65 µm, Supelco) fibre was used as it had been successfully applied in our previous analyses of volatile compounds from urine, including cresols [18]. Before the usage of the SPME fibre, it had been conditioned following the recommendation of the manufacturer at a temperature of 250 °C in the injection port. In the present work, extraction was carried out with no agitation of the solution, as most of the analytes were abundant in the headspace, and their concentrations were sufficient for SPME technique application [13]. After collection of volatiles, the fibre was immediately injected into the GC port, and volatile compounds were desorbed in split-splitless (splitless for 2 min) mode.

A Clarus 500 gas chromatograph (Perkin Elmer, USA) equipped with FID was used for chromatographic analyses. The mixture of cresols was separated on an Elite Wax fused silica capillary column (30 m × 0.25 mm × 0.25 µm film thickness) using hydrogen carrier gas at a flow rate of 1.5 mL/min. The column oven temperature was programmed as follows: the initial temperature 40 °C for 1 min, then it was increased at the rate of 7 °C/min to 240 °C, and the final temperature was held for 1 min. The injector and detector temperatures were 210 °C and 240 °C, respectively, except when the effect of desorption temperature was investigated.

RESULTS AND DISCUSSION

Desorption conditions

Thermal desorption in the injection port of GC can be affected by several parameters such as the temperature of the GC injector and desorption duration of analytes from a fibre. Part of the compounds under study can remain in the fibre coating and appear in subsequent analyses (carryover effect). In general, the injector temperature was set at the maximum temperature level still suitable to maintain the stability of the fibre coating and close to the boiling temperature of the analytes. Cresols are semivolatile compounds (boiling temerature of o-cresol is ~190 °C and of m- and p-cresol ~202 °C), therefore the injector temperature in a range from 200 to 240 °C was tested. The most intensive desorption (both of o- and m-cresols) was registered when the temperature was increased up to 210 °C (Fig. 1). No significant changes in the peak area of any of the cresols tested were observed within the temperature range from 210 to 230 °C. Peak areas of m- and p-cresols increased more considerably again at a temperature of 240 °C. Thus, 210 °C was selected as the optimal temperature for desorption, and it was employed in all optimization experiments.

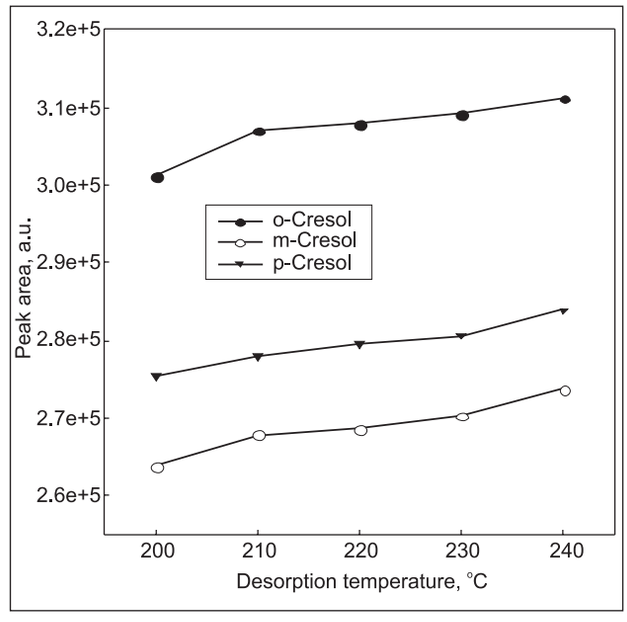

Fig. 1. Peak areas of cresols as a function of desorption temperature. Concentrations of o-, m- and p-cresols analysed were 1.75, 1.90 and 2.20 mg/L, respectively. Extraction was carried out at 40 °C for 60 min in the presence of 0.3 g/mL of sodium chloride. Chromatographic conditions: temperature of the detector was 240 °C, the GC oven temperature was programmed from 40 °C (isothermal for 1 min) and increased to 240 °C at a rate of 7 °C/min, and the final temperature was kept for 1 min, desorption duration 60 s

The following desorption durations were examined with the aim to reveal the most appropriate desorption conditions: 20, 40, 60, 80 and 120 seconds (Fig. 2). The peak areas of the cresols increased insignificanty when desorption duration was over 60 s. To determine the optimum desorption duration, the carryover effects of the analytes were examined in a particular optimization experiment by injecting the blank PDMS / DVB fibre in the GC injection port immediately after completing each extraction–desorption procedure. None of the cresols appeared in blank runs, thus the 60 s duration was selected as the optimum desorption at 210 °C.

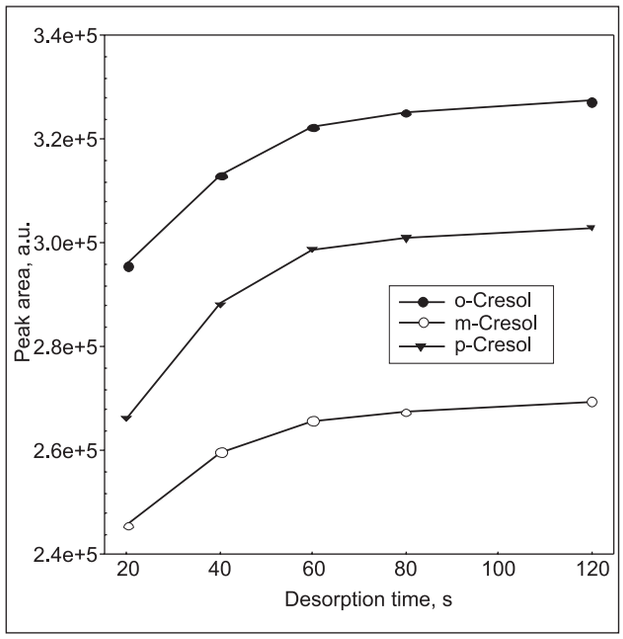

Fig. 2. Peak areas of cresols as a function of desorption duration. Extraction and chromatographic conditions were the same as in Fig. 1, temperature of the injector was 210 °C

Extraction conditions

Extraction is affected by the temperature, duration and ionic strenght of the solution. Adding salt to a sample enhances the extracted amount of the analyte as it increases the ionic strength of the sample. The increase in the ionic strength decreases the solubility of the analyte in the aqueous phase and enhances its diffusion towards the fibre. This effect is not general and depends mainly on the characteristics of the analyte, the fibre and the sample. To increase the ionic strength, sodium chloride was added. This salt is most often used for this purpose. The effect of the salt added to the solution analyzed is shown in Fig. 3. Chromatographic response was the highest when the concentration of sodium chloride in an aquatic sample reached 0.3 g/mL. An extra amount of the salt did not increase the peak area of the cresols. The rest parameters influencing the sampling and analysis of cresols were investigated in the presence of 0.3 g/mL of sodium chloride in the solution.

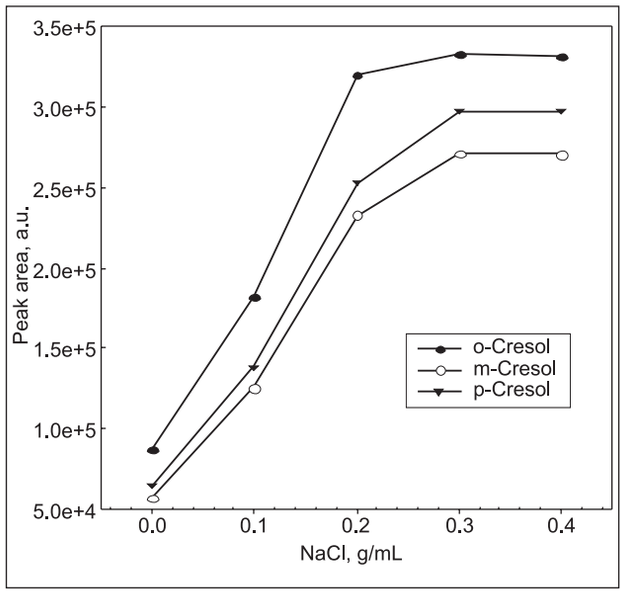

Fig. 3. Peak areas of cresols as a function of sodium chloride amount added to sample solution. Extraction from headspace was carried out at 40 °C for 60 min. Chromatographic conditions were the same as in Figs. 1 and 2

Extraction temperature influences the SPME process in two opposite ways. An increase in temperature during extraction enhances the diffusion of analytes towards the fibre. Besides, in the HS-SPME sampling mode, temperature enhances the transfer of analytes to the headspace. On the other hand, increase in temperature decreases the distribution constant of the analytes because the absorption is an exothermic process, and any increase in sampling temperature decreases

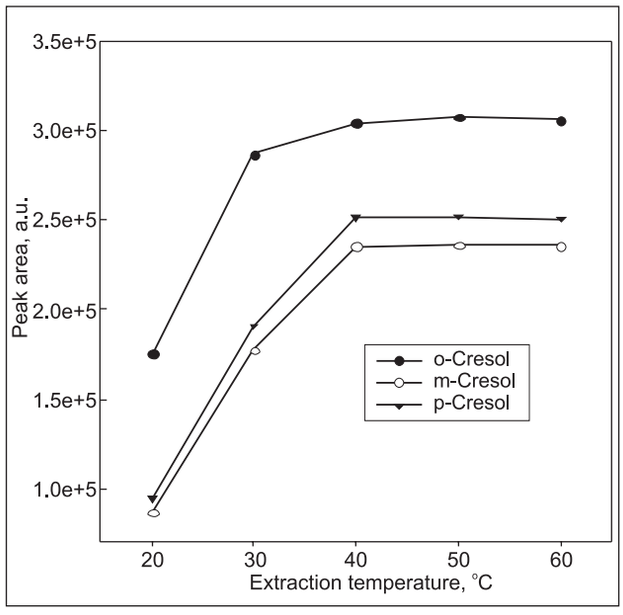

Fig. 4. Peak areas of cresols as a function of extraction temperature. Extraction was carried out for 60 min in the presence of 0.3 g/mL of sodium chloride. Chromatographic conditions were the same as in Figs. 1 and 2

analyte recovery. With this in mind, the extraction temperature was changed within 20 to 60 °C, and the extraction lasted for 60 min. Data presented in Fig. 4 demonstrate that the extraction reached the highest efficiency at a temperature of 40 °C. With the further temperature increase there was no significant increase in the efficiency.

The amount of cresol registered at 40 °C when the extraction varied from 20 to 120 min is presented in Fig. 5. Approximately twice higher chromatographic responses were registered when the extraction lasted for 40 min in comparison to those of 20 min. The results demonstrated that extraction during 60 min was sufficient the for analysis of cresols because extraction efficiency was similar to that of 120 min extraction.

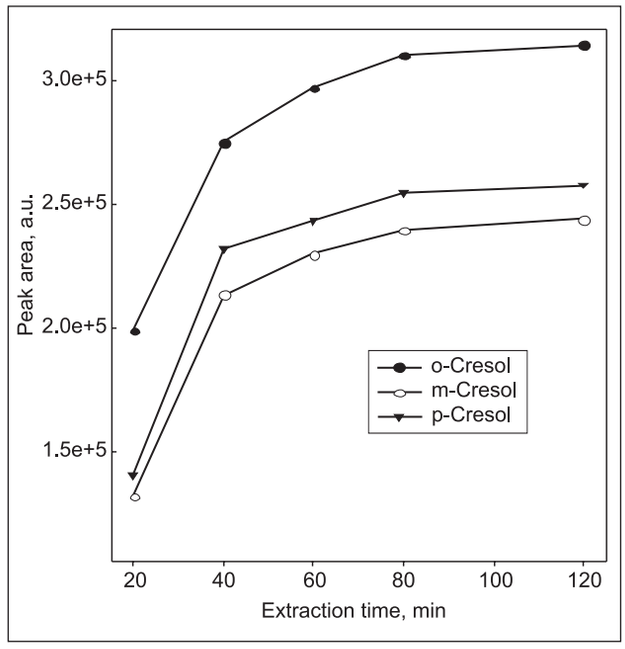

Fig. 5. Peak areas of cresols as a function of extraction duration. Extraction was carried out at 40 °C in the presence of 0.3 g/mL of sodium chloride. Chromatographic conditions were the same as in Figs. 1 and 2

All experiments carried out with the aim to optimise both desorption and extraction conditions were performed using 1.75, 1.90 and 2.20 mg/L solutions of o-, m- and p-cresols, respectively. Considering the results, we conclude that 40 °C and a 60 min duration in the presence of 0.3 g/mL were optimal conditions for cresol extraction with a PDMS / DVB fibre, and 210 °C and 60 s were optimal for desorption.

Analytical performance

The repeatability of the analytical procedure (relative standard deviation, RSD %) was estimated based on six replicates of two different concentrations of cresols under optimal conditions. Relative standard deviations are presented in Table.

Table. **Repeatability of cresol analyses (p = 0.95)**

Compound	Concentration, mg/L	RSD, %	
		within a day (n = 6)	within a month (n = 25)
o-Cresol	1.75	5.9	22.4
	7.0	5.8	23.1
m-Cresol	1.90	3.3	21.4
	7.6	3.7	22.0
p-Cresol	2.20	3.6	22.1
	8.8	3.5	22.8

Higher RSD values were obtained in a month's study compared to those obtained during one-day study. In our opinion, this occurred since the solutions were kept at 4 °C in a refrigerator and were taken many times, thus slight objective changes in the concentration could occur. Some changes in the characteristics of the fibre could occur as well. For a better repeatability, it is desirable to prepare solutions and to perform analyses the same day.

It should be noted that the method showed a good linearity with the regression coefficient 0.997 within the ranges of concentrations from 1.5 to 10 mg/L for all cresols analysed. The limits for detection based on the signal-to-noise ratio of S / N = 3 for o-, m- and p-cresols were 0.68, 0.96 and 1.31 μg/L, respectively.

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DUJŲ CHROMATOGRAFINIS KREZOLIŲ ANALIZĖS VANDENINIUOSE TIRPALUOSE METODAS, NAUDOJANT KIETAFAZĘ MIKROEKSTRAKCIJĄ

S a n t r a u k a

Parengtas paprastas ir jautrus krezolių mišinio vandeniniuose tirpaluose analizės metodas, naudojant kietafazę mikroekstrakciją iš viršerdvio ir dujų chromatografiją su liepsnos jonizacijos detekcija. Krezolių mišinys skirstytas Elite Wax kolonėlėje. Nustatyta temperatūros, laiko ir natrio chlorido įtaka krezolių ekstrakcijai, taip pat temperatūros ir laiko įtaka junginių desorbcijai. Optimalios krezolių analizės sąlygos yra šios: ekstrakcija iš viršerdvio vykdoma 60 min 40 °C temperatūroje, esant tirpale 0,3 g/mL natrio chlorido, desorbcija – 60 s 210 °C temperatūroje. Aptikimo ribos, nustatytos po sorbcijos PDMS-DVB strypelyje, buvo 0,68 µg/L o-krezoliui, 0,96 µg/L m-krezoliui ir 1,31 µg/L p-krezoliui. Santykinis standartinis matavimų nuokrypis (RSD) buvo ≤5,9 %. Chromatografiniai atsakai kinta tiesiškai krezolių koncentracijų intervale nuo 1,5 iki 10,0 mg/L ($R^2 = 0,997$).