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Determination of alkylphenols in water samples using liquid chromatography–tandem mass spectrometry after pre-column derivatization with dansyl chloride

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ABSTRACT

The present study describes an effect of reaction condition of pre-column derivatization of alkylphenols (APs): bisphenol A (BPA), 4-tert-octylphenol (4-t-OP), 4-octylphenol (4-OP), 4-n-nonylphenol (4-n-NP), and isomers of 4-nonylphenol (iso-NP) with 5-(dimethylamino) naphthalene-1-sulfonyl chloride (dansyl chloride, DNSC) on their LC–ESI–MS/MS determination in water samples. Chemical derivatization improves the sensitivity and selectivity of LC–MS/MS analysis. In principle, alkylphenols can be analyzed by LC–MS/MS without derivatization. However, pre-column derivatization of APs increases the sensitivity up to 1000 times in comparison with the analysis of underivatized alkylphenols. Reaction conditions affecting formation of the DNSC-derivatives, such as various solvent, reaction temperature, reaction time, DNSC concentration and pH values were tested. The most suitable conditions, in terms of achieving a high sensitivity, resulting from this study are: acetonitrile as reaction solvent, 60 min as reaction time, 60 °C as reaction temperature, pH values 10.5, 0.5 mg mL^{-1} as DNSC concentration. Calibration curves are linear at least in the range of 1–1000 ng mL^{-1}, limits of detection (LOD) and limits of quantification (LOQ) ranging from 0.02 to 0.25 pg/injection and from 0.08 to 0.83 pg/injection, respectively. The improved procedure was successfully applied for the analysis of APs and BPA in real water samples. The median concentration of BPA and iso-NP obtained in bottled waters was 4.7 ng L^{-1} and 33.5 ng L^{-1}, respectively. The median concentration of 4-t-OP was 1.3 ng L^{-1}.

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1. Introduction

Alkylphenols (APs) are toxic, xenobiotic substances classified as endocrine disruptors, which have a negative effect on the hormonal system of many organisms including humans. APs are among the ubiquitous environmental contaminants and are frequent contaminants in wastewater [1,2]. These compounds are also discharged into the environment as metabolites of alkylphenol ethoxylates (APE) mainly by biodegradation in sewage treatment plants. Bisphenol A (BPA) is used as a monomer in the preparation of epoxide resins, polycarbonate plastics and as an antioxidant or stabilizer in polyvinylchloride. BPA can be released into the environment from packaging materials, food and feed as a result of (i) diffuse environmental pollution and direct uptake by animals via food or air and potential bioaccumulation and transfer through the food web, (ii) food processing by contact with plastics, resins, lacquers, surfactants, containers, and (iii) migration from packaging and bottling material, and printer ink [3]. The presence of alkylphenols residues in bottled water is attributed to (i) water contamination in the bottling plant, (ii) migration of plasticizers from the bottle material to the water since quality may vary depending on the raw material as well as the technology used in bottle production [4]. Alkylphenols including linear octylphenol (OP) and nonylphenol (NP) are widely used as intermediates in production of surfactant (anionic and non-ion surfactants) and as a stabilizers of ethylcellulose resin, oil-soluble, phenols and esters. Octylphenol (log K_{OW} 4.12), nonylphenol (log K_{OW} 4.48) and bisphenol A (log K_{OW} 3.32) are lipophilic compounds. Therefore, they can easily contaminate foods of animal origin (e.g., meat, milk, cheese), which are thought to represent the most important source of human exposure to many organic pollutants [3,5].

BPA, OP and NP are nominated in the contaminant candidate list for compounds that may require regulations under the Safe Drinking Water Act [6]. Total allowable concentration (TAC) for BPA is 100 μg L^{-1} in drinking water [7]. The Integrated Risk Information System (IRIS) of US EPA proposed values of

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Table 1
Comparison of instrumental methods used for analyses of APs and BPA with and without derivatization reagents.

Analytes	Derivatization agents	Detection	LOD	Reference
OP, NP	–	HPLC-UV	NP 0.2 μg L^{-1} OP 0.1 μg L^{-1}	[22]
APs, BPA	–	HPLC-UV	2–6 μg L^{-1}	[23]
APs, BPA	BSTFA + 10% TMCS	GC–MS	0.001–0.02 μg L^{-1}	
NP	–	GC–MS	27 ng L^{-1}	[16]
BPA	–	GC–MS	<0.3 μg L^{-1}	[24]
NP, OP	–	HPLC-DAD	NP 0.06 ng μL^{-1} OP 0.04 ng μL^{-1}	[25]
APs, BPA	BSTFA	GC–MS	BPA 0.15 ng L^{-1} NP 0.60 ng L^{-1} OP 1.50 ng L^{-1}	[19]
APs	Pentafluororyridine	GC–MS	NP 85.2 ng L^{-1} OP 12.5 ng L^{-1}	[15]
BPA, NP, OP	MTBSTFA	GC–MS	4–6 ng L^{-1}	[26]
APs, BPA	–	HPLC–ESI/MS/MS	*12–240 ng mL^{-1}	[18]
APs, BPA	DNSC	HPLC–ESI/MS/MS	*4–197 pg mL^{-1}	[27]

BSTFA, N,O-bis (trimethylsilyl)trifluoroacetamide; TMCS, trimethylchlorosilane; MTBSTFA, N-methyl-N-(tert-butyl-dimethylsilyl)-trifluoroacetamide; DNSC, 5-(dimethylamino) naphthalene-1-sulfonyl chloride.
* IDL, instrument detection limit.

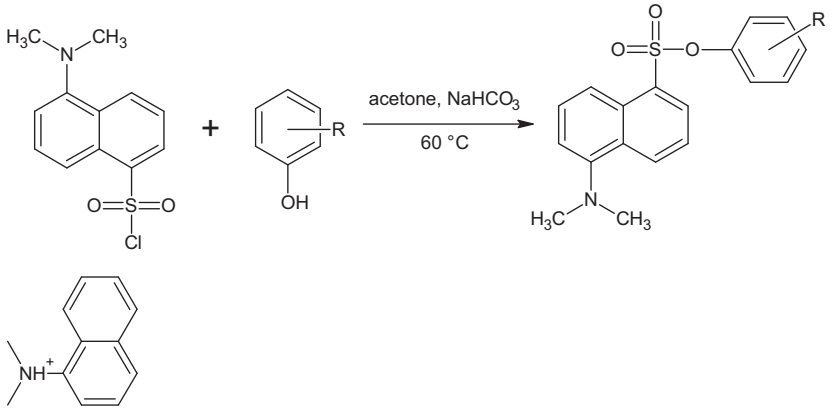

Fig. 1. Reaction scheme of dansylation of alkylphenols. Ion used for MS/MS quantification [5].

reference dose for chronic oral exposure (RfD) based on chronical health hazard assessment for non-carcinogenic effects for BPA 5×10^{-2} mg/kg bw/day [8,9].

High concentrations of BPA, OPs and NPs can be found in wastewater at the outlet of the wastewater treatment plant (WWTP). Acute toxicity levels for BPA, defined as the concentration at which half of the organisms survive (LC_{50}), have been measured in a variety of aquatic organisms, including freshwater and saltwater algae, invertebrates (daphnia and mysid shrimp) and fish. LC_{50} values range from 1 to 20 mg L^{-1} [10]. Similar study describes the values for nonylphenol toxicity to aquatic organisms; LC_{50} values for nonylphenol are in range from 20 to 1590 μg L^{-1}. For instance, NPs at concentrations below 350 μg L^{-1} did not have any toxic effect (EC_{50}) on *Caenorhabditis elegans* (a sediment-dwelling nematode) but enhanced its growth and reproduction [11].

In order to assess the risk of contamination of environment by alkylphenols it is necessary to have sensitive and selective methods for their quantitative analyses. Current requirements for analytical methods include the high sensitivity, high throughput, ease of use, precision, accuracy and automation. Although instrumentation meets some of these requirements, sample preparation that takes place prior to instrumental analysis is still under active research and development [12]. Chromatographic methods are used for determination of alkylphenols, for example micellar liquid chromatography (MLC) [13], gas chromatography [14–16] and liquid chromatography [17–19]. The alkylphenols and bisphenol A can be analyzed directly and/or after pre-column derivatization with various agents as shown in Table 1. Analytical derivatization of target analytes can substantially improve chromatographic separation as well as the sensitivity and selectivity of detection [12]. Dansyl chloride has been widely used as a derivatizing reagent for fluorescence detection and for facilitating the MS detection of phenols and amines, but not for general alcohols [20]. The general derivatization reaction of alkylphenols with DNSC is described in Fig. 1. The reaction occurs in mildly alkaline solution acetone-aqueous medium at multiple excess of reagent. Both degree of derivatization and hydrolysis of derivatives increase with increasing pH of the reaction medium. The recommended pH of reaction medium is pH 9.5–10. Derivatization is carried out exclusively before LC–MS analysis as a pre-column procedure. The disadvantage of dansylderivation may be long reaction time (2–60 min) and high reaction temperature (60–100 °C) [18,21].

The dansylation of phenolic hydroxyl groups introduces ionizable basic nitrogen that enhances the mass spectrometric response of the target analytes. The MS/MS analysis of such a derivative utilizes the formation of product ion with *m/z* 171 corresponding to the protonated 5-(dimethylamino) naphthalene (Fig. 1). LC–ESI–MS/MS analysis based on this product ion is highly selective and sensitive and is commonly used for MS/MS detection of dansylderivatives of phenolic compounds [28,29]. The efficiency of electrospray ionization (ESI–MS/MS) of APs without pre-column derivatization is much lower than those of derivatized APs. The difference in LOQ reaches up to 3 orders [18]. Due to different conditions of derivatization used and presented in various studies, the

Table 2
Precursor and product ions and collision energies of MRM transitions used for BPA and APs analyses.

Analyte	BPA	4-t-OP	iso-NP	4-OP	4-n-NP
Transitions monitored	695.3 → 171 695.3 → 461	440.3 → 171 440.3 → 156	454.3 → 171 454.3 → 156	440.3 → 171 440.3 → 156	454.3 → 171 454.3 → 156
Collision energy (eV)	44	38	34	34	44

verification of derivatization procedure of alkylfenols has to be carried out in order to achieve the highest possible yield of the reaction. The main aim of our study was to test the effect of reaction conditions of dansylderivatisation on the sensitivity of LC–ESI–MS/MS analysis of the most frequently monitored alkylphenols. The reason was because of limited information on the effect of individual parameters on efficiency of derivatization reaction found in published papers. Bisphenol A, 4-*tert*-octylphenol, 4-octylphenol, mixture of nonylphenol isomers called also as technical nonylphenol and 4-n-nonylphenol were selected for these purposes. The usability of improved derivatization reaction is demonstrated on LC–ESI–MS/MS analysis of alkylphenols and bisphenol A in drinking water and wastewater samples.

2. Experimental

2.1. Reagents and chemicals

Analytical standards (Sigma–Aldrich, Germany): 4-n-nonylphenol (99.9%), bisphenol A (>99%), 4-octylphenol (99%), 4-tert-octylphenol (97%), nonylphenol – mixture of ring and chain isomers (iso-NP, analytical grade), solvents: acetone (99.8%), dichloromethane (DCM, 99.8%), toluene (99.8%) (LAB-SCAN, Poland), acetonitrile (ACN, 99.9%), methanol (MeOH, for pesticide residue analysis), water for LC/MS (impurities <0.0001%, Fluka, Sigma–Aldrich, Germany). Derivatizing agent dansyl chloride (Fluka, Sigma–Aldrich, Germany), pH adjusting agents and additives for HPLC-MS: sodium bicarbonate, sodium hydroxide, formic acid (Fluka, Sigma–Aldrich, Germany). Methanol for mobile phase preparation (LC-MS, Biosolve, Netherlands).

2.2. Derivatization conditions

The efficiency of dansyl derivatization depends on experimental conditions. The conditions of dansylderivatization of APs were tested using various solvents for preparation of standard solutions, reaction time, reaction temperature, pH value of reaction mixture and concentration of DNSC. Optimal parameter was always used in a test of the next parameter. Derivatization reaction was carried out in a 2 mL amber glass sample vial with 200 μL standard solution of alkylphenols (1 ng mL^{-1} each AP) in selected reaction solvent. Fifty microliters of 100 mM $NaHCO_3$ in water were added and the content was mixed (vortex mixer) for 1 min. Then 200 μL of DNSC in acetone (0.5 mg mL^{-1}) were added and mixed again. Subsequently the mixture was incubated for selected time at selected temperature. The reaction mixtures were then cooled to room temperature and evaporated to dryness under gentle stream of nitrogen. The residue was redissolved in 1 mL of methanol and mixed. The solution was injected into the LC–ESI–MS/MS. The derivatization parameters were tested in the order: effect of reaction solvents (acetone, ACN, MeOH, water, toluene, DCM), reaction time (1, 3, 10, 30, 60, and 120 min), reaction temperature (5, 20, 40, 60, 80, and 100 °C), pH value in the range from 8.25 to 11.9, and concentration of DNSC in the range from 0.0005 to 5 mg mL^{-1}. The changes of LC–ESI–MS/MS peak area were used for assessment of effect of different derivatization conditions.

Table 3
Characteristics of samples of bottled drinking water.

	A	B	C	D	E	F
Bottle type	PET	PET	PET	PET	PET	PET
Colour	Clear	Clear	Clear	Dark blue	Light green	Clear
Volume (L)	1.5	1.5	1.5	1.5	1.5	1.5
Water type[a]	BW	MSW	NMW	NMW	NMW	MSW
Σ Cations (mg L^{-1})	79.5	104.7	36.6	213.6	179.4	110.7
Σ Anions (mg L^{-1})	259.0	386.1	114.9	981.1	580.5	361.1

[a] BW, baby water; MSW, mountain spring water; NMW, natural mineral water; PET, polyethylene.
Σ Cations: Na^+; K^+; Mg^{2+}; Ca^{2+}.
Σ Anions: SO_4^{2-}; HCO_3^-; Cl^-; NO_3^-.

2.3. LC–ESI–MS/MS analysis

Chromatographic separations were performed using an Agilent 1200 Infinity Series (Agilent Technologies, Santa Clara, CA, USA) with chromatographic column ACE 5 C18, 150 mm × 4.6 mm i.d., 5 μm particle size (ACE, Scotland, UK). The column temperature was maintained at 40 °C and the injection volume was 10 μl. Methanol (A) and water containing 7 mM formic acid (B) were used as a mobile phase. The isocratic elution of 90% (A) + 10% (B) was used at flow rate 0.5 mL min^{-1}. The Agilent 6410 Triple Quadrupole (Agilent Technologies, Santa Clara, CA, USA) was used for MS/MS analysis. The instrument was operated in the ESI-positive MRM mode. Two MS/MS transitions were used for MS analyses. Fragment ion *m*/*z* 171 was chosen for the final quantification, fragment ion *m*/*z* 156 was used for confirmation, except of BPA (Table 2). The structure of quantification ion is described in the Fig. 1. The optimized instrument conditions were as follows: drying gas temperature 350 °C; capillary voltage 2 kV; nebulizer gas pressure 50 psi. LC–MS/MS parameters are summarized in Table 2 [5].

2.4. SPE procedure

The following procedure was used for treatment of water samples for alkylphenols and bisphenol A analyses. The SPE columns (octadecyl C 18, 500 mg) were conditioned with (2 mL of methanol and 2 mL of water (purity for LC–MS). The water samples (100 mL) were passed through the SPE columns using vacuum manifold flowrate 5 mL min^{-1}. After extraction, the columns were dried with air for 5 min. Elution was performed with ACN (5 mL). The eluate was evaporated and converted into mini-vial derivatized with optimized dansyl chloride procedure and analyzed by LC/ESI/MS/MS [27].

2.5. Water samples

Six samples of drinking water packed in 1.5 L PET bottle were purchased in the wholesale network in December 2014 for determination of alkylphenols and bisphenol A. Water samples were lettered A–F, to avoid the legal dispute (Table 3). At the same time a composite water sample was taken at effluent of the wastewater treatment plant in Brno – Modřice for direct comparison of the

content of alkylphenols and BPA in bottled drinking water and water leaving the treatment plant. The samples were stored at 4 °C in cool box until analyzed.

2.6. QA/QC

Because the BPA and/or APs are present in various laboratory equipment, the use of any plastic and rubber material was avoided to minimize possible contamination of the samples during sample collection, storage, transport, treatment, analyte extraction, derivatization procedure, calibration and LC/MS/MS analyses and also volume measurements and weighing. Only glass and/or stainless steel materials were used. No detergents were used for glassware cleaning. All glassware was rinsed with water, acetone, methanol and acetonitrile purity for LC/MS. The composite samples were collected into amber glass bottle treated by the same procedure as for other glassware.

Solvent blanks and standard solutions were analyzed in sequence with each set of analyzed samples for control of background contamination. Blanks were processed in the same way as the samples. All of the standard solutions used for the calibration curves were injected before and after each analysis of a set of samples to check for instrumental sensitivity and reproducibility. To assess the efficiency of the SPE method used for extraction of BPA and APs from water samples, preliminary experiments were performed. BPA and APs were spiked in to same volume of water for LC/MS as the volume of analyzed real water samples. Only glass SPE columns were used for extraction of analytes from water samples. The recovery calculated from analysis of six parallel spiked water samples was used for correction of data obtained from LC/MS/MS analyses of real samples [27].

In order to obtain high selectivity and sensitivity of LC/MS analyses, multiple-reaction-monitoring (MRM) was chosen as a data acquisition mode. Three identification ions (one precursor ion and two products ions) were used for each analyte. The relative response between the two MRM transitions together with retention times was used as a criterion for the identification of the compounds. Agilent Mass Hunter quantitation software was used for these purposes. The uncertainty of the ratio of the quantification ion to the qualifier ion of individual analytes was set at 20% of the expected values according MassHunter standard evaluation procedure of MassHunter software.

Quantitation of analytes was performed using a seven-point calibration curve in the concentration range of 1–1000 ng L^{-1}. Internal standard 4-n-NP–d8 was added to all samples and calibration point s. Instrumental limits of quantification was determined from the injected amount of standard solutions that provide signal-to-noise ratio of S/N = 10. The limits of detection was determined from the amount of analytes that provides signal-to-noise ratio of S/N = 3. Repeated analysis (n = 6) of standard solutions was carried out for these purposes.

Accuracy and precision of the method used for the LC/MS/MS determination of alkylphenols were calculated for six parallel samples and three levels of concentrations (50 ng L^{-1}, 250 ng L^{-1}, 1000 ng L^{-1}). The concentration levels were chosen based on the content of alkylphenols commonly present at the outlet of the water treatment plants.

Single factor analysis of variance ANOVA was used to compare the differences of experimental data obtained from the study of the effect of investigated parameter of derivatization reaction on LC/MS/MS signal (peak area) for each of compound. The null hypothesis was that investigated parameter has no effect on LC/MS/MS signal; it means that the yield of derivatization reaction is not dependent on parameter in tested region. If the probability (p-value) <0.05 then the null hypothesis was rejected.

3. Results and discussion

3.1. Effect of reaction conditions on the yield of derivatization reaction of alkylphenols

3.1.1. Effect of a sample solvent

The first factor, which can affect the derivatization reaction, is a solvent in which sample and/or standard are dissolved. Six different solvents (acetone, ACN, MeOH, water, DCM and toluene) were used for these purposes. Different solvents were used to prepare a standard solution of analytes. The ANOVA analysis was used for testing the differences in peak areas obtained for individual analytes when different solvents were used (see ANOVA result in Section 3.3). As shown in the Fig. 2A, regarding the sum of the detector responses (peak area) of individual analytes, acetonitrile is the most suitable solvent for the mixture of alkylphenols. Water could be preferable as the reaction solvent if only iso-NP have to be analyzed. Completely unsuitable sample solvent is methanol. Selection of the appropriate sample solvent may depend on the method used for extraction and/or purification of extracts of alkylphenols from various types of matrices. From the results of the test of suitability of selected solvents follows that methanolic SPE eluates from purification processes have to be converted into another solvent after evaporation, preferably into acetonitrile [27]. The similar effect was described in the case of study focussed on dansylderivatisation of fluorotelomers. The decrease of sensitivity of derivatization reaction in methanol was assigned to the reaction of DNSC with methanol. Due to reactivity of DNSC preferably with primary amino, hydroxyl and carboxylic groups the differences in effect of other tested solvents cannot be associated with reaction of solvents with DNSC [30].

3.1.2. Effect of reaction time

Reaction time is one of the most important factors in derivatization procedures. In our study reaction time was tested in the range of 1–120 min; the results you can see in Fig. 2B. Changes of reaction time from 30 to 120 min do not lead to significant changes in peak areas (see ANOVA result in Section 3.3). Based on these results, 60 min was selected for further experiments. The shorter time is recommended in several studies [18,27]. However shorter reaction time especially in the range of 1–10 min may cause poor repeatability due to possible variability of time delays between the individual steps of sample derivatization.

3.1.3. Effect of reaction temperature

To evaluate which value of recommended temperature is optimal in terms of yield of derivatization reaction and the time of sample preparation for analysis, the effect of a wide range of temperatures on the yield of derivatization was tested. The starting point was below the laboratory temperature (5 °C); the last point (100 °C) was slightly above boiling point of acetonitrile chosen as reaction solvent (82 °C), in order to test the whole temperature range recommended in the literature. Sixty minutes were used as the reaction time. As is apparent from Fig. 2E, the suitable reaction temperature is 60 °C for the whole mixture of alkylphenols. No significant differences were found in the region of 60–100 °C, except for iso-NP (see ANOVA result in Section 3.3). The different shape of iso-NP dependence curve could be caused by different physico-chemical properties of individual isomers in the mixture. The rigorous explanation could be carried out only if standards of individual isomers will be available.

3.1.4. Effect of pH

The pH of the reaction mixture affects the efficiency of derivatization due to the degree of dissociation of phenolic hydroxyl (pK_a of alkylphenols ranged from 9.9 to 10.9 pK_a of BPA is lower – approx.

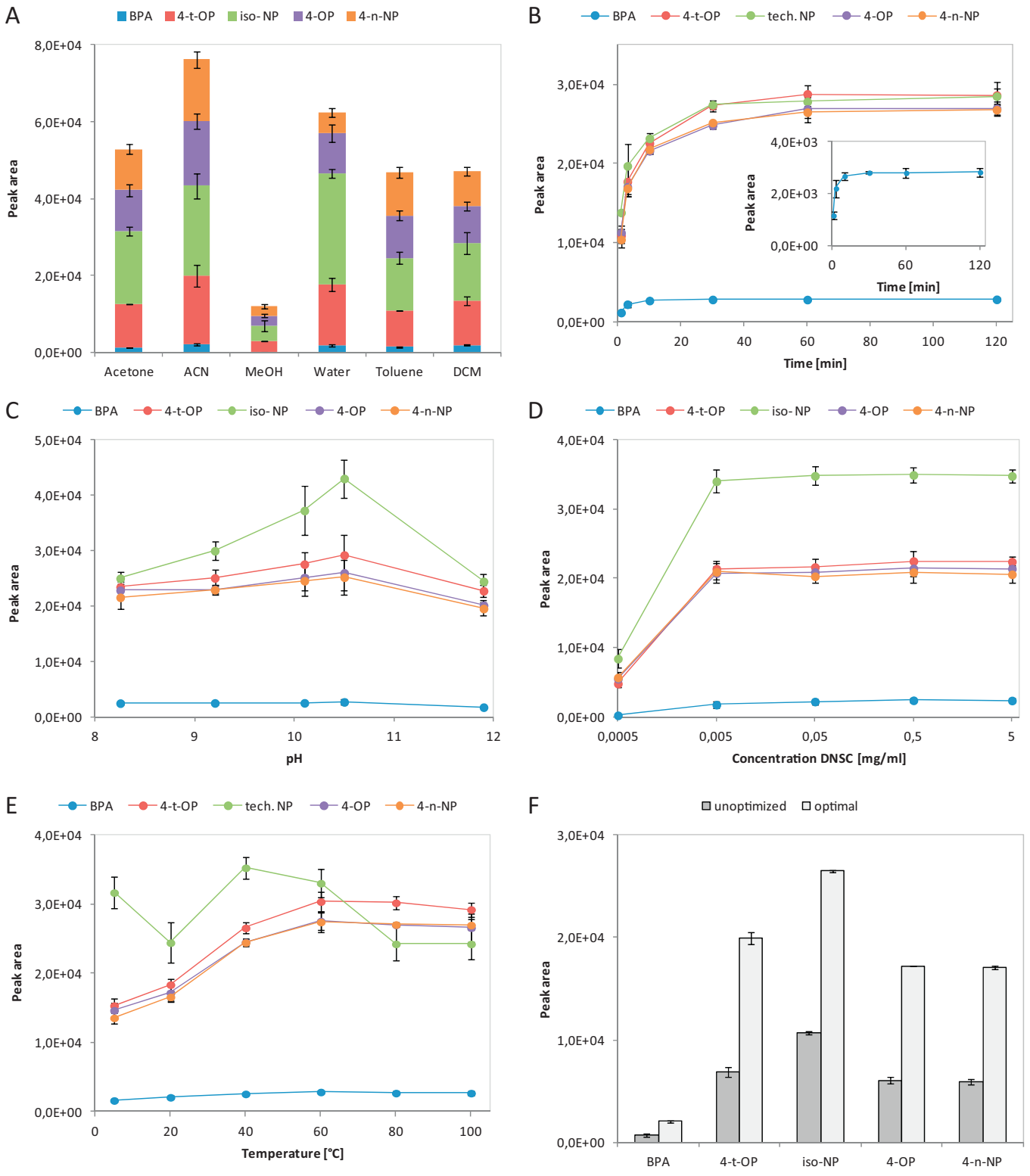

Fig. 2. Effect of reaction properties on the yield of derivatization of APs and BPA expressed as chromatographic peak areas of LC/MS/MS analysis. Effect of: (A) solvent, (B) reaction time, (C) pH values, (D) DNSC concentration and (E) reaction temperature. (F) compares the yield of optimized derivatization reaction with unoptimized.

8.5–9.2 depending on environment) and the speed of hydrolysis of dansyl-derivatives [21]. Series of experiments were carried out to determine the effect of pH in the following manner. The sodium bicarbonate at a concentration of 100 mM was adjusted using 3 M NaOH to a pH in the range of 8.25–11.9 usually recommended in the literature. The significant effect of pH on the peak area was confirmed by ANOVA for all investigated analytes (Section 3.3). The highest effect was obtained for the mixture of iso-NPs that can have different pK_a values depending on chain structure. The maximum peak area was obtained at pH 10.5, as you can also see in Fig. 2C.

Based on these facts, the pH of the sample was adjusted to pH 10.5 in the subsequent experiments.

3.1.5. Effect of concentration of dansyl chloride

The effect of the concentration of dansyl chloride on the yield of derivatization procedure was investigated in the range of concentrations 0.0005 and 5 mg mL^{-1} which are recommended for determination of alkylphenols on the level of ng mL^{-1} and lower. Concentration of each alkylphenols used for this study was 1 ng mL^{-1}, typical for environmental samples, such as wastewater, soil, sediments. As is shown in Fig. 2D the DNSC concentration of 0.005 mg mL^{-1} (i.e. 5000 times greater for each alkylphenols) provides maximum yield of derivatization reaction, that did not change with additional surplus. No significant differences were found in the region of 0.005–5 mg mL^{-1} (see ANOVA result in Section 3.3). As to concentration of alkylphenols expected in environmental samples and as to the content of other compounds which can be present in the matrix the DNSC concentration of 0.5 mg mL^{-1} was chosen as sufficient excess.

If above optimized derivatization conditions are used for determination of alkylphenols under investigation, two times higher responses can be obtained comparing to conditions of most often recommended for derivatization of phenolic compounds (Fig. 2F). As can be seen especially in the case of effect of temperature and reaction time, the use of appropriate values in the plateau of the curves (Fig. 2B and E) allow decreasing the experimental errors caused by eventual fluctuation of these reaction conditions outside of plateau. The following "unoptimized" conditions were used for comparison in our study: 100 mM solution of $NaHCO_3$ in water, acetone as reaction solvent, 0.5 mg mL^{-1} dansyl chloride solution in acetone, reaction time 3 min, reaction temperature 60 °C [18].

3.2. Chromatographic analysis of derivatized alkylphenols

The chromatographic analysis of derivatized alkylphenols obtained under above specified conditions selected on the basis of previous experiments is shown in Fig. 3. As you can see poor separation was obtained for the mixture of nonylphenol isomers (iso-NP). A focus on a better resolution of mixture of iso-NP is not necessary because, as is common, iso-NP quantification is based on the sum of signals of isomers in the mixture [14,31]. The mixture of ring and chain nonylphenol isomers (iso-NP) present in Sigma–Aldrich standard mixture was used for quantification of nonylphenols except the linear nonylphenol (n-NP). The length of chromatogram can be modified depending on the aim of chromatographic analyses and properties of sample matrix. Especially an increase of column temperature can be used for effective time shortening up to one half.

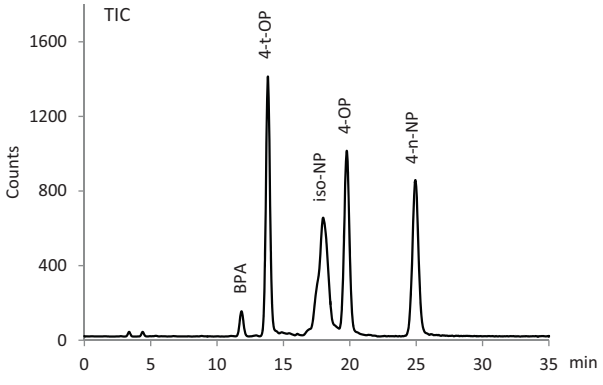

Fig. 3. Chromatogram of the dansylderivatives of the alkylphenolic compounds (c = 1 ng mL^{-1}).

Table 4
Validation parameters of selected alkylphenols and bisphenol A.

Compounds	Regression equation	R^2	[pg/inj]		t_R [min]
			LOD	LOQ	
BPA	$y = 8270x$	0.9933	0.25	0.83	11.8
4-t-OP	$y = 39788x$	0.9988	0.02	0.08	13.8
iso-NP	$y = 48426x$	0.9989	0.06	0.20	17.9
4-OP	$y = 72487x$	0.9995	0.06	0.19	19.7
4-n-NP	$y = 79749x$	0.9996	0.04	0.14	24.9

Table 5
p-values of ANOVA of effect of parameters of derivatization reaction on peak area of LC/MS/MS analyses of alkylphenols.

	pH	Temperature	Time	DNCS concentration	Solvents
BPA	0.044	0.663	0.973	0.235	1.08E−03
4-t-OP	0.021	0.472	0.338	0.513	6.68E−05
iso-NP	2.72E−05	4.38E−03	0.261	0.821	1.34E−05
4-OP	0.031	0.719	0.085	0.765	9.19E−04
4-n-NP	4.28E−03	0.843	0.131	0.931	1.78E−05

3.3. Method validation

Validation parameters for selected alkylphenols and bisphenol A investigated under optimized conditions are listed in Table 4. Good linearity of calibration curves was obtained in the whole concentration range. Instrumental limits of quantification and limits of detection determined from the injected amount of individual analytes ranged from of 0.08 to 0.83 pg/injection and 0.02–0.25 pg/injection, respectively.

Accuracy and precision of the method used for the LC/MS/MS determination of alkylphenols in water samples involving SPE procedure (Section 2.4) and derivatization of analytes in the preconcentrated sample, ranged from 71 to 99% and from 16 to 3%, respectively.

Uncertainties of the results of the study of effect of individual reaction conditions and results of analysis of real samples expressed as RSD ranged for selected analytes as follows: BPA 0.6–34.9%, 4-t-OP 0.3–16.1%, iso-NP 0.9–31.8%, 4-OP 0.9–21.7%, and 0.6–23.7% for 4-n-NP. The highest values were found only for experiments with methanol as reaction medium, which is not suitable for derivatization of alkyphenols and also for lowest concentration of DNSC which represents insufficient surplus of DNSC. Uncertainties of LOQ expressed as RSD were in the range of 4.5–14.8%.

Table 5 lists p-values of ANOVA of effect of reaction parameters for individual compounds. The significant difference was indicated for effect of pH of reaction mixture and reaction solvents because p-values are <0.05. The p-values of effect of reaction time, DNSC concentration and reaction temperature were higher than 0.05 for values in the plateau of the experimental curves (regions of 30–120 min, 60–100 °C and 0.005–5 mg mL^{-1}, respectively) therefore the effect of that parameters is non-significant in mentioned region. The p-value of effect of temperature for iso-NP was an exception (for explanation see Section 3.1.3).

3.4. Determination of alkylphenols in water samples

Improved method was used in our study for analysis of the content of alkylphenols and bisphenol A in various commercially available drinking waters. The method was used also for analyses of bisphenol A, nonylphenols and octylphenols in wastewater collected as 24 composite samples at the output from wastewater treatment plant (WWTP). Three parallel samples were analyzed in each case (Fig. 4).

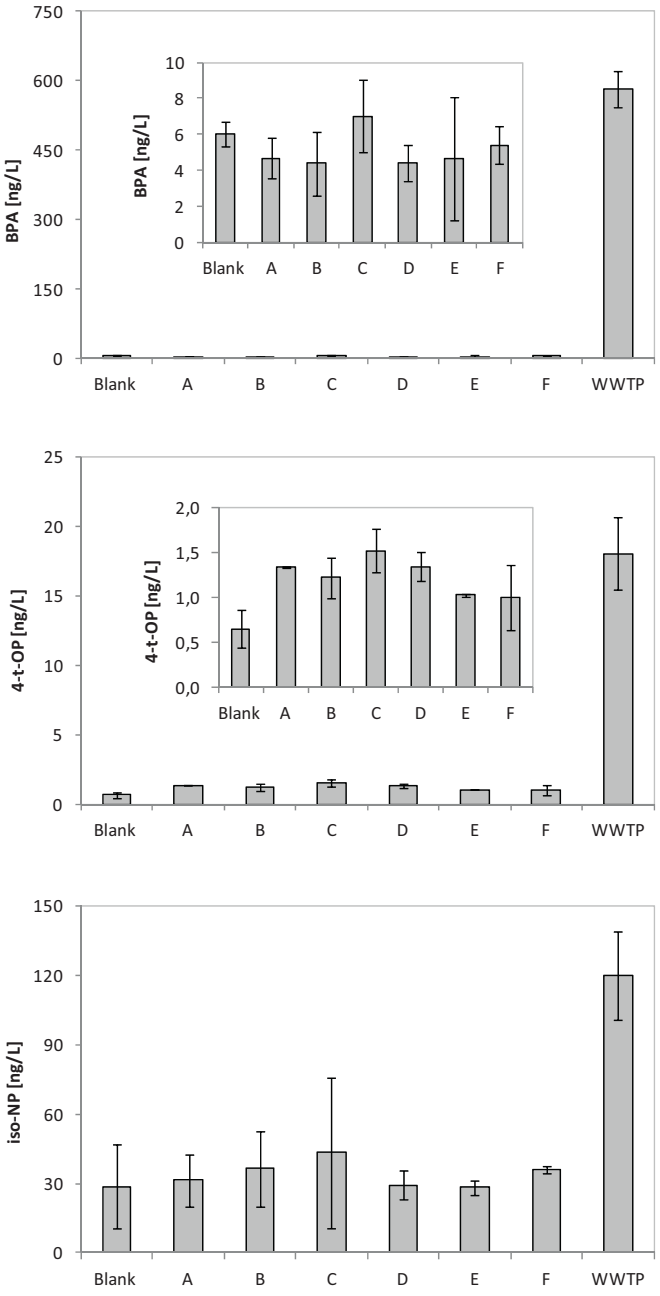

Fig. 4. Content of APs and BPA in drinking and wastewater ($n = 3$).

The median concentration of BPA and iso-NP obtained for bottled waters was 4.7 ng L^{-1} and 33.5 ng L^{-1}, respectively. These values are not distinguishable from the blanks in case of all samples. The median concentration of 4-t-OP was 1.3 ng L^{-1} approximately two times higher than blank.

A relatively high level of alkylphenols and bisphenol A in blank samples comparing to water samples is caused by common contamination of samples coming from laboratory air, septa vials, and solvents and chemicals used. This can be a problem especially in determination of alkylphenols and bisphenol A at low concentrations, as in mineral water.

Concentration of BPA and alkylphenols in determined in bottled drinking waters was well below the guideline values for drinking waters. Daily intake of these compounds from drinking bottled water will be low and does not exceed the acceptable value of income relative to the reference doses.

The daily intake of these compounds via consumption of drinking water is low, contributing to respective tolerable intakes with compared to reference doses. Equally, there is no special hazard arising from the presence of alkyphenols and BPA in the effluent of water treatment plants relative to the reference values. Equally, no risk of water presents in the effluent of a wastewater treatment plant in comparison with the reference values. The linear alkylphenols such as 4-OP and 4-n-NP in real samples did not appear in any of the cases.

4. Conclusions

The aim of our study was testing the conditions of derivatization reaction of selected analytes (BPA, 4-t-OP, iso.-NP, 4-OP and 4-n-NP) with dansyl chloride.

The study of effect of conditions of derivatization reactions of alkylphenols using dansyl chloride reagent was carried out, because limited information on the effect of individual parameters on efficiency of derivatization reaction can be found in published papers. Improved conditions enable to obtain more reproducible results comparing to results obtained by previously recommended method. Especially the use of appropriate value of temperature and reaction time is critical, due to need to reduce errors caused by changes during derivatization procedures of samples. Experimental results have shown, that the developed method enables to improve limits of detection and limits of quantification comparing to recommended method.

The improved method was used for the determination of analytes in real samples. The concentration of BPA and APs in bottled drinking waters and wastewater analyzed in our study was below the maximum safe doses for human and other organism exposure. The relatively high levels of alkylphenols and bisphenol A in blank samples are a serious problem in the analysis. Especially for analyses of trace levels of alkylfenols and BPA occurring in environmental samples, further research focussed on sample treatment and final analytical procedures is necessary. Elimination or at least decrease of sources of contamination of treated sample by APs and BPA in laboratory materials and chemicals will be the aim of following research.

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