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Membrane-assisted solvent extraction of seven phenols combined with large volume injection–gas chromatography–mass spectrometric detection

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Abstract

Membrane-assisted solvent extraction (MASE) was applied for the determination of seven phenols (phenol, 2-chlorophenol, 2,4-dimethylphenol, 2,4-dichlorophenol, 4-chloro-3-methylphenol, 2,4,6-trichlorophenol and pentachlorophenol) with log K_{ow} (octanol–water-partition-coefficient) between 1.46 (phenol) and 5.12 (pentachlorophenol) in water. The extraction solvents cyclohexane, ethyl acetate and chloroform were tested and ethyl acetate proved to be the best choice. The optimisation of extraction conditions showed the necessity of adding 5 g of sodium chloride to each aqueous sample to give a saturated solution (333 g/L). The pH-value of the sample was adjusted to 2 in order to convert all compounds into their neutral form. An extraction time of 60 min was found to be optimal. Under these conditions the recovery of phenol, the most polar compound, was 11%. The recoveries of the other analytes ranged between 42% (2-chlorophenol) and 98% (2,4-dichlorophenol). Calibration was performed using large volume injection (100 μL injection volume). At optimised conditions the limits of detection were between 0.01 and 0.6 μg/L and the relative standard deviation ($n = 3$) was on average about 10%. After the method optimisation with reagent water membrane-assisted solvent extraction was applied to two contaminated ground water samples from the region of Bitterfeld in Saxony-Anhalt, Germany. The results demonstrate the good applicability of membrane-assisted solvent extraction for polar analytes like phenols, without the necessity of derivatisation or a difficult and time-consuming sample preparation.

Keywords: Membrane-assisted solvent extraction; Phenols; Chlorophenols; Extraction conditions; Large volume injection; Ground water samples

1. Introduction

Phenolic compounds are present in the aquatic environment due to their industrial application. These compounds are generated in the production of plastics, dyes, drugs, pesticides, antioxidants, paper, and in the petrochemical industry. For example, pentachlorophenol is used as a wood preservative, phenol is emerged from lignin degradation in the production of paper and chlorophenols can be produced from phenols in the chlorinating of drinking water. These processes often lead to waste water and ground water contamination, hence the phenolic compounds are included in the list of priority pollutants of both the U.S. Environmental Protection Agency (EPA) and the European Union.

The determination of phenols and chlorophenols is normed by the EPA method 625, which involves liquid–liquid extraction with dichloromethane, drying and concentration of the extract and analysis with GC–MS. The achieved limits of detection (LODs) range between 1.5 μg/L (phenol) and 3.6 μg/L (pentachlorophenol) [1]. Besides the time consumption of liquid–liquid extraction, the method requires a large volume of sample and of toxic organic solvent and is difficult to automate. Solid phase extraction (SPE) is another often-applied technique for the extraction of phenols [2–4]. Additionally to carbon and silica based material there is a trend towards the usage of polymeric and modified polymeric sorbents. For example, Castillo et al. described polymeric liquid–solid extraction (LSE). Three different sorbents

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based on polystyrene–divinylbenzene polymers were applied for a variety of phenolic compounds prior to analysis with LC-UV. LODs between 0.05 μg/L (2,4-dichlorophenol) and 0.8 μg/L (pentachlorophenol) were achieved [5]. The usage of pyrole based polymers as sorbents in solid-phase extraction in combination with RP-HPLC-UV for the analysis of phenols and chlorophenols led to LODs in the ng/L range and to relative standard deviation (R.S.D.) values lower than 7% ($n = 5$) [6]. Fontanals et al. synthesized a hydrophilic polymeric resign based on 4-vinylpyridine–divinylbenzene for solid-phase extraction of polar compounds from water. SPE was combined with LC-UV and detection limits were 0.2 μg/L for phenol [7]. Compared to liquid–liquid extraction the SPE techniques are easier to automate but still they require a certain amount of organic solvents and can have a series of many different steps like drying and conditioning of the cartridges. Another alternative to extract phenols and chlorophenols in water is Solid Phase Micro Extraction (SPME), which eliminates the need of solvents. SPME has been applied to the determination of chlorophenols in landfill leaches and in wastewater [8–10]. A more polar fibre (polyacrylate) is preferred for the analysis of polar compounds [9–11].

In GC–MS analysis of polar analytes, derivatisation can be carried out. Derivatisation may lead on the one hand to better efficiency and chromatographic behaviour, since the polarity of the compounds is reduced. On the other hand, derivatisation means an additional step in the sample preparation procedure, which can be a source of errors. The combination of SPE with derivatisation generally requires complicated procedures such as purification, extraction and concentration [12,13]. In the case of acetylation of the phenols, the pH value has to be controlled carefully in order to reach optimal extraction yields [14]. Another possibility is the conversion of the phenols into methylated phenols. However, this method requires the use of diazomethane, which is carcinogenic and explosive [15].

Membrane-based extraction methods are more and more applied as sample preparation methods [16]. Main advantages are the high degree of selectivity and cleanup from complicated matrices, the very small solvent consumption and the possibility for automation and on-line coupling to analytical instruments. Jönsson and Mathiasson [17,18] developed supported liquid membrane extraction (SLM). A porous membrane, which is soaked with an organic solvent, separates the aqueous donor phase from the aqueous acceptor phase. The pH values of the two aqueous phases are different to prevent the back-extraction of the analytes into the donor phase. For supported liquid membrane extraction of phenols in water a system with *n*-undecane was used and the membrane set-up was coupled to an LC with electrochemical detection. Detection limits in the ng/L range were achieved [19].

Membrane extraction methods are also suitable for samples with high matrix contents. Phenols in crude oil were analysed using silicone membranes as a separation barrier prior to the introduction of the sample into the chromatographic system [20,21].

The method of membrane-assisted solvent extraction (MASE) is described in this paper. The membrane system is on-line coupled to the inlet of a programmed-temperature–vaporizer of a gas chromatograph with mass-selective detection. MASE has been successfully applied for the determination of non-polar compounds, such as polychlorinated biphenyls (PCBs) and semi-polar compounds (triazines, organochlorine and organophosphorus compounds) [22–26]. The purpose of this work was to optimise this fully automated extraction technique for the determination of the very polar phenols (phenol: $\log K_{ow}$: 1.46) and chlorophenols and to extract these analytes from real water samples.

2. Experimental

2.1. Chemicals and standards

An EPA phenolic standard, consisting of the seven phenols listed in Table 1, with a concentration of 500 mg/L of each phenol was obtained from Supelco (Bellfonte, PA, USA). The calibration standard was diluted to a concentration of 10 mg/L in methanol and used to spike 15 mL water samples at the μg/L to ng/L level. The solvents methanol and ethyl acetate were obtained from Merck (Darmstadt, Germany). Reagent water for optimisation and validation consisted of deionised tap water prepared from an ion-exchange cartridge.

2.2. Samples

Two ground water samples of the Bitterfeld region, Saxony-Anhalt, Germany were analysed. After the collection the samples were kept in darkness at 10 °C. For quantification the samples had to be diluted 1:10, 1:50 and 1:100 (corresponding to the calibration range) with reagent water.

2.3. Membrane-assisted solvent extraction

The device of membrane-assisted solvent extraction is produced by Gerstel (Mühlheim, Germany) and is described in several papers [23–26]. The extraction cell consists of a conventional 20 mL headspace-vial and is filled with 15 mL

Table 1
The seven phenols with their K_{ow} values and the selected SIM ions

Compound	$\log K_{ow}$	*m/z*
2-Chlorophenol	2.15	128, 139
Phenol	1.46	94, 136
2,4-Dimethylphenol	2.30	107, 122
2,4-Dichlorophenol	3.06	162, 164
4-Chloro-3-methylphenol	3.10	107, 142
2,4,6-Trichlorophenol	3.69	196, 198
Pentachlorophenol	5.12	266, 268

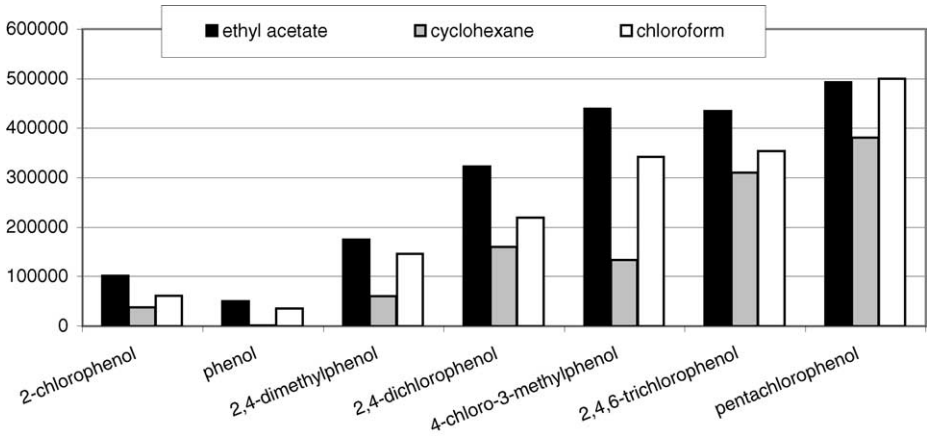

Fig. 1. Optimisation of extraction solvent (c = 6.7 μg/L, extraction: 50 min, 45 °C, 750 rpm).

of the aqueous sample. The membrane bag is 4 cm long, has a wall thickness of 0.03 mm and an internal diameter of 6 mm. It is attached to a metal funnel and fixed with a PTFE ring. The material of the membrane bag is dense polypropylene. This synthetic polymer is resistant to most organic solvents and stays stable during agitation. The membrane bag is placed into the vial, which is then closed with a metallic crimp cap. All further steps are carried out automatically with the multi purpose sampler (MPS 2, Gerstel). The membrane bag is filled with 800 μL of organic solvent and the vial is transferred into an agitator. After the optimised agitation time, the organic phase is withdrawn with a syringe from the membrane bag and transferred to a 2 mL autosampler vial. Then large volume injection is performed.

2.4. Large volume injection (LVI)–GC–MS

Chromatographic analyses were performed on an HP 6890 gas chromatograph with an HP 5973 mass selective detector (Agilent Technologies, Waldbronn, Germany) equipped with an MPS 2. Large volume injection was carried out with a temperature-programmable injector (CIS 4, Gerstel) provided with a septum-less head. One hundred microlitres of the extracted sample were injected with a 1000 μL syringe. The injection speed was optimised to 0.8 μL/s. During large volume injection the inlet temperature was maintained at 30 °C by cooling with liquid nitrogen. The vent pressure was reduced to 5 kPa and the split vent was set to 100 mL/min. After 3.6 s the split valve was closed for 1.6 min and the liner was heated at a rate of 12 °C/s to 280 °C. This temperature was held for 1 min, then the split valve was opened and heating was continued with 12 °C/s to a final temperature of 330 °C (cleaning step). The separation was carried out using a 30 m × 0.25 mm × 0.25 μm fused silica column (HP 35, USA). Helium was used as carrier gas at a flow rate of 1 mL/min (constant flow) and an initial pressure of 53 kPa. The oven temperature program was as follows: 50 °C (6 min), 5 °C/min to 75 °C, 20 °C/min to 280 °C. The ion source temperature of the mass selective detector was set to 230 °C, the quadrupole to 150 °C and the transfer line was kept at 280 °C.

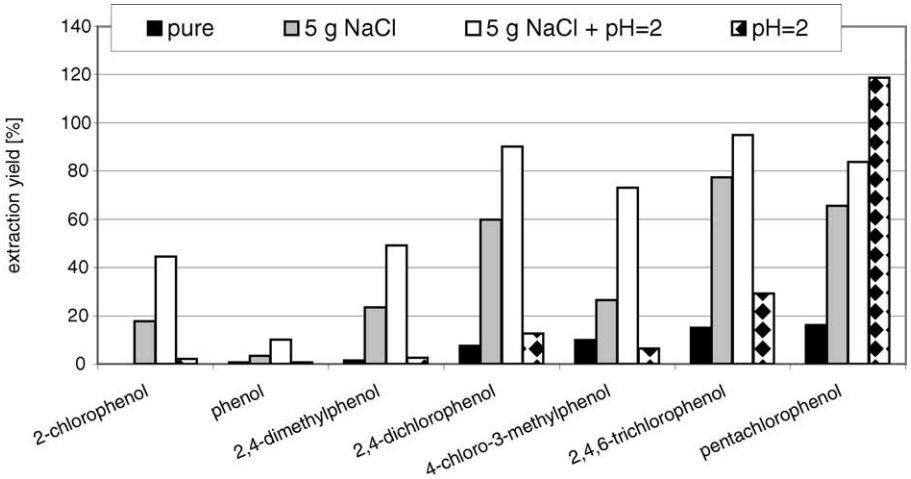

Fig. 2. Different extraction conditions (c = 6.7 μg/L, extraction: 50 min, 45 °C, 750 rpm).

The MS operated at 70 eV with electron ionisation. Samples were analysed in the full scan mode (35–400 u) for ion selection and determination of the background and in single ion monitoring mode (SIM) for optimisation and quantification.

2.5. Data processing

All data were recorded in triplicate. For optimisation studies as well as for the determination of the precision and the recovery an aqueous standard spiked at a concentration of 6.7 μg/L was used and 100 μL of the organic extract were injected. For all experiments the extraction temperature was set at 45 °C and the stirring rate at 750 rpm. The extraction yields were calculated by spiking the same amount of standard used for the preparation of the aqueous standard directly into 800 μL ethyl acetate. Calibration was performed at seven concentration levels: 0.17, 0.34, 0.67, 1.34, 3.35, 6.67, 6.67 and 13.4 μg/L. The precision was determined by a threefold extraction using three different membrane bags. Determination of the detection limits was carried out by measuring blanks consisting of reagent water six times. The detection limit was defined as the concentration corresponding to the mean blank value plus three times the standard deviation.

3. Results and discussion

3.1. Optimisation of the working parameters

3.1.1. Comparison of different types of liners

Empty baffled glass liners were used for large volume injection. The baffles, which are arranged on one half of the liner extend the surface and lead to a better adhesion of the compounds during LVI. The commercially available glass liners were used in the reverse direction with the baffles pointing towards the syringe. These liners were compared with self-made continuously baffled liners, which have an even larger surface area. For the analysis of organophosphorus pesticides the enrichment of the analytes was on average about 10% greater when the continuously baffled liners were used [25]. Also for the phenols and chlorophenols larger peak areas were achieved applying the self-made continuously baffled liners, so they were used in all further experiments.

3.1.2. Preconditioning of the membrane bags

The membrane bags underwent a preconditioning step in order to remove interfering compounds such as alkanes and phthalates, which can be coextracted from the membrane material. Before application, a twofold extraction at room temperature using cyclohexane was performed. As it was shown in a former work the membrane bags can be reused up to seven times without losing efficiency [24].

3.1.3. Optimisation of extraction solvent

Since the lowest possible extraction temperature in the agitator is 35 °C, the boiling point of the solvent should be higher than this temperature. On the other hand, the solvent has to be volatile enough to be removed through the split outlet during the large volume injection. For the determination of PCBs, triazines, organochlorine and organophosphorus compounds cyclohexane proved to be an optimal extraction solvent [23–26], so it was tested first. Using cyclohexane, the most polar compound, phenol, could not be extracted. Obviously a more polar solvent was needed. In a former work, it was noticed, that a water miscible solvent (methanol) was not suitable, because it diffuses through the membrane into the aqueous phase and the volume of the organic phase is strongly reduced after the agitation process [25]. Therefore, ethyl acetate and chloroform were tested as extraction solvents. As shown in Fig. 1 ethyl acetate gave the best results.

3.1.4. Impact of salt and pH-value

The presence of salt and a change in pH can have a significant influence on the extraction of organic compounds. It was shown that the addition of salt gave rise to a larger extraction yield. Also acid conditions to make sure that the phenols and chlorophenols are in their neutral form can improve the extraction yield. Using SPME for the determination of chlorophenols, salt addition and a pH of 2 led to an increase of extraction yield [8–11]. When the phenols were first transferred into their acetates and then analysed with HS-SPME it was also observed that salt addition resulted in higher extraction yields [27]. Applying MASE, a strong salting out effect was determined for organophosphorus pesticides and triazines [23,25,26]. Hence, both the impact of salt addition and a low pH value was tested. As expected the best results were achieved using a saturated salt solution with a pH value of 2 (Fig. 2). Only in the case of pentachlorophenol (log K_{ow}:

Table 2
Validation data for membrane-assisted solvent extraction

Compound	R.S.D. (%), $n = 3$	Recovery (%)	LOD (μg/L)	R^2
2-Chlorophenol	8.0	42.7	0.036	0.9844
Phenol	18.3	10.7	0.049	0.9866
2,4-Dimethylphenol	7.0	52.4	0.013	0.9911
2,4-Dichlorophenol	6.4	98.4	0.015	0.9944
4-Chloro-3-methylphenol	10.6	73.8	0.134	0.9923
2,4,6-Trichlorophenol	11.5	95.7	0.009	0.9921
Pentachlorophenol	12.7	95.4	0.595	0.9774

Table 3
Results of the ground water samples (Bitterfeld and Antonie) extraction: 60 min, 45 °C, 750 rpm, 5 g NaCl, pH 2

Compound	Nr.	Bitterfeld			Antonie		
		Dilution	c (μg/L)	R.S.D. (%), $n = 3$	Dilution	c (μg/L)	R.S.D. (%), $n = 3$
2-Chlorophenol	1	1:10	79.74	17.0	–	–	–
Phenol	2	1:100	866.14	16.2	1:10	92.60	9.9
2,4-Dimethylphenol	3	1:100	304.01	2.6	1:50	127.42	13.5
2,4-Dichlorophenol	4	1:10	103.21	5.7	1:50	70.13	7.5
4-Chloro-3-methylphenol	5	1:10	113.91	4.2	1:10	88.21	12.9
2,4,6-Trichlorophenol	6	1:10	54.64	2.7	1:50	216.20	2.5
Pentachlorophenol	7	1:10	79.50	11.8	1:10	3.53	14.8

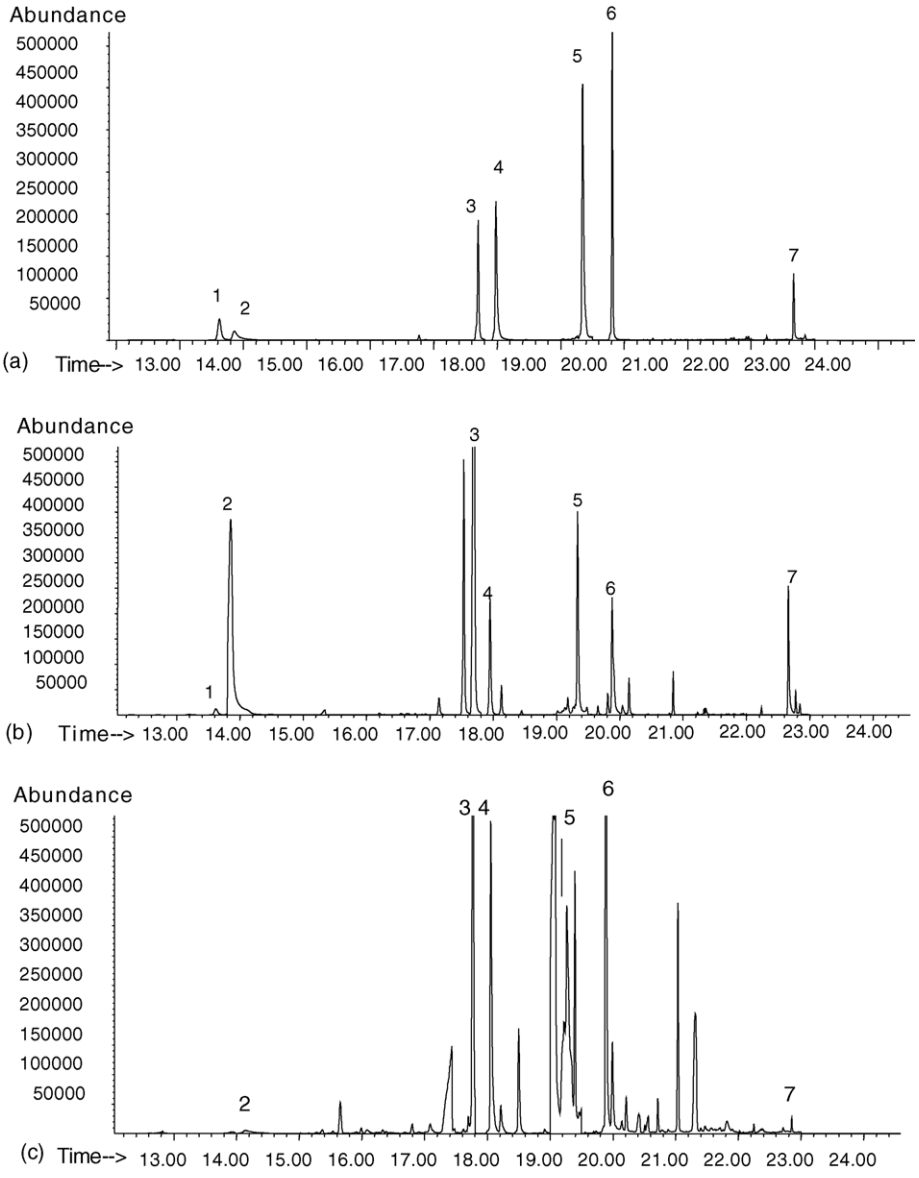

Fig. 3. Chromatograms of (a) the standard ($c = 6.7$ μg/L), (b) the first ground water sample (Bitterfeld, dilution 1:10) and (c) the second ground water sample (Antonie, dilution 1:10) extraction: 60 min, 45 °C, 750 rpm, 5 g NaCl, pH 2.

5.12) the addition of salt decreased the extraction yield compared to a pH 2 solution without salt (Table 1).

3.1.5. Extraction time

In MASE an intensive stirring is known to shorten the extraction time. Using the MPS 2 the highest possible stirring rate is 750 rpm. Applying this stirring rate and 45 °C extraction temperature, the extraction time was varied between 5 and 150 min. A strong increase from 5 to 60 min for the peak areas of all components was noticed. After 60 min the peak areas increased only slowly or even decreased a bit and reached the 60 min level again at 150 min. An extraction time of 60 min was chosen resulting in extraction yields between 10.7% (phenol) and 98.4% (2,4-dichlorophenol).

3.2. Method validation

Sample analysis was performed under the optimised conditions: addition of 5 g NaCl to each sample, pH value of 2 and 60 min extraction time. The results concerning precision, recovery, detection limits, and calibration data are summarized in Table 2. The standard deviations of the peak areas of the phenols and chlorophenols range from 6 to 18%. The correlation coefficient of the calibration graph (R^2) is between 0.9774 and 0.9944. R^2 could probably be further improved using a surrogate standard, which is suitable for the investigated analytes. The detection limits between 0.01 and 0.6 μg/L are comparable with those obtained with other extraction methods for phenols [5,7,8,10]. Considering the recovery of 94% for pentachlorophenol and 74% for 4-chloro-3-methylphenol the detection limits of 0.6 and 0.1 μg/L are relatively high, respectively. This is due to the elevated noise in this part of the chromatogram. The relatively poor recovery for phenol and chlorophenol is due to the high water solubility of these compounds. Applying SPME for the extraction of phenols and chlorophenols in wastewater the recovery is even less; 1% for phenol and 3% for 2-chlorophenol [9]. In another SPME-application, the recovery is 12% for phenol and 0.3% for 2-chlorophenol. These results are explained due to the low octanol–water-partition-coefficient of the compounds and therefore their low affinity to the SPME phase [10].

3.3. Ground water samples

After the method development based on reagent water, the method was applied to real samples. Since phenols and chlorophenols are often found in water, two contaminated ground water samples of the Bitterfeld region in Saxony-Anhalt, Germany were analysed under optimised conditions. In order to give consideration to the calibration range the samples were diluted 1:10, 1:50 and 1:100. Quantification was carried out using the calibration data for reagent water. The obtained extracts of the ground water samples were very clear and free of particles. In Table 3, the average results of three measurements are listed and Fig. 3 shows the chromatograms of a standard and the two ground water samples. To test for matrix influences, the only compound which could not be detected in the second ground water sample (2-chlorophenol) was added to the water sample at a concentration of 6.7 μg/L. Then membrane-assisted solvent extraction and the GC–MS analysis was performed and a concentration of 7.2 μg/L for 2-chlorophenol was determined using the calibration data for reagent water. The deviation is only 7%, which is by all means acceptable.

4. Conclusion

This application shows that membrane-assisted solvent extraction combined with large volume injection GC–MS is a suitable method for the analysis of polar compounds in water samples. Also for real water samples the results are reliable, as was proved by spiking one ground water sample with a compound, which was not contained in the sample. Derivatisation is not necessary. Problems of liquid–liquid-extraction can be overcome since only a small amount of organic solvent is required (800 μL) and formation of emulsions during the extraction process cannot occur.

The method is fast, fully automated and easy to perform. The low-cost polypropylene membrane bags are robust, easy to handle and after a simple cleaning procedure they can be re-applied for different matrices. The extraction yields of the phenols and chlorophenols lie in the range of 11–98% using the optimised conditions. Detection limits between 0.01 and 0.6 μg/L were achieved and are lower than those required by EPA 625.

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