# The Use of Polytetraflouroethylene Multi-Capillary Trap Extraction for Isolation of Octylphenol and its Short-Chained Oxyethylates from the Water Matrix

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## Abstract

**A method for the determination of octylphenol and short-chained octylphenol oxyethylates in water samples using isolation in a polytetrafluoroethylene (PTFE) multi-capillary trap and high-performance liquid chromatography with fluorescence detection was developed. The analytes were adsorbed on the inner surface of the PTFE capillary trap, from which they were eluted by a small amount of acetonitrile. Detection limit of 0.1 µg/L was achieved for each of the analyte. Acceptable recovery results (90–100%) were obtained for spiked river water samples.**

## Introduction

Oxyethylated alkylphenols (Figure 1) are one of the most frequently used classes of non-ionic surfactants. Nevertheless, their use is limited to industrial and agricultural applications because of their difficult biodegradation (1). The hydrophilic part of oxyethylated alkylphenols is easily biodegraded by most bacteria (1,2). However, the lipophilic part is accumulated in the environment. Its biodegradation is limited as its branched alkyl chain makes β-oxidation problematic. Moreover, the presence of the phenyl ring reduces the possibility of the central scission biodegradation mechanism (1). Primary biodegradation of oxyethylated alkylphenols leads to a shortening of its hydrophilic chain. As a result, three main biodegradation products can be found in the environment–alkylphenol and its mono- and dioxyethylated derivatives (3,4). These compounds are known to demonstrate estrogenic activity (5,6). Monitoring of nonylphenol and its oxyethylates became necessary because of European law regulations (directive 2003/53/EC). Nevertheless, no European regulation can be found for octylphenol and its oxyethylates. Lack of regulations concerning these compounds can be connected with their low concentrations in surface waters and sewage treatment plant effluents (4). However, low concentration of octylphenol and its oxyethylates can be ascribed to their higher cost in comparison to nonylphenol and its oxyethylates. The European regulations banning the use of nonylphenol surfactants certainly will give rise to higher use of octylphenol surfactants, thus both octyl- and nonylphenol, as well as their oxyethylates should be monitored in the environment.

Analysis of alkylphenols and their oxyethylates is a difficult task. Both low concentration and a complex matrix can be a problem. So far, alkylphenols were isolated from the water matrix with the use of several analytical techniques. Here, solid phase extraction is a widely used technique. Both octadecyl silica (7,8) and graphitised carbon black (9,10) were used as sorbents leading to recovery higher than 80%. Two other techniques were used for the isolation of alkylphenols: liquid–liquid extraction (3,11) and solid phase microextraction (12).

This paper presents an isolation technique using adsorption of analytes on the inner surface of polytetrafluoroethylene (PTFE) capillaries. It was described in the literature that PTFE tends to demonstrate adsorption of surfactants (13). This property was used for isolation of surfactants from the water matrix (13,14). Adsorption of other compounds on PTFE surface is also possible and PTFE was used for sample preparation by several researchers. Here, pesticides (15), polycyclic aromatic compounds (16), phtalate esters (17), and even metals (18) were isolated from the water matrix using different types of PTFE traps. The proposed technical solutions for trapping analytes include a PTFE membrane filter (14), PTFE turnings packed in a column (17,18) and a single PTFE capillary (13,15,16). This paper presents the use of the multicapillary PTFE trap for isolation of short-chained oxyethylated octylphenol and octylphenol from the water matrix.

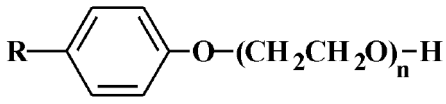


**Figure 1.** Structure of oxyethylated alkylphenol, R – alkyl chain, n – number of oxyethylene groups.

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## Experimental

### Reagents and chemicals

4-tert-octylphenol was from Sigma-Aldrich (Seelze, Germany). Triton X-15 and Triton X-100 (both polydispersal mixtures) were from SERVA Feinbiochemica (Heidelberg, Germany) and Merck (Darmstadt, Germany) respectively. Oxyethylated dodecanole (also polydispersal) was from Sigma-Aldrich. Tetrahydrofurane, methanol and acetonitrile of high-performance liuid chromatography grade were from J.T. Baker (Deventer, Netherlands). HPLC-grade water was prepared by reverse osmosis in a Demiwa system from Watek (Ledec nad Sazavou, Czech Republic), followed by triple distillation from a quartz apparatus.

### Sample preparation

A water sample was passed through a polytetrafluoroethylene multi-capillary trap. The trap was constructed from an old degassing unit taken from a Hewlett Packard (Palo Alto, CA) chromatograph model 1050. A bunch of PTFE capillaries was taken out from the degassing unit and connected to an HPLC isocratic pump from Dionex (Hvidovre, Denmark). The trap consisted of 38 capillaries. Each capillary was 2.5 m long with an inner diameter of 0.35 mm. The sample flow-rate was 1 mL/min. After the sample was passed through the capillaries, the trap was dried with nitrogen. The analytes adsorbed on the inner walls of the trap were eluted in back-flush mode with acetonitrile. The eluate was evaporated to 1 mL under a nitrogen purge. The eluted analytes were subjected to HPLC analysis.

### LC

A Dionex chromatographic system consisting of the P 580 A LPG gradient pump, the ASI-100 autosampler, the STH 585 oven and the RF 2000 fluorescence detector was used for the experiments. Fifty microliter volumes of samples and standards were injected on a 250 × 4.6 mm i.d. analytical column packed with 5 µm Hypersil BDS C18 (ThermoQuest, Runcorn, United Kingdom) with a guard column packed with 3 µm Hypersil BDS C18 (ThermoQuest). The mobile phase consisted of water–tetrahydrofurane mixture (1:1) at a flow-rate of 1 mL/min at 30°C. Signal responses were measured by fluorescence detection at wavelengths set at 225 nm for excitation and 300 nm for emission.

### Method validation

The analytical method was subjected to validation process. Linearity of the method was tested in a wide range for all the analytes. Calculations were performed using Microsoft Excel. The REGLINP function was used for calculation of all the required parameters (slope, intercept, errors of these parameters, and correlation coefficient of the regression line).

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 were tested for real river water sample spiked with octylphenol and its oxyethylates. The spiked sample was forced through the PTFE multicapillary trap and subjected to HPLC analysis according to the procedure previously described. Precision was calculated from the recovery test results.

## Results

### Method development

Octylphenol and oxyethylated octylphenol were analyzed with the use of HPLC with fluorimetric detection. Separation of the analytes was tested in mobile phases consisting of methanol–water, acetonitrile–water, and tetrahydrofurane–water mixtures. Satisfactory results were achieved only in mobile phases consisting of methanol–water and tetrahydrofurane–water mixtures. However, separation in methanol–water mobile phase was unacceptable due to long and unrepeatable retention times. Thus, tetrahydrofurane–water mobile phase was chosen for further experiments. Appropriate proportion of tetrahydrofurane to water in the mobile phase was chosen to enable chromatographic separation of octylphenol and its oxyethylates having one to three oxyethylene groups (Figure 2). Higher homologues were deliberately eluted without complete separation, as their influence on the environment was lower (6). Before testing of the PTFE multi-capillary trap a series of tests was performed including linearity test and determination of instrumental limits of quantitation and detection. As a result linearity range was established and instrumental limits of quantitation and detection were evaluated (Table I).

A series of tests was performed to assure conditions for proper adsorption of these analytes

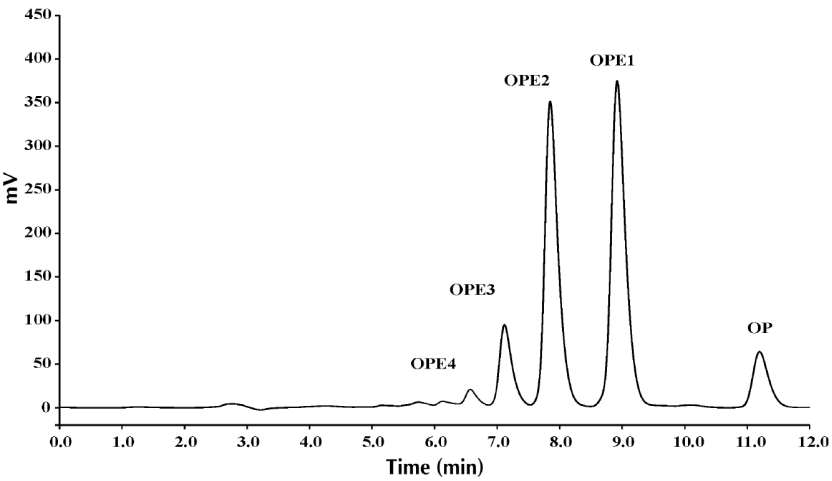


**Figure 2.** Chromatographic separation of octylphenol and its short-chained oxyethylates on Hypersil BDS C18 5 µm, 250 × 4.6 mm i.d. column with Hypersil BDS C18 3 µm guard column. The mobile phase was tetrahydrofurane–water (1:1) at a flow rate of 1 mL/min at 30°C. Injection was 50 µL and fluorescence detection was at 300 nm with excitation at 225 nm.

inside the PTFE trap and to obtain complete desorption of the analytes. A mixture of octylphenol and Triton X-15 (containing short-chained octylphenol oxyethylates–from one to three oxyethylene groups) was taken for experiments. The water sample containing these analytes was passed through the PTFE capillaries and then the analytes were eluted with acetonitrile. Influence of sample flow-rate on adsorption of the analytes was tested in the range 0.5–3 mL/min. An optimal flow-rate was established on basis of the recovery of the analytes. The mean recovery of the analytes ranged from 55% to 97% and maximum was found at 1 mL/min. Thus, the flow-rate of 1 mL/min was chosen for further experiments.

Volume of eluent needed for complete desorption of the analytes was established in a simple test. The analytes were eluted with three 1 mL portions of acetonitrile and the eluates were tested for presence of the analytes. As only the first two portions of the eluates contained the analytes, optimal volume of eluting solvent was set to two milliliters.

The influence of sample volume on the recovery of the analytes was tested in a range from one to one hundred millilitres (Figure 3). Concentrations of OP in these samples ranged from 10 to 500 ng/mL and concentrations of Triton X-15 ranged from 12 to 600 ng/mL. The concentrations and volumes of the samples were matched to introduce constant amount of analytes into the trap. As much as 1 µg of OP and 1,2 µg of Triton X-15 were introduced into the capillaries. On the basis of this experiment a volume of 20 mL was taken for further work. Higher volumes of the sample led to significant loss of octylphenol, though the breakthrough volume for octylphenol oxyethylates was higher than 100 mL.

The influence of other surfactant on analytes recovery was also tested. Oxyethylated dodecanol was used as a model surfactant. The influence of oxyethylated dodecanol on octylphenol and oxyethylated octylphenol recovery was tested at three different concentration levels. The results (Figure 4) proved the relatively low influence of other surfactant on the recovery of the analytes. Recovery of octylphenol and oxyethylated octylphenol tends to be higher with increasing oxyethylated dodecanol concentration (Figure 4). On the other hand addition of oxyethylated dodecanol led to small loss of octylphenol di- and trioxyethylates. Relative standard deviation obtained in all the measurements ranged from 1% to 13% (Figure 4). However, the average relative standard deviation was only 5%. The precision obtained here was found to be high enough for using multi-capillary extraction to isolate octylphenol and its oxyethylates from the water matrix.

On the basis of performed experiments a final version of the method was established. In the final method, 20 mL of the water sample was forced through the PTFE multi-capillary trap. Then the sample was eluted with 2 mL of acetonitrile and evaporated to 1 mL. A 50 µL aliquot of this sample was injected into the chromatographic column. This procedure allowed to obtain LODs of the method: 0.07, 0.05, 0.07, and 0.06 µg/L for octylphenol, octylphenol mono-, di- and trioxyethylate, respectively. The obtained LOQs of the method for all the analytes were 0.2 µg/L.

**Table I. Linearity and Instrumental LOD and LOQ Obtained for Octylphenol and its Short-chained Oxyethylates**

| Analyte | OP | OPE1 | OPE2 | OPE3 |
|---|---|---|---|---|
| Calibration curve range (µg/L) | 5–485 | 3–393 | 5–344 | 3–84 |
| Number of data points | 8 | 9 | 8 | 7 |
| Correlation coefficient ($r^2$) | 0.9997 | 0.9997 | 0.9997 | 0.9991 |
| Slope | 0.55 | 0.56 | 0.57 | 0.57 |
| Slope standard deviation | 0.004 | 0.004 | 0.004 | 0.007 |
| Intercept | 2.99 | 1.31 | 1.13 | 0.32 |
| Intercept standard deviation | 0.95 | 0.70 | 0.66 | 0.29 |
| Standard error | 1.96 | 1.52 | 1.42 | 0.56 |
| Instrumental LOQ (µg/L) | 4.4 | 3.0 | 4.7 | 3.8 |
| Instrumental LOD (µg/L) | 1.3 | 0.9 | 1.4 | 1.1 |

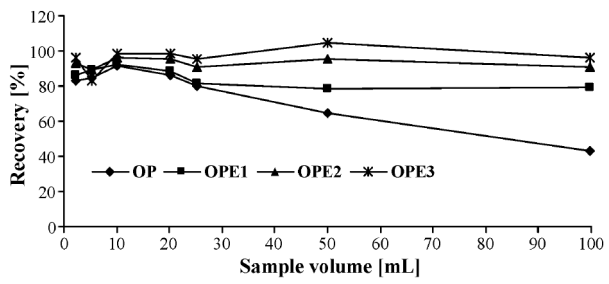


**Figure 3.** Influence of sample volume purged through the PTFE multi-capillary trap on the recovery of octylphenol and its short-chained oxyethylates. Sample flow-rate through the trap was 1 mL/min. Elution was performed with 2 mL of acetonitrile.

**Method usage**

A water sample was taken from the Warta River (Poznań, Poland) and tested for presence of octylphenol and its short-chained oxyethylates. Neither octylphenol nor its oxyethylates were detected in the sample taken from the Warta River. The same sample was taken for recovery experiment in a real environmental matrix. Octylphenol was recovered at 92.4% (RSD 2.0%). Recoveries of octylphenol oxyethylates were at 102.8% (RSD 0.2%), 100.7% (RSD 2.2%), and 101.0% (RSD 0.8%) for octylphenol mono-, di-, and trioxyethylate, respectively.

The developed analytical method was used in monitoring of octylphenol and its short-chained oxyethylates in a biodegradation test performed in a sewage treatment plant (STP). The STP

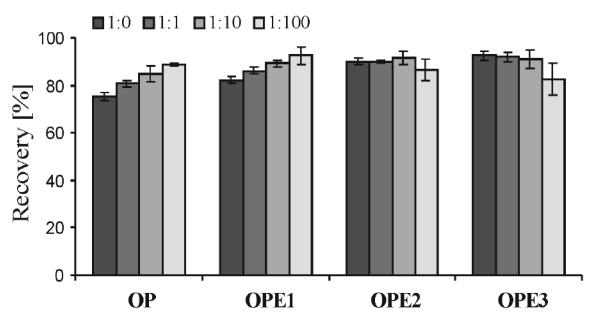


**Figure 4.** Influence of oxyethylated dodecanole on the recovery of octylphenol and its short-chained oxyethylates using the PTFE multi-capillary trap. The ratio of octylphenol and its oxyethylates concentration to oxyethylated dodecanole concentration was from 1:0 to 1:100. Sample flow-rate through the trap was 1 mL/min. Elution was performed with 2 mL of acetonitrile.

was to receive sewage delivered from a large-scale commercial production process using Triton X-100. Thus, it was decided to test if this kind of sewage could be safely biodegraded in the STP. Octylphenol and oxyethylated octylphenol were analysed in raw sewage and sewage treated in a sewage treatment plant. At the beginning of the test only higher homologues of oxyethylated octylphenol were detected in raw sewage and neither octylphenol nor its oxyethylates were detected in treated sewage. Then raw sewage was enriched with a high amount of Triton X-100. This process was performed continuously for 24 h. Triton X-100 and short-chained oxyethylated octylphenol homologues, as well as octylphenol were analysed in raw and treated sewage. The obtained results (Table II) confirmed fast primary biodegradation of Triton X-100. However, substantial amounts of octylphenol and its short-chained oxyethylates were detected in treated sewage.

## Discussion

### Method development

Development of a method for isolation of octylphenol and its short-chained oxyethylates from the water matrix with the use of the PTFE trap was the main aim of this work. The use of PTFE for isolation of surfactants was already reported (13,14). However, short-chained octylphenol oxyethylates demonstrated weak surface-active properties, and octylphenol does not belong to surfactant class. Thus, low adsorption of the analytes on the PTFE surface was supposed to be a potential problem in their isolation from the water matrix. This problem of low break-through volume for different analytes adsorbed on the PTFE surface was already reported in the literature (16). Thus, it was decided to establish maximum allowable sample volume that can be used for adsorption of octylphenol and its oxyethylates. The obtained results (Figure 3) demonstrated a substantial difference between octylphenol and its oxyethyletes. Break-through volume of surface active octylphenol oxyethylates was over 100 mL, but for octylphenol it was considerably lower. This result was attributed to lower adsorption properties of octylphenol.

A study on co-adsorption of octylphenol and its oxyethylates proved that both octylphenol and octylphenol monooxyethylate tend to demonstrate this effect with oxyethylated dodecanole. However, no co-adsorption was observed for octylphenol di- and trioxyethylates. It was supposed that adsorption of these two analytes on the PTFE surface was easier because of their higher surface-active properties.

Nevertheless, both low surface-active properties of octylphenol and the necessity of using a sample volume of 20 mL enabled obtaining satisfactory LODs for all the analytes. The reported LODs were similar to those reported in the literature. Careri et al. reported LOD of 0.08 µg/L and LOQ of 0.15 µg/L for octylphenol (19). Cespedes et al. reported LOD of 0.06 µg/L for octylphenol (20). Jahnke et al. reported LOD of 4, 12, and 0.1 ng/L for octylphenol, octylphenol monooxyethylate, and octylphenol dioxyethylate, respectively (21).

**Table II. Analysis Results of OP and OPE Having 1–3 Oxyethylene Groups and Triton X-100 in Sewage Treatment Plant Influent and Effluent**

| | | Analyte concentration (µg/L) | | | | |
|---|---|---|---|---|---|---|
| Sewage type | Day | OP | OPE1 | OPE2 | OPE3 | Triton X-100 |
| Raw | 0 | – | – | – | – | 22.5 |
| | 1 | – | – | – | 24.6 | 5725.0 |
| | 2 | – | 3.4 | 0.6 | 1.3 | 120.3 |
| | 3 | 2.2 | 4.7 | 0.9 | 1.2 | 55.3 |
| Treated | 0 | – | – | – | – | – |
| | 1 | – | – | – | – | – |
| | 2 | 0.4 | 8.1 | 1.3 | 1.2 | – |
| | 3 | – | 1.7 | – | – | – |

### Method usage

The developed method was tested for applicability for the real samples. Although no analytes were found in the water sample taken from the Warta River, high recoveries were obtained in this experiment. The obtained values of the recoveries were slightly higher than those obtained for the samples prepared in HPLC-grade water. This small increase can be ascribed to co-adsorption of the analytes with unknown river water matrix components. Thus, the usefulness of the developed method in real sample analysis was proved.

The results of the biodegradation test performed in the STP confirmed the difficult biodegradation of octylphenol oxyethylates reported in the literature (1). Although primary biodegradation of Triton X-100 was completed, considerable amounts of octylphenol and its short-chained oxyethylates were formed during this test. Thus, on the basis of the results obtained in this test, the STP management decided not to take sewage containing a large amount of Triton X-100. A different procedure of utilization of the sewage is under consideration.

Usefulness of the developed method in analysis of real samples was proved. Moreover, co-adsorption with model non-ionic surfactant can be potentially useful in a different application. Octylphenol is probably not the only contaminant which demonstrates co-adsorption with non-ionic surfactants. Thus, the PTFE trap could potentially be used for isolation of many different analytes from the water matrix. Addition of non-ionic surfactant to the matrix can lead to co-adsorption of some analytes and improve their isolation from the water matrix. Thus, the PTFE trap could be used in a wider area of applications.

## Conclusions

The developed method is suitable for the determination of octylphenol and oxyethylated octylphenol in the water matrix. Moreover, the PTFE multi-capillary trap can be used over 100 times which significantly reduces the cost of sample preparation in comparison to a widely used solid phase extraction. Also, the PTFE trap enables low solvent consumption per analysis.

The developed method was used in solving a real problem of biodegradation of Triton X-100 in a sewage treatment plant. This way it was possible to avoid contamination of the environment with octylphenol and its short-chained oxyethylates.

The PTFE trap can potentially be used for isolation of other analytes. Here, the co-adsorption of analytes with a non-ionic surfactant can lead to their satisfactory adsorption on the inner walls of the trap if the surfactant is added to the water matrix.

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