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HPLC–ED of low-molecular weight brominated phenols and tetrabromobisphenol A using pretreated carbon fiber microelectrode

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ABSTRACT

Electrochemically pretreated carbon fiber microelectrode was used to develop a simple, fast and sensitive HPLC–ECD method for the determination of brominated phenols. In addition to simple mono-, di- and tri-bromophenols (4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol, 2,4,6-tri-bromophenol) the possibility of electrochemical detection of 3,3′,5,5′-tetrabromobisphenol A in oxidation mode is reported for the first time. The isocratic separation was achieved within 14 min using ternary mobile phase consisting of 50 mM-phosphate buffer (pH 3.5), acetonitrile and methanol (35/15/50, v/v), and detection potential of $E=+1450$ mV (vs. Ag/AgCl). The carbon fiber microelectrode permitted to use high anodic potentials (up to +1800 mV vs. Ag/AgCl), the optimum analytical response was achieved at +1450 mV vs. Ag/AgCl. The limits of detection (LOD) for the studied analytes were within the range of 1.8–56.6 ng mL^{-1}. The developed method was applied to determination of brominated phenols in spiked water samples. Furthermore, after simple extraction with methyl *tert*-butyl ether, it was possible to quantify tetrabromobisphenol A (TBBA) in a piece of CRT monitor plastic casing. The found amount of TBBA was 10.22 mg kg^{-1} ($\pm$0.43).

1. Introduction

Brominated phenols (BPs) are a rather heterogeneous class of organohalogen derivatives including compounds of both anthropogenic and natural origin. BPs of anthropogenic origin represent commercially important compounds used as flame retardants and antifungal agents [1]. As the main function of flame retardants is to impede or suppress the combustion process, brominated flame retardants (BFRs) have routinely been added to consumer products for several decades in a successful effort to improve their resistance to fire, reduce fire-related injury and property damage [1]. With the rapidly growing production of synthetic polymeric materials in recent years, the use of BFRs has also dramatically increased. This trend has led to increased levels of BFRs in the environment. Many of the BFRs are considered toxic, persistent and bioaccumulative [2].

It has been widely reported that residues of BPs can be found in body tissues of piscivorous animals [3,4]. Recently, BPs have been found to act as endocrine disruptors [5], therefore their toxicological impacts are considered as significant; other toxicological characteristics (such as neurotoxicity and reproduction-related effects) are still under investigation.

It has been discovered that some BPs are also produced naturally by marine organisms [6]. Particularly, simple mono and dibromophenols can be found in fishes, algae and other sea products [7]. As the BPs are characterized by a typical distinctive odor, its presence strongly affects the flavour of the sea food [8]. The proper role of the brominated naturally occurring phenolic compounds has not been yet fully understood, it is supposed they may play a protective role [9]. However, it has been reported that BPs both present in marine organisms and in industrial flame retardants, disturb cellular Ca^{2+} signaling in neuroendocrine cells [10].

3,3′,5,5′-Tetrabromobisphenol A (TBBA), a brominated derivative of bisphenol A, is among the most extensively used BFRs today [11]. It is introduced as a reactive or, less frequently, as an additive agent into miscellaneous polymers, such as ABS and epoxy and phenolics resins. It has been reported that TBBA acts as thyroid hormone agonist with neurotoxic and immunotoxic effects [11]. Traces of TBBA have been found in home and office dust [12], and have also been detected in blood plasma of persons working in the IT environment [13]. The TBBA has also been detected in air [14], human serum and body fluids [15].

Analytical characteristics, techniques used for sample treatment (clean-up, extraction), and instrumental techniques used for

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analysis of miscellaneous BFRs as well as brominated natural compounds are reviewed by Covaci et al. [16]. For determination of various BPs in environmental matrices, separation techniques are by far the most frequently used, namely GC–MS [17,18], CZE and ITP-CZE [19,20], HPLC with UV spectrophotometric [12,21,22], and recently predominantly with mass spectrometric [23–25] detection.

The use HPLC with electrochemical detection (HPLC–ED) is limited to compounds exhibiting electroactivity. On the other hand, it has several significant advantages over conventional spectrophotometric detection techniques, including typically higher selectivity and sensitivity. Moreover, it can be easily adapted to miniaturized HPLC systems, whose popularity has been continuously growing in recent years.

It is generally known that phenols are readily electrooxidizable compounds; BPs are thus suitable candidates for electrochemical detection (ED) [26]. Despite these facts, and in contrast to their chlorinated counterparts [27,28], there exist few reports employing ED to detect BPs [29]. Quintana et al. used a thin-layer cell equipped with a glassy carbon electrode for the HPLC–ED determination of 2-bromophenol, 3-bromophenol and 4-bromophenol, as a complementary assay to UV-spectrophotometric detection of set of six brominated organic pollutants in river water samples [30].

While the electroactivity of simple BPs is known, no report exists in the literature concerning the electrochemical behaviour of TBBA. The only electrochemical study dealing with TBBA does not consider TBBA as electroactive compound, but rather uses electrochemical method (ferro/ferricyanide electrochemistry) to monitor attachment of TBBA molecules onto molecularly imprinted polymer [31].

In this paper, the unique sensing properties (low noise, low background currents, resistance to electrode fouling, suitable size enabling to work in microenvironments) of electrochemically pretreated cylindrical carbon fiber microelectrodes (CFMEs) are utilized in HPLC sensing of selected BPs, including TBBA. Recently, we have shown the outstanding performance of electrochemically pretreated CFME used for HPLC–ED analysis of phenolic acids [32]. The performance of electrochemical detector employing electrochemically pretreated cylindrical CFME as a working electrode are in some aspects, which are discussed below, comparable to a latter-day popular boron doped diamond electrode. Electrochemical pretreatment of CFMEs, i.e. subjecting the fibers to rapid excursions of potentials towards positive (anodic) direction is a common way of increasing the responses and modulating the sensing properties of CFMEs. It is mainly used in sensing neurotransmitters in neurophysiology [33] and in construction of nitric oxide sensors [34]. The electrochemical pretreatment removes carbon material from the fiber surface (fiber thinning) and at the same time introduces surface oxygen containing moieties e.g. carboxyl and hydroxyl groups, often denoted as graphitic oxide [35]. Despite the presence of chloride anions in the electrolyte producing chlorine at positive potentials used in the course of pretreatment procedure it is highly probable that the main cause for fiber thinning is the electrolysis of water, producing reactive hydroxyl radicals, attacking the electrode material:

$$H_2O \rightarrow HO^{\bullet} + H^+ + e^- \quad (1)$$

The electrochemical production of hydroxyl radicals according to (Eq. (1)) and their interaction with the electrode have been extensively studied at boron doped diamond electrode (e.g. [36]). The sp^3 regions of diamond crystallites on BDD have been found to be particularly inert towards the $HO^{\cdot}$ attack while the amorphous sp^2 regions have proven readily oxidizable [37]. The electrochemical pretreatment of BDD electrode was studied by Sekioka et al. at anodic potentials similar to those used by us for CFME pretreatment [38]. An increase in BDD surface hydrophilicity caused by the formation of surface oxygen-containing groups was observed. After pretreatment, the enhancement of electrochemical responses of analytes sensitive to the presence of oxygen-containing groups occurred for BDD (e.g. $[Fe(CN)_6]^{2+/3+}$ [38] or dopamine [39]), while the response of analytes with density of electron-state dependent electrochemistry (e.g. $[Ru(NH_3)_6]^{2+/3+}$ [38]) remained nearly unaltered. Similar behaviour has been observed for CFME where the pretreatment protocol for a given CF material can be optimized by altering the electrolyte composition, potential limits, frequency, waveform shape and total pretreatment time, in order to achieve optimum sensitivity for an analyte as shown by us on the example of dopamine [40]. The optimum dopamine sensitivity for polyacrylonitrile-based CF is achieved by applying short pretreatment times, thereby exposing only the thin layer where parallel orientation of graphene sheets prevails. The extended length of the procedure leads to some decrease in the sensitivity for cationic species while the sensitivity towards neutral and anionic analytes is increased due to the formation of dislocations which remain uncovered by the oxidic layer, and which exhibit the character of graphite edge sites [41].

2. Materials and methods

2.1. Reagents

The standards of brominated phenols, 4-bromophenol (4-BP), 2,4-dibromophenol (2,4-DBP), 2,6-dibromophenol (2,6-DBP), 2,4,6-tribromophenol (2,4,6-TBP), 3,3′,5,5′-tetrabromobisphenol A (TBBA), and methyl *tert*-butyl ether (MTBE) were purchased from Fluka (Fluka AG, Buchs, Switzerland). Methanolic stock solutions of standards (1.0 mg mL^{-1}) were stored at −20 °C. Working standard solutions were prepared by diluting the stock solutions with the mobile phase. For the mobile phase preparation, sodium dihydrogen phosphate (TraceSelect purity) obtained from Fluka (Fluka AG, Buchs, Switzerland), and gradient grade acetonitrile and methanol (LabScan, Dublin, Ireland), were used.

2.2. Chromatographic system

The HPLC system consisted of an ESA isocratic pump (Model 582), (ESA Inc., Chelmsford, MA, USA) with a pulse damper, a Rheodyne manual injector (Rheodyne, Cotati, CA, USA) equipped with a 10 μL loop. Electrochemical detection was performed using an ESA coulometric detector Coulochem III (ESA Inc., Chelmsford, MA, USA), an amperometric cell equipped with a carbon fiber working electrode, an L-Chem silver-silver chloride micro electrode as a reference (L-Chem, Czech Republic, www.lchem.cz). An ESA 5020 guard cell was placed prior to the injector, (ESA Inc., Chelmsford, MA, USA).

The Clarity chromatographic station (DataApex, Prague, Czech Republic) was used for chromatogram recording and handling. The samples were introduced into the system using a glass 25 μL syringe (Hamilton, Reno, NV, USA). HPLC separations were performed on a Supelco reversed phase column C18 3 μm, 250 × 2.1 mm I.D. (Supelco, Bellefonte, PA, USA). All fittings and connecting tubings were made of PEEK™. The final mobile phase consisted of 50 mM-NaH_2PO_4 (pH 3.5)/MeOH/ACN (35/15/50, v/v). The mobile phase was vacuum-filtered through a 0.2 μm porous filter (Supelco, Bellefonte, PA, USA) and degassed by helium sparging before use. The flow rate was 0.3 mL min^{-1}. The working potential was maintained at +1450 mV (vs. Ag/AgCl). The set-up detector gain ranged from 1 nA V^{-1} to 10 nA V^{-1}. The guard cell potential was set to +800 mV (vs. Pd/H_2).

2.3. Electrochemical measurements

Cyclic voltammetry, amperometric measurements and electrochemical pretreatment of carbon fiber microelectrodes were performed using Nanoampere electrochemical workstation (L-Chem, Czech Republic). The electrochemical experiments were performed in three-electrode setup, employing RE-5b reference electrode (BASi, U.S.A), platinum wire as auxiliary electrode and studied carbon fiber microelectrodes as working electrodes. In the course of amperometric measurements, the aliquots of samples were introduced into the stirred solution of mobile phase using an auto sampler (Titronic Basic, Schott, Germany interfaced to PC using a custom made software).

2.4. Sample preparation

To obtain a suitable sample of plastics for the subsequent BP extraction, outer plastic parts of a 1995 made CRT monitor casing were finely filed and 100 mg of the resulting powder were suspended with 2 mL of *tert*-butyl ether (MTBE). An aliquot volume (1 mL) was evaporated and dried under a stream of nitrogen. The post-evaporation residue was reconstituted with 200 μL of mobile phase and directly analyzed by HPLC. The identification and quantification were performed using the standard addition method.

Sample of local tap water was withdrawn after 10 min of water flow and filtered through a 0.2 μm porous filter. The resulting sample was then divided and subjected to analysis (blank), or spiked with BP standards from their stock solutions at concentration level of 25 ng mL^{-1} (25 ppb), respectively.

2.5. Carbon fiber based HPLC–EC detector

2.5.1. Preparation of CFMEs

A polyacrylonitrile-based carbon fiber (7–8 μm Courtaulds XA-S, type α, Courtaulds Ltd., UK) was obtained from a local distributor (Havel Composites Inc., Czech Republic). The procedure for microelectrode preparation was as follows: carbon fiber was glued using conductive silver epoxy (Epotek H20E, Polytec, Germany) onto a copper wire, the junction was then hardened at 170 °C for 10 min. The fiber with copper contact attached was fitted into a glass capillary and about 5 mm of the fiber was left protruding from its contracted end. Both ends of the capillary were sealed using epoxy resin (CHS Epoxy 1200, Sindat Pilsen, Czech Republic). The fiber end of the electrode was briefly sonicated in dichloromethane in order to clean the fiber and to remove grease. Prior to use, the microelectrode was electrochemically activated using the following pretreatment procedure: microelectrode was placed into electrochemical cell containing 1% (w/w) NaCl and cycled between 0 and 2.9 V vs. Ag/AgCl for 20 s, 50 Hz sinusoidal wave, followed by 5 s at constant potential of –0.8 V and 5 s at 1.5 V.

2.5.2. Assembly of HPLC–EC detector

An HPLC detector was constructed from a piece of Teflon plastics, allowing to accommodate inlet and outlet PEEK tubing and electrode system (Fig. 1). The pretreated carbon fiber microelectrode was inserted into the end of a PEEK (70 μm ID) capillary so that 3–4 mm of the fiber protruded inside the capillary. A miniature Ag/AgCl reference electrode (filled with 3 M-KCl) and stainless steel auxiliary electrodes were located at the bottom of the detector.

3. Results and discussion

3.1. Electrochemical pretreatment of the working microelectrode and electrochemical behaviour of brominated phenols

In this work, the analytical performance of pretreated CFME-based detector in chromatographic setup was studied for following brominated phenols: 4-bromophenol (4-BP), 2,4-dibromophenol (2,4-DBP), 2,6-dibromophenol (2,6-DBP), 2,4,6-tri-bromophenol (2,4,6-TBP) and 3,3′,5,5′-tetrabromobisphenol A (TBBA). First, the electrochemical behaviours of the tested brominated phenols were evaluated. Cyclic voltammograms (Fig. 2), recorded with pretreated CF-microelectrode as the working electrode were performed in the mobile phase the composition of which ensures their effective chromatographic separation. Under identical conditions, the electrodes without electrochemical pretreatment do not give reproducible voltammograms (not shown). Irreversible peak-shaped voltammograms with peak potentials between 900 and 1200 mV vs. Ag/AgCl are found for all studied species with the exception of 2,4,6-tribromophenol. The peak shape and irreversible character of voltammograms is caused by the formation of insulating polymer films during electrooxidation, as indicated by almost complete disappearance of voltammetric peak at second CV cycle (as demonstrated for 2,6-dibromophenol, Fig. 2B). TBBA provides peak shaped irreversible voltammograms, but with ca. five times lower first peak current compared to other bromophenols. In the case of 2,4,6-tribromophenol (Fig. 2C) a wave-shaped voltammogram is found and much less pronounced difference in currents at first and second CV cycle is observed, indicating that polymeric product is not formed and deposited on the electrode by the electrooxidation of this species.

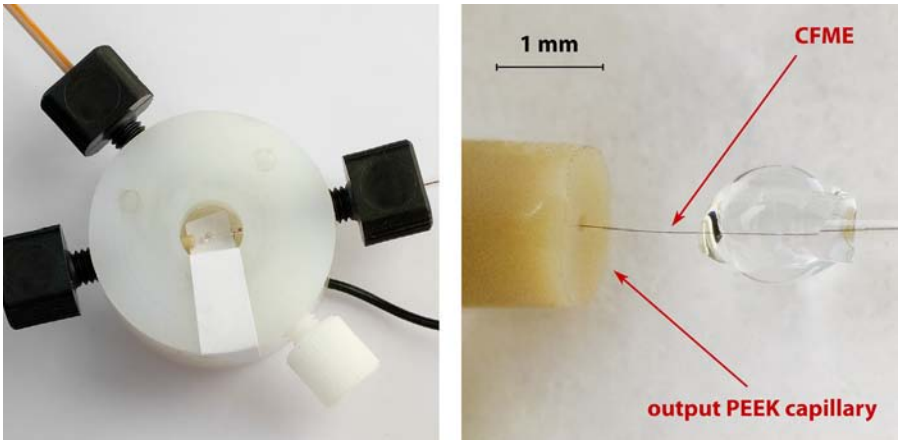

Fig. 1. Assembly of CFME based HPLC detector, left: open detector with PEEK inlet—CFME connection. A piece of white paper was inserted below the CFME to make the connection visible; right: close view.

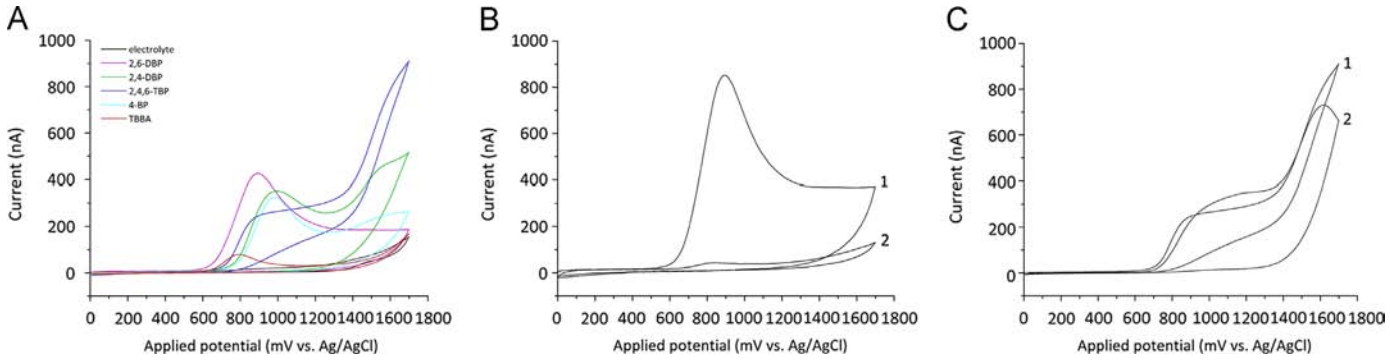

Fig. 2. Cyclic voltammograms of 2,6-DBP, 2,4-DBP, 2,4,6-TBP, 4-BP, TBBA (A); comparison of first and second cycle CVs of 2,6-DBP (B) and 2,4,6-TBP (C). *Conditions:* 1 mM solutions of BPs in the electrolyte containing 50 mM-NaH_2PO_4 (pH 3.5)/MeOH/ACN (35/15/50, v/v), CF as working electrode, scan rate: 10 mV/s.

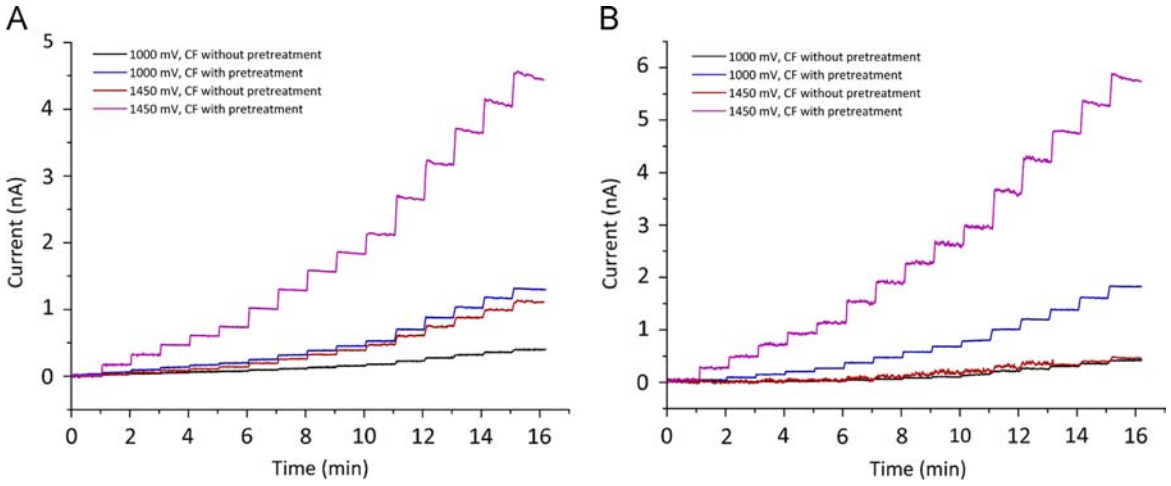

Fig. 3. Amperograms of 2,4-DBP (A) and 2,4,6-TBP (B) measured in stirred solution (300 rpm.) containing 50 mM-NaH_2PO_4 (pH 3.5)/MeOH/ACN (35/15/50, v/v), native and pretreated CF as working electrodes, applied potentials 1000 and 1450 mV. Five additions each corresponding to increase in the concentration of the studied analyte in 5×10^{-7} M steps, followed by 5×1.10^{-6} M and 5×2.10^{-6} M.

It is worth mentioning that pretreated carbon fiber microelectrodes provide steady state voltammograms of potassium ferricyanide electrochemical standard, indicating that radial diffusion is the prevailing mode of transport during electrolysis (not shown).

Cyclic voltammetry experiments are carried out at high (1 mM) concentrations of tested analytes inducing severe electrode fouling. It is, however, expectable that the graphite oxide layer makes the electrode surface particularly resistant towards fouling by products of electrooxidation reactions. This was checked by amperometric experiments, carried out for 2,4-dibromophenol and 2,4,6-tribromophenol employing micromolar concentrations of these species. The amperograms for two working potentials of 1000 and 1450 mV vs. Ag/AgCl were recorded using microelectrodes both with and without electrochemical pretreatment (Fig. 3). It follows from the amperograms that despite the observed formation of insulating polymers upon electrooxidation, brominated phenols provide good quality amperograms when CFMEs are used working electrodes. The responses are higher at 1450 mV compared to 1000 mV, however, in order to precisely determine optimum working potential for the selected compounds under chromatographic conditions, hydrodynamic voltammograms (HDVs) were measured.

3.2. Hydrodynamic voltammetry

As shown in Fig. 4, simple BPs gave high analytical currents past +1400 mV range (vs. Ag/AgCl) while TBBA provided a relatively flat curve profile across the potential interval measured. It can be speculated that the symmetric substitution at bisphenol rings by bromine atoms hinders the electrooxidation process.

TBBA can be, therefore, regarded as an irreversibly oxidizable BP. Nevertheless, as shown further, despite the lower oxidation efficacy and thus lower analytical currents, TBBA can be electrochemically detected using the CFME detector. For the simultaneous detection of BPs, working potential of +1450 mV (vs. Ag/AgCl) provided the best general analytical response for all the analytes of interest.

3.3. Mobile phase composition. HPLC separation of standards

Mobile phase composition and effects of acetonitrile and methanol as organic modifiers were tested with the aim of achieving a satisfactory separation of the analytes under isocratic conditions. A mobile phase containing phosphate buffer of pH 3.5 offered reasonable retention and selectivity for the selected BPs. It is not surprising that acetonitrile as an organic modifier provided a markedly better peak shape and resolution than methanol based environment. Acetonitrile-water based mobile phases exhibit generally lower viscosity than those with methanol, resulting in higher separation efficiency and thus narrower peaks. It has been observed, however, that chromatographic selectivity was favourably affected by addition of methanol (up to 15%, v/v) into the mobile phase (Fig. 5). For that reason, a ternary mixture of phosphate buffer, acetonitrile and methanol was used for the separation of BP standards (Fig. 5). The

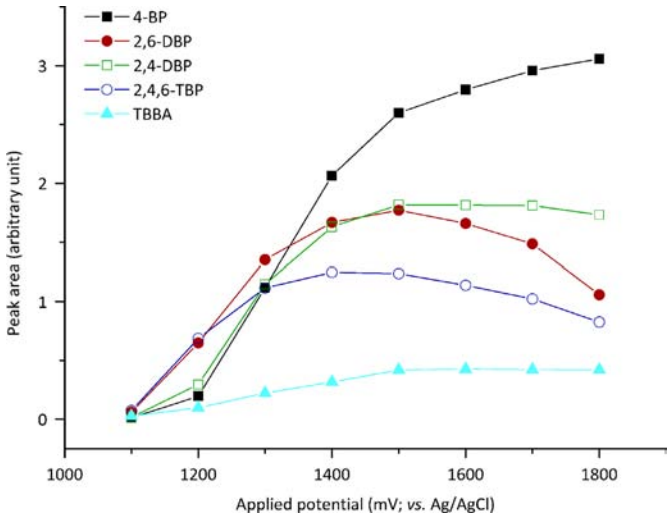

Fig. 4. Hydrodynamic voltammogram of BPs selected.

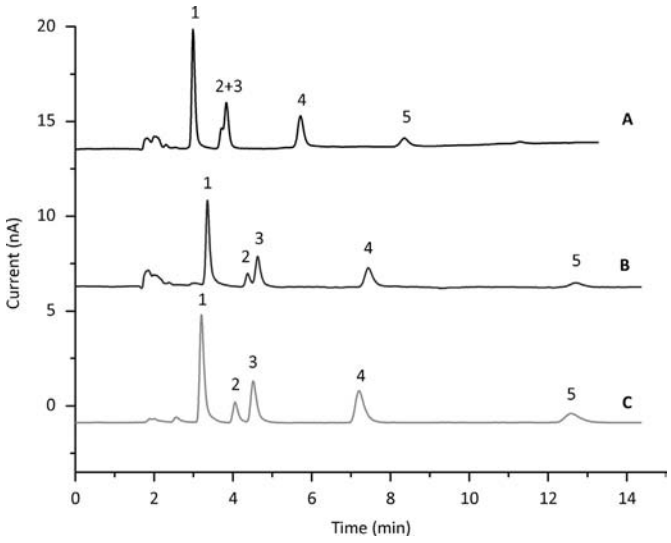

Fig. 5. Effect of methanol addition on HPLC separation of selected BPs: (1) 4-BP; (2) 2,6-DBP; (3) 2,4-DBP; (4) 2,4,6-TBP; (5) TBBA. *Conditions:* Supelcosil C18, 250 × 2.1 mm, 5 μm; flow-rate 0.3 mL min^{-1}; Detection potential $E = +1450$ mV (vs. Ag/AgCl). Analyte concentration: 1 mg L^{-1} (1–3), 2 mg L^{-1} (4, 5), injection 10 μL. Mobile phase composition: (A) 50 mM-NaH_2PO_4/ACN (35/65, v/v), pH 3.5, (B) 50 mM-NaH_2PO_4/ACN (40/60, v/v), pH 3.5, (C) 50 mM-NaH_2PO_4/MeOH/ACN (35/15/50, v/v), pH 3.5.

chromatographic resolution (R_S) between isomeric 2,6-DBP and 2,4-DBP improved significantly from 0.67 (Fig. 5, record A) to 1.79 (Fig. 5, record C) if appropriate portion of ACN was replaced by methanol. Decreasing the ACN content (Fig. 5, record B) was found to slightly improve the resolution ($R_S = 1.12$) as a result of increased capacity, however, at the cost of prolonged analysis time. Chromatographic analysis of BP model mixture under optimum conditions is shown in Fig. 6.

3.4. Calibration, LOD, LOQ, linearity and repeatability

Determination of the analytes was based on peak area. Calibration was carried out at six concentration points ranging from 25 to 500 ng mL^{-1} for 4-BP, 2,4-DBP, 2,6-DBP, 2,4,6-TBP, or 200–1000 ng mL^{-1} for TBBA, respectively. Calibration curves expressed as peak area vs. concentration of corresponding analyte were plotted and basic parameters for the partial validation of the method were calculated (Table 1). Linear regression equations

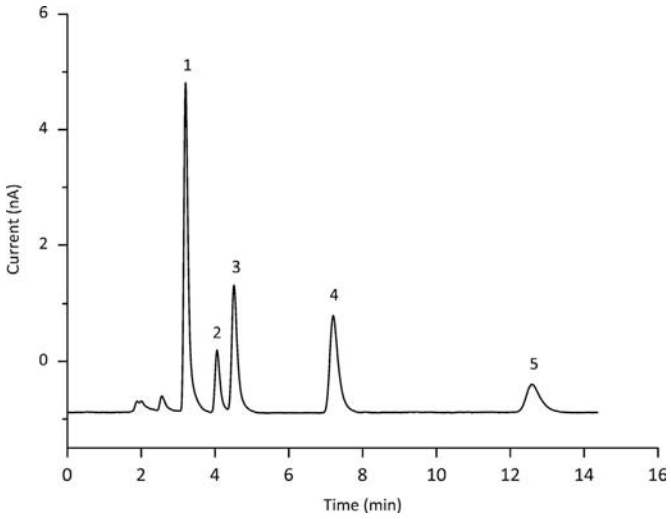

Fig. 6. A chromatogram of BP standard mixture at final experimental conditions. Analyte concentration: 1.0 mg L^{-1} (1, 2 and 3); 2.0 mg L^{-1} (4 and 5). Legend: (1) 4-BP; (2) 2,6-DBP; (3) 2,4-DBP; (4) 2,4,6-TBP; 5) TBBA *Conditions:* Supelcosil C18, 250 × 2.1 mm, 50 mM-NaH_2PO_4/MeOH/ACN (35/15/50, v/v), pH 3.5; flow-rate 0.3 mL min^{-1}, $E = +1450$ mV (vs. Ag/AgCl).

relating the concentration to peak area listed in Table 1, together with their correlation coefficients for the determination of BPs, indicate that the linearity of the method is acceptable.

LOD and LOQ for the studied compounds measured under final experimental conditions were obtained from equations LOD = $3.3\sigma/b$ and LOQ = $10\sigma/b$, where σ was the standard deviation of the mean value for 6 signals using blank and b represented the slope of the corresponding calibration curve.

To check the linearity of the detector response, a linear regression analysis of peak areas versus concentration of the BPs was performed. The linearity was determined by the square correlation coefficients of the calibration curves using triplicate injections of standards at six concentration levels.

The analytical curves were linear for the bromophenols studied over the concentration ranges measured, as shown in Table 1. The square correlation coefficients R^2 of the calibration curves were higher than 0.99, confirming the linearity of the method developed. The calibration curves of the studied BPs were found to be linear within the range measured. The results also indicate good linearity of this method for the intra- and inter-day assays. The achieved LODs and LOQs also confirm that the proposed method is suitable for the determination of bromophenols in plastics and other real samples.

The corresponding peak areas as well as the retention times obtained from HPLC analyses of standard mixtures were used to estimate the precision. The intra-day repeatability was calculated using the RSD of six injections performed on the same day, while the inter-day repeatability was based on RSD of analyses carried out on five consecutive days. Retention time repeatability expressed as RSD ranged between 0.5–0.7% for the intra-day, and 1.7–2.7% for the inter-day, respectively. Peak area repeatability ranged between 1.6–2.5% for the intra-day, and 5.9–6.3% for the inter-day, respectively. The linearity and repeatability parameters for the compounds studied are summarized in Table 1.

3.5. Application of the method

Brominated phenols are known to be environmental pollutants detrimental to aquatic life, and numerous papers dealing with their analysis in water matrices were published [17,19,20,30].

To test the applicability of the method, determination of BPs in spiked tap water was carried out. For this purpose, tap water sample (prepared as described in experimental) was spiked with

Table 1
Calibration parameters, limits of detection (LOD) and quantification (LOQ) and repeatability of BPs.

Analyte	Intra-day[a]		Inter-day[b]		LOD	LOQ	Slope	Intercept	R^2
	Area RSD (%)	t_R RSD (%)	Area RSD (%)	t_R RSD (%)	(ng mL^{-1})	(ng mL^{-1})			
4-BP	2.0	0.6	5.9	1.7	1.8	5.4	31.627	355.3	0.9992
2,4-DBP	1.9	0.7	6.1	1.8	4.3	13.2	18.004	74.4	0.9993
2,6-DBP	1.6	0.6	6.0	2.1	5.8	17.6	12.054	−18.1	0.9986
2,4,6-TBP	2.0	0.6	6.2	2.5	7.7	23.4	10.066	−126.2	0.9943
TBBA	2.5	0.5	6.3	2.7	56.6	171.4	4.197	21.4	0.9982

Calibration range: 25–500 ng mL^{-1} (4-BP, 2,4-DBP, 2,6-DBP, 2,4,6-TBP); 200–1000 ng mL^{-1} (TBBA).
Sensitivity gain: 1 nA V^{-1}.
[a] (n=6, c=1 μg mL^{-1}; for 2,4,6 TBP and TBBA c=2 μg mL^{-1}).
[b] (n=5, c=1 μg mL^{-1}; for 2,4,6 TBP and TBBA c=2 μg mL^{-1}).

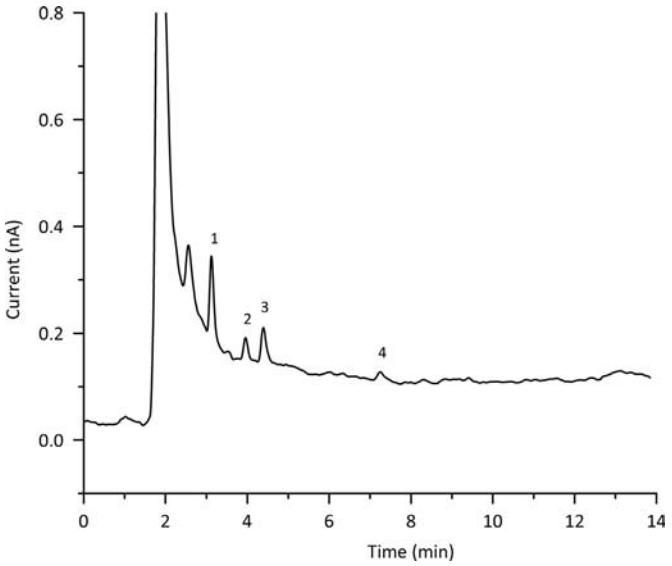

Fig. 7. A chromatogram of water spiked with BPs studied. The final concentration of all analytes is 25 mg L^{-1}. Legend: (1) 4-BP; (2) 2,6-DBP; (3) 2,4-DBP; (4) 2,4,6-TBP *Conditions*: See experimental.

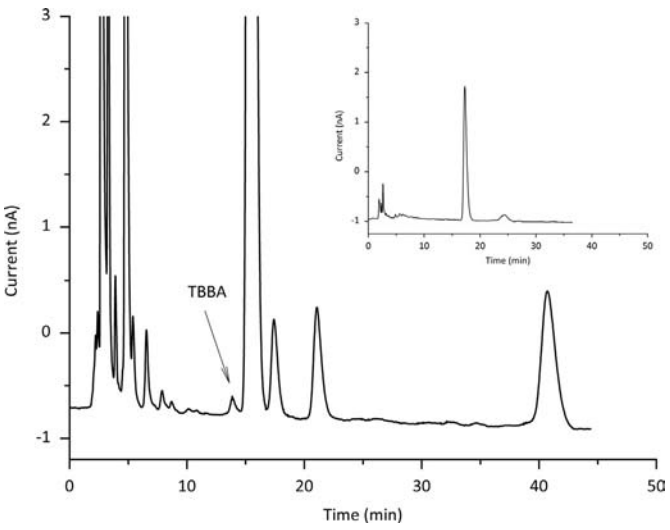

Fig. 8. A chromatogram of a PC monitor plastic. TBBA (3,3′,5,5′-tetrabromobisphenol A) marked by an arrow (5 times diluted sample shown). Inset: chromatogram of blank extract. *Conditions*: See experimental.

selected BP standards from their stock solutions at concentration of 25 ng mL^{-1}, and the samples were analyzed using the described method. Recoveries found for the BPs tested were as follows: 4-BP (97.1%), 2,4-DBP (107.3%), 2,6-DBP (111.7%), 2,4,6-TBP (113.5%). The method proved suitable for the determination of BPs in the range of tens of ppb for the BPs studied except for TBBA, where the expected limits are rather higher (Fig. 7).

The method was also applied to determination of BPs in plastics. The standard addition method is a common way of eliminating the undesired matrix effects. One of the major drawbacks of the standard addition method is that each sample has to be calibrated separately and thus the method may be inconvenient and more time-consuming particularly for large series of samples. Standard addition method is, however, extremely useful in cases when no blank sample is available.

Among several different kinds of plastics tested and processed as described in Section 2.4, significant amounts of TBBA were found in an external plastic material of a computer monitor, (Fig. 8). As was mentioned in the Introduction, the plastics of the IT environment have typically been doped by BFRs to eliminate the combustion. The peak identity was confirmed by standard addition, as well as by comparing the electrochemical response of analyte vs. standard. Based on the calibration curve, the calculated amount of TBBA found in plastics was 10.22 mg kg^{-1} (±0.43).

4. Conclusions

We have developed a straightforward HPLC–ED method for the determination of the most common BPs (4-bromophenol, 2,4-dibromophenol, 2,6-dibromophenol, 2,4,6-tri-bromophenol and 3,3′,5,5′-tetrabromobisphenol A) based on their amperometric detection at +1450 mV (vs. Ag/AgCl). The isocratic HPLC analysis separates all brominated phenols within 14 min. The used amperometric HPLC detector based on CFME is characterized by low noise, low background currents, resistance to electrode fouling, broad potential window, fast reacquisition to the working potential, and minimum costs. Using the described arrangement, the method developed has been applied to analysis of bromophenols in spiked tap water and plastics.

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