DETERMINATION OF PCP IN GROUND/RIVER WATER SAMPLES BY STIR BAR SORPTIVE EXTRACTION–THERMAL DESORPTION–GAS CHROMATOGRAPHY-TRIPLE QUADRUPOLE MASS SPECTROMETRY

Ivana Deršek Timotić[1], Mirjana Balać[1], Katarina Nedeljković[1], Daliborka Popadić[1], Zoran Stojanović[1], Vojislava Bursić[2], Dušan Marinković[2]

[1]Serbian Environmental Protection Agency, Kneza Višeslava 66, 11000 Belgrade, Serbia
[2]University of Novi Sad, Faculty of Agriculture,21000 Novi Sad, Trg Dositeja Obradovica 8, Serbia
e-mail: ivana.dersek@sepa.gov.rs

Abstract

Pentachlorophenol (PCP) use to be in wide usage as insecticide, fungicide and contact-herbicide, but primarily as wood preservative and for industrial purposes. PCP is classified as extremely toxic substance for humans and environment. Systematic investigation of trace amounts PCP in surface water in Serbia has initiated in 2009. For analysis of surface water samples, described in this paper, method using stir bar sorptive extraction (SBSE) after derivatization with acetic acid anhydride followed by thermal desorption (TD) – triple quadrupole gas chromatography mass spectrometer with negative chemical ionization (GC/MS/MS-NCI) analysis was applied. Obtained LOQ of PCP – $6.25x10^{-4}$ µg/L is much lower than environmental quality standard (EQS) provided by the Water Frame Directive 2013/31/EU (WFD) for this compound – 0,4 µg/L, what makes this method appropriate for determination of PCP in water samples. Recovery and corresponding RSD of PCP is 82.47%, and 8.7%, respectively. Method showed good linearity over the concentration range of 0.6 - 50 pg/L PCP with coefficient of correlation R^2 of 0.999. PCP was detected in all samples and measured amounts were bellow the set EQS values. Minimum ammount of PCP in samples was 5.55 $x10^{-3}$ µg/L, maximum ammount was 111.74 $x10^{-3}$ µg/L and the average was $11.62x10^{-3}$ µg/L.

Introduction

PCP use to be in wide usage as insecticide, fungicide and contact-herbicide, but primarily as wood preservative. After PCP was recognized as extremely toxic substance for humans and environment its usage was reduced and now PCP is permitted only for industrial purposes. Agricultural and domestic uses are prohibited.

PCP is classified as a highly hazardous pesticide, considered to be a carcinogen and a tumor promoter. The persistence of PCP in soil and water and apparent widespread use has resulted in significant exposure to animals. Studies on relation of exposure to PCP and cancer (on both humans and animals) led to EPAs classification of PCP as a Group B2, probable human carcinogen. Also, a significant amount of hexachlorobenzene is metabolized in animal tissues to PCP [1, 2].

Emission of PCP in water bodies was detected on locations with facilities for water purification or drop-off of dangerous waste. Therefore, PCP was put on a list of priority substances in DIRECTIVE 2013/39/EU as pollutant which presence in environment needs to be monitored. Serbian Water Laws were first are harmonized with Water Framework Directive (2000/60/EC) requirements in 2012, and since than surface water status monitoring in Serbia follows the EUs legislative.

Many analytical methods for the determination of PCP have been reported in literature including derivatization and SBSE followed by gas chromatography (GC) with mass spectrometry (MS).

M. Kawaguchi et al. (2004) investigated trace analysis of phenolic xenoestrogens such as 2,4-dichlorophenol (2,4-DCP), 4-*tert*-butylphenol (BP), 4-*tert*-octylphenol (OP), 4-nonylphenol (NP), pentachlorophenol (PCP) and bisphenol A (BPA) in water samples [3]. L. Montero et al. (2005) assesed presence of phenols and selected chlorphenols in lake and ground water samples [4] Same approach was applied by J. Llorca-Porcel et al. (2009) on anaysis of chlorophenols, bisphenol-A, 4-tert-octylphenol and 4-nonylphenols in soil, etc [5].

Experimental

Water samples.

Surface water samples were collected and analyzed on presence of PCP. Samples were collected in glass containers (approximately 3L), refrigerated at 4^{o}C and stored in the dark from the time of collection until extraction. Samples were extracted within 7 days of collection and completely analyzed within 40 days of extraction [7].

Sample extraction.

A 100 ml of sample was weighed into 250 ml erlenmayer flask, 1g potassium bicarbonate was added and well mixed. After that, solution was spiked with certain amount of internal standard (2, 3, 5, 6–tetrachlorophenol) and 1 ml acetic anhydride was added as the derivatization reagent. After derivatization (15 minutes), 80ml of that solution was transfered in 100 ml erlenmayer flask containing 20g of sodium chloride and one stirbar "Twister" was added. Stir bar "Twister" (polydimethylsiloxane-PDMS coated stir bar, 1cm length/1 mm film thickness) must be preconditioned. Then erlenmayer flask was put on magnetic stirrer and extraction was performed at room temperature for 2 hours while stirring at 1100 rpm. Stir bar was easily removed from erlenmayer flask with magnetic stick, rinsed with deionized water, dried with lint-free tissue and placed in a special TDU tube for GERSTEL Twister® stir bar.

Sample analyses.

Prepared special TDU tube is put on TDU tray of GERSTEL MultiPurpose Sampler, (MPS2) with Thermal Desorpton Unit (TDU2) which through fully automated procedure inserts tube with stir bar in TDU for thermal desorption.

The temperature of TDS 2 was programmed to increase from 30 °C (held for 0.5 min) to 295 °C (held for 10 min) at a rate of 720 °C/min.

After desorption, for chromatography separation, an Agilent 7890B GC with analytical column HP-5MSI 30 m, 0.25 mm, 0.25 µm was used. Applied temperature program is described in Table 1. Helium was used as the carrier gas at a flow rate of 1.5 ml/min.

Table 1. Temperature program of the GC oven

	Rate ^{0}C/min	Value ^{0}C	Hold Time min	Run Time min
(Initial)		70	0.1	0.1
Ramp 1	600	270	5	23.667
*				

For the mass spectrometric analysis, an Agilent 7000C Triple-Quad MS system was used. The mass spectrometer operated in the multiple reaction monitoring (MRM) mode. Three

transitions were monitored: 307.7→265.0 *m/z* as transition for quantification and 307.7→230.8 *m/z* and 264.7→228.7 *m/z* for qualification.
MassHunter Workstation Software version B.04. was applied for the method acquisition and data processing.

Validation.
The method was validated according to regulations [8]. The limit of detection-LOD was determined as the lowest concentration of PCP in spiked real sample where ratio of PCP signal and noise is three (S/N=3). Limit of quantification-LOQ was determined as the lowest concentration of PCP in spiked real sample where ratio of PCP signal and noise is larger then ten (S/N>10). The ratio signal/noise in the obtained chromatograms for the LOD and LOQ was calculated by MassHunter Qualitative Analysis B 07.00 Software.
The recovery and precision of the method were determined by replicate analysis (n=6) of different samples spiked with surrogate standards at 2.5 and $10.0x10^{-3}$pg/L. The non-spiked and spiked samples were analyzed using the same procedure as for calibration standards and samples. The recovery was calculated by subtracting the results for the non-spiked samples from those for the spiked samples. The results were calculated by using calibration curves obtained from standard solutions with surrogate standards.

Results and discussion
Assesment of obtained results for validation and analysis showed that applied method is appropriate for trace analysis of PCP in surface water samples, because all relevant parameters are within established performance criteria for the application of this method.
Obtained LOQ of PCP-$6.25x10^{-4}$pg/L is much lower than environmental quality standard (EQS) provided by the Water Frame Directive 2013/31/EU (WFD) for this compound-0.4μg/L. Method showed good linearity over the concentration range of 0.6/2.5/5.0/10.0/ 17.5/25.0/37.5 and $50.0x10^{-3}$pg/L PCP with coefficient of correlation higher than R^2 of 0.999. Recovery and corresponding RSD of PCP is 82.47%, and 8.7%, respectively.
The presence of PCP was detected in all samples and measured amounts were bellow the set EQS values. Minimum ammount of PCP in samples was 5.55 $x10^{-3}$ μg/L, maximum ammount was 111.74 $x10^{-3}$ μg/L and the average was $11.62x10^{-3}$ μg/L (Table 2).
Typical MRM chromatogram of river water samples are shown in Figure 1.

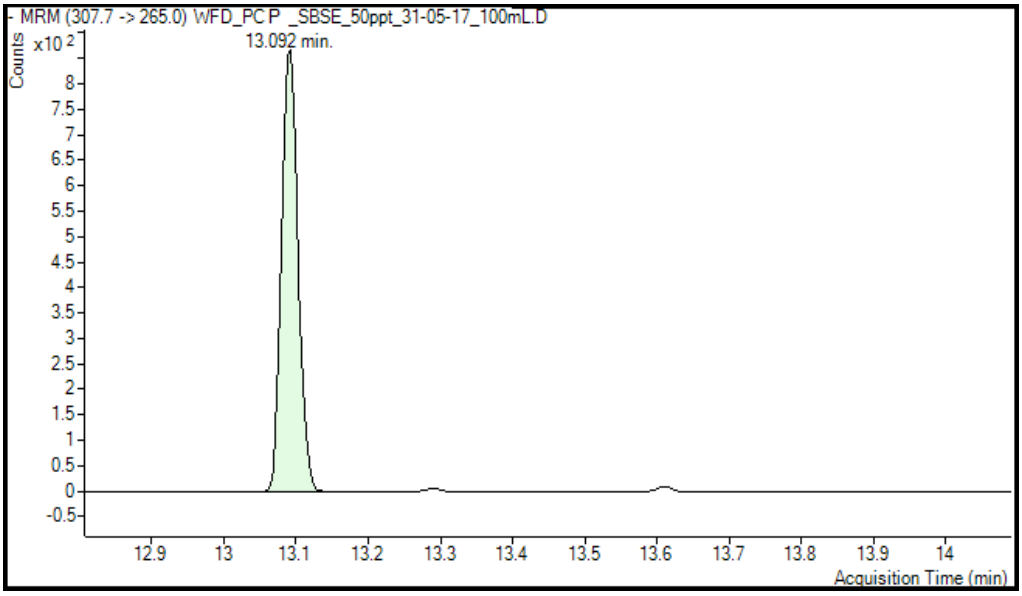

Figure 1. Chromatogram of river water sample

Table 2. PCP residue detected in analyzed samples of surface water samples

Compound	**AA EQS** (µg/L), WFD2013/39/EU	**Min conc** (µg/L), real samples National lab-SEPA	**Max conc** (µg/L), real samples National lab-SEPA	**Number of postive results,** real samples National lab-SEPA
Pentachlorphenol	0.400	0.005	0.100	101

Conclusion

Method used for determination of trace amounts PCP in surface water with derivatization followed by SBSE and triple quadrupole gas chromatography mass spectrometer with negative chemical ionization (GC/MS/MS-NCI) analysis is simple and accurate. The method has been validated with good sensitivity and selectivity for PCP and is applied for routine analysis of PCP. It has a potential for application on different types of water samples. We detected presence of PCP in different types of surface water in concentration ranges from 5.55 x10-3 µg/L to 111.74 x10-3 µg/L.

PCP was found in all samples and detected amounts were significantly lower the set EQS values.

Acknowledgements

This work was founded and a part of the project IPA 2012 Establishment of an integrated environmental monitoring system for Air and Water Quality capacity of air and water monitoring in Serbia.

References

[1] U.S. Department of Health and Human Services Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological Profile for Pentachlorophenol, (1999) str

[2] U.S. Environmental Protection Agency, Integrated Risk Information System (IRIS) on Pentachlorophenol, (1999) 163.
[3] M. Kawaguchi, K. Inoue, M. Yoshimura, N. Sakui, N. Okanouchi, R. Ito, Y. Yoshimura, H. Nakazawa, Trace analysis of phenolic xenoestrogens in water samples by stir bar sorptive extraction with in situ derivatization and thermal desorption–gas chromatography–mass spectrometry, (2004) 19.
[4] L. Montero, S. Conradi, H. Weiss, P. Popp, Determination of phenols in lake and ground water samples by stir bar sorptive extraction–thermal desorption–gas chromatography–mass spectrometry (2005).
[5] J. Llorca-Pórcel, M. Martínez-Parreno, E. Martínez-Soriano, I. Valor, Analysis of chlorophenols, bisphenol-A, 4-tert-octylphenol and 4-nonylphenols in soil by means of ultrasonic solvent extraction and stir bar sorptive extraction with in situ derivatisation, Spain, (2009) 5955.
[7] U.S. Environmental Protection Agency, Federal Register, Vol. 49, No. 209, (1984) 61.
[8] Guidance document on analytical quality control and method validation procedures for pesticides residues analysis in food and feed - SANTE/11945/2015.