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Membrane-assisted solvent extraction of polychlorinated biphenyls in river water and other matrices combined with large volume injection–gas chromatography–mass spectrometric detection

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Abstract

Membrane-assisted solvent extraction was applied to the determination of polychlorinated biphenyls (PCBs) in aqueous samples. The apparatus of membrane-assisted solvent extraction consisted of a 20 ml headspace vial which was filled with 15 ml of the aqueous sample. The membrane bag was placed into the vial and the extraction took place in an agitator. After extraction, the analytes were transferred into the inlet of a gas chromatograph by large volume injection. A mass-selective detector was used. The whole procedure was fully automated. The work included optimization of the extraction conditions (stirring rate and extraction time) and the influence of matrix effects like salt addition and the presence of organic solvents was studied. Calibration was performed using injection volumes of 100 and 400 μl. Several parameters like linearity and reproducibility of the procedure were determined. At optimized conditions detection limits in the ng/l range were achieved. The effectiveness of the method towards real samples was tested by analyzing river water, white wine and apple juice.

Keywords: Water analysis; Environmental analysis; Wine; Fruit juices; Food analysis; Membrane-assisted solvent extraction; Extraction methods; Large volume injection; Polychlorinated biphenyls

1. Introduction

Monitoring the presence of polychlorinated biphenyls (PCBs) in the environment is still important as they had a widespread use due to their chemical and physical properties. PCBs are inert, have a good chemical resistance and small electrical conductibility. They were applied as oil in condensers, additives in glue and pigments and, furthermore, as plasticisers and non-flammable materials. Because of their persistence these toxic compounds can be found in the environment and in food [1]. Hence, there is still an interest for investigating methods for determining PCBs, especially in complex matrices.

Analysis of PCBs in aqueous samples is mostly performed by conventional methods like liquid–liquid extraction [2,3] and solid-phase extraction [4] combined with a chromatographic system. These preparation methods, especially liquid–liquid extraction, are time consuming, require a large amount of organic solvents and are difficult to automate. Using sample

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volumes between 1 and 10 l, limits of detection (LODs) in the range of 0.1 μg/l can be achieved [5].

The determination of PCBs is also possible by applying newer, solvent-free or solvent-reduced methods. One way is the use of solid-phase microextraction (SPME) for analyzing PCBs in water samples. When applying 30 and 100 μm PDMS fibers, limits of detection between 50 and 100 ng/l are achieved [6,7]. The reproducibility, expressed by the relative standard deviation (R.S.D.) is between 8 and 14%. Problems can also occur concerning carry over effects. Therefore, blank extractions between each sample are necessary to check the performance of the used fiber [6]. The method of stir bar sorptive extraction (SBSE) reaches detection limits in the ppt range [8]. Maximum recovery values vary around 50–60% for each PCB. In-vial extraction and direct injection of water samples combined with large volume injection lead to LODs from 30 to 50 ng/l [9].

Using membrane materials for extraction of organic compounds has become more and more popular during the last years. Major advantages are the small amount of solvent required and the little time consumption of this procedure. There is no formation of emulsions, a problem which can occur in conventional liquid–liquid extraction. Jönsson and Mathiasson presented the technique of supported liquid membrane extraction (SLM) [10,11]. The system is based on a three phase system with an organic phase between two aqueous phases. The organic phase consists of a porous membrane which is soaked with an organic solvent. The acceptor solution has a different pH value according to the donor phase. This makes sure that the analytes in the acceptor phase are ionized and can not pass the membrane again in direction to the donor phase. In this way a high enrichment of the analytes is achieved. This method is used for polar compounds and has, for instance, been applied to carboxylic acids [12] and amino acids [13,14]. Another membrane system also described by Jönsson and Mathiasson, is the microporous membrane liquid–liquid extraction (MMLLE) [10]. In this modification the extraction process takes place via a hydrophobic membrane which is situated between an aqueous donor phase and an organic acceptor phase. The membrane is wetted with the organic solvent of the acceptor phase which fills the pores of the membrane. There is a direct contact between the organic and the aqueous phase close to the surface of the membrane and the mass transfer is proceeded at that surface [15,16]. MMLLE offers a good possibility to handle non-polar compounds. The membrane methods can easily be combined with chromatographic systems [17–20] and capillary electrophoresis [21,22].

Membrane-assisted solvent extraction combined with large volume injection with gas chromatography and mass spectrometric detection was recently described by Hauser et al. [23]. Organic compounds, which are dissolved in an aqueous sample, are diffusing through a nonporous membrane into an organic solvent. The method has been applied in two different modifications for chlorobenzenes and triazines [23,24]. Since the analytes are enriched by the transfer into a small organic volume and in the inlet of the gas chromatograph, no further preconcentraction steps are necessary. The purpose of this work was to optimize the membrane-assisted solvent extraction for PCBs in order to determine these hydrophobic compounds in water and other complex samples.

2. Experimental

2.1. Chemicals and standards

Analytical grade cyclohexane and acetonitrile as well as sodium chloride were obtained from Merck (Darmstadt, Germany). Reagent water for optimization and validation consisted of deionized tap water. One standard mix of PCBs in acetonitrile (10 ng/μl) and one in *iso*-octane (10 ng/μl) as well as the internal standard 2,3′,4,4′,5-pentachlorobiphenyl were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Spiking different volumes of the undiluted and diluted stock solution, two batches were prepared for the calibration curve: one in 10 ng/l to 20 μg/l range (for the 100 μl injected volume) and another in the range of 1 ng/l to 5 μg/l (for the 400 μl injected volume), then a fixed volume of internal standard was added to each sample to give a final concentration of 1.5 μg/l.

2.2. Samples

Water samples were collected at the river “Weisse Elster” in Leipzig in March 2003. White wine “Müller Thurgau” (Rheinsberg Kellerei, Bingen, Germany)

and apple juice (Libehna Fruchtsaft, Raguhn, Germany) were bought in a supermarket. All samples were kept in darkness at 10 °C.

2.3. Membrane extraction

The principle of membrane-assisted solvent extraction is the transport of organic compounds through a membrane bag into a small amount of organic solvent. In this application the material of the membrane is dense polypropylene. This synthetic solid polymer has a good stiffness and it stays stable even during the highest possible agitation speed. Moreover, polypropylene is resistant to most organic solvents. The device of membrane-assisted solvent extraction produced by Gerstel (Mühlheim, Germany) is shown in Fig. 1. A 20 ml headspace vial is filled with 15 ml of the aqueous sample. Then the membrane bag (4 cm long with a wall thickness of 0.03 mm and an internal diameter of 6 mm) which is attached to a steel funnel and fixed with a PTFE ring is placed into the vial and filled automatically with 800 μl of cyclohexane. Cyclohexane was chosen because it has a low solubility in water, it is not diffusing through the membrane into the aqueous phase. Besides, it is volatile enough to be removed through the split outlet during large volume injection. The extraction takes place inside an agitator. After a fixed agitation time, the organic phase is withdrawn with a syringe from the membrane bag (contained inside of the headspace vial) and automatically injected into the inlet of the gas chromatograph.

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

Chromatographic analyses were performed on HP 6890 gas chromatograph equipped with a HP 5973 mass-selective detector (Agilent Technologies, Waldbronn, Germany). The separation was carried out with a fused silica column (SPB 5, Supelco, Bellefonte, USA), 30 m × 0.25 mm I.D. and 0.25 μm thickness coating. 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 was programmed at 15 °C/min from 50 to 200 °C, held for 1 min isothermally, then programmed at 8 °C/min to a final tesmperature of 300 °C, which was held for 3 min isothermally. The MS conditions were as follows: the ion source temperature was set to 230 °C, the quadrupole to 150 °C and the transfer line was kept at 300 °C. The instrument operated at 70 eV with electron ionization. Samples were analyzed in full scan mode for ion selection and determination of the

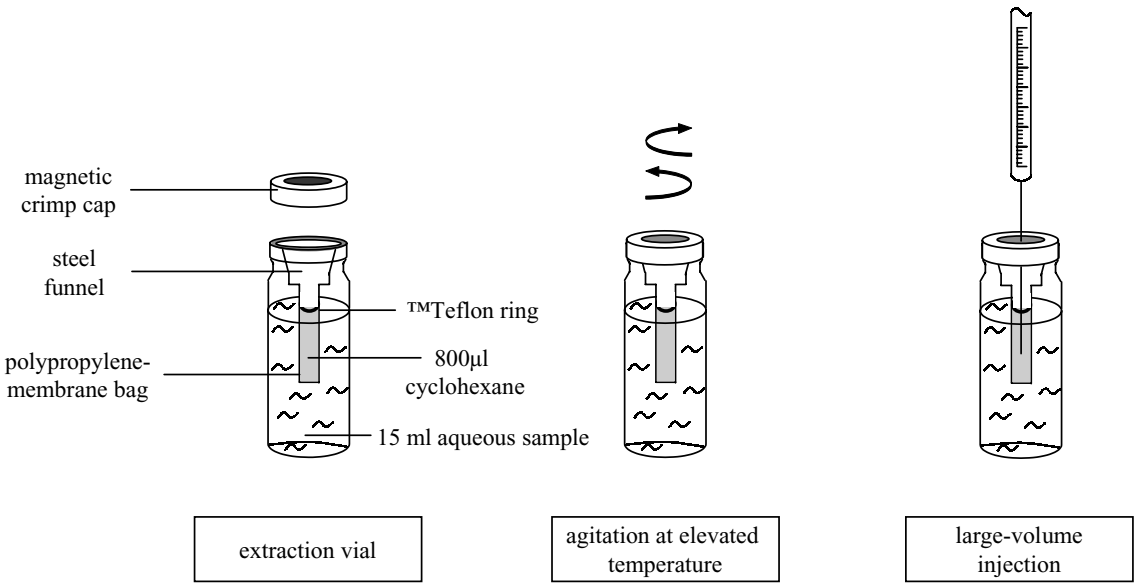

Fig. 1. Device of membrane-assisted solvent extraction.

Table 1
The six PCBs and the internal standard with their octanol–water partition coefficients ($K_{o/w}$), water solubilities and the selected SIM ions

PCB [25]	log $K_{o/w}$	Structure	Water solubility at 25 °C (mg/l) [26]	SIM ions
28	5.74	2,4,4′-Trichlorobiphenyl	0.65	256, 186, 75
52	6.25	2,2′,5,5′-Tetrachlorobiphenyl	0.26	292, 220, 110
101	6.85	2,2′,4,5,5′-Pentachlorobiphenyl	0.099	326, 254, 127
138	7.00	2,2′,3,4,4′,5′-Hexachlorobiphenyl	0.038	360, 290, 145
153	7.44	2,2′,4,4′,5,5′-Hexachlorobiphenyl	0.038	360, 290, 145
180	8.28	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	0.014	394, 324, 162
118 Internal standard	6.25	2,3′,4,4′,5-Pentachlorobiphenyl	0.099	326, 254, 184

background and in single ion monitoring (SIM) mode (Table 1) for quantification. Large volume injection was performed using a multi purpose sampler (MPS 2) from Gerstel. The injection system consisted of a septumless head and a temperature-programmable injector (cooled injection system, CIS 4) provided with an empty baffled glass liner. A 1000 μl syringe was used and 100 μl of the extracted sample were injected. For calibration the injection volume was increased to 400 μl. The injection speed for both volumes was set at 0.8 μl/s. During large volume injection the inlet temperature was maintained at 45 °C by cryocooling with liquid nitrogen. The vent pressure was reduced to 5 kPa and the split vent was set to 100 ml/min. After 0.08 min the split valve was closed for 1.6 min and the liner was heated at 12–250 °C/s. This temperature was held for 1 min, then heating was continued with 12 °C/s to a final temperature of 330 °C (cleaning step).

2.5. Data processing

All data were recorded in triplicate. For optimization an aqueous standard spiked at a concentration of 1 μg/l was used and 100 μl were injected. The extraction temperature was set at 45 °C for all experiments. The extraction yields were calculated by spiking the same amount of standard used for preparation of aqueous standard directly into 800 μl cyclohexane. The reproducibility for the membrane bags was determined by six-fold extraction using three different membrane bags. Furthermore the standard deviation for the method was calculated by three-fold extraction using the same membrane bag. Calibration graphs were based on peak areas versus the peak area of the internal standard 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118). Determination of the detection limits was carried out measuring samples of reagent water six times. The detection limit was defined as the peak area at the retention time of each PCB in the blank corresponding to the mean plus three times the standard deviation.

3. Results and discussion

3.1. Optimization of the extraction parameters

3.1.1. Preconditioning of the membrane bags

The membrane bags underwent a preconditioning step in order to remove interfering compounds like alkanes and phthalates, which were coextracted from the membrane material. Before application a two-fold extraction at room temperature using cyclohexane was performed. The stirring rate was set to 60 rpm. A seven-fold extraction using the same membrane bag each time proved that the membrane bags could be reapplied without losing efficiency (Fig. 2).

3.1.2. Optimization of the stirring rate

For improving the transport of the analytes through the membrane the extraction vials were stirred in the agitator at different stirring rates between 250 and 750 rpm. For all PCBs increasing stirring rates gave rise to a larger extraction yield (between 25 and 40%). Hence, the highest possible stirring rate of 750 rpm was chosen.

3.1.3. Optimization of extraction time

The extraction time was varied between 5 and 70 min. A significant increase of the extraction yield from 5 to 30 min was observed. After 30 min the equilibrium for all analytes is nearly achieved and the

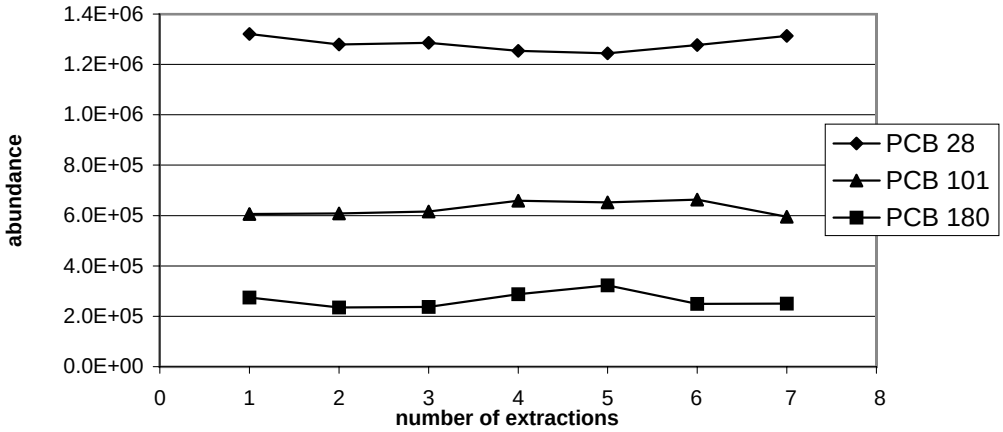

Fig. 2. Seven-fold extraction, shown for three PCBs, same membrane bag, 1 μg/l each compound, extraction conditions: 45 °C, 30 min, injection volume: 100 μl.

extraction yields lie between 39 and 82%. Therefore, 30 min extraction time was chosen for all further analysis.

3.2. Influence of matrix compounds

The influence of different matrix compounds on membrane extraction was studied (Fig. 3). The addition of the sodium salt of humic acid had a relatively low impact on extraction yields, thus there is no big influence of organic matter. A salting out effect was not observed and was not expected because of the low water solubility of the analytes. The saturation of the sample with sodium chloride even decreased the extraction yields of the PCBs. The presence of methanol led to a higher enrichment of the compounds. This effect has been observed before and can be explained by decreased glass adsorption of the PCBs when an organic solvent is added to the aqueous sample [8].

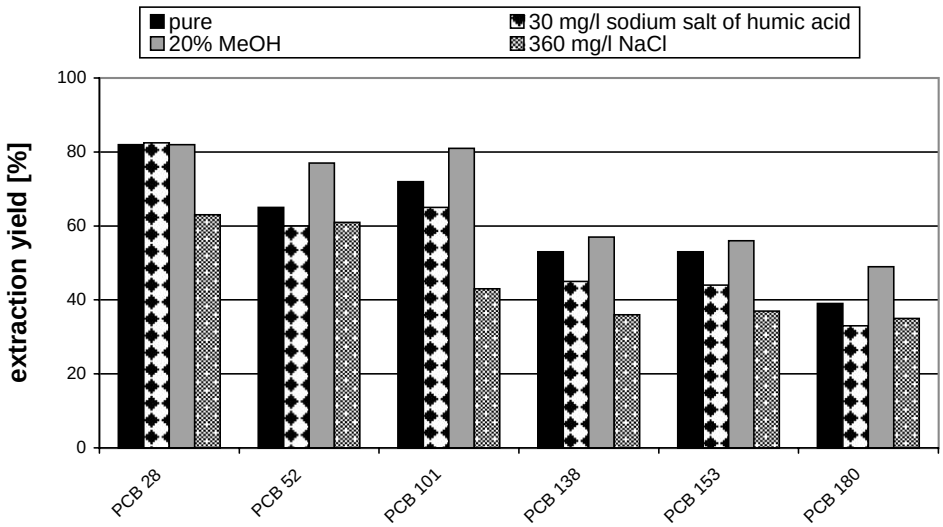

Fig. 3. Influence of matrix compounds on the extraction yield, spiked to 1 μg/l of each PCB, 30 min extraction time, 45 °C, 750 rpm, injection volume: 100 μl.

Table 2
Validation data for membrane-assisted solvent extraction

PCB	100 μl injection			400 μl injection		
	LOD (ng/l)	Linear dynamic range (μg/l)	Correlation coefficient	LOD (ng/l)	Linear dynamic range (μg/l)	Correlation coefficient
28	4	0.004–25	0.9986	2	0.002–5	0.9971
52	11	0.011–25	0.9995	3	0.003–5	0.9987
101	9	0.009–25	0.9988	3	0.003–5	0.9994
138	21	0.021–25	0.9956	9	0.009–5	0.9981
153	22	0.022–25	0.9946	10	0.010–5	0.9999
180	27	0.027–25	0.9948	10	0.010–5	0.9981

3.3. Validation of the procedure

Membrane-assisted solvent extraction was performed using optimized conditions: 750 rpm and 30 min extraction time. The results of calibration data, detection limits and linear dynamic range are listed in Table 2. When injecting 100 μl the linear range is situated between 0.004 and 25 μg/l. For 400 μl injection volume the extracted amount was linear between 0.002 and 5 μg/l. The correlation coefficient was 0.995 or better for 100 and 400 μl injection volumes. For an injection volume of 400 μl detection limits between 2 and 10 ng/l were achieved. This demonstrates the potential and the sensitivity of the method for determining organic analytes in aqueous samples.

In Table 3 the reproducibility of the membrane bags and of the method are presented. The relative standard deviations using the same bag are very low (below 2%). Using different membrane bags R.S.D. was between 3.7 and 12.2%, indicating that the measurements can be performed under these conditions, too.

Table 3
Relative standard deviation, 1 μg/l each compound, 100 μl injection volume

PCB	R.S.D. (same bag) (%, $n = 3$)	R.S.D. (three different bags) (%, $n = 6$)
28	1.1	7.8
52	0.7	4.3
101	1.2	3.7
138	1.0	7.3
153	1.2	9.1
180	1.7	12.2

3.4. Environmental and food samples

In order to test the efficiency of the method for samples with complex matrices, river water, white wine and apple juice were analyzed. No PCBs were found in the samples. Therefore the samples were spiked with the selected PCBs to a concentration of 0.5 ng/ml

Table 4
Results of the spiked samples

PCB	Spiked amount (ng/ml)	River water		White wine		Apple juice	
		Detected amount (ng/ml)	Recovery[a] (%)	Detected amount (ng/ml)	Recovery[a] (%)	Detected amount (ng/ml)	Recovery[a] (%)
28	0.50	0.44	88	0.57	114	0.49	98
52	0.50	0.44	88	0.53	107	0.50	100
101	0.50	0.45	92	0.53	105	0.53	106
138	0.50	0.48	96	0.52	105	0.52	104
153	0.50	0.44	88	0.51	101	0.52	104
180	0.50	0.50	100	0.46	92	0.47	94

[a] Percentage values obtained considering extraction recoveries in reagent water (Fig. 3) as 100%.

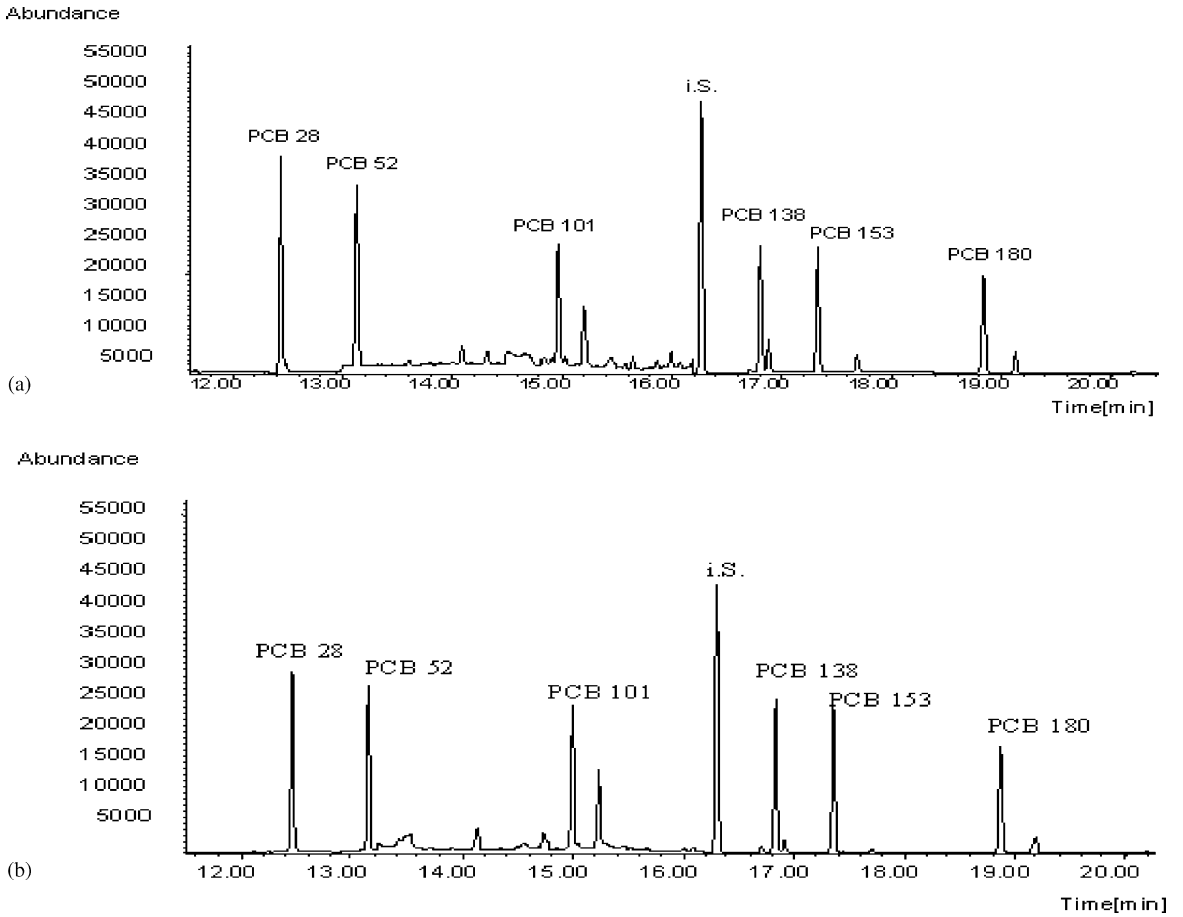

Fig. 4. Chromatograms of spiked water and apple juice samples, 30 min extraction time, 45 °C, 750 rpm, injection volume: 400 μl. (a) Pure water spiked to 0.5 μg/l. (b) Apple juice spiked to 0.5 μg/l.

for each PCB. Quantification of the spiked samples was performed according to the described procedure, 400 μl were injected. The concentration of the PCBs represent an average of three measurements for each sample.

The results in Table 4 show recoveries between 88 and 114%. When extracting the three samples the membrane showed a sufficient exclusion of particles, as was reflected in obtaining clear organic extracts. This is also shown in the received chromatograms. A sample of apple juice, spiked to 0.5 μg/l is presented in Fig. 4. These results prove the independence of the method towards matrix compounds in complex systems.

4. Conclusion

Membrane-assisted solvent extraction is a simple, solvent-reduced technique. Polypropylene membranes have the advantage of low cost, easy handling and after a simple cleaning procedure, they can be reused for different matrices. The whole procedure including filling the membrane bag, agitating the sample and large volume injection can be fully automated. Due to enrichment of the analytes in the organic phase and during large volume injection detection limits in the ng/l range can be obtained. For real samples a good recovery is achieved indicating that the method is almost independent of

the matrix of the samples. Thus, the method has a promising applicability for environmental and food analysis.

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