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Solid-phase extraction combined with headspace single-drop microextraction of chlorophenols as their methyl ethers and analysis by high-performance liquid chromatography-diode array detection

Nisha Sharma, Archana Jain, Vandana Kumari Singh, Krishna K. Verma *

Department of Chemistry, Rani Durgavati University, Pachpedi, Jabalpur 482001, Madhya Pradesh, India

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ABSTRACT

Solid-phase extraction (SPE) of phenol and chlorophenols, their derivatization to methyl ethers, headspace single-drop microextraction (HS-SDME) of methyl ethers using 1-butanol as extraction solvent, and direct transfer of the drop into the injector for high performance liquid chromatography with diode array detection (HPLC-DAD) have been reported. A flanged-end polytetrafluoroethylene sleeve, 3 mm × 0.5 mm, placed at the tip of the syringe needle, allowed the use of 10 μL solvent drop for extraction. The procedure has been optimized for variables involved in SPE and HS-SDME. A rectilinear relationship was obtained between the amount of chlorophenols and peak area ratio of their methyl ethers/internal standard (4-methoxyacetophenone) in the range 0.01–10 mg L^{-1}, correlation coefficient in the range 0.9956–0.9996, and limit of detection in the range 1.5–3.9 μg L^{-1} when HS-SDME alone was used for sample preparation. When using coupled SPE and HS-SDME, the linear range obtained was 0.1–500 μg L^{-1}, correlation coefficient in the range 0.9974–0.9998, and the limit of detection in the range 0.04–0.08 μg L^{-1}. Spiked real samples have been analyzed with adequate accuracy, and application of the method has been demonstrated for the analysis of chlorophenols formed upon bamboo pulp bleaching.

1. Introduction

Phenol and substituted phenols are priority pollutants and considered to be one of the most obnoxious contaminants even when present at low concentrations [1,2]. Chlorophenols are extensively used as preservatives, fungicides, pesticides, disinfectants, and phenol derivatives are widely used as intermediates in the synthesis of plastics and dyes. Chlorophenols are generated from phenols during treatment of tap water with chlorine. All pulp and paper mills use chlorine in one of the multiple bleaching steps, and produce a number of chlorophenols in its effluents [3]. Chlorophenols deteriorate taste of water and produce unfavorable smell. Sediments can adsorb the phenolic compounds in relatively high concentrations and affect the aquatic lives. Furthermore, chlorophenols accumulate in the environment and are considered to be serious health hazardous materials. Chlorophenols have been usually detected in human urine [2,4]. The toxicity of chlorophenols depends on the pH and the number of chlorine atoms in the molecule; pentachlorophenol being the most toxic of chlorinated phenols [5]. Most chlorophenols are listed by the US Environmental Protection Agency (US EPA) [6], and European Community [7] as priority pollutants. The standard permissible limit for total chlorophenols in drinking water is stated to be 0.5 μg L^{-1} while each phenol should not exceed 0.1 μg L^{-1}.

Owing to their highly toxic, carcinogenic, and poorly biodegradable nature in the environment, it is essential to develop rapid, sensitive and reliable analytical methods to determine chlorophenols in aqueous samples. Since these compounds are present in trace quantities, analytical procedures usually include a pre-concentration step prior to chromatography. Furthermore, derivatization of chlorophenols is recommended in many GC methods in order to reduce their tendency to give broad and tailed peaks owing to native polarity. The phenolic compounds were extracted from acidic aqueous donor phase to organic solvent, and then into the alkaline acceptor phase by a process called liquid–liquid–liquid microextraction; the extract was analyzed by high-performance liquid chromatography [8,9]. A high throughput system consisted of multi-syringe flow injection solid-phase extraction and on-line separation on monolithic column without analyte derivatization [10]. Acetylation of phenolic compounds has been most commonly used method of derivatization to reduce polarity, and to enhance volatility and extractability of derivatives. This technique has been used during one or another step in the determination of phenols such as purge-and-trap of acetyl derivatives and gas chromatography [11,12]; extraction of phenolics with an organic solvent, stir bar sorptive extraction of acetyl derivatives, and analysis by gas chromatography [13,14]; or headspace solid-phase extraction of acetylated analytes and

* Corresponding author. Tel.: +91 761 2605103; fax: +91 761 2403252.
E-mail address: arichna@sancharnet.in (K.K. Verma).

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gas chromatography [15]. Chlorophenols were extracted from a large volume of aqueous sample (100 mL) by solid-phase extraction, eluted with acetone, and the eluate subjected to dispersive liquid–liquid microextraction with in situ acetylation, and analysis by gas chromatography [16]; the method was reported to result in high enrichment of analytes. The advantage of acetylation is applicability of acetic anhydride in aqueous solution and rapidity of derivatization reaction in carbonate medium. Silyl reagents are less stable in aqueous medium, among other reagents *N*-(*tert*-butyldimethylsilyl)-*N*-methyl-trifluoroacetamide was found relatively resistant to hydrolysis. This reagent was incorporated in organic solvent drop which was immersed into the aqueous sample for both derivatization and extraction to occur in a single step [17], or the whole process was completed in two discrete steps of headspace solid-phase microextraction of phenolic compounds, and subsequent exposure of fibre to the reagent vapours for derivatization [18]. Ethyl chloroformate [19] and benzoyl chloride [20] are still other reagents that have been used for phenols and amines; a secondary reaction with pentafluoropropionic anhydride was necessary for amines to avoid peak tailing [19]. Two gas chromatography based methods involved methylation of phenols either using phase-transfer catalysis in water–dichloromethane and analysis of a portion of organic phase for spiked lake water samples [21], or solid-phase microextraction of methylated derivatives for analysis of odorous trichlorobromophenols in drinking water [22]. Solid-phase microextraction is a method of choice, however, high cost of fibres, their fragility and analyte carryover are some of its limitations. Extraction of phenols by pressurized liquid extraction from leather [23] or liquid–liquid–liquid extraction from water [8] and high-performance liquid chromatography-diode array detection have been reported. Few liquid chromatographic methods involved derivatization of phenols with reagents such as coumarin-6-sulphonyl chloride [24] or 2-(9-carbazole)-ethyl-chloroformate [25] whereas many others were based on chromatography of underivatized phenols [26–29].

Over the past decade, liquid-phase microextraction (LPME) has emerged as a novel sample preparation technique, which is simple, cost effective and rapid, involves little sample and solvent consumption [30,31]. This technique is based on passive distribution of analytes between the microlitre volumes of organic phase and the aqueous phase. Single-drop microextraction (SDME) has evolved from this technique in which the extraction phase is a drop of water-immiscible solvent suspended in or placed in the headspace of the stirred aqueous sample. Because of its extreme simplicity, many successful applications of SDME have been reported [32,33]. However, major disadvantage of this technology is the instability of a drop. Ionic liquids at room temperature have been used to overcome this problem [34,35]. Here, the tip of the microsyringe needle was enclosed with a 3-mm-long silicon rubber tube (0.2 mm i.d. and 2.8 mm o.d.) or a 3 mm long polytetrafluoroethylene (PTFE) tube (0.6 mm i.d. and 1.8 mm o.d.) for pre-concentrating polycyclic aromatic hydrocarbons or chlorinated anilines from water samples, and a reasonable sensitivity was obtained. Using this kind of sheathed needle, it was observed that the problem of drop easy detachment still exists and the suspended volume is not larger than 7 μL [36], especially for common organic solvents which have relatively low viscosity. On-line coupling of ionic liquid-based SDME has also been used with capillary electrophoresis of phenols [37]; the limit of detection was in range 5–50 $\mu g\,L^{-1}$ of phenols. It is of great importance to develop a new design to accommodate bigger extraction drop of organic solvents which are commonly used for SDME and to overcome the instability of the drop.

The aim of this work is to achieve high enrichment factor, and thus sensitivity of detection, by using solid-phase extraction (SPE) in combination with headspace-SDME (HS-SDME), and to assemble a small extraction device to increase the drop volume and stabilize it for enhanced pre-concentration of analytes in SDME. The practicability of this coupled extraction technique has been tested on chlorophenols involving SPE, derivatization to methyl ethers and subsequent HS-SDME, and analysis by HPLC-DAD.

2. Experimental

2.1. Standards, reagents and solvent

The following chemical standards (purity over 99%) were obtained from Aldrich (Milwaukee, WI, USA), phenol, 4-chlorophenol, 2,6-dichlorophenol, 2,3-dichlorophenol, 3,4-dichlorophenol, 4-chloro-2,6-dimethylphenol, 3,4-dichlorophenol, 2,3,6-trichlorophenol, and pentachlorophenol. A mixed standard solution containing 50 $\mu g\,mL^{-1}$ of each analytes was prepared in methanol. Working standards were prepared by sequential dilution of the stock solution with methanol. 4-Methoxyacetophenone, 0.5 $\mu g\,mL^{-1}$ in methanol, was used as internal standard. The derivatization reagent dimethyl sulphate (99%), 1-butanol and HPLC grade methanol were purchased from Merck, Mumbai. Standard solutions were kept in refrigerator when not in use. An all glass 0.45-μm membrane filter unit (Millipore, Bedford, MA, USA) was used for filtration of HPLC solvents. Millipore laboratory water system (Millipore, Bangalore) was used to obtain HPLC grade water.

2.2. Instrumentation and chromatographic conditions

The HPLC system (Waters, MA, USA) included two 515 HPLC reciprocating pumps, a pump control module II, a 2998 photo diode array detector (DAD) with a 8 μL (1 cm path length) flow-through cell, and a 7725i Rheodyne manual injection valve with 20 μL sample loop. Separations were performed on Waters analytical column, 25 cm × 4.6 mm i.d., stainless steel tube packed with octadecylsilane (ODS), 5 μm particle size. Chromatographic data were processed with Waters Empower 2 software. DAD scanning range was 200–300 nm with resolution of 1.2 nm. 4-Methoxyacetophenone was used as internal standard. The peak area ratio of analytes to the internal standard was used for quantification. All peak areas were integrated at 220 nm.

Methanol and water in different composition with a total flow rate of 1 $mL\,min^{-1}$ were tried as eluting solvent in both isocratic and gradient modes to achieve good separation in minimum possible time. The compromise between a good peak resolution and a reasonably short analysis time was achieved with a gradient elution program beginning with methanol–water, 50:50% (v/v), and the methanol portion was raised to 60% in 6 min, 70% in 8 min, 80% in 10 min and finally 100% in 20 min.

Off-line SPE cartridges (10 mm × 3 mm i.d.) were home packed with a slurry of PLRP-S sorbent (styrene-divinylbenzene copolymer, particle size 8 μm, Polymer Lab Ltd., UK) in methanol. Cartridge packing tools and column holder were obtained from the Free University, Amsterdam, The Netherlands. Shimadzu HPLC (LC-5A) pump was used for the activation of SPE sorbent, sample loading and washing.

The flanged sleeve was constructed with Easy Flange™ Tubing Flanger Kit (Alltech Associate, Sweden, Part-No. 201530) consisting of two parts, first a two-pieces slippery fluted polymeric cuboids to hold PTFE tube by clamping them together, and the second a disc holder which houses a small metal pin to be inserted into the PTFE tube hole and a small cone tip at the center to flatten it. To prepare the flanged sleeve, a PTFE tube of 0.5 mm i.d. was securely held between the pair of cuboids protruding 2 mm portion of the tube outside. The metal pin mounted on disc was emphatically compressed into the projected tube and the pin was rotated till the tube end appeared as a symmetric bell-mouthed shape.

Then, about 4 mm length of PTFE tubing from the flanged end was cut. This flanged sleeve was used for extractions. A flat-end 25 μL Hamilton syringe (Supelco, New Delhi) was used for HS-SDME and introduction of analytes into the HPLC injector loop. After each extraction the PTFE flanged sleeve and the syringe were washed separately with methanol to avoid analyte carryover. An in-house made syringe stand was used for holding the syringe upright during the extraction. Extraction vials, 4 mL, with PTFE silicon septum and screw cap (Supelco), were used. Thermostatic magnetic heating plate (Kumar Equipments, Mumbai, India) was used for heating and stirring the sample.

2.3. Sample collection

Environmental water samples (Ganga, Varanasi, and Narmada, Jabalpur), Jabalpur city tap water and ground water samples were analyzed. The samples were collected in amber glass bottles with polypropylene screw caps having PTFE septa, treated with 1 mL of 0.1 M ascorbic acid (for every 100 mL of water sample) to reduce any free chlorine, and stored at 4 °C. The analyses were done within 2 days of sample collection.

2.4. SPE of chlorophenols

The SPE cartridge (10 mm × 3 mm) containing 100 mg of sorbent (polystyrene–divinylbenzene) was activated with 3 mL of methanol, and conditioned with 3 mL of water, both at a flow rate of 1 mL min^{-1}. A 50-mL portion of sample was mixed with about 1 mL of 1 M sulphuric acid, and a 40-mL aliquot was passed through the SPE cartridge at a flow rate of 4 mL min^{-1}. During this period nitrogen (30 mL min^{-1}) was passed through the air loop (Fig. 1A). After sample loading, the sorbent in the SPE cartridge was washed with 2 mL of water (flow rate 1 mL min^{-1}). The switching valve V3 was actuated to flush the cartridge with nitrogen (30 mL min^{-1}) for 5 min to remove water. The solvent loop (200 μL) was manually filled with ethyl acetate, thereafter, V1 and V2 valves were switched to elute the analytes from SPE cartridge. Water delivered by HPLC pump pushed nitrogen in the air loop that in turn propelled ethyl acetate through the SPE cartridge. The large air loop avoided chances of mixing of water with ethyl acetate. Eluate was collected in a 4 mL screw cap vial having PTFE-lined cap with silicon septum.

2.5. Derivatization and HS-SDME of chlorophenols

The typical device used for HS-SDME is shown in Fig. 1B. Into the 4 mL screw-cap vial, containing the eluate from previous step, was placed a PTFE coated magnetic stirring bar, 0.4 mL of 8 M potassium hydroxide, 0.1 mL of dimethyl sulphate, and 1.5 mL of de-ionized water. The flat-end needle of a 25 μL Hamilton microsyringe, filled with 15 μL of 1-butanol (containing 5 μg L^{-1} of 4-methoxyacetophenone as the internal standard), was penetrated through the septum of the cap (a hole already drilled with a bevel end needle), fitted with flanged PTFE sleeve, and the vial was capped with it. The vial was heated at 70 °C by immersing it up to the level of reaction mixture into the thermostated water. After 15 min, a 10 μL extraction drop was configured about 0.8 cm above the sample, stirred at about 1000 rpm, and held for 20 min. Thereafter, the drop was withdrawn into the syringe and immediately injected into the liquid chromatograph for analysis.

3. Results and discussion

3.1. The derivatization reaction

Methylation of phenol and chlorophenols was chosen as method of derivatization because it was found to be efficient, simple and fast reaction that converts phenols to volatile methyl ethers. The derivatization reaction worked well in aqueous medium at high pH when the phenoxide ion eventually reacted with dimethyl sulphate. Methylation of phenols obviously competes with alkaline hydrolysis of dimethyl sulphate, but the latter process is a slow reaction. The retention time of derivatives was confirmed by methylating individual phenols and their HPLC under the same conditions. Authentic samples of methyl ethers were synthesized in the laboratory by using standard procedure [38].

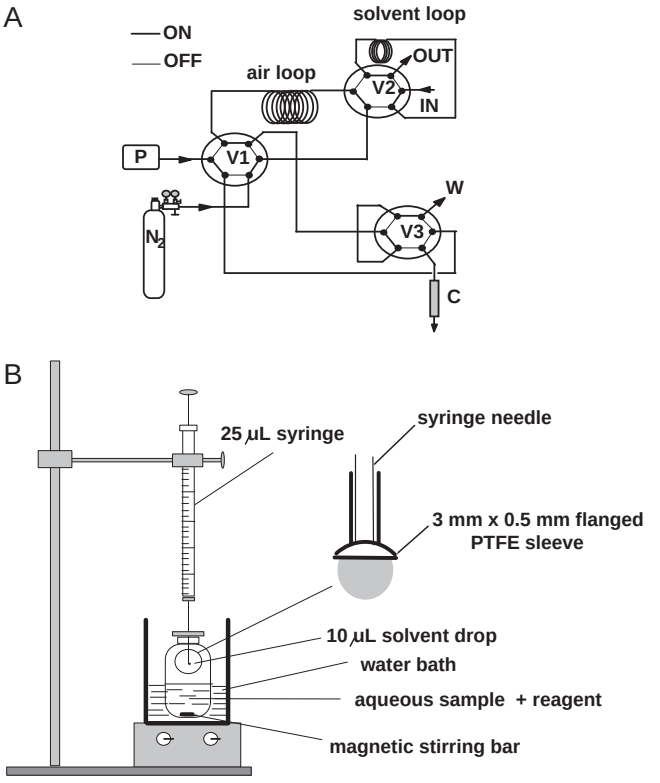

Fig. 1. (A) Experimental set-up for the off-line SPE of phenol and chlorophenols. V1–V3 = Rheodyne 7010 6-port valves; P = HPLC pump; C = SPE (10 mm × 3 mm i.d.) cartridge packed with PLRP-S (polystyrene–divinylbenzene, 8 μm particle size); and W = waste. Solvent loop was 200 μL, and air loop was PTFE tubing, 7 m × 0.8 mm i.d. (B) Typical device for headspace single drop microextraction with in drop derivatization showing 10 μL drop of the organic solvent suspended at the tip of a 25 μL microsyringe needle with flanged sleeve PTFE tubing fitted; the drop kept above the aqueous sample solution for headspace extraction. The panel in the right shows the solvent drop suspended from the PTFE sleeve fixed at the needle tip.

The polarity of phenol and chlorophenols results in inadequate separation and tailing peaks in reversed-phase liquid chromatography. Thus, the only way to avoid these inconveniences was to reduce their polarity by utilizing the hydroxyl groups in derivatization reaction such as methylation. Derivatization reactions in chromatographic methods are most frequently found in the literature as they are selective and applicable to a wide variety of analytes [39,40]. Methyl iodide has been used for methylation of phenols, however, it is expensive, volatile and water immiscible reagent that necessitates use of phase-transfer agents [21]. Recently, Microwave-assisted methylation of phenols with tetramethylammonium chloride in the presence of potassium or caesium carbonate has been reported [41], but the reaction has not been utilized for analytical purpose. Dimethyl carbonate is inflammable liquid, and is able to methylate anilines, phenols and carboxylic acids [42]. Many of these reactions are conducted in autoclave. The boiling point of reagent is another factor in HS-SDME since volatile reagents are likely to be extracted. Dimethyl sulphate (boiling point 188 °C) is effective and affordable reagent for methylation of phe-

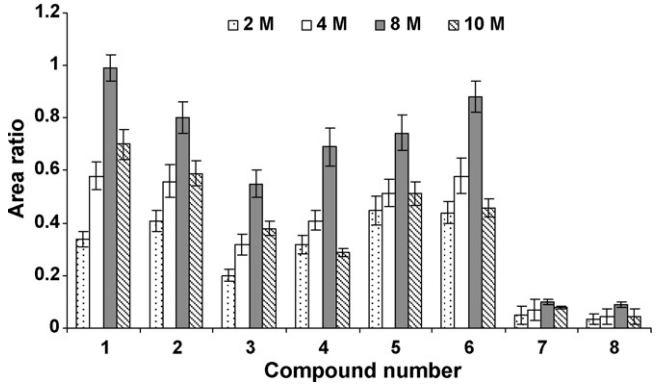

Fig. 2. Effect of potassium hydroxide concentration on the methylation reaction; 2.5 mg L^{-1} each phenol. Reaction/extraction temperature, 70 °C, reaction time 15 min, extraction time 15 min, and extraction of methyl ethers with 10 μL of 1-butanol. Compounds identification as: 1 = phenol, 2 = 4-chlorophenol, 3 = 2,6-dichlorophenol, 4 = 2,3-dichlorophenol, 5 = 3,4-dichlorophenol, 6 = 4-chloro-2,6-dimethylphenol, 7 = 2,3,6-trichlorophenol, 8 = pentachlorophenol.

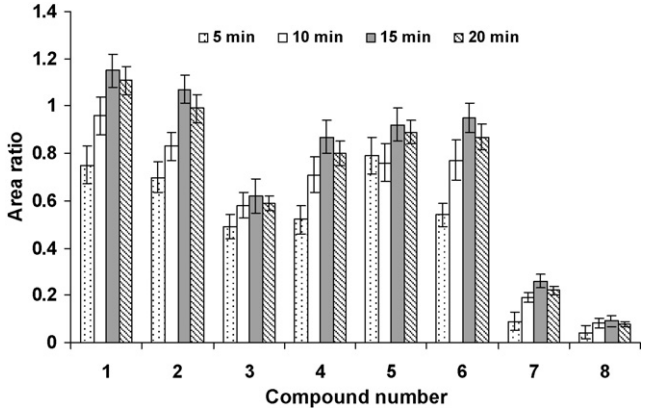

Fig. 3. Effect of time on the methylation reaction; 2.5 mg L^{-1} each phenol. Reaction/extraction temperature, 70 °C, extraction of methyl ethers with 10 μL of 1-butanol, extraction time 15 min. Compounds identification as in Fig. 2.

nols. Typically, one methyl group of the reagent is transferred more quickly in methylation via S_N2 mechanism than the second, and the yield of methyl ethers is high.

3.2. Optimization of derivatization conditions

Preliminary experiments were conducted in triplicate at 70 °C for derivatization reaction and using 10 μL of 1-butanol for headspace extraction. Different volumes, range tested 0.1–0.4 mL, of dimethyl sulphate as derivatizing reagent were taken to derivatize chlorophenols when the peak areas were optimum with 0.2 mL of the reagent, and thereafter found to decrease. Ostensibly, excess of methyl sulphate acted as solvent for methyl ether derivatives and decreased their headspace concentration. Upon increasing potassium hydroxide concentration, the peak area of methylated phenols increased steadily from 2 M and reached to optimum at 8 M. The peak areas of methyl ethers began to decrease at higher alkali concentration; at 10 M potassium hydroxide the decrease was about 15–25% from optimum (Fig. 2). This effect that was believed to be due to instability of phenols in highly alkaline medium at elevated temperatures. In the subsequent experiments 8 M potassium hydroxide was used. Temperature and length of derivatization reaction were two important factors in the method development. As the temperature of the analysis vial increased from 50 °C to 60 °C, the peak areas for methyl ethers increased 17–62%, and a further increase of 10 °C gave another increment of 8–28%. At 80 °C peak areas decreased considerably (9–48%) and the repeatability of the method deteriorated (RSD, 20%). Therefore 70 °C was considered to be the optimum temperature for methylation. The effect of reaction time was examined in the range 5–20 min at 70 °C. The peak areas increased with increasing reaction time until 15 min, which was taken as optimum (Fig. 3). After 15 min, the system was basically in a steady state and no significant increase was observed with additional reaction time.

3.3. Optimization of extraction conditions

Initial experiments were done by HS-SDME without using any SPE. For selecting solvent for HS-SDME and subsequent liquid chromatography of extract, the major points of consideration were low volatility of solvent, drop stability in the headspace, affinity for analytes, miscibility with the mobile phase, and transparency in UV detection. Thus, 1-butanol, 1-propanol and ethylene glycol were tested for extraction when optimum performance was found with 1-butanol. Micro-drop volume of 1–3 μL can be conveniently used for extraction using a general bevel tip Hamilton syringe (as used in gas chromatography). Drops larger than 3 μL are difficult to handle because of their tendency for dislodgement from the syringe needle. However, by putting PTFE flanged sleeve at the end of the commercially available blunt 25 μL Hamilton microsyringe (as used with model 7725i Rheodyne and similar injectors in high-performance liquid chromatography), it was possible to use as large as 10 μL drop of butanol in the headspace of sample vial and keep it stable for more than 20 min. The extraction efficiency using drop volume of 3,5,7 and 10 μL was studied. A total volume of 15 μL of extracting solvent was used, a 10 μL portion was configured as drop and the remaining solvent was left within the needle. An increase in the signal is expected on increasing drop volume but bigger solvent drops require extended equilibration times as mass transfer inside the drop is by diffusion [43]. Drop volumes greater than 10 μL were not used since they were difficult to handle in the present system. Maximum extraction and reproducible results were obtained when 10 μL drop volume was used (Fig. 4). A series of extraction times were investigated in range 5–20 min. Shorter periods are best for extraction, but equilibrium was not attained even after 20 min. To keep a reasonable sample throughput, and on account of enrichment factor attained, an extraction time of 15 min was chosen as optimum. The overall extraction efficiency was evaluated by the enrichment factor (EF), which was calculated using the equation [44], $EF = C_{drop}/C_{sample}$, where, C_{drop} is the analyte concentration in 1-butanol drop after extraction, and C_{sample} is the original analyte concentration in the sample. At optimum conditions, analyte con-

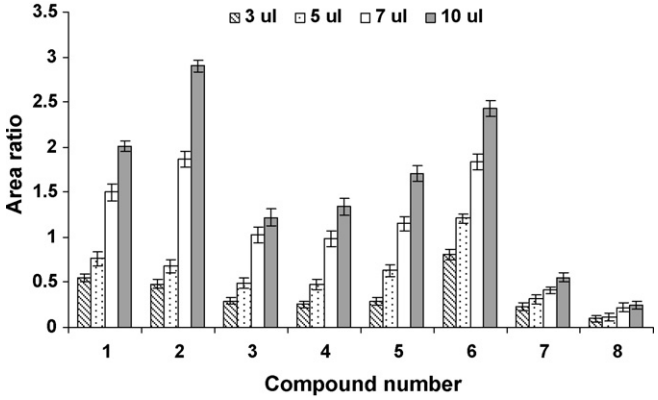

Fig. 4. Effect of drop volume of 1-butanol for the extraction of methyl ethers of chlorophenols, 5 mg L^{-1} each phenol. Reaction/extraction temperature, 70 °C, reaction time 15 min, and extraction time 15 min. Compounds identification as in Fig. 2.

centrations of 0.2, 1 and 5 mg L^{-1} gave an average enrichment of 130-fold in 15 min. Continuous stirring of sample at about 1000 rpm was performed during extraction.

Solid-phase extraction of phenols on styrene-divinylbenzene copolymer, which was found an efficient sorbent in earlier experiments for polar compounds [10,20], was used in the present work in combination with HS-SDME. The sample solution was acidified to avoid dissociation of phenols and to enhance retention. Aliquots (10–100 mL) of sample aqueous solution containing chlorophenols in the calibration range was pre-concentrated on polystyrene–divinylbenzene when practically no breakthrough of analytes was observed up to 60 mL of sample. Sample loading was tested over the range 1–10 mL min^{-1}. The analytical signal was constant up to 6 mL min^{-1}, thereafter it started to decrease; at a flow rate of 10 mL min^{-1} the decrease was about 25%. In the optimized method 40 mL of sample was pre-concentrated at a flow rate of 4 mL min^{-1} to keep reasonable the sampling time. Ethyl acetate was found a good eluent to recover the retained analytes, and was easy to handle; a 200 μL aliquot of eluent gave optimum recovery. The coupled SPE and HS-SDME technique on application to standards containing 1, 10 and 100 μg L^{-1} of phenols in the optimum conditions gave an average enrichment of 2400 for phenol and chlorophenols.

3.4. Method validation

Phenol, monochlorophenols, dichlorophenols, trichlorophenol and pentachlorophenol were determined under the optimized experimental conditions. The calibration results of Table 1 were obtained on standard solutions made by spiking Narmada river water that was found not to contain any of the tested analytes, nor having any interfering peaks. Using HS-SDME alone as sample preparation technique, a linear calibration graph was obtained in range 0.01–10 mg L^{-1} of phenols (after derivatization to their methyl ethers) with an average correlation coefficient of 0.9983 (range found 0.9956–0.9997). The average limit of detection was 2.54 μg L^{-1} (range found 1.5–3.9 μg L^{-1}) estimated at three times the standard deviation in the six replicate analyses of lowest concentration of phenolic compounds in the linear range of determination. Using combined SPE and HS-SDME, the linear range obtained was 0.1–500 μg L^{-1}, the average correlation coefficient of 0.9984 (range found 0.9974–0.9998), and the average limit of detection of 0.05 μg L^{-1} (range found 0.04–0.08 μg L^{-1}). Results of both methods, HS-SDME and combined SPE–HS-SDME are given for comparison and improved performance of latter method. Relative standard deviations were calculated for five replicate injections of 400, 100, 10, 1.0 and 0.5 μg L^{-1} of phenolic compounds when the average RSD was 4.1% (range found 3.1–4.9%).

3.5. Application

The present procedure was applied to real water samples of the Ganga river water, Narmada river water, lake water, and Jabalpur city tap water spiked at 100, 10, 1.0 and 0.5 μg L^{-1} of phenolic compounds (Table 2). The overall recovery of four replicate analyses of each sample at each concentration level was 101.2% (range found 91.7–112.5%). A typical chromatogram obtained for spiked Ganga river water, which was considered most polluted among the samples analyzed, is shown in Fig. 5 and showed no interference from co-existing compounds.

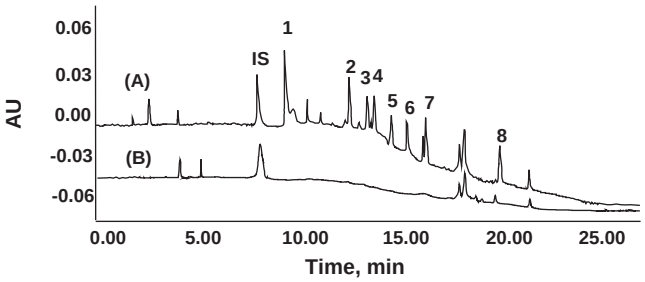

Fig. 5. The combined SPE–HS-SDME and HPLC-DAD of 10 μg L^{-1} of each phenol spiked to Ganga river water (A), and the river water blank spiked with the internal standard (B). Derivatization and extraction conditions as in the text. Peaks identification as in Fig. 2; IS = 4-methoxyacetophenone.

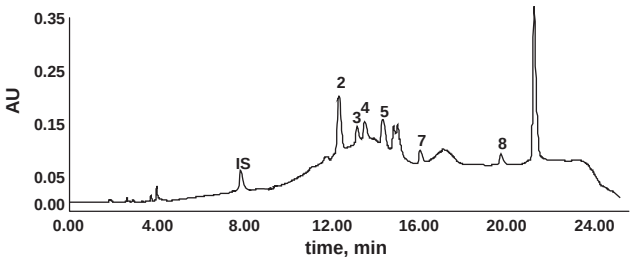

Fig. 6. The HPLC-DAD of phenols obtained from chlorinated bamboo pulp extract after combined SPE–HS-SDME. Derivatization and extraction conditions as in the text. Peaks identification as in Fig. 2; IS = 4-methoxyacetophenone.

Bamboo (after removing the outer green layer) was cut into small pieces, and about 250 g of it was soaked in 1 L of deionized water for 24 h. The pieces were blended in an electric mixer to produce a fine pulp. The pulp was mixed with 500 mL of deionized water, acidified to about pH 2 with hydrochloric acid, and chlorine was passed through it under hood for about 20 min with occasional shaking. The pulp, saturated with chlorine, was allowed to stand for 48 h in a closed glass container. Thereafter, the excess chlorine was reduced by addition of portions of solid ascorbic acid, treated with 1 M sodium hydroxide to adjust between pH 11–12, and stirred for 30 min. The fibrous matter was removed by centrifugation and the supernatant was used for identification of halo-phenols by the present method. A typical chromatogram obtained is given in Fig. 6; all peaks were characterized by matching their retention time and electronic spectra of standards. The degree of chlorination and amounts of chlorophenols formed during chlorination depends

Table 1
Determination of phenols as their methyl ethers by HS-SDME/SPE–HS-SDME and HPLC-DAD.

Compound	HS-SDME			Combined SPE–HS-SDME		
	Linear range (mg L^{-1})	r^2	LOD (μg L^{-1})	Linear range (μg L^{-1})	r^2	LOD (μg L^{-1})
Phenol	0.01–10	0.9970	1.5	0.2–500	0.9984	0.06
4-Chlorophenol	0.01–10	0.9991	1.6	0.1–500	0.9989	0.05
2,6-Dichlorophenol	0.03–10	0.9991	1.9	0.1–500	0.9984	0.04
2,3-Dichlorophenol	0.03–10	0.9956	2.3	0.1–500	0.9974	0.04
3,4-Dichlorophenol	0.03–10	0.9981	2.0	0.1–500	0.9980	0.05
4-Chloro-2,6-dimethylphenol	0.03–10	0.9997	3.3	0.2–500	0.9989	0.06
2,3,6-Trichlorophenol	0.03–10	0.9979	3.8	0.4–500	0.9998	0.06
Pentachlorophenol	0.03–10	0.9996	3.9	0.5–500	0.9976	0.08

Table 2
Recovery (%) of phenols spiked to real water samples by combined SPE–SDME.

Compound	Recovery (%)[a]			
	Ganga river water[b]	Jabalpur city tap water[c]	Lake water[d]	Narmada river water[e]
Phenol	92.5	96.0	109.9	93.9
4-Chlorophenol	98.3	112.5	103.2	98.9
2,6-Dichlorophenol	97.6	110.2	101.8	108.8
2,3-Dichlorophenol	103.0	108.2	109.5	99.7
3,4-Dichlorophenol	96.8	102.9	104.2	98.9
4-Chloro-2,6-dimethylphenol	91.7	109.0	102.6	98.8
2,3,6-Trichlorophenol	96.6	95.8	93.3	107.2
Pentachlorophenol	98.8	97.2	108.0	93.5

[a] Results are averages of 4 determinations.
[b] Spiking level, 10 $\mu g\,L^{-1}$ of each phenol.
[c] Spiking level, 100 $\mu g\,L^{-1}$ of each phenol.
[d] Spiking level, 1.0 $\mu g\,L^{-1}$ of each phenol.
[e] Spiking level, 0.50 $\mu g\,L^{-1}$ of each phenol.

on the concentration (pressure) of chlorine and length of its contact with bamboo pulp. In our experiments the chlorophenols ($mg\,kg^{-1}$) formed were 4-chlorophenol (65.9), 2,6-dichlorophenol (22.6), 2,3-dichlorophenol (29.3), 3,4-dichlorophenol (45.8), 2,3,6-trichlorophenol (31.3) and pentachlorophenol (15.7).

4. Conclusions

Combined SPE and HS-SDME is a rapid and sensitive method for the analysis of phenol and chlorophenols after their methylation with dimethyl sulphate and HPLC-DAD. A flanged PTFE sleeve was a successful device for holding as large as 10 μL of extracting solvent drop for as long as 20 min in the headspace of the sample. Polystyrene–divinylbenzene was efficient sorbent for pre-concentration of chlorophenols in SPE, and 1-butanol was suitable for HS-SDME as it gave stable drop, had excellent extraction suitability for methyl ethers of phenolic compounds, and was compatible with HPLC mobile phase for chromatography.

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References

[1] WHO, Chlorophenols in Drinking-water, WHO/SDE/WSH/03.04/47, 2003, Geneva.
[2] H. Kontsas, C. Rosenberg, P. Pfaffli, P. Jappinen, Analyst 120 (1995) 1745–1749.
[3] H.-B. Lee, T.E. Peart, J. Chromatogr. 636 (1993) 263–270.
[4] P. Bartels, E. Ebeling, B. Krämer, H. Kruse, N. Osius, K. Vowinkel, O. Wassermann, J. Witten, C. Zorn, Fresen. J. Anal. Chem. 365 (1999) 458–464.
[5] WHO, Pentachlorophenol in Drinking-water, WHO/SDE/WSH/03.04/62, 2003, Geneva.
[6] EPA Method 604, Phenols, Part VIII, 40 CFR Part 136, U.S. Environmental Protection Agency, Washington, DC, 1984, p. 58.
[7] Directive 80/77/CE 157-1990, Off. J. Eur. Commun., 30-8-1990, European Community, Brussels, 1990.
[8] C.-Y. Lin, S.-D. Huang, J. Chromatogr. A 1193 (2008) 79–84.
[9] W. Pan, H. Xu, Y. Cui, D. Song, Y.-Q. Feng, J. Chromatogr. A 1203 (2008) 7–12.
[10] H.M. Oliveira, M.A. Segundo, J.L.F.C. Lima, V. Cerda, Talanta 77 (2009) 1466–1472.
[11] J.W. Blythe, A. Heitz, C.A. Joll, R.I. Kagi, J. Chromatogr. A 1102 (2006) 73–83.
[12] R.-S. Zhao, C.-G. Cheng, J.-P. Yuan, T. Jiang, X. Wang, J.-M. Lin, Anal. Bioanal. Chem. 387 (2007) 687–694.
[13] J. Llorca-Porcel, M. Martinez-Parreno, E. Martinez-Soriano, I. Valor, J. Chromatogr. A 1216 (2009) 5955–5961.
[14] P. Tolgyessy, B. Vrana, M. Bartal, Z. Krascsenits, K. Silharova, Chromatographia 69 (2009) 389–392.
[15] J. Regueiro, E. Becerril, C. Garcia-Jares, M. Llompart, J. Chromatogr. A 1216 (2009) 4693–4702.
[16] N. Fattahi, S. Samadi, Y. Assadi, M.R.M. Hosseini, J. Chromatogr. A 1169 (2007) 63–69.
[17] X. Wang, L. Luo, G. Louyang, L. Lin, N.F.Y. Tam, C. Lan, T. Luan, J. Chromatogr. A 1216 (2009) 6267–6273.
[18] Y.-P. Pan, S.-W. Tsai, Anal. Chim. Acta 624 (2008) 247–252.
[19] M.-J. Paik, Y. Choi, K.-R. Kim, Anal. Chim. Acta 560 (2006) 218–226.
[20] S. Mishra, V. Singh, A. Jain, K.K. Verma, Analyst 126 (2001) 1663–1668.
[21] Y.C. Fiamegos, C.G. Nanos, G.A. Pilidis, C.D. Stalikas, J. Chromatogr. A 983 (2003) 215–223.
[22] A. Diaz, F. Ventura, M.T. Galceran, Anal. Bioanal. Chem. 386 (2006) 293–298.
[23] G. Favaro, D. De Leo, P. Pastore, F. Magno, A. Ballardin, J. Chromatogr. A 1177 (2008) 36–42.
[24] F.E.O. Suliman, S.S. Al-Kindi, S.M.Z. Al-Kindy, H.A.J. Al-Lawati, J. Chromatogr. A 1101 (2006) 179–184.
[25] L. Zhang, L. Zhang, W. Zhang, Y. Zhang, Anal. Chim. Acta 543 (2005) 52–57.
[26] M. Castillo, D. Puig, D. Barcelo, J. Chromatogr. A 778 (1997) 301–311.
[27] R. Wissiack, E. Rosenberg, M. Grasserbauer, J. Chromatogr. A 896 (2000) 159–170.
[28] R. Wissiack, E. Rosenberg, J. Chromatogr. A 963 (2002) 149–157.
[29] L. Yang, Z. Wang, L. Xu, J. Chromatogr. A 1104 (2006) 230–237.
[30] F. Pena-Pereira, I. Lavilla, C. Bendicho, Spectrochim. Acta B 64 (2009) 1–15.
[31] A.N. Anthemidis, K.-I.G. Ioannou, Talanta 80 (2009) 413–421.
[32] M.A. Jeannot, A. Przyjazny, J.M. Kokosa, J. Chromatogr. A 1217 (2010) 2326–2336.
[33] S. Dadfarnia, A.M.H. Shabani, Anal. Chim. Acta 658 (2010) 107–119.
[34] J.F. Liu, G.B. Jiang, Y.G. Chi, Y.Q. Cai, Q.X. Zhou, J.T. Hu, Anal. Chem. 75 (2003) 5870–5876.
[35] J.F. Peng, J.F. Liu, G.B. Jiang, C. Tai, M.J. Huang, J. Chromatogr. A 1072 (2005) 3–6.
[36] A.K.K.V. Pillai, K. Gautam, A. Jain, K.K. Verma, Anal. Chim. Acta 632 (2009) 208–215.
[37] Q. Wang, H. Qiu, J. Li, X. Liu, S. Jiang, J. Chromatogr. A 1217 (2010) 5434–5439.
[38] B.S. Furniss, A.J. Hannaford, V. Rogers, P.W.G. Smith, A.R. Tatchell, Vogel's Textbook of Practical Organic Chemistry, Longman, London, 1978, pp. 755, 756.
[39] K. Blau, J.M. Halket (Eds.), Handbook of Derivatives for Chromatography, John Wiley & Sons, Chichester, 1993.
[40] H. Lingeman, W.J.M. Underberg (Eds.), Detection-oriented Derivatization Techniques in Liquid Chromatography, Marcel Dekker, New York, 1990.
[41] M. Nenad, P. Slovenko, K. Marijan, Tetrahedron 64 (2008) 11618–11624.
[42] W.-C. Shieh, S. Dell, O. Repic, J. Org. Chem. 67 (2002) 2188–2191.
[43] A.L. Theis, A.J. Waldack, S.M. Hansen, M.A. Jeannot, Anal. Chem. 73 (2001) 5651–5654.
[44] Y. He, A. Vargas, Y.-J. Kang, Anal. Chim. Acta 589 (2007) 225–230.