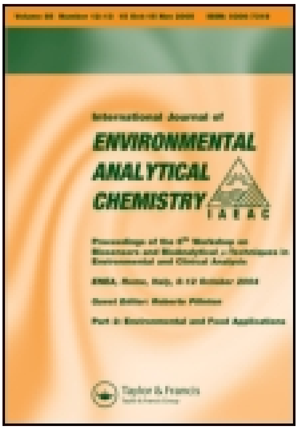

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Agnieszka Zgoła-Grześkowiak [a] & Tomasz Grześkowiak [a]
[a] Institute of Chemistry and Technical Electrochemistry, Poznan University of Technology, Poznań, Poland

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Determination of alkylphenols and their short-chained ethoxylates in Polish river waters

Agnieszka Zgoła-Grześkowiak* and Tomasz Grześkowiak

Institute of Chemistry and Technical Electrochemistry,
Poznan University of Technology, Poznań, Poland

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A simple and robust analytical method for analysis of octyl- and nonylphenol as well as their short-chained ethoxylates in river water was proposed. Quantification of these analytes was performed by high-performance liquid chromatography with fluorescence detection after isolation using solid phase extraction with polytetrafluoroethylene sorbent. The method allowed one to obtain about 80–100% recovery for octylphenol and its ethoxylates and 70–80% for nonylphenol and its ethoxylates. Also, there was no need for additional sample cleaning before chromatographic analysis. The limit of detection was $0.01\,\mu g\,L^{-1}$ for octylphenol and its ethoxylates and $0.03\,\mu g\,L^{-1}$ for nonylphenol and its ethoxylates. The proposed method was used for quantitation of octyl- and nonylphenol together with their short-chained ethoxylates. Nonylphenol, nonylphenol mono- and diethoxylates were detected at concentrations ranging from 0.12 to $0.53\,\mu g\,L^{-1}$. Octylphenol, octylphenol mono- and diethoxylates were detected in four out of eleven samples at concentrations ranging from 0.03 to $0.17\,\mu g\,L^{-1}$. High concentrations of nonylphenol and its ethoxylates were found in the samples, despite the fact that their use in European countries was forbidden several years ago.

Keywords: alkylphenols; alkylphenol ehoxylates; polytetrafluoroethylene sorbent; river water

1. Introduction

Octylphenol (OP) and nonylphenol (NP) belong to chemical compounds produced in large amounts. Interest in analysis of these compounds in environmental samples has increased in recent years when endocrine disrupting properties of NP have been found [1]. Also, short-chained alkylphenol ethoxylates and their carboxylic derivatives were discovered to be endocrine disrupting compounds [2,3]. Moreover, it was found that endocrine disrupting properties are dependent on alkylphenol structure. Octylphenol was found to have more estrogenic character than nonylphenol [2,3]. Estrogenic activity of short-chained NP derivatives was found similar or higher than NP [2,3].

Production of OP in the European Union in 2001 amounted to about 23,000 tonnes. About 98% of this was used for production of phenyl-formaldehyde resins where OP can be found mostly in non-soluble polymer. The rest of OP production (2%) was used for production of octylphenol ethoxylates (OPEOs). In 2001 as much as

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*Corresponding author. Email: civ@o2.pl

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400 tonnes of OP was changed into 1000 tonnes of OPEOs, which were used mostly for emulsion polymerisation and also in the production of paints, textiles and pesticides [4,5].

Nonylphenol production in the European Union in 1997 amounted to over 70,000 tonnes, of which 60% was used for the production of almost 120,000 tonnes of nonylphenol ethoxylates (NPEOs). The main area of NPEOs usage was in industrial and institutional cleaning (30%), while usage in other applications was considerably lower: textiles, 10%; synthesis of nonylphenol ether sulphates and phosphates, 9%; leather, 8%; agriculture, 6%; and paints, 5% [6,7]. Production and use of NP and NPEOs were restricted in the European Union by European Directive 2003/53/EC [8]. However, no restrictions were introduced in other countries. In the USA, about 120,000 tonnes of NP is still being produced, of which about 80% is used for the production of NPEOs [9,10].

Concentrations of NP and NPEOs in environmental samples before European Directive 2003/53/EC [8] became mandatory were usually significantly higher than concentrations of OP and OPEOs [11–13]. This was an obvious consequence of the structure of alkylphenol ethoxylates production. According to a DEFRA report [4], production and use of OPEOs should not increase in the European Union after withdrawal of NPEOs. Nevertheless, even the latest reports from European countries show considerable environmental contamination caused by OP, NP and their ethoxylates [14–16].

There are many different analytical methods that can be used for determination of alkylphenols and their ethoxylates. However, most researchers report the use of SPE for sample isolation [11–14,17–25] and LC-MS for analyte quantitation [13,14,16–19,23]. Some papers reported usage of LC with fluorescence detection [11,12,15,20,24–27], but none of them reported simultaneous measurement of both OP and NP with their ethoxylates. Fewer papers reported usage of GC-MS [20–22,28] or GC-FID [20].

Among the SPE sorbents reported in the literature, mostly octadecylsilica [17–20] and polymeric sorbents [11,13,14,17] were used for isolation of alkylphenols and their ethoxylates from the water matrix. Also graphitised carbon black sorbents [21,22] were used for that purpose. However, the above-mentioned SPE sorbents are usually used only once which greatly increases the cost of the analysis. In past years a few papers presented the use of polytetrafluoroethylene (PTFE) for analyte isolation. There, among different kinds of sample isolation techniques, a PTFE capillary was used for isolation of surfactants from the water matrix [29] and a knotted PTFE capillary was used for isolation of pesticides [30] and polycyclic aromatic compounds [31]. Another isolation technique used PTFE turnings placed in a column, which was successfully used for isolation of phthalate esters [32] and even arsenic(III) [33] from the water matrix. The usefulness of a multicapillary PTFE trap for isolation on nonylphenol and its ethoxylates was also proved [15].

This paper presents the use of PTFE microspheres as an SPE sorbent. The PTFE microspheres with a 100 μm diameter were packed in an SPE tube and used for isolation of alkylphenols and alkylphenol ethoxylates from the water matrix. The isolated analytes were subsequently quantified by LC with fluorescence detection. The proposed procedure was successfully used for monitoring of alkylphenols and alkylphenol ethoxylates in several Polish rivers.

2. Experimental

2.1 *Reagents and chemicals*

Standards of 4-tert-octylphenol and nonylphenol were from Aldrich (St. Louis, MO, USA). Alkylphenol Target Analyte Mix containing octylphenol, nonylphenol and their mono- and diethoxylates used for peak identification was from Fluka (Buchs, Switzerland). Two mixtures of alkylphenol ethoxylates were used together with alkylphenol standards for recovery studies. The first mixture containing octylphenol ethoxylates with an average ethoxylation degree 1.5 was obtained from Serva Feinbiochemica GmbH & Co (Heidelberg, Germany) as Triton X-15. The second mixture containing nonylphenol ethoxylates with an average ethoxylation degree 1.5 was purchased from Aldrich as Igepal CO-210. HPLC-grade acetonitrile and methanol were from J.T. Baker (Deventer, The Netherlands). The HPLC-grade water was prepared by reverse osmosis in a Demiwa system from Watek (Ledec nad Sazavou, Czech Republic), followed by double distillation from a quartz apparatus. Only freshly distilled water was used. Polytetrafluoroethylene powder (100 µm particle size) was from Aldrich.

2.2 *Sampling sites*

The river water samples were taken from four main Polish rivers (Wisla, Odra, Warta and Narew) and from four smaller rivers (Notec, Nysa Luzycka, Bzura and Ner). Two samples were collected from Wisla, Odra and Warta and one sample was from Narew and the four small rivers. The sampling sites are marked on the map (Figure 1). The sampling was performed in March and April 2009.

2.3 *Sample preparation*

The water samples were filtrated through medium (3h) filtering paper from Munktell & Filtrak (Bärenstein, Germany). Two hundred and fifty millilitres of sample were passed through a 6 mL polypropylene SPE cartridge containing 5 g of PTFE powder, which was previously conditioned with 7 mL of methanol and washed with 7 mL of water. After the sample was percolated through the cartridge, the cartridge was dried and then the adsorbed analytes were eluted with 2 mL of acetonitrile and evaporated with nitrogen to 1 mL. The eluted fractions were subjected to HPLC analysis. After usage the SPE cartridge with PTFE sorbent was eluted with 10 mL of methanol to remove possible contaminants still being adsorbed on PTFE microspheres. Then the cartridge was reused.

2.4 *Liquid chromatography*

A chromatographic system from Dionex (Sunnyvale, CA, USA) consisting of a P580 A LPG gradient pump, an ASI-100 autosampler, an STH 585 oven and an RF 2000 fluorescence detector was used. One hundred microlitres of samples were injected into a 50 mm × 4.6 mm I.D. analytical column packed with 3 µm Inertsil ODS3 from Phenomenex (Torrance, CA, USA) with a guard column packed with 4 µm C18. The mobile phase used for the analysis consisted of methanol, acetonitrile and water (50 : 15 : 35). The time of separation was 25 min in isocratic elution mode, at a flow-rate

Figure 1. Localisation of sampling sites.

of 1.5 mL min^{-1} at 35°C. The signal response was measured by fluorescence detector at wavelengths set at 225 nm for excitation and 300 nm for emission.

2.5 *Method validation*

The analytical method was subjected to a validation process. Linearity of the method was tested in a wide range for all analytes. Octylphenol was injected at concentrations: 10, 25, 50, 100, 150, 250 and 350 μg L^{-1}, Triton X-15 at concentrations: 20, 50, 100, 200, 300, 500, 700 μg L^{-1}, NP at concentrations: 15, 38, 75, 150, 230, 380, 530 μg L^{-1} and Igepal CO-210 at concentrations: 30, 75, 150, 300, 450, 750 and 1050 μg L^{-1}. The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated on the basis of signal to noise (S/N) ratio. The S/N = 3 was used for calculation of LOD and the S/N = 10 for calculation of LOQ.

Recoveries of the analytes from the river water samples were tested. The samples of river water were first analysed without spiking them and then after spiking them with octylphenol, Triton X-15 (containing desired short-chained octylphenol ethoxylates), nonylphenol and Igepal CO-210 (containing desired short-chained nonylphenol ethoxylates). Recoveries at two levels of spiking were performed – at high concentration ($4\,\mu g\,L^{-1}$ of OP and OPEOs and $12\,\mu g\,L^{-1}$ of NP and NPEOs) and at low concentration ($0.4\,\mu g\,L^{-1}$ of OP and OPEOs and $1.2\,\mu g\,L^{-1}$ of NP and NPEOs). Three samples were prepared for each of the recovery test. The spiked samples were passed through the SPE cartridge and subjected to chromatographic analysis according to the procedure described above.

3. Results and discussion

The method of chromatographic determination of OP, NP and their short-chained ethoxylates was subjected to validation tests proving the possibility of its use in the analysis of real samples. The results of validation are presented in Table 1. Linearity of

Table 1. Linearity, limit of detection, limit of quantitation, recovery and precision (calculated as relative standard deviation – RSD) obtained during validation of the method of analysis of octylphenol (OP), octylphenol monoethoxylate (OPEO1), octylphenol diethoxylate (OPEO2), nonylphenol (NP), nonylphenol monoethoxylate (NPEO1) and nonylphenol diethoxylate (NPEO2). S – error of slope or intercept.

Parameters	OP	OPEO1	OPEO2	NP	NPEO1	NPEO2
Calibration curve range [$\mu g\,L^{-1}$]	10–355	10–334	8–295	15–529	16–564	29–403
Correlation coefficient (r^2)	0.9998	0.9998	0.9996	0.9994	0.9993	0.9995
Slope	0.68	0.80	0.82	0.79	0.88	0.82
S_{slope}	0.004	0.005	0.01	0.01	0.01	0.01
Intercept	0.04	0.02	−0.82	−4.47	−3.35	−2.53
$S_{intercept}$	0.78	0.80	1.04	2.29	3.03	2.00
Instrumental limit of quantitation [$\mu g\,L^{-1}$]	9	8	9	23	25	25
Instrumental limit of detection [$\mu g\,L^{-1}$]	3	3	3	7	7	7
Limit of quantitation [$\mu g\,L^{-1}$]	0.03	0.03	0.04	0.09	0.10	0.10
Limit of detection [$\mu g\,L^{-1}$]	0.01	0.01	0.01	0.03	0.03	0.03
Recovery [%]*	81	84	85	74	73	77
Precision (RSD) [%] ($n = 3$)*	1	3	2	1	3	2
Recovery [%]**	81	95	103	79	71	70
Precision (RSD) [%] ($n = 3$)**	2	4	4	4	5	9
Mean recovery [%]	81	89	94	76	77	74
Precision (RSD) [%] ($n = 6$)	2	8	11	5	7	8

Notes: *Recovery and precision for OP, OPEO1, OPEO2 added at $4\,\mu g\,L^{-1}$ and for NP, NPEO1, NPEO2 added at $12\,\mu g\,L^{-1}$.
**Recovery and precision for OP, OPEO1, OPEO2 added at $0.4\,\mu g\,L^{-1}$ and for NP, NPEO1, NPEO2 added at $1.2\,\mu g\,L^{-1}$.

calibration graphs is satisfactory in a wide range. The calculated LOD and LOQ levels for OP and OPEOs are three times lower than those calculated for NP and NPEOs. This can be ascribed to the wide chromatographic peaks of NP and NPEOs which is a result of existence of many different NP isomers (Figure 2). Moreover, no interference from unknown sample constituents can be observed on the sample chromatograms.

Recoveries of the analytes from river water were tested at two different concentration levels. At both levels the used concentration of NP and NPEOs was three times higher than the concentration of OP and OPEOs. This was connected with both the higher LOD of NP and NPEOs and the higher expected environmental concentration of NP and NPEOs than OP and OPEOs. The precision of measurements was satisfactory as well as the results of recovery test of all tested analytes at both concentration levels.

The results presented here proved the possibility of using PTFE microspheres as a new SPE sorbent for isolation of alkylphenols and their ethoxylates from the water matrix. Thus, the use of standard SPE equipment is possible and a few samples can be processed simultaneously. This is a considerable improvement on our previous method of sample isolation in which only one sample could be passed through the PTFE capillary at the same time [15].

The possibility of multiple usage of the SPE cartridge containing PTFE microspheres was tested. For this purpose recoveries of the analytes from spiked river water samples were tested. It was observed that recoveries of the analytes during 10 consecutive tests on the same cartridge were constant (Figure 3). Also methanol used for cartridge washing between experiments was tested for presence of contaminants. The last 1 mL out of 10 mL of methanol was analysed and no peaks were observed in the chromatogram. Thus, it was

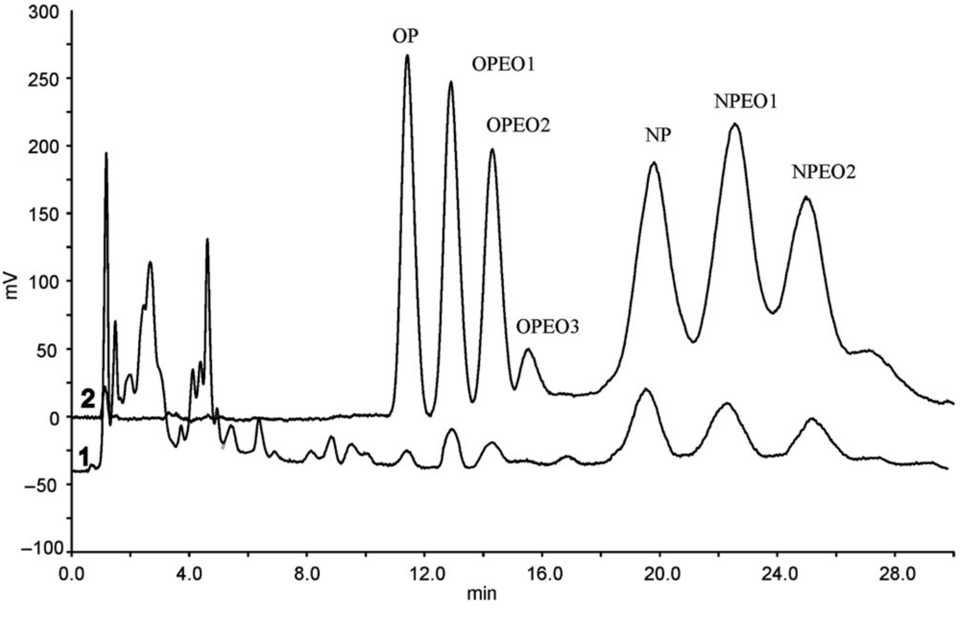

Figure 2. Chromatograms of (1) typical sample solution and (2) solution containing octylphenol at 100 μg L^{-1}, Triton X-15 at 200 μg L^{-1}, nonylphenol at 300 μg L^{-1} and Igepal CO-210 at 600 μg L^{-1}.

concluded that the cartridge with the PTFE sorbent can be used repeatedly at least 10 times which substantially reduces the cost of the analysis.

The validated method was used for the determination of OP, NP and their short-chained ethoxylates in several Polish rivers. The results are presented in Table 2. Nonylphenol was found in all tested samples at concentrations ranging from 0.2 to 0.5 µg L^{-1}. The short-chained NPEOs were found in most but not all of the tested river water samples. The concentrations of the two NPEOs were slightly lower than the concentration of NP. Also, the concentrations of NP and NPEOs reported here are similar to those reported in other European rivers (Italian [14,19,24,34] and Spanish [18,23]).

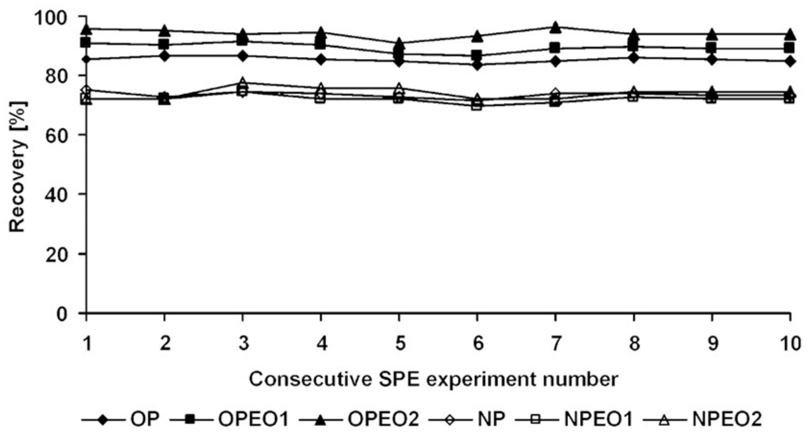

Figure 3. Recoveries of the analytes during 10 consecutive tests on the same cartridge.

Table 2. Concentrations of octylphenol (OP), octylphenol monoethoxylate (OPEO1), octylphenol diethoxylate (OPEO2), nonylphenol (NP), nonylphenol monoethoxylate (NPEO1) and nonylphenol diethoxylate (NPEO2) found in Polish river waters. One 2.5 L sample was taken in each sampling site.

River (sampling site)	Concentration of analytes [µg L^{-1}]					
	OP	OPEO1	OPEO2	NP	NPEO1	NPEO2
Odra (Slubice)	–	–	–	0.36	0.12	< LOQ
Odra (Krosno Odrzanskie)	–	–	–	0.37	–	–
Nysa Luzycka (Gubin)	–	–	–	0.19	0.17	0.21
Notec (Santok)	–	–	–	0.18	–	–
Warta (Poznan)	–	–	–	0.22	–	–
Warta (Konin)	0.04	0.15	0.08	0.53	0.42	0.29
Ner (Poddebice)	–	–	–	0.38	0.17	0.29
Bzura (Leczyca)	< LOQ	0.07	0.06	0.36	0.23	0.30
Wisla (Plock)	0.04	0.17	0.11	0.42	0.14	0.13
Wisla (Nowy Dwor Maz.)	–	–	–	0.32	0.16	0.23
Narew (Nowy Dwor Maz.)	0.03	0.17	0.05	0.34	0.13	0.12

Only the concentrations of NP measured in Belgian rivers [14] and in lower parts of Spanish rivers [23] were considerably higher.

Octylphenol and OPEOs were found only in four tested samples. Here, the concentrations of OP were slightly lower than those found for OPEOs. The concentrations of OP were similar to those reported by Loos *et al.* [14] in Italian rivers but lower than those presented by Céspedes *et al.* [18,23] in Spanish rivers. However, the results obtained by Céspedes *et al.* [18,23] are also higher than those obtained by other researchers [14,17] and may be indicating a rather high usage of OP and OPEOs in Spain.

As was expected the concentrations of OP and OPEOs were considerably lower than those calculated for NP and NPEOs. This confirmed previous results [14,16] presenting higher concentration of NP and NPEOs than OP and OPEOs in the European environmental samples several years after introduction of the European Directive 2003/53/EC [8]. In this context the obtained results are similar to those reported before the year 2003 [28] or to reports from non-European countries [17]. Thus, if ultimate biodegradation of NPEOs is possible in 128 days, as was suggested by Naylor *et al.* [35], our results would suggest that NPEOs are still being discharged to the environment in Polish rivers.

4. Conclusions

The developed method is suitable for the determination of alkylphenols and their short-chained ethoxylates in environmental samples. The SPE cartridge with PTFE sorbent used for isolation of the analytes allows one to obtain clean samples and no further cleaning is needed. One PTFE cartridge can be used many times, which significantly reduces the cost of sample preparation in comparison to the widely used solid phase extraction with octadecylsilica cartridge.

The water samples from the tested rivers contained substantial amounts of NP and NPEOs. The concentrations of OP and OPEOs were considerably lower. Moreover, OP and OPEOs were detected only in less than half the samples. The proportion of NP and NPEOs to OP and OPEOs is still similar to that reported before the European Directive 2003/53/EC became mandatory.

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