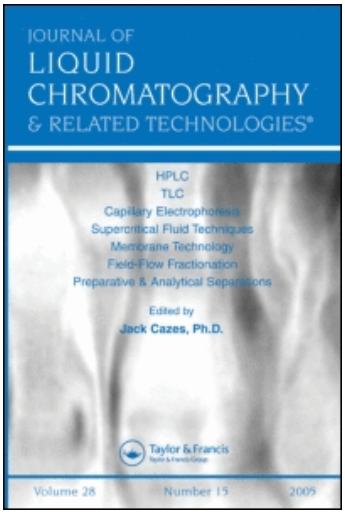

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Isolation and Determination of 4-Nonylphenol in Environmental Samples Using Combined Chromatographic Techniques

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Isolation and Determination of 4-Nonylphenol in Environmental Samples Using Combined Chromatographic Techniques[#]

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ABSTRACT

The aim of the present study was to develop a new procedure for isolation, enrichment, and determination of 4-nonylphenol (4NP) from aqueous matrices (surface waters: rivers, fishponds) and sewage. An attempt was focused to find selective sorbents for nonylphenol isolation by solid-phase extraction (SPE), solvents selection for elution of this

[#]This paper is dedicated to memory of Prof. Csaba Horváth.

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group of compounds isolated from aqueous samples, and the optimum composition of HPLC mobile and stationary phases for final determination of 4NP. New-generation chromatographic packings were used whose structure resembles that of natural biological systems. The results obtained were verified by employing GC-MS as a comparative method.

Key Words: 4-Nonylphenol; SPE; Chromatography; Environmental samples.

INTRODUCTION

One of the main problems connected with municipal and industrial sewage treatment is incomplete degradation of surface-active compounds. An important group of such compounds are nonylphenol ethoxylates (NP*n*EO), used among others, in the textile and pulp-and-paper industry, and for detergent production. The global production of surface-active compounds is expected to increase by over 3.5% of its present level by the year 2001.[1] Wastewater treatment is a very complex process, dependent upon many factors, and NP*n*EOs are not easily and ultimately biodegradable. When degraded by de-ethoxylation, they form metabolites characterized by higher persistency and toxicity (toxicity of NP*n*EO increases as the ethoxylate chain becomes shorter). 4-Nonylphenol (4NP) and other products of NP*n*EO degradation may cause disturbances in the endocrine gland functions in fish. Both in vivo and in vitro assays concerning the estrogenic activity of nonylphenols confirm their adverse effects on the development and reproduction of some fish species.[2–8] Systems simulating natural biological ones (tissues, cells, or biofilms) are more and more commonly applied to explain the phenomena taking place in living organisms at the cell level, as a result of the activity of these xenobiotics. An example may be HPLC columns and packings, as well as liquid–solid extraction adsorbents. Commercially available materials characterized by selective properties are amide[9] and cholesterol[10] phases, as well as phospholipid packings referred to as immobilized artificial membrane (IAM).[11,12]

Due to the progress observed in sample preparation methods and analytical techniques (developing combined techniques), more and more authors deal with these problems.[1,8,13–15] Methods for the detection and isolation of 4NP and their metabolites are constantly being improved.

In the present study, an attempt was made to use sorbents with selective properties for nonylphenol extraction from liquid samples, and to find the optimum composition of HPLC mobile and stationary phases for nonylphenol

determination. The results obtained by RP-HPLC were verified employing gas chromatography coupled with mass spectrometry (GC/MS).

EXPERIMENTAL

Materials and Reagents

The following solvents were used for chromatographic analysis (HPLC) and solid-phase extraction (SPE): methanol, acetonitrile, ethyl acetate, dichloromethane, cyclohexane, and acetone (HPLC-grade, J.T. Baker, Deventer, Holland). The aqueous mobile phase was prepared with water purified by passing it through a Mili-Q apparatus (Milipore, El Passo, TX). Acetic, hydrochloric, and sulfuric acids (HPLC-grade, J.T. Baker, Deventer, Holland) were used for preparing solutions for column conditioning, analyte elution, and liquid sample acidification. A 4-nonylphenol solution (Sigma Aldrich, Steinhaim, Germany) in methanol at a concentration of 1 mg/L was applied as a internal standard.

A series of SPE cartridge with different packings (carbon, polymeric, and octadecyl) were used. Table 1 presents the physicochemical parameters of packings used for 4NP of SPE.

Apparatus

Chromatographic measurements were taken using HP-1050 liquid chromatograph system (Hewlett Packard, Waldbronn, Germany) equipped with a quaternary gradient pump, an autosampler (injection volume 20 mL) and a diode detector with a measuring cell with a volume of 8 μL, and ChemStation for data collection and control over the process. In all chromatographic investigations, the flow rate was 1 mL/min. Separations were performed on three different columns: SUPELCOSIL LC-C18-DB (Supelco^{TM}, Bellefonte, PA) (250 × 4.6 mm^2 I.D.; $d_p = 5$ μm particle size), SG-MIX (125 × 4.6 mm I.D.; $d_p = 5$ μm particle size),[16] SG-AP (I.D. 250 × 4.6 mm^2, $d_p = 5$ μm ziarno),[9] and SG-CHOL (I.D. 250 × 4.6 mm^2, $d_p = 5$ μm).[10] SG-MIX, SG-AP, and SG-CHOL were home-made and their structures are presented in Table 2.

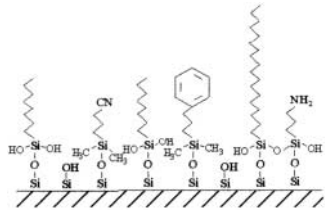
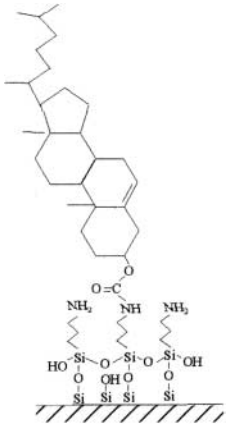
Gas chromatograph (GC/MS): Autosystem XL (Perkin Elmer) with a capillary column SB1 (WGA, Düsseldorf, Germany) 60 m × 0.25 mm × 0.25 μm and MS Turbomass (Perkin Elmer); data acquisition system: Turbomass software (Perkin Elmer); analytical conditions: GC: 100°C (3 min), ramping 7°C/min to 295°C (15 min); MS: electron impact ionization (EI), energy 70 eV, ion source and transfer line temperatures—250°C, acquisition mode—full scan in the mass range 35–500 amu.

Table 1. Characteristics of some common commercial SPE silica, carbon, and polymeric sorbents.

Sorbents	Manufacturer	Structure	Surface area (m^2/g)	Pore diameter (Å)	d_p (μm)	Carbon content (%)	Nitrogen content (%)
Carbon sorbents							
Carbograph™	Alltech Associates, Inc.	GCBs[a]	100		38–125		
SAN1, SAN2	Polymer Institute (SAN, Bratislava)	GCBs[a]		<15			
Polymeric sorbents							
Isolute ENV +	IST	PS-DVB	1000	100	90		
Abselut Nexus	Varian Associates, Inc.	Dual function[17]	500–650	100	65–80		
Strata™-X	Phenomenex, Inc.	PS-DVB	800	85	33		
Speedisk DVB	J.T. Baker	PS-DVB	700	n.a.	150		
Silica sorbents							
Zorbax SPE C18 (EC)	Agilent Technologies	Tri[b] + EC		80	50	14.8	
Isolute C_{18}	IST	Tri[b]	500	55	50	16	
Isolute C_8	IST	Tri[b]	500	55	70	12	
Bakerbond C_{18} Polar Plus	J.T. Baker	Tri[b]	328	60	47–60	16	
Speedisk®Column Octadecyl C18	J.T. Baker		290–350			16.5	
Isolute SAX	IST		500	55	50		
Bakerbond Amino (NH_2)	J.T. Baker		529	60	40	6.8	2.3

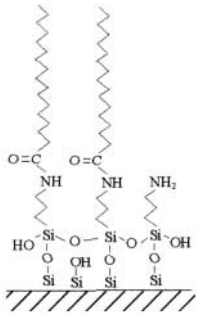
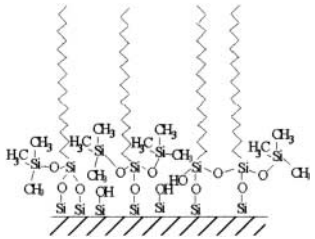
Note: n.a., not available in data supplied by manufacturers; EC, end-capping.
[a]Graphitized carbon blacks.
[b]Silane function.

Table 2. Parameters determining column efficiency for 4-nonylphenol as a test substance.

Stationary phase	Structure of stationary phase	Capacity factor k	Asymmetry coefficient f_{AS}	Number of theoretical plates N_T (per 25 cm of column)
SG-MIX		1.4	1.60	7210
SG-CHOL		4.99	1.06	5638

(*continued*)

Table 2. Continued.

Stationary phase	Structure of stationary phase	Capacity factor k	Asymmetry coefficient f_{AS}	Number of theoretical plates N_T (per 25 cm of column)
SG-AP		4.8	0.98	6925
Supelcosil LC-C18-DB		8.8	1.10	10155

SPE of all samples was performed using a commercial SPE-12 G (J.T. Baker, Groß-Gerau, Germany) vacuum set.

RESULTS AND DISCUSSION

4NP Extraction from Aqueous Matrices

The efficiency of nonylphenol isolation from aqueous matrices was determined for various types of packings silica with octadecyl, porous carbons, and polymers recommended for SPE (Table 1). The non-polar or low-polarity sorbents used in the experiment are characterized by different hydrophobicity, absorption capacity, and porosity represented by specific surface area, which enables specific interactions between the analyte and the adsorbent. Due to the variety of sorbents used, the SPE process required different procedures [octadecyl—Fig. 1(A); carbon—Fig. 1(B); polymeric—Fig. 1(C)]

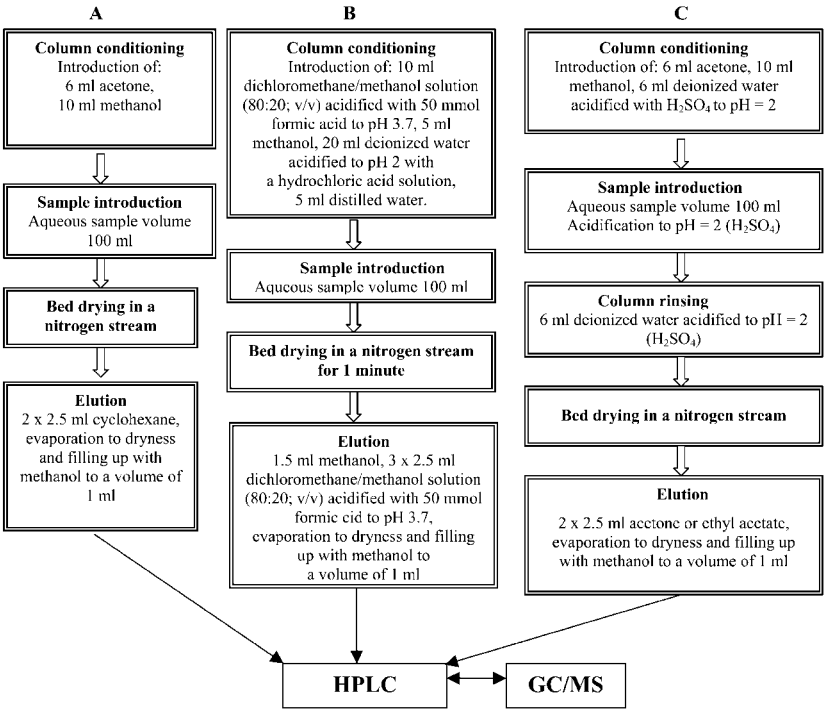


Figure 1. SPE of a nonylphenol sample using: (A) octadecyl packing (C_{18}), (B) carbon packing, and (C) polymeric packing.

of sample preparation (isolation and enrichment) for final analyte determination.

Analysis of the results obtained for porous polymeric packings on the basis of styrene–divinylbenzene shows that the recovery was the highest for Speedisk DVB ($R = 58.9\% \pm 4.2\%$) (Fig. 2). Much lower values of recovery were reported for nonylphenol isolated by Isolute ENV+ ($R = 35.9\% \pm 2.2\%$), Abselut Nexus ($R = 49.2\% \pm 2.2\%$), and StrataX ($R = 51.0\% \pm 2.6\%$), with ethyl acetate as eluent. It was probably connected with specific and non-specific interactions, as well as minor effects of polar interactions (dipole and hydrogen bonds). Using acetone as eluent always results in lower recoveries, by 10–15% (Fig. 2). This is associated with higher volatility of acetone, whose contact with the bed is shorter due to analyte elution under vaccum.

For octadecyl packing (C_{18}), the highest level of recovery was noted for the sorbent Zorbax SPE C18 ($R = 60.55\%$). Unfortunately, the process reproducibility was low standard deviation (SD = $\pm$ 9.41%). By far, the better results were obtained for Bakerbond C_{18} Polar Plus ($R = 62.2\% \pm 1.34\%$) and Isolute C_{18} ($R = 64.1\% \pm 3.54\%$) (Fig. 3). Values of carbon percentage for all investigated octadecyl adsorbents are situated between 12–16% (Table 1). It shows that the effectiveness of blocking of undersurface silanol groups during formation of chemically bonded phase is good. Slightly lower recoveries were observed for the columns Speedisk column C_{18} ($53.54\% \pm 0.52\%$) and Isolute C_8 ($R = 55.1\% \pm 3.54\%$). In all cases of octadecyl packing application, the values of SD were low (except the phase Zorbax SPE C18). Homogeneity and thickness of the undersurface structure of the chemically bonded phase of the parameters (pore diameters, specific surface porosity), which through recovery rate, have an influence on the

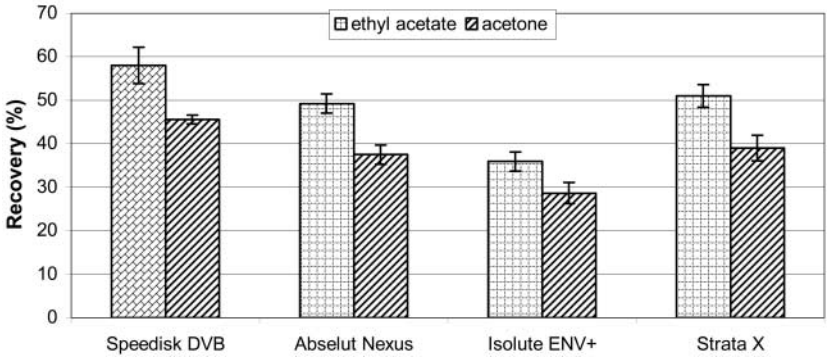


Figure 2. Nonylphenol recoveries for polymeric packing.

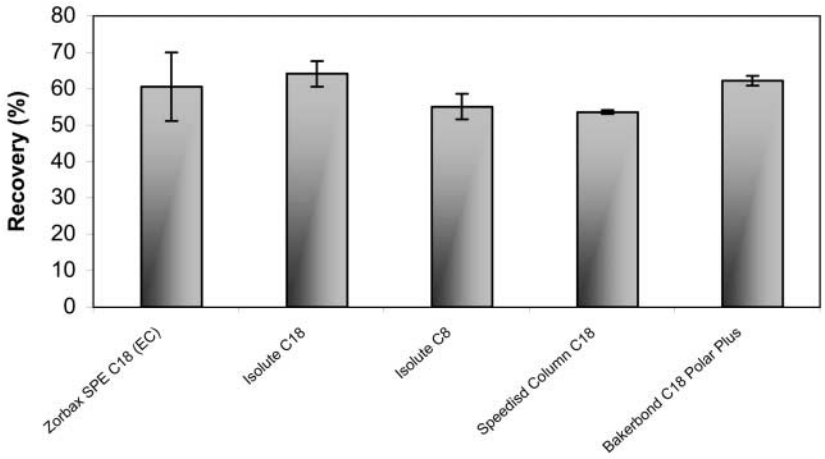


Figure 3. Results of 4-nonylphenol extraction for octadecyl packing.

reproducibility of the sorption of analytes, which are isolated and enriched from matrices.

Another group of adsorbents were carbon materials (Table 1). In this case, the highest recovery and process reproducibility were achieved for the sorbents prepared at the Polymer Institute (SAN, Bratislava) ($R_{SAN2} = 83.6\% \pm 4.6\%$; Fig. 4). Lower (by ca. 10%) R-values were obtained for carbon packings of the Carbograph type ($R = 72.4\% \pm 4.7\%$) and the adsorbent SAN1 ($R = 70.9\% \pm 3.43\%$; Fig. 4). Due to the carbon–oxygen bonds (positively charged region) in graphitized carbons, these sorbents can

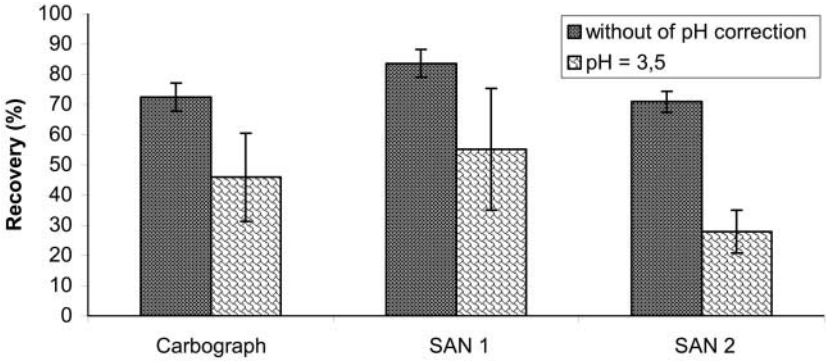


Figure 4. 4NP recoveries for carbon packing.

act as anion exchangers in acidified aqueous samples. That is why an attempt was made to analyze the effects of sample acidification on the recovery levels for columns with carbon packings ($R_{SAN1} = 55.2\% \pm 20.2\%$ and $R_{SAN2} = 27.8\% \pm 7.1\%$) and for a commercial SPE column with Carbograph ($R = 45.9\% \pm 14.53\%$). Polymeric packings (e.g., copolymer styrene–divinylbenzene—Speedisk DVB) and graphitized carbons (Carbograph and SAN1) are characterized by larger adsorption surface areas and higher hydrophobicity than silica sorbents (C_{18} packings) (Table 1). A decrease in the solution pH from 5.7 to 3.5 reduces nonylphenol recovery from aqueous matrices by 30–40%. High values of standard deviation (SD from 7 to 20) indicate nonreproducibility of the extraction process (Fig. 4).

With regard to carbon packings, the sorbent should be washed with methanol after sample introduction to remove sample remainders and impurities adsorbed on carbon. Therefore, it was necessary to examine the influence of this solvent on 4NP recovery. Standard solutions containing 0.05, 0.2, and 1 μg/L of 4NP were prepared. One series of these standard solutions was evaporated to dryness, the other to a volume of 1 mL. It was found that low intensity of methanol flow causes nonylphenol elution and reduces the level of recovery. An increase in the rate of methanol flow through the bed to 20 mL/min allows avoiding analyte loss. The effects of eluate evaporation to dryness and eluent change before chromatographic analysis, according to the procedures of sample preparation, were also determined (Fig. 2). It was found that evaporation to dryness does not cause analyte loss.

In the case of 4NP isolation from aqueous matrices, the highest recovery and the best SPE reproducibility were achieved for carbon packings. Due to their better efficiency, such polymeric packings and graphitized carbons are often used as sorbents for trace amounts of organic compounds. Cross-linked aromatic rings enable electron-donor interactions between the sorbents and the $\pi-\pi$ bonds of the substance isolated, which additionally intensifies the analyte–sorbent interactions. Another advantage of aromatic sorbents is their selective interaction with the aromatic rings of the analyte.[18]

In order to determine the breakthrough volume (V_e), curves were plotted following the introduction of 10, 20, 50, 75, 100, 150, 200, and 500 mL of a solution containing 0.1 mg/L of 4NP on selected sorbents (Fig. 5). Taking into account the results obtained on the basis of sorbent selection for NP*n*EO isolation, breakthrough curves were plotted for two carbon sorbents, considering the highest recovery level and reproducibility. The curves show that the maximum sample volume (V_e) is about 200 mL (Fig. 5). Analyzing the run of the breakthrough curves, it can see that the equilibrium was obtained after elution of twice higher volume of the sample (V_e). It suggests that Carbograph SPE column is characterized by a more homogenous porous structure of carbon than the similar absorbent prepared by SAN (Fig. 5).

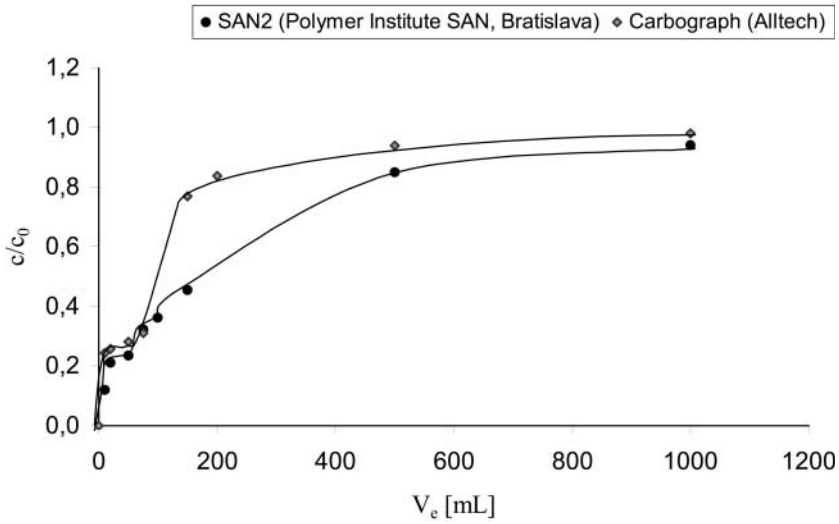


Figure 5. Breakthrough curves for selected carbon sorbents.

Chromatographic Determination 4NP

Analysis of earlier prepared samples obtained from particular extractions and tested for nonylphenol was preceded by analysis of packings, which differed in the near-surface structures of chemically bonded phases. These materials were characterized by different hydrophilic and/or hydrophobic properties (Table 1). In order to optimize the chromatographic process aimed at nonylphenol determination, a mixture of acetontrile and water was used as a mobile phase, whereas home-made new-generation columns SG-MIX, SG-AP, SG-CHOL were used as a stationary phase. They were compared with a commercial octadecyl column, Supelcosil LC-18-DB (Table 2). Due to nonylphenol structure, it was necessary to search for packings offering various types of interactions. The symmetry coefficient (f_{AS}) of the 4NP peak obtained for C_{18} phase was very similar to the Gaussian shape, obtaining theoretical (N_T) value ($f_{AS} = 1.00$) (Table 2). The highest number of theoretical plates was also noted for this phase. This could be connected with good screening of residual silanols of the under-surface effect (secondary silanization). Attaining satisfactory results is often difficult and the substances are poorly separated. Resolution could be improved if functional groups of analytes interact specifically with various functional groups of the stationary phase materials (interaction between analit-packing) for better selectivity. Consequently, the number of theoretical plates and selectivity play important

roles in chromatographic analysis. This limited analyte access to residual groups $\equiv$Si–OH on the silica support surface and reduced the participation of hydrogen interactions (analyte $\Leftrightarrow$ stationary phase). Unfortunately, the asymmetry coefficient for the SG-MIX phase is very high ($f_{AS} = 1.6$) (Table 2). Mixed phases (contained: –CN, $-NH_2$, –phenyl, $-C_8$, and C_{18} groups) are more polar than typical hydrophobic C_{18} phases, with much lower participation of interactions between the non-polar part of 4NP and carbon chains, and higher contribution of selective interactions of the $\pi \cdots \pi$ type between the polar fragment of the analyte molecule, and phenolic and cyanic groups on the packing surface.

It is obvious that amino groups containing free pairs of electrons and residual hydroxyl groups on the silica gel surface participate in the resolution process. This is confirmed by well-visible tailing of the analyte peak. The other two columns are characterized by even lower selectivity. Peak tailing in the case of the SG-CHOL phase may also indicate too strong $\pi \cdots \pi$ interactions between the nonylphenol molecule and stationary phase, with considerable contribution of non-polar interactions. For phases SG-CHOL and SG-MIX the values of the asymmetry coefficients are very high [$f_{AS(SG\text{-}CHOL)} = 1.06$ and $f_{AS(SG\text{-}MIX)} = 1.6$]. Moreover, the number of theoretical plates obtained for the SG-MIX column was unsatisfactory ($N_T = 7210$; calculation for column length; $L = 250$ mm). In such columns there exist strong $\pi \cdots \pi$ interactions between the nonylphenol molecule and stationary phase. Due to a low number of theoretical plates and very asymmetric analyte peaks, these phases were not used for quantitative determination of the analyzed compounds.

Quantitative analysis of 4NP in environmental samples was preceded by fixing the limit of quantification (LOQ = 3.76 μg/L) and the limit of detection (LOD = 0.72 μg/L). The results obtained show that liquid chromatography with UV-VIS detection at a wavelength $\lambda = 226$ nm may be applied for determining the analyte at low concentrations.

Due to high recovery and reproducibility of extraction, columns with carbon packing (Carbograph®) were used for nonylphenol isolation from aqueous samples from the Municipal Sewage Treatment Plant in Toruń. The samples were prepared according to the procedure shown in Fig. 2. Analysis was performed by means of HPLC (Fig. 6) and verified employing GC/MS (Fig. 7). The chromatograms obtained show that the retention time for a 4NP standard ($m/z = 107$) is $t_R = 12.2 \pm 0.5$ min [Fig. 7(A)]. A peak with a time of $t_R = 12.1$ min is visible in a sample of treated sewage, which may confirm the presence of this compound. Due to too low nonylphenol concentrations (small peak areas) in the samples examined, definite qualitative determination was impossible. That is why the results obtained with HPLC were verified using GCMS. A peak with the retention time $t_R = 14.79$ min

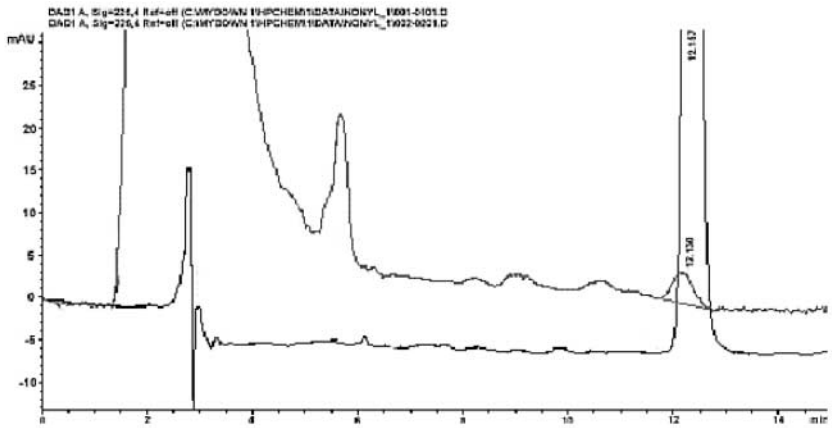


Figure 6. HPLC chromatograms for the standard solution of 4NP with concentration 10 ppm (A) and sewage sample examined (B). Chromatographic conditions: Supelcosil LC-18-DB (250 × 4.6 I.D.; $d_p = 5\,\mu m$), gradient elution: 1 min 50/50 ACN/H_2O; 10 min 100% ACN; 15 min 100% ACN; 20 min 50/50 ACN/H_2O; 30 min 50/50 ACN/H_2O, and flow rate 1 mL/min.

was obtained for the standard solution [Figure 7(A)], and a peak with a similar retention time ($t_R = 14.85$ min) for a treated sewage sample [Figure 7(B)]. GC/MS is more sensitive than HPLC, as its LOD is 0.01 μg/L (option SIM for ion $m/z = 220$). The content of the analyte tested in sewage samples was $4.17 \pm 0.25\,\mu g/L$. The conformity of mass spectra for the standard and sample (Fig. 7) confirms the presence of 4NP in treated aqueous samples. The 4NP spectrum is the most abundant ion $m/z = 107$, corresponding to $C_7H_7O^+$.

CONCLUSIONS

Among the sorbents for nonylphenol enrichment and isolation from aqueous matrices, tested in the present study, the highest recovery level (ca. 80%) and reproducibility of extraction were obtained for carbon packings. Sample acidification reduces the value of recovery by ca. 15%, and the extraction process is no longer reproducible. For the carbon packings tested, the maximum sample volume, determined on the basis of breakthrough curves, is 200 mL. It was found that in the case of nonylphenol isolation with polymeric packings ethyl acetate is a more selective solvent than acetone.

The application of packings with built-in molecules, which can simulate natural systems, did not bring the expected results. That is why an octadecyl

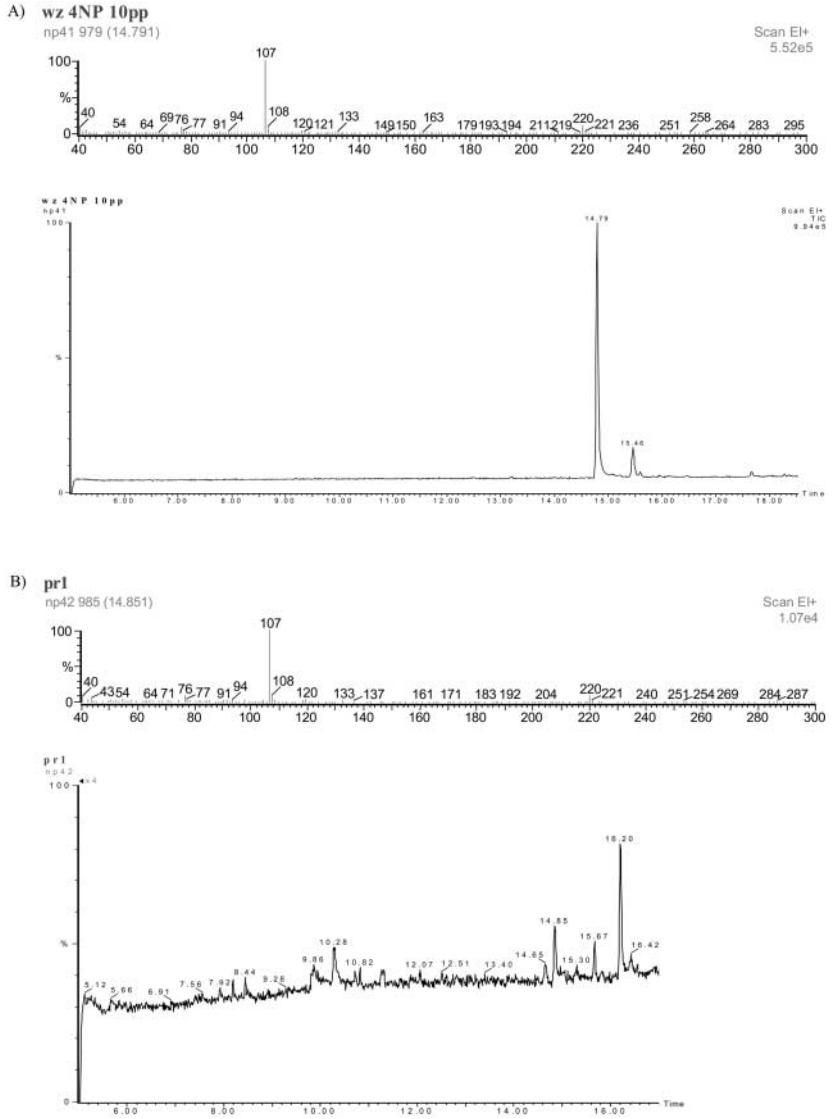


Figure 7. GC chromatograms for the 4NP standard (10 ppm) (A) and sewage sample (B) with mass spectra. Chromatographic conditions: column PE 5 msec (Perkin Elmer) 30 m × 0.25 mm × 0.25 μm. Temperature program: 110°C for 3 min, heating 12°C/min to 190°C (1 min), then heating 5°C/min to 220°C (0 min), then heating 10°C/min to 290°C (5 min). Injector: 280 C, ion source: 280 C, carrier gas: helium—30 cm/sec, splitless 0.4 min, mass range: 40–500 amu, scanning time: 0.5 sec.

phase with secondary silanization was chosen for HPLC determination of nonylphenol; the mobile phase was a mixture of acentronitrile and water. The results obtained by HPLC were verified using GC/MS, due to its higher sensitivity and the possibility of identification on the basis of mass spectra. The 4NP content of sewage was $4.17 \pm 0.25\ \mu g/L$.

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