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Solid phase extraction and gas chromatographic – mass spectrometric analysis of phthalates in surface water: method development and validation[1]

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The aim of the research was to apply and optimize the solid phase extraction (SPE) and gas chromatographic – mass spectrometric (GC–MS) method for the analysis of phthalates in surface water and carry out the necessary method validation steps.

Two standard mixtures of phthalates were used: the self-composed diethyl phthalate (DEP) and di(*n*-butyl) phthalate (D*n*BP) standard mixture (EBS), and EPA506 standard mixture consisting of seven phthalates. Detection limits for EPA506 varied in the range 45–500 ng/L, depending on the compound. The linear dependence of the EPA506 standard mixture components was in the range 0.1–54 μg/L. SPE was performed using a column filled with C-18 silicagel adsorbent in a system isolated from atmospheric contamination. The recovery values of EBS components when using a 1 : 1 mixture of ethyl acetate and dichlormethane as an eluent were 79 ± 5% for DEP and 97 ± 11% for D*n*BP. The background signals of D*n*BP and di(2-ethylhexyl) phthalate were determined. The method was applied for the analysis of samples from the Venta river (Lithuania) in 2010 and 2011. The variation of pollutant concentrations was determined. D*n*BP in December 2010, January, March, and April 2011 were 0.14 μg/L, 2.7 μg/L, 1.45 μg/L, and 2.2 μg/L, respectively; DEHP concentration in January 2011 was 2.1 μg/L, in April 0.23 μg/L, and DEP concentration in March 2011 was 0.61 μg/L.

Key words: surface water, phthalates, solid phase extraction, gas chromatography – mass spectrometry

INTRODUCTION

In the last two decades, it has been revealed that certain compounds, referred to as environmental estrogens or endocrine disrupting compounds, can interfere with the endocrine system of hormone production and transmission. They are comprised of different classes of organic compounds. Recently, phthalates, which are widely applied as plasticizers in the production of floor coverings, rubber items, and paints [1], have been undergoing intensive research. In plastics, phthalates do not have chemical bonds; therefore, under favourable conditions, they can migrate from plastics and spread in the environment. Despite its harmful effect, the consumption of plastics has increased almost 20 times during the last 50 years. Over 9 billion kilos of phthalates, half the amount of which are di(2-ethylhexyl) phthalates [2], are used annually in the world in polyvinylchloride production. Plasticizers enter surface water together with rain water and sewage from water treatment plants. Their concentration in natural waters is very low; therefore, sensitive methods of modern analysis, such as solid phase extraction (SPE) and gas chromatography – mass spectrometry, are usually used. For SPE columns or extraction discs, the embedded silicagel with hydrophobic octadecyl group (C18) matrices are applied [3].

Effective analyte desorption from adsorbent is one of the most important steps in the process of sample preparation. Fatoki and co-authors optimised the conditions of SPE by applying a mixture of methanol and dichlormethane solvents

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of different ratio for phthalate desorption. The highest recovery was obtained using solvents of equal mass fractions (1 : 1 *m*/*m*) [4]. In the standard EPA method issued in the USA, the recommended phthalate SPE extractant is a 1 : 1 mixture of ethyl acetate and dichloromethane. In the standard method provided in ISO 18856 : 2004, it is recommended to use only the ethyl acetate non-chlorinated solvent [5–7].

The preconcentration of determinable phthalate compounds in water depends on the efficiency of the SPE method. Jara et al. carried out studies with several different concentrations of a standard solution of phthalate and using a polystyrene–divynilbenzene (PS-DVB) adsorbent, and determined that the recovery of branched phthalates and phthalates with long alkyl chain, di(2-ethylhexyl) phthalate and di(*n*-octyl) phthalate (DEHP and D*n*OP) was highest when the concentration of such compounds in water samples did not exceed 3 μg/l [8]. With increasing the amounts of analytes 100 and more times, the recovery was only up to 36–20%. This fact is explained by the low phthalate solubility in water; therefore, it was suggested to add methanol into a sample before extraction. The recovery of phthalates with a short alkyl chain and low-branched di(n-butyl) phthalate and butyl benzyl phthalate (D*n*BP and BBzP) was 85–99% [8].

When preparing samples, an important factor is the background contamination by phthalates. Tiepont and co-authors, while performing routine analyses of phthalate, determined that the background air was mostly polluted with diisobutyl phthalate (DIBP), D*n*BP and DEHP [9]. A group of USA scientists analysed a blank sample applying the USA EPA method 525.2 [7] and for sample preparation using SPE and solid phase microextraction (SPME). They have found that the background contamination of the adsorbent is lower using the SPE, when the amounts of solvents used to condition the SPE cartridge do not exceed 5 ml. It is worth mentioning that in almost all samples traces of D*n*BP were found [10].

For separation of analytes, gas or liquid chromatography coupled to mass spectrometry or flame ionisation detection are used. The method of liquid chromatography is applied when performing the analysis of phthalate ester isomers, since, according to David [3], gas chromatography is not effective in separation of these compounds. The method of selected ion monitoring (SIM) is applied in mass spectrometry to analyse the composition of samples.

The aim of the present research was to apply and optimize the solid phase extraction and gas chromatographic – mass spectrometric method for the analysis of phthalates in surface water and to perform the required method validation steps.

EXPERIMENTAL

Standards and reagents

The 506 Laboratory Performance Check Mix in purge-and-trap grade methanol (99.9%), (EPA506) was purchased from Restek (USA): dimethyl phthalate (DMP) 100 μg/ml, diethyl phthalate (DEP) 100 μg/ml, di(*n*-buthyl) phthalate (D*n*BP) 100 μg/ml, buthyl benzyl phthalate (DMP) 250 μg/ml, di(2-ethylhexyl) adipate (DEHA) 1200 μg/ml, di(2-ethylhexyl) phthalate (DEHA) 250 μg/ml, di(*n*-octyl) phthalate (D*n*OP) 650 μg/ml. The standards DEP and D*n*BP, were purchased from Sigma-Aldrich (USA). Lichrosorb RP-18 silicagel, 7 μm particles, 100Å pore size, methanol LiChrosolv (CH_3OH) (99.9%, HPLC) were from Merck (Germany), ethyl acetate ($CH_3COOC_2H_5$) (analytical grade) (99.5%), dichlormethane (CH_2Cl_2) (GC purity grade) (99.5%), sodium sulphate (Na_2SO_4) (analytical grade) (99.0%), sulphuric acid (analytical grade) (95–97%) were purchased from Sigma-Aldrich. Nitrogen gas, N_2, (99.9%) was purchased from ELME MESSER (Lithuania).

Standard EPA506 and EBS stock solutions prepared in methanol were stored at +4 °C.

Sample collection

Samples of surface water were taken from the Venta river flowing across the Kuršėnai town (Šiauliai region, Lithuania), below the outlet of a biological water treatment plant. Sampling in a stream, manually, at a depth of ca. 30 cm was carried out. Samples were poured into amber glass bottles, acidified to pH 2 and placed into a mobile refrigerator. At the laboratory, the samples were filtered through 0.8 μm membrane filters and stored at +4 °C until extraction.

Extraction

To optimize the solid phase extraction (SPE) method, three solutions of different concentrations were tested. In the mixture of two standard compounds (EBS), the concentration of both phthalates was 12.5 μg/L. In one EPA506 solution, the standard concentrations of the compounds varied from 1.23 to 15 μg/L and in another solution from 0.45 to 5.4 μg/L. The extraction system isolated from the environment with a 50 ml column filled with 1000 mg of Lichrosorb C18 adsorbent was used.

The adsorbent was rinsed with CH_3OH, then with $CH_3COOC_2H_5$ and a 1 : 1 mixture of $CH_3COOC_2H_5$ and CH_2Cl_2 (*v*/*v*), dried in vacuum for ca. 1 minute, and conditioned with the sample water (with methanol additive). The extraction flow speed was 1 drop/s. After extraction, the adsorbent was rinsed twice with 5 ml of 10% CH_3OH and dried for ca. 3 min in vacuum. For desorption, 2.5 ml of $CH_3COOC_2H_5$ and 2.5 ml of 1 : 1 mixture of $CH_3COOC_2H_5$ and CH_2Cl_2 (*v*/*v*) was added twice. Traces of moisture were collected with water-free Na_2SO_4. The extract was rinsed twice with 0.75 ml of CH_2Cl_2 and concentrated with N_2 gas.

GC–MS analysis

Standard solutions and extracts were analysed by GC–MS: a GC–2010 Shimadzu (Japan) apparatus with a capillary column (30 m × 0.25 mm × 0.25 mm) (DB-5ms) coated with non-polar 5% phenyl methylpolysiloxane stationary phase was used for separation of the analytes. Samples were injected employing an AOC-20i auto-injector (Japan); 1 μl of a sample was injected in a split mode (split ratio 10 : 1). The

flow rate of the carrier gas (He) was 1 ml/min. The injection temperature was 270 °C. The column temperature gradient was as follows: 80 °C (5 min) to 260 °C at 18 °C/min and then to 300 °C at 6 °C/min. The total duration of the analysis was 18 minutes; the retention time of each compound is provided in Table 2.

Electron ionization was performed at 70 eV. To determine the composition of the compounds, a GC MS–QP2010 Shimadzu mass spectrometer was used in a selected ion monitoring (SIM) mode. Ion characteristics for the analytes are provided in Table 2.

RESULTS AND DISCUSSION

Comparison of extraction protocols and method validation

To apply SPE using an EBS standard mixture, several optimization steps were performed: 7.5 ml of solvents was estimated to be sufficient for an effective desorption of the analytes. Upon replacing the preconcentration of extracts by air with nitrogen gas, the duration of the process decreased from 2.5 hours to 45 minutes.

SPE is affected by the phthalates present in the bacground air of the laboratory. When analysing such compounds, their concentration in the ambient air increases even more [5]. In order to reduce the influence of the background, an extraction system isolating the sample from environmental exposure was set. Recovery values obtained when using the EBS standard for SPE optimization were close to the recommended values (Fig. 1).

The further steps of optimization were performed with an EPA506 mixture of standards by testing two different concentrations of the analytes.

To assess the accuracy of the results, each extraction of water with a respective spike was performed at least three times. The repetitions were carried out day after day, without changing the extraction conditions and extraction setup.

Extraction of the internal standard, in which compound concentrations vary from 0.45 to 5.4 µg/L, showed that the recovery of many compounds corresponded to ISO and USA

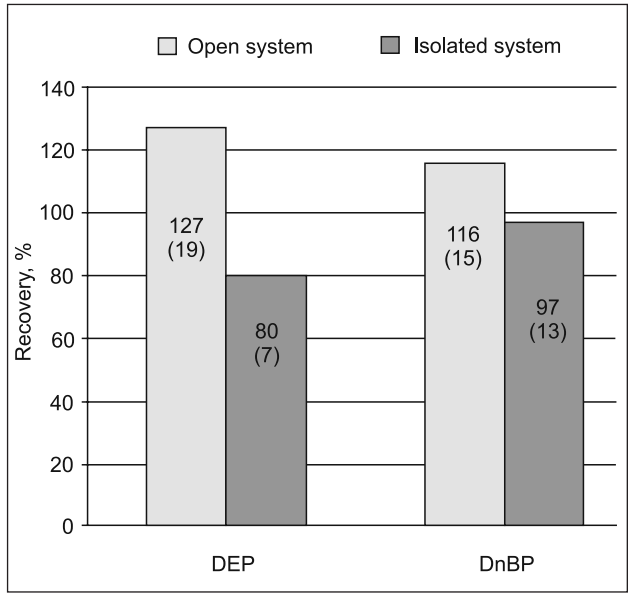

Fig. 1. Recovery of solid phase extraction of EBS standard mixture compounds and RSD, %, n = 3

EPA standard requirements and varied from 102 ± 20% for BBzP to 110 ± 22% for DEHP; however, the obtained RSDs were high – from 26% for DMP to 34% for BBzP. The D*n*BP and DEHP analysis was rather complicated, i. e. the recovery of D*n*BP was 165 ± 64% and of DEHP 124 ± 69%. Relative standard deviations for these compounds were 107% and 74%, respectively. The latter phthalates are the main components of background contamination: therefore, the resultant dispersion affects the accuracy of the analysis (Table 1).

The average recovery of D*n*OP was 51% and RSD was 45%. This compound is considered in ISO 18856 : 2004 standard in which the permitted D*n*OP recovery is 60–75% [5].

The problems of accurate measurement of small volumes are regarded as one of the reasons for excessive RDS values.

When using the concentrations of standard compounds from 1.25 (DEP) to 15.0 (DEHA) µg/L (EPA506 mixture of phtalates) to optimize the SPE, the results, complying with

Table 1. **SPE recovery and accuracy data (250 ml water of the analysis, pH 2, with 5 ml of CH_3OH spiked with EPA 506 mixture of standards)**

Compound	DMP	DEP	D*n*BP	BBzP	DEHA	DEHP	D*n*OP
EPA506 (4.5 µl)							
True value, µg/l	0.450	0.450	0.450	1.125	5.400	1.125	2.925
Mean, µg/l	0.323	0.338	0.743	1.104	4.115	1.396	1.503
SD, µg/l	0.116	0.194	0.483	0.545	1.518	0.828	1.267
RSD, %	26	43	107	34	28	74	45
Recovery, %	72	75	165	98	76	124	51
EPA506 (12.5 µl)							
True value, µg/l	1.25	1.25	1.25	3.13	15.00	3.13	8.13
Mean, µg/l	1.38	1.45	1.65	3.59	14.5	3.77	5.10
SD, µg/l	0.12	0.15	0.35	0.51	0.61	0.88	2.80
RSD, %	8	10	20	18	14	22	36
Recovery, %	110	116	132	115	97	120	63

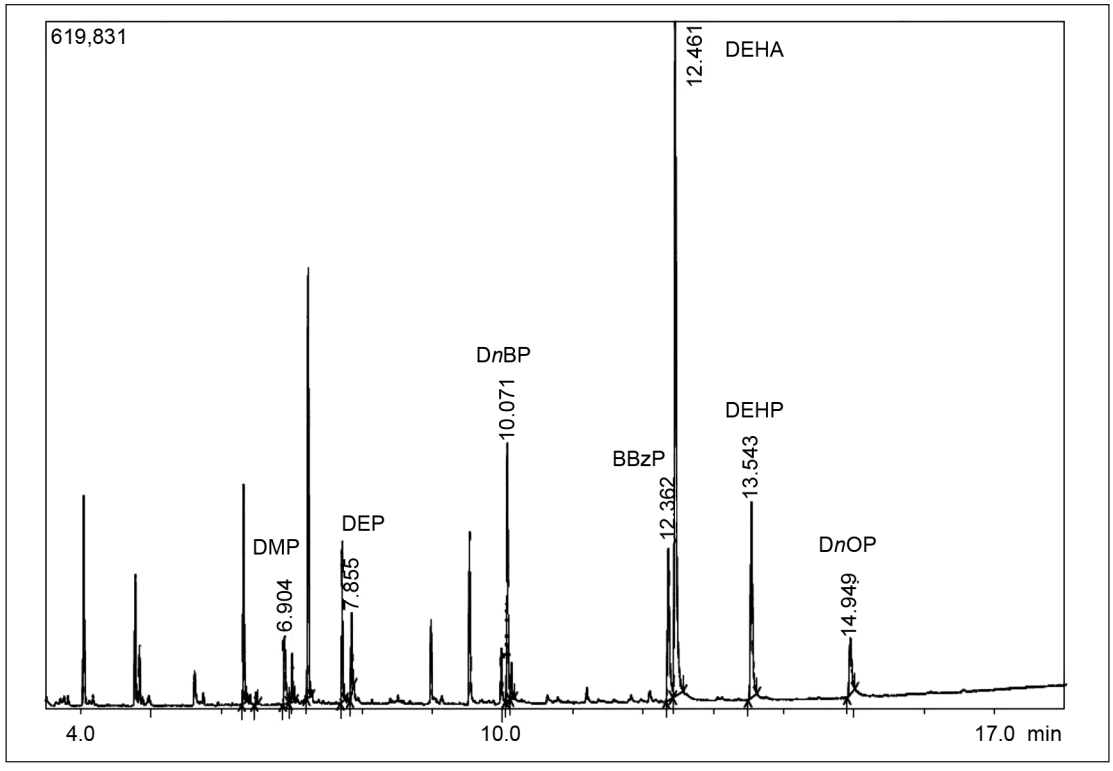

Fig. 2. Chromatogram of standard mixture EPA506 (1.25–15.9 µg/L) after extraction

the ISO standard for eluted compounds, were obtained before the thirteenth minute (Fig. 2).

The recovery of DEHP was 120% and of RSD 22% (Table 1).

The obtained results quite well coincide with interlaboratory analysis results claimed in the standard [5]. Casajuana in [11] reported the problem of DEHP determination.

The results for estrogen D*n*OP with the longest alkyl chain did not improve upon increasing the test concentration: ca. 40% RSD was obtained.

The background contamination with phthalates is unavoidable; however, it was controlled for each SPE series by analysing a blank sample. The background concentration of D*n*BP when using an isolated extraction system decreased from 0.9 to 0.3 µg/L and for DEHP from 0.74 to 0.27 µg/L.

All extraction solvents showed traces of D*n*BP concentration of ca. 0.15 µg/L. Tienpont and co-authors [9] have also determined the main background contamination to consist of D*n*BP and DEHP.

To assess the suitability of the SPE and GC–MS analysis methods, the limits of detection and determination as well as the linear range of determination were evaluated. The limit of detection (LOD) calculated at a signal-to-noise ratio of 3 varied from 45 ng/l (for D*n*BP) to 500 ng/l (for D*n*OP).

To produce the calibration graphs, eight EPA506 standard solutions of different concentrations were prepared. The lower limit of the linear determination range, depending on the compound, varied from 0.1 to 0.625 µg/l, and the maximum values were up to 4.5–54 µg/l. The calculated correlation coefficients R^2 were within 0.986–0.995 (Table 2).

Irrespective of analyte structure, the compounds accurately elute according to their retention indices. The standard deviation for elution time is only parts of millisecond (0.0001–0.03 min), i. e. within the requirements of the ISO 18856 : 2004 standard method (±0.03 min) [5].

Table 2. **Compounds studied, retention time (R_t), ions monitored (quantification ion in bold), detection limits (LOD), linear range and correlation coefficients**

Analyte	R_t, min	Main ions, m/z	LOD, ng/l	Linear range, µg/l	R^2
DMP	6.88	**163**, 77, 223	100	0.3–4.5	0.992
DEP	8.84	**149**, 105, 177	100	0.3–4.5	0.994
D*n*BP	11.06	**149**, 93, 205	45	0.1–4.5	0.995
BBzP	12.34	**149**, 91, 206	250	0.625–11.25	0.992
DEHA	12.44	**129**, 57, 147	300	0.54–54	0.993
DEHP	13.52	**149**, 57,167	60	0.1–11.25	0.991
D*n*OP	14.99	**149**, 57, 279	500	0.625–29.25	0.986

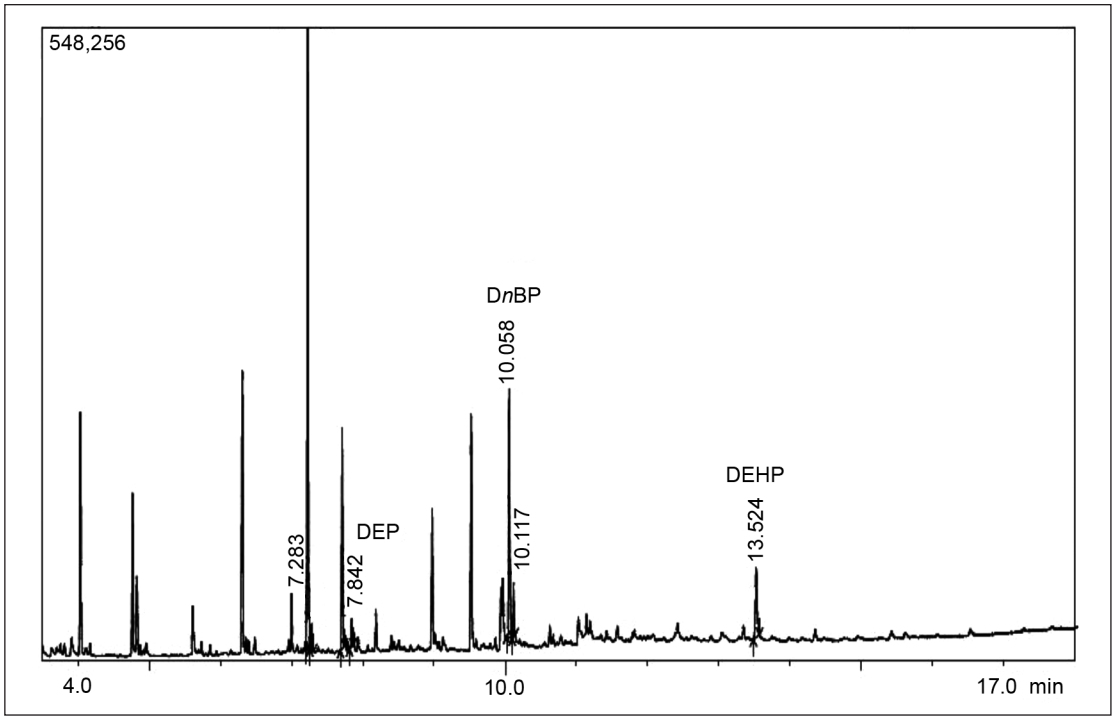

Fig. 3. GC–MS analysis of the Venta river water; sample prepared using SPE without internal standard, March 2011

Analysis of samples from the Venta river

The method of determining phthalate esters in surface water was applied for the analysis of samples from the Venta river. At least 4 litres of surface water were taken. The analyses were carried out according to the internal standard (the concentrations of compounds were 1.25 to15 μg/L) and without the internal standard.

Analyses carried out in December to April showed that phthalate esters presumably entered the river together with sewage (Fig. 3). The main contaminant was D*n*BP: the minimum pollution by DnBP was 0.14 μg/L in December, whereas in April its concentration was almost 16 times higher, i. e. 2.2 μg/L (Fig. 4).

A very high pollution with phthalates was determined in January: the D*n*BP concentration was 154 μg/L and the DEHP concentration 2.1 μg/L. A strong signal of diisobutyl phthalate was obtained, which had not been validated in the method but was recognised according to quantitative ions and mass spectral library. In another sample taken in January, the concentration of D*n*BP was 2.7 μg/L. DEP was detected in this sample, but its concentration was below the detection limit.

In March, the D*n*BP concentration was 1.45 μg/L, the DEP concentration 0.61 μg/L, and the DEHP concentration 0.23 μg/L (Fig. 3).

The determined concentrations of phthalates may be regarded as a concentrated pollution. Further the contaminants are undoubtedly diluted with the clean water of the river, and presumably water biota is not affected by toxicity; however, of major concern is the fact that this river of the Baltic Sea basin is contaminated with environmental estrogens.

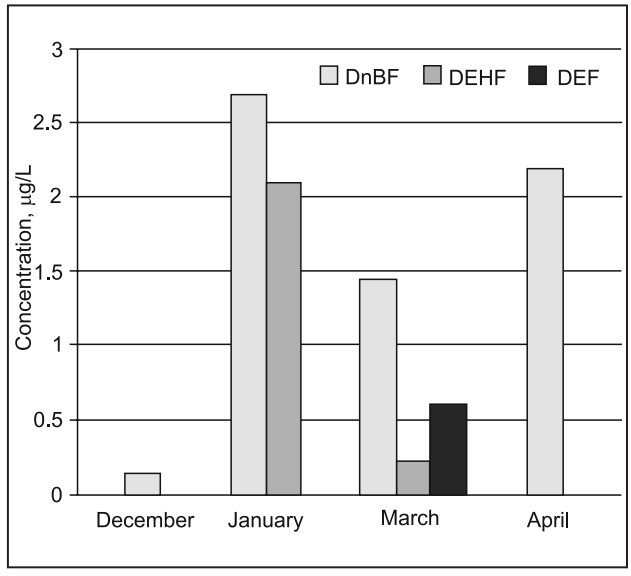

Fig. 4. Phthalate distribution in the Venta river during December to April (2010 / 2011)

CONCLUSIONS

The maximum recovery of SPE was obtained using the sample extraction setup, which is isolated from the environment, and 7.5 ml of the 1 : 1 mixture of ethyl acetate and dichloromethane as a desorption solvent for the analytes and nitrogen gas for conditioning the extraction cartridge.

High SPE recoveries and accurate quantitative data while performing gas chromatographic – mass spectromet-

ric analysis were obtained for phthalates with the shorter alkyl chain: for DMP, DEP, BBzP the recovery varied within 72–116%.

D*n*BP and DEHP were determined as the main components of background contamination. Background contamination with D*n*BP of ca. 0.15 μg/L was determined for the extraction solvents.

The variation of D*n*BP, DEP and DEHP pollutants from December to April 2010 / 2011 in the river Venta was determined. DnBP concentrations in December 2010, January, March and April 2011 were 0.14, 2.7, 1.45, 2.2 μg/L, respectively; DEHP concentration in January 2011 was 2.1 μg/L, in April 0.23 μg/L, and the DEP concentration in March 2011 was 0.61 μg/L.

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FTALATŲ NUSTATYMAS PAVIRŠINIAME VANDENYJE KIETAFAZĖS EKSTRAKCIJOS IR DUJŲ CHROMATOGRAFIJOS–MASIŲ SPEKTROMETRIJOS METODU: ANALIZĖS METODO DIEGIMAS IR ĮTEISINIMAS

S a n t r a u k a

Šio darbo tikslas buvo pritaikyti ir optimizuoti kietafazės ekstrakcijos ir dujų chromatografijos – masių spektrometrijos metodą ftalatų analizei paviršiniame vandenyje ir atlikti būtinus šio metodo įteisinimo veiksmus. Šiuo tikslu buvo naudojami du standartų mišiniai: laboratorijoje paruoštas dietilftalato (DEP) ir di(n-butil) ftalato (DnBP) standartų mišinys (EBS) ir EPA506 standartų mišinys, sudarytas iš septynių ftalatų. Apskaičiuotos metodo aptikimo ir nustatymo ribos. EPA506 standartui, atsižvelgus į junginį, aptikimo ribos kito nuo 45 ng/l iki 500 ng/l, tiesinė priklausomybė buvo intervale nuo 0,1 μg/l iki 54 μg/l. Kietafazei ekstrakcijai atlikti naudota C18 silikageliu pakrauta kolona ir taikyta izoliuota nuo aplinkos ekstrakcijos sistema. EBS standarto išgava, naudojant 1 : 1 etiloacetato ir dichlormetano mišinį, buvo 79 ± 5 % DEF ir 97 ± 11% D*n*BF. Nustatytas D*n*BP ir di(2-etilheksil)ftalato foninės taršos signalas. Įteisintas metodas pritaikytas ftalatų analizei Ventos upės paviršiniame vandenyje. Taršos kaita matuojant 2010–2011 m. D*n*BF buvo gruodį, sausį, kovą, balandį atitinkamai 0,14 μg/l, 2,7 μg/l, 1,45 μg/l, 2,2 μg/l; DEHF– sausį 2,1 μg/l, kovą 0,23μg/l ir DEF kovą 0,61 μg/l.