Journal of Chromatography A, 1513 (2017) 42–50

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A hybrid material as a sorbent phase for the disposable pipette extraction technique enhances efficiency in the determination of phenolic endocrine-disrupting compounds

Gabriela Corazza, Josias Merib, Hérica A. Magosso, Otávio R. Bittencourt, Eduardo Carasek*

Departamento de Química, Universidade Federal de Santa Catarina, Florianópolis, 88040-900, SC, Brazil

ARTICLE INFO

Article history:
Received 5 April 2017
Received in revised form 12 July 2017
Accepted 13 July 2017
Available online 14 July 2017

Keywords:
DPX
Sample preparation
Sorbent phase
Phenolic compounds

ABSTRACT

In this study, the hybrid material 3-*n*-propyl(3-methylpyridinium) silsesquioxane chloride ($Si3Py^+Cl^-$) was synthesized and investigated as a novel sorbent phase for the disposable pipette extraction (DPX) technique coupled to high-performance liquid chromatography-florescence detection. This sorbent phase was characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). Aqueous samples containing the phenolic endocrine-disrupting compounds bisphenol A (BPA), 17α-ethynylestradiol (EE2), 4-*tert*-octylphenol (4-t-OP), 4-octylphenol (4-OP) and 4-nonylphenol (4-NP) were subjected to DPX procedures and a series of optimizations was performed to determine the ideal extraction conditions using this approach. The proposed sorbent phase exhibited higher extraction efficiency than DPX-RP (reversed phase tips containing styrene-divinylbenzene), commonly used for the determination of the phenolic endocrine- disrupting-compounds under study. Satisfactory analytical performance was achieved with linear ranges from 2 to 100 $\mu g\,L^{-1}$ for 4-t-OP and 1–100 $\mu g\,L^{-1}$ for the other analytes. Limits of detection of 0.60 $\mu g\,L^{-1}$ for 4-t-OP and 0.30 $\mu g\,L^{-1}$ for other analytes, RSDs ranging from 1 to 20% and relative recoveries of 83–116% were obtained. Based on these satisfactory results, this sorbent phase represents a valuable alternative for the extraction of compounds with polar moieties in their structure.

1. Introduction

Phenolic compounds are widely used in a number of different applications and exhibit a broad range of applicability in the chemical industry. Subsequently, due to the fact that they are often not totally degraded in effluent treatment plants [1], these compounds have been found in different environmental matrices [2–5]. The main concern in this regard is associated with the fact that they are endocrine-disrupting, which can interfere in the human endocrine system causing dysfunction of reproduction systems, a decrease in immune function and enhanced incidence of cancer [6–9]. Therefore, the presence of these pollutants in different environmental matrices needs to be monitored.

The determination of phenolic compounds is usually carried out by chromatographic techniques, including gas chromatography (GC) and liquid chromatography (LC), coupled to different detectors. However, due to the complexity of some environmental matrices and the analytes often being present in low concentrations, a suitable sample preparation technique needs to be applied prior to the chromatographic analysis. An appropriate sample preparation technique allows the preconcentration of the analytes, a decrease in the matrix effect through the removal/separation of the analytes from interferents in the matrix, and the transfer of the analytes to a medium compatible with the analytical instrumentation [10]. Several extraction and microextraction techniques have been developed and applied as sample preparation tools for the determination of a number of compounds from different chemical classes.

Solid-phase extraction (SPE) has been widely employed for the extraction of phenolic compounds from water. However, this technique is associated with long extraction times and the use of relatively large volumes of solvents [11–14]. Other reported techniques are solid-phase microextraction (SPME) [15,16], hollow-fiber liquid phase microextraction (HF-LPME) [17], magnetic solid-phase extraction (MSPE) [18], dispersive

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* Corresponding author.
E-mail addresses: ecarasek@hotmail.com, eduardo.carasek@ufsc.br (E. Carasek).

http://dx.doi.org/10.1016/j.chroma.2017.07.050
0021-9673/

liquid–liquid microextraction (DLLME) [19,20] and single-drop microextraction (SDME) [20].

One well-known sample preparation technique is disposable pipette extraction (DPX), which is an extraction technique based on SPE [21]. The standard procedure consists of the following steps: (*i*) conditioning to activate the sorbent sites; (*ii*) sample aspiration followed by air aspiration to allow the dynamic mixing between sorbent and sample; (*iii*) cleaning using solvents to remove matrix interferences; and (*iv*) desorption of the analytes using a solvent or a mixture of solvents [22]. The dynamic mixing of the solid sorbent with the sample using air aspiration provides fast equilibrium between the analyte and sorbent and thus the extraction time is reduced. Another advantage is the lower consumption of solvent and sorbent phase compared to SPE.

The choice of the sorbent to be used for the DPX procedure is dependent on the analytes and constituents of the sample matrix. A variety of DPX devices with different extraction phases is commercially available: DPX-RP – reversed phase tips that contain styrene-divinylbenzene as a sorbent, ideal for non-polar and slightly polar compounds. DPX-WAX – weak anion exchange tips that contain a sorbent with poly-amino groups for adsorption interactions with acidic compounds as well as a strong reversed phase for many drugs and metabolites. DPX-CX – cation exchange tips that contain a sorbent with sulfonic acid groups for strong adsorption interactions with basic compounds. DPX-WCX – weak anion exchange tips that contain a sorbent with poly-carboxylate groups for the adsorption of basic compounds. DPX-SI – tips that contain silica gel for the rapid cleanup of environmental samples. DPX-C18 – tips that contain C18 sorbent for rapid sample cleanup to remove matrix interferences [22–24]. The main applications of these commercially available DPX devices are in the extraction of non-polar compounds and acid and basic drugs and also for cleanup procedures. However, based on the available literature, there appears to be a need for further studies involving the determination of phenolic compounds in water samples using commercial-DPX. This sample preparation technique enables the use of alternative materials as sorbent phases. In this study, the polymer 3-*n*-propyl(3-methylpyridinium) silsesquioxane chloride ($Si3Py^+Cl^-$) was used for the first time as the extraction phase for DPX. This material possesses functional groups that can interact selectively with the more polar moieties of the analytes. Thus, the matrix interference can be reduced and enhanced sensitivity can be achieved.

The aim of this study was to propose a novel sorbent phase for the analysis of BPA, EE2, 4-t-OP, 4-OP and 4-NP using DPX with $Si3Py^+Cl^-$. The extraction conditions were optimized using both univariate and multivariate strategies and the analytical parameters of merit were determined in environmental water samples with separation/detection by high-performance liquid chromatography coupled to fluorescence detection. In addition, the chemical interactions between the new sorbent employed in this study and the analytes were evaluated and discussed.

2. Experimental

2.1. Reagents and materials

The phenolic compounds BPA (99%), EE2 (98%), 4-t-OP (97%), 4-OP (99%) and 4-NP (99.8%) were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Individual stock solutions at a concentration of 1000 mg L^{-1} were prepared in methanol (MeOH). Working solutions containing a mix of the analytes at a concentration of 100 mg L^{-1} in MeOH were prepared by appropriate dilution of the stock solution. Plastic pipette tips (1 mL, without sorbent material) were acquired from DPX Labs (Columbia, SC, USA). Acetonitrile (ACN) and MeOH were supplied by JT Baker (Mallinckrodt, NJ, USA). Water was purified in an ultrapure water system (Mega Purity, Billerica, USA). Tetraethylorthosilicate (TEOS), ethanol, chloropropyltrimethoxysilane (CPTS), toluene and 3-methylpyridine were also obtained from Sigma-Aldrich (Milwaukee, WI, USA). Phosphate dibasic, citric acid, hydrochloric acid and sodium chloride were obtained from Vetec (Rio de Janeiro, Brazil). EPA 525 PAH mix B was obtained from Sigma-Aldrich (Milwaukee, WI, USA) with 500 μg mL^{-1} of each compound in acetone.

2.2. Instrumentation and chromatographic conditions

Chromatographic analysis was performed on a Shimadzu Prominence LC 20AT series HPLC system (Shimadzu, Kyoto, Japan) equipped with a fluorescence detector (RF 20A series) with a 20 μL loop and Rheodyne 7725i manual injector (Rohnert Park, CA, USA). Chromatographic separations were performed in reversed phase mode using a C18 column (ZORBAX Eclipse XDB®, 250 mm × 4.6 mm i.d., 5 mm film thickness, Agilent, CA, USA). The injection volume was 20 μL at a flow rate of 1 mL min^{-1} of mobile phase in gradient mode. The mobile phase consisted of a mixture of ACN (A) and water (B) in the following gradient compositions: 0–5.5 min A 60% and B 40%; 5.5–7.5 min A 80% and B 20% and isocratic mode held up to 25 min; 25–26 min return to the initial conditions which were held up to 30 min. The fluorescence detector was set to analyze the excitation wavelength of 277 nm and the emission wavelength of 307 nm. The chromatographic data were evaluated with LCsolution software (Shimadzu, Kyoto, Japan).

2.3. Synthesis of the sorbent phase

The polymer $Si3Py^+Cl^-$ was prepared by the sol-gel processing method. Tetraethylorthosilicate (TEOS), ethanol and an aqueous HCl solution were mixed in a round-bottomed flask, and the resulting solution was stirred for 2.5 h at room temperature (298 K). A solution of 3-*n*-chloropropyltrimethoxysilane (CPTS) was then added and the solution was stirred for 2 h at room temperature. The temperature of the solution was increased to 328 K and the mixture was allowed to stand for 60 h open to the ambient atmosphere until the gelation process occurred. The resulting gel was powdered, washed with ethanol and then dried under vacuum (133×10^{-3} Pa) at room temperature. The dry gel was immersed in a round-bottomed flask containing a solution prepared by mixing pure 3-methylpyridine and dry toluene. Each mixture was heated at the reflux temperature of the solvent for approximately 3 h. The solids were filtered, washed with ethanol and dried for 2 h under vacuum (133×10^{-3} Pa) at room temperature [25]. The chemical structure of this synthesized compound is represented in Fig. 1.

2.4. Characterization of the sorbent phase

Structural information was obtained by Fourier transform infrared spectroscopy (FTIR), using a Varian 3100 spectrometer (Santa Clara, CA, USA). FTIR spectra were generated from KBr pellets. The morphology of the polymer $Si3Py^+Cl^-$ was evaluated by scanning electron microscopy (SEM), using a Hitachi TM 3030 microscope (Tokyo, Japan).

2.4.1. Optimization of DPX procedure

A series of experiments were carried out to optimize the DPX procedure. Extractions were performed using 700 μL of ultrapure water spiked with the analytes at a concentration of 30 μg L^{-1} for each compound. The desorption step was performed using 200 μL of organic solvent. The sample volume was kept constant at 700 μL to allow a satisfactory dynamic mixture between the sorbent phase and the aqueous sample inside the pipette (1 mL

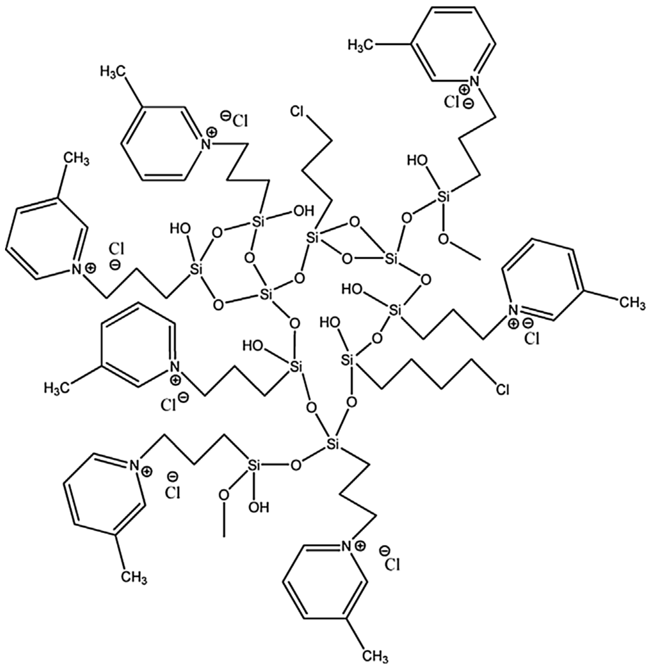

Fig. 1. Chemical structure of the polymer $Si3Py^+Cl^-$ obtained by sol-gel method [25].

capacity). For the desorption step, the volume of organic solvent was kept constant (200 μL). In relation to the time of each extraction/desorption cycle, this was fixed at 30 s. These parameters were chosen and fixed based on previously reported studies in which DPX was used as the sample preparation technique [26–30]. Important parameters related to the extraction and desorption steps were optimized, including the mass of sorbent phase, type of desorption solvent, number of cycles of the extraction and desorption steps (withdraw/release), sample pH, ionic strength, cleaning step and stability of the proposed sorbent phase toward the organic solvents. Multivariate and univariate strategies were employed and the experimental data obtained from the multivariate optimizations were processed using Statsoft Statistica 8.0 software (Statsoft, USA).

2.4.2. Optimization of the mass of sorbent phase

Initially, the mass of sorbent phase that provided the best extraction efficiency was evaluated. In this optimization, 30, 50 and 70 mg of $Si3Py^+Cl^-$ were placed into the DPX pipette tip and extractions were performed in triplicate applying the univariate approach.

2.4.3. Optimization of desorption solvent

The desorption solvent was optimized using MeOH, ACN, water, and mixtures of these solvents according to a simplex-lattice design containing 10 experiments (including a triplicate at the central point). In this procedure, 200 μL of solvent and 5 cycles of withdraw/release with the same aliquot were employed.

2.4.4. Optimization of the number of extraction/desorption cycles

Prior to the extractions, the sorbent phase was conditioned applying 3 cycles with MeOH:ACN (50:50, v/v) and 2 cycles with ultrapure water. For each cycle, a new aliquot of solvents was drawn into the pipette. The optimization of the number of extraction cycles (from 1 to 7) and desorption cycles (from 1 to 7) was carried out using a Doehlert design with 9 experiments (including a triplicate at the central point). In this study, a new aliquot of ultrapure water containing the analytes was withdrawn for each extraction cycle. The number of desorption cycles was optimized using the same aliquot (200 μL) of organic solvent.

2.4.5. Optimization of pH and ionic strength

The influence of the sample pH (4, 5 and 6) and ionic strength (0, 15 and 30% w/v of NaCl) on the extraction efficiency was evaluated. These variables were studied using a univariate procedure in triplicate. Phosphate dibasic, citric acid and aqueous solutions of hydrochloric acid (1 mol L^{-1}) were used for the sample pH adjustments. Sodium chloride was employed for the ionic strength evaluation.

2.4.6. Optimization of the cleaning step

Following the above-described optimizations, the cleaning procedure was optimized to avoid a carryover effect by varying the number of cycles and the volume of solvents as follows: (a) 1 cycle of 200 μL of MeOH:ACN (50:50 v/v) + 2 cycles of 200 μL of H_2O; (b) 2 cycles of 200 μL of MeOH:ACN (50:50 v/v) + 2 cycles of 200 μL H_2O; (c) 3 cycles of 200 μL MeOH:ACN (50:50 v/v) + 2 cycles of 200 μL H_2O; and (d) 1 cycle of 400 μL MeOH:ACN (50:50 v/v) + 1 cycle of 400 μL H_2O. In this study, a univariate procedure was performed in duplicate.

2.5. Evaluation of the sorbent stability and extraction efficiency of DPX-$Si3Py^+Cl^-$ for the extraction of phenolic compounds

The stability of $Si3Py^+Cl^-$ was evaluated by performing 15 complete cycles (extraction/desorption/cleaning) using the same aliquot of sorbent phase under the optimized conditions previously determined. The material was considered stable since no loss of extraction efficiency was observed.

The extraction efficiency obtained for the proposed sorbent phase was compared to that of the commercial styrene-divinylbenzene phase (DPX-RP). The choice of DPX-RP was based on the fact that styrene-divinylbenzene contains aromatic rings in its structure, which can interact with analytes such as phenolic compounds through π–π interactions. In this step, extractions using ultrapure water spiked with the analytes (BPA, EE2, 4-t-OP, 4-OP and 4-NP) at 50 μg L^{-1} were carried out.

In addition, a study to assess the behavior of the proposed sorbent phase in the presence of possible interfering compounds was performed. Polycyclic aromatic hydrocarbons (PAHs) were used for this evaluation, and the chromatographic responses corresponding to the analytes were monitored with the addition of 50 μg L^{-1} of PAHs and without the addition of these compounds.

2.5.1. Analytical figures of merit and analysis of real samples

The analytical figures of merit were determined based on the guidance document on analytical quality control and method validation procedures provided by the European Commission [31]. Calibration curves were obtained using river water samples from the Rio Itajaí (Itajaí, Santa Catarina, Brazil) spiked with the analytes at concentrations from 1 to 100 μg L^{-1}. The extractions were performed in triplicate using DPX-$Si3Py^+Cl^-$. Correlation coefficients (r) were calculated based on the calibration curves. The linearity of the calibration curves was verified using analysis of variance (ANOVA). The limit of quantification (LOQ) was adopted as the first value of the linear range and the limit of detection (LOD) was obtained by dividing the LOQ by 3.3, based on previous studies [32,33]. Accuracy and precision (repeatability) were assessed by performing extractions in river water samples spiked with the analytes at three different concentrations (2; 20 and 100 μg L^{-1}), in triplicate. Accuracy was evaluated as the percentage of relative recovery considering an acceptance criterion of 70–120% [31]. In addition, the *t*-test was applied for each spiked concentration to evaluate if the recovery results were statistically different from

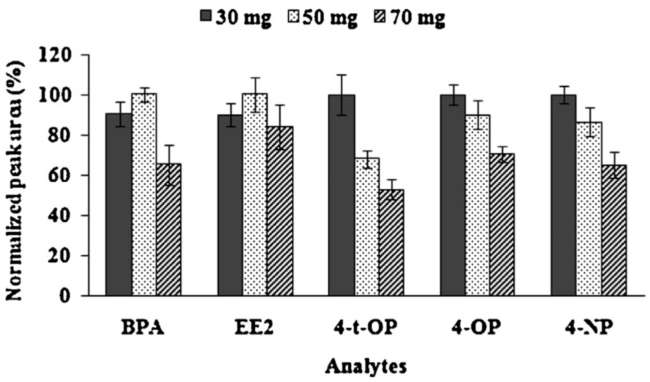

Fig. 2. Optimization of the mass of Si3Py$^+$Cl$^-$ for the extraction of BPA, EE2, 4-t-OP, 4-OP and 4-NP.

100%. Repeatability was calculated based on the relative standard deviation (RSD) considering a precision lower than 20% as the acceptance criterion.

The developed method was applied in the analysis of river water samples collected from three rivers in Santa Catarina State, southern Brazil: sample A collected from Rio Itajaí (Itajaí); sample B from Rio São Francisco do Sul (São Francisco do Sul) and sample C from Rio Cubatão (Palhoça). Samples were stored in glass bottles, properly sealed and kept under refrigeration at 4 °C until analysis.

3. Results and discussion

3.1. *Characterization of the Si3Py$^+$Cl$^-$*

The sorbent phase (Si3Py$^+$Cl$^-$) was characterized by FTIR to evaluate the functional groups in the molecule and the results can be found in the Supplementary information (Fig. S-1). A broad peak at 3434 cm^{-1} is assigned to OH stretching (νOH) of the silanol group and to adsorbed water. The peak observed at 1633 cm^{-1} is due to the ring coupled stretching vibration (νCC + νCN). A medium intensity peak at 2959 cm^{-1} is related to stretching of the methyl group (νCH$_3$). The peak between 1000 and 1250 cm^{-1} corresponds to the SiO stretching (νSi–O) of Si–OH and Si–O–Si bonds [25,34].

3.2. *Micrographs at different magnifications obtained by*

SEM are shown in Fig. 2-S of the Supplementary information. According to these images, a particulate material with a large surface area was obtained, with particles of different sizes and shapes. This is of particular interest with regard to the DPX procedure, since the dispersion of the sorbent phase can easily be performed and a large surface area can enhance the mass transfer of the analytes from the sample to the extraction phase.

3.3. *Optimization of DPX procedure*

3.3.1. *Optimization of sorbent phase mass*

The sorbent phase mass is an important parameter in the DPX procedure. By increasing the mass it is possible to increase the amount of extracted analytes [35]. However, the sorbent phase mass must be compatible with the volume of the pipette (in this case 1 mL) to allow dynamic mixing between the sorbent phase and the aqueous sample containing the analytes [22]. The bar graph in Fig. 2 shows the normalized peak areas for each analyte using 30, 50 and 70 mg of sorbent phase.

According to the bar graph, better extraction efficiencies were obtained using 30 and 50 mg of the sorbent phase. This is because, with the use of a larger mass of sorbent, effective mixing between the sorbent phase and the sample containing the analytes was not achieved, possibly due to the formation of small agglomerates of the sorbent phase. The results obtained using 30 and 50 mg were very similar. However, in the case of 4-t-OP the efficiency with the use of 30 mg was around 40% higher than that obtained with 50 mg. Therefore, 30 mg was adopted for subsequent optimizations.

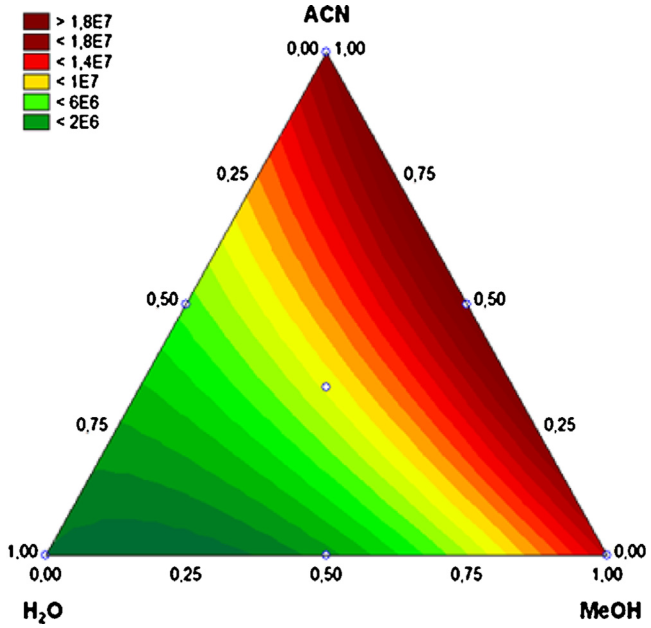

Fig. 3. Triangular response surface obtained for the optimization of the desorption solvent.

3.3.2. *Optimization of desorption solvent*

The solvent used for the liquid desorption needs to be able to interrupt the sorbent-analyte interaction [22]. Therefore, to ensure good desorption efficiency, the best solvent (or mixture of solvents) was determined, aiming to achieve the complete desorption of the analytes from the Si3Py$^+$Cl$^-$. The analytes were eluted applying 5 cycles of withdraw/release and a solvent volume of 200 µL was employed. Experiments were carried out as described in the experimental section, and the triangular response surface shown in Fig. 3 was obtained using the geometric means of the chromatographic peak areas for the analytes.

As can be seen in Fig. 3, the results obtained using the organic solvents MeOH and ACN exhibited better performance compared to H$_2$O. More precisely, MeOH:ACN in the proportions of 50:50 (v/v) presented very satisfactory results for the desorption of the analytes and these were used for further experiments.

3.3.3. *Optimization of number extraction/desorption cycles*

The number of cycles of the extraction and desorption steps is also an important parameter to be evaluated in the DPX procedure. In this step, 1–7 extraction/desorption cycles were evaluated and 30 s was adopted for each cycle. Extraction cycles were performed with a new aliquot of sample (700 µL) to improve the preconcentration of the analytes. Desorption cycles were performed with the same aliquot of sample (200 µL of 50:50 (v/v) of MeOH:ACN). A Doehlert design with 9 experiments (including a triplicate at the central point) was adopted. The response surface obtained using the geometric means of the chromatographic peak areas for the analytes is shown in Fig. 4.

From Fig. 4, it can be observed that increasing the number of extraction cycles also increases the mass of extracted analyte. This is due to the use of a new sample aliquot in each extraction cycle, which allows the insertion of a new portion of analytes suitable for extraction by the sorbent phase. This profile must be maintained

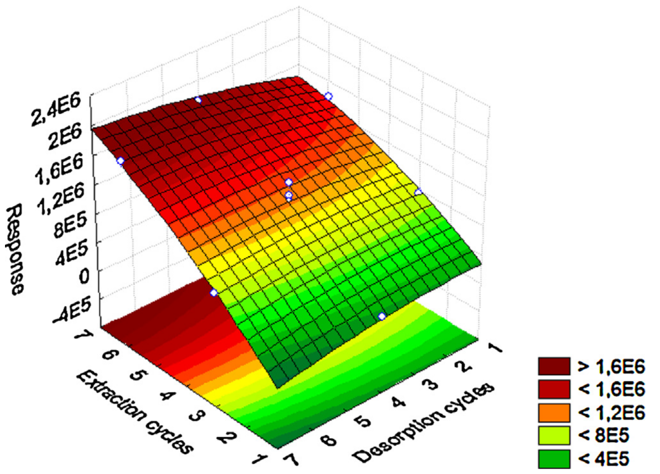

Fig. 4. Response surface obtained using a Doehlert design for the number of extraction cycles *versus* number of desorption cycles for DPX-Si3Py$^+$Cl$^-$.

until the saturation of the material. As the responses for 5, 6 and 7 extraction cycles exhibited similar results, the number of extraction cycles chosen was 5. With respect to the number of desorption cycles, it can be observed that 2 cycles allowed satisfactory desorption of the analytes. Therefore, 5 extraction cycles and 2 desorption cycles were applied in subsequent experiments.

3.3.4. *Optimization of pH and ionic strength*

The extraction efficiency can be strongly affected by the form in which a compound is present in aqueous samples, since the extraction behavior for the dissociated form differs from that of the neutral form [2]. In this case, the analytes in the neutral form are preferred since this allows greater interaction with the sorbent phase. For acid species, the sample pH should be at last 1.5 units lower than the pKa of the analytes [10]. In this study, the pH values evaluated were 4, 5 and 6. These were selected considering that the phenolic compounds under examination are weak organic acids and their pKa values are 9.78, 10.33, 10.23, 10.31 and 10.30 for BPA, EE2, 4-t-OP, 4-OP and 4-NP, respectively. Similar results were observed for all analytes at pH 4, 5 and 6. Therefore, pH 6 was selected for further experiments, since this pH is similar to that of the ultrapure water samples and real river water samples.

Another parameter that can affect the DPX extraction efficiency is the ionic strength of the sample. On increasing the ionic strength of an aqueous sample, the solubility of organic compounds can decrease, facilitating their migration toward the sorbent phase. This effect is observed principally for polar to medium-polarity compounds [36,37]. Another effect observed when a high salt concentration is used is the formation of particles that can migrate to the sorbent phase, masking the active sites. Thus, the active sites are less available to interact with the analytes. In this study, concentrations of 0, 15 and 30% w/v of NaCl were evaluated and a bar graph representing the normalized peak areas is shown in the Supplementary information (Fig. S-3).

In this study higher extraction efficiencies were obtained with no addition of salt. This is probably due to the relatively low polarity presented by the analytes (log P ≥ 3.90) leading to a less pronounced salting-out effect. Therefore, no salt was used in subsequent experiments.

3.3.5. *Optimization of the cleaning step*

After the desorption of the analytes, the procedure used for the cleaning of the sorbent phase was also evaluated. Optimizations consisting of varying the number of cycles and volume of solvents were performed according to the experimental plan described in the experimental section. MeOH:ACN (50:50, v/v) was used as the organic solvent based on the desorption solvent optimization. Ultrapure water was used to remove the excess of solvent and to maintain the sorbent phase conditions the same as those prior to the extraction procedure. The procedure which provided efficient cleaning with no observed carryover consisted of 3 cycles with 200 μL of MeOH:ACN + 2 cycles with 200 μL of H_2O.

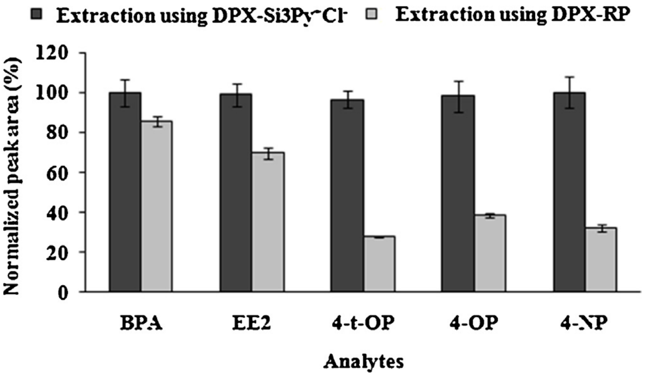

Fig. 5. Comparison of the extraction efficiency using DPX-Si3Py$^+$Cl$^-$ and DPX-RP.

After all of the optimization steps had been completed, the ideal experimental conditions were identified as: 30 mg of sorbent phase; 5 extraction cycles using different aliquots of the sample (700 μL each aliquot) followed by 2 desorption cycles using the same aliquot of MeOH:ACN 50:50 (v/v); pH = 6; and no salt addition.

3.4. *Evaluation of the sorbent stability and possible interfering compounds for Si3Py$^+$Cl$^-$*

To verify the stability of the proposed sorbent phase after successive extractions, 15 complete cycles (extraction/desorption/cleaning) were employed. It was noted that the sorbent phase extracted similar amount of analytes, which provides important evidence that this material can be reused for at least 15 extractions with no loss of efficiency, this being adequate for the intended application. This characteristic is very attractive, since in some sample preparation techniques, such as SPE, the cartridges are used for only one extraction.

The extraction efficiency for the developed method using DPX-Si3Py$^+$Cl$^-$ was compared to that using the commercially-available DPX-RP. In this experiment, the procedure with DPX-RP was also optimized. The best conditions for the commercial phase were 6 cycles of extraction using new 700 μL aliquots of the sample, 2 cycles of desorption using 200 μL of ACN, sample pH adjusted at 6 and no salt addition. The extractions were performed employing the optimized conditions for DPX-Si3Py$^+$Cl$^-$ and DPX-RP and the results are shown in Fig. 5.

According to Fig. 5, DPX-Si3Py$^+$Cl$^-$ provided excellent results for the extraction of phenolic compounds and exhibited higher efficiency than DPX-RP, mainly for 4-t-OP, 4-OP and 4-NP. This behavior can be attributed to the different chemical structures of these sorbents. The sorbent styrene-divinylbenzene contains aromatic rings that can interact with analytes through π-π interactions. On the other hand, the polymer comprised of Si3Py$^+$Cl$^-$ contains silanol groups (Si-OH) which can strongly interact with the hydroxyl moieties of the analytes through hydrogen interactions. For this reason, the extraction efficiency for molecules containing polar moieties can be enhanced, this representing a promising alternative as the sorbent phase for DPX applications.

In addition, a study to verify the influence of potentially interfering compounds in the determination of the analytes was performed using DPX-Si3Py$^+$Cl$^-$. For this particular case, PAHs were chosen

Table 1
Linear range, correlation coefficients, and limits of detection and quantification for the proposed method using DPX-Si3Py$^+$Cl$^-$.

Compound	Equation	Correlation coefficient (r)	Linear range (μg L^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})
BPA	y = 86994x – 26154	0.9973	1–100	0.30	1
EE2	y = 64143x – 3434.7	0.9982	1–100	0.30	1
4-t-OP	y = 71005x – 69874	0.9960	2–100	0.60	2
4-OP	y = 64602 – 35490	0.9960	1–100	0.30	1
4-NP	y = 49502 – 22845	0.9970	1–100	0.30	1

since these compounds are emerging contaminants that are frequently found in water samples [32,33]. Their structures contain aromatic rings that can interact with Si3Py$^+$Cl$^-$ through π-π interactions. In this evaluation, water samples spiked with the analytes at 50 μg L^{-1} were subjected to extraction in the presence of PAHs at 50 μg L^{-1} and without the addition of these compounds. The normalized peak areas were compared, as shown in Supplementary information (Fig. S-4).

It was observed that the extraction efficiency for the phenolic compounds was not affected by the presence of PAHs. This provides important evidence that this sorbent phase exhibits satisfactory performance even with other classes of compounds present in the water samples.

3.5. Analytical figures of merit

Applying the optimized conditions for DPX-Si3Py$^+$Cl$^-$, calibration curves were constructed using river water samples spiked with the analytes at concentrations of 1–100 μg L^{-1}. The analytical figures of merit are shown in Table 1.

Table 1 The correlation coefficients ranged from 0.9960 for 4-t-OP and 4-OP to 0.9982 for EE2, which indicates good linear correlations for the data. In addition, the linearity of the calibration curves was verified using analysis of variance (ANOVA). All of the calibration curves exhibited very small *p*-values, providing strong evidence of correlations between the independent and dependent variables. Also, a good linear relationship is observed since the mean square is much greater than residual mean square [38] (ANOVA tables corresponding to the calibration curve for each of the studied compounds can be seen in Table S-1 of the Supplementary information). In relation to BPA, EE2, 4-OP and 4-NP, the LOD and LOQ were 0.30 and 1 μg L^{-1}, respectively, while for 4-t-OP the corresponding values were 0.60 and 2 μg L^{-1}.

The relative recoveries were used to determine the accuracy of the method at three different analyte concentrations. The values for the relative recovery and RSD are shown in Table 2 and a chromatogram obtained applying the optimized DPX Si3Py$^+$Cl$^-$ procedure to a river water sample spiked with 100 μg L^{-1} of each analyte is shown in Fig. 6. All analytes exhibited satisfactory relative recoveries (83–116%) and repeatability (RSD ≤ 20%), in accordance with validation guidelines [31]. In addition, the relative recovery results were evaluated by applying the *t*-test and these are included in Table S-2 of the Supplementary information. From the evaluation of the critical t-values and experimental values it is possible to confirm that the relative recoveries were not statistically different from 100%. These results highlight the good reliability and potential of the DPX-Si3Py$^+$Cl$^-$ method for the extraction of the phenolic compounds under study.

Table 2
Results obtained for concentration ± confidence interval, relative recovery (%) and relative standard deviation (%) for the analytes in river water using the optimized DPX-Si3Py$^+$Cl$^-$ procedure.

Compound	Spiked concentration (μg L^{-1})	Found concentration ± confidence interval	Relative recovery (%)	RSD (%)
BPA	2	2.10 ± 0.26	105	12
	20	18.4 ± 0.13	88	7
	100	94.2 ± 2.91	94	3
EE2	2	1.66 ± 0.22	83	13
	20	18.5 ± 1.17	92	6
	100	98.3 ± 2.27	98	2
4-t-OP	2	2.33 ± 0.46	116	20
	20	18.9 ± 1.11	95	6
	100	103 ± 1.12	104	1
4-OP	2	1.96 ± 0.12	98	6
	20	17.8 ± 1.43	89	8
	100	96.5 ± 1.39	91	1
4-NP	2	1.90 ± 0.31	95	16
	20	18.4 ± 1.63	92	9
	100	95.5 ± 0.68	95	1

In addition, a comparison of the results obtained in this study using the proposed method (DPX-Si3Py$^+$Cl$^-$) with others reported

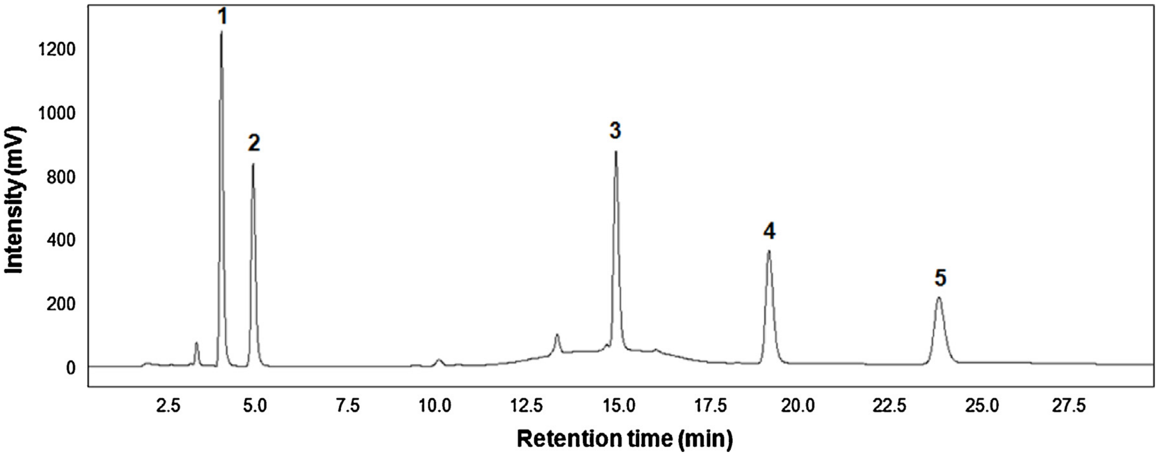

Fig. 6. Chromatogram for river water sample spiked with the analytes BPA, EE2, 4-t-OP, 4-OP and 4-NP at 100 μg L^{-1} obtained with DPX-Si3Py$^+$Cl$^-$ as the sorbent phase. Elution order: 1) BPA, 2) EE2, 3) 4-t-OP, 4) 4-OP and 5) 4-NP.

Table 3
Comparison of the proposed DPX-Si3Py$^+$Cl$^-$ method with others reported in the literature for the same analytes. All studies employed liquid chromatography as the separation technique and FLD^a or MS/MS^b as the detector.

Method	Matrix	Linear range (μg L^{-1})					Total extraction time (min)	Ref.
		BPA	EE2	4-t-OP	4-OP	4-NP		
This study^a	River water	1–100	1–100	2–100	1–100	1–100	3.50	
ILs-DLLME^a	Ultrapure water Mineral water Wastewater	–	1.20–21 1.80–12 1.30–13	–	–	–	–	[46]
SFE-PTFE^a	River water	–	–	–	10–355	15–529	–	[40]
PPP-HF-LPME^a	River water Tap water	–	–	–	0.50–200	0.50–200	2	[41]
Temperature- controlled ionic liquid dispersive liquid phase microextraction^a	Water samples	1–100	–	–	3–300	1.50–150	20	[47]
VALLME^a	Tap water and river water	0.05–100	–	–	0.05–100	0.05–100	4	[48]
FPSE^a	Urine, samples water and hospital effluent		1–500				28	[49]
DLLME^a	Water samples	2.50–250	2–125				–	[50]
SPE-HPLC–MS/MS (column switching)^b	Human milk	0.30 –< 100					–	[51]
SPE-HPLC–MS/MS (column switching)^b	Serum	0.30 –≤ 100					–	[52]
Liquid-liquid extraction^b	Urine	0.20–50		0.20–50			21	[42]
HM-PAN nanofiber mat-based SPE^b	Water samples	0.05–20			0.1–20	0.1–20	–	[53]

in the literature is presented in Table 3. It can be observed that the proposed method provided a relatively short extraction time (only 3.50 min) and wide linear range (1–100 μg L^{-1} for BPA, EE2, 4-OP and 4-NP and 2–100 μg L^{-1} for 4-t-OP), which is particularly advantageous for high-throughput analysis for a wide range of concentrations. Also, can be observes that the first concentration level of the linear range for the proposed method is higher than the values generally obtained using LC–MS/MS. Therefore, this powerful analytical instrument (LC–MS/MS) is an impressive tool, able to increase the sensitivity for a number of different analytes. On the other hand, the LODs obtained in this study are similar to those previously obtained employing the same detector (fluorescence detector). Thus, in spite of the lower sensitivity compared to LC–MS/MS instruments, the results achieved using the proposed method are adequate for the determination of phenolic endocrine-disrupting compounds with aid of analytical instrumentation associate with lower costs compared with LC–MS/MS.

3.6. *Analysis of real samples*

The developed method was applied in the extraction of analytes from three water samples collected from rivers in Santa Catarina State, southern Brazil: sample A from Rio Itajaí (Itajaí), sample B from Rio São Francisco do Sul (São Francisco do Sul) and sample C from Rio Cubatão (Palhoça). Bisphenol A, EE2 and 4-t-OP were detected at concentrations lower than the limits of quantification for samples A, B and C. On the other hand, 4-OP and 4-NP were not detected in the evaluated samples.

The Directive 2013/39/EU of the European Parliament and of the Council establishes annual average (AA – total concentration of all isomers) and maximum allowable concentrations (MACs) of OPs and NPs in superficial waters. For OPs the AA is 0.1 μg L^{-1} and the MAC is not applicable. For NPs the AA is 0.3 μg L^{-1} and the MAC is 2.0 μg L^{-1}. There is no legislation for the other compounds under study [39]. Based on this information, the LOD of the proposed method is adequate for 4-NP analysis. In previous studies reported in literature the linear range for the same compounds were between tens of ng L^{-1} to tens of μg L^{-1} [40–42]. Thus, this LODs, LOQs and linear range observed in this study are acceptable. The analyte concentrations obtained for the water sample were 45.65 μg L^{-1} for 4-NP [2], 1.03 μg L^{-1} for NP and 0.81 μg L^{-1} for BPA [3], 9.10 μg L^{-1} for 4-t-OP and 9.00 μg L^{-1} for 4-NP [43]. In some cases, the presence of the analytes in the sample was not detected [18,41,44,45].

4. Conclusions

A new sorbent phase for DPX analysis comprised of Si3Py$^+$Cl$^-$ was successfully applied as a valuable tool for the extraction of phenolic compounds from environmental aqueous samples. This extraction phase showed very satisfactory chemical and mechanical stability employing organic solvents typically used in DPX analysis. It also exhibited higher extraction efficiency for the determination of some phenolic compounds compared to a commercially-available sorbent phase (styrene-divinylbenzene). The procedure described herein shows satisfactory analytical performance, with a short extraction time (3.50 min) and small volumes of organic solvents were used to complete the procedure. Based on these characteristics, the proposed sorbent phase offers a powerful alternative for the extraction of analytes containing polar moieties. Further studies need to be carried out to evaluate this material using other extraction and microextraction techniques. In addition, further studies will be carried out to evaluate this material as a potential sorbent phase for the extraction of more polar compounds from complex matrices, including urine and waste water.

Acknowledgements

The authors are grateful to the Brazilian governmental agency “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” for financial support which made this research possible. J.M. is grateful to CAPES “Coordenação de Afeiçoamento de Pessoal do Ensino Superior” for a post-doc fellowship.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2017.07.050.

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