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Membrane-Assisted Solvent Extraction of Triazines and Other Semi-volatile Contaminants Directly Coupled to Large-Volume Injection / GC-MS

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Introduction

We can manage without crude oil, but not without water. Hence, it is obvious that we should test water for possible contamination; not just drinking water but especially waste and surface water, in order to discover any contamination. The analysis can be complicated if the water contains a large proportion of suspended substances and foreign particles. Membrane extraction promises simplification in this case: a novel procedure for sample preparation developed by the Environmental Research Centre Leipzig-Halle, which has been licensed and brought into and perfected for series production by GERSTEL.

Numerous standard sample preparation procedures for chromatographic methods, according to accepted laboratory procedures, are based on liquid-liquid extraction (LLE), even though this technique is marred by several disadvantages: many steps are difficult to automate and relatively large volumes of, sometime toxic, organic solvent are involved. The extract must sometimes be cleaned up in several stages, concentrated and evaporated, in order to achieve adequate

detection limits. Existing alternatives to LLE, in effect, do not exist, they still do not comply with many of the users wishes. A change is in sight:

Membrane-assisted solvent extraction has recently been introduced [1]. It is based on small-scale LLE with a flat, low density polyethylene (LDPE) membrane separating the aqueous sample from the organic solvent. Like liquid phase micro-extraction (LPME) membrane-assisted extraction is carried out off-line in a vial, from which the organic extract is transferred to a sample vial; this is followed by large-volume injection (LVI).

The extraction device for the membrane-assisted LLE was modified for this investigation, so that it fitted a conventional 20 mL headspace vial. For this purpose a tube with an external diameter (OD) of 6 mm was hot-sealed from nonporous polypropylene membrane 0.05 mm thick and converted into a membrane sac, which was attached to a stainless steel funnel and placed in a 20 mL glass vial, which contained 15 mL of the aqueous sample. GERSTEL now supplies ready-to-use membrane sacs with walls only 0.03 mm thick.

A GERSTEL MultiPurposeSampler MPS 2 is used, this is capable of filling the membrane sac with 500 µL of an organic solvent, agitating at defined temperature and carrying out an LVI of the extract directly.

METHOD

Preparation of the standard. Methanol solutions were prepared of: pure standard substances at 1 µg/µL, mixed working standards of 0.05, 0.5, 5 and 50 ng/µL. Methanolic solutions of simetryn (50 ng/µL) and pentachlorobenzene (20 ng/µL); simetryn was used as internal standard for optimization of the extraction parameters and added directly to the extraction solvent hexane.

Pentachlorobenzene was used for the calibration of the membrane-assisted LLE and added to the aqueous samples before extraction. The direct calibration of LVI/GC-MS was carried out with mixed standards: 1 - 500 pg/µL in hexane for 10 µl injection; 0.01 - 100 pg/µL for 100 µL injection.

Aqueous standards were prepared for membrane-assisted LLE: suitable aliquots of methanol mixed standard were diluted with 15 mL water, whereby the methanol content did not exceed 0.2 percent by volume. Each water sample was treated with 5 g sodium chloride in order to promote extraction of the triazines.

Membrane-assisted solvent extraction. Figure 1 illustrates the device for membrane-assisted solvent extraction. Conditioning is carried out by extracting 8 to 10 membrane sacs three times with 50 mL portions of hexane at room temperature. The vial was filled with 15 mL of an aqueous sample, the membrane sac was attached to the metal funnel with a Viton ring and the funnel was suspended in the mouth of the vial. The membrane sac was then filled with 500 µL hexane to which had been added 1 µL of the simetryn internal standard and the vial was sealed with a metal crimp cap.

The vials were placed in the agitator of the MPS for extraction and agitated at a defined temperature for a fixed time. They were then automatically removed from the MPS and transferred to the sample tray. A microlitre syringe was used for the manual removal of the organic extract from the membrane sac and its transfer to 2 mL sampling vials.

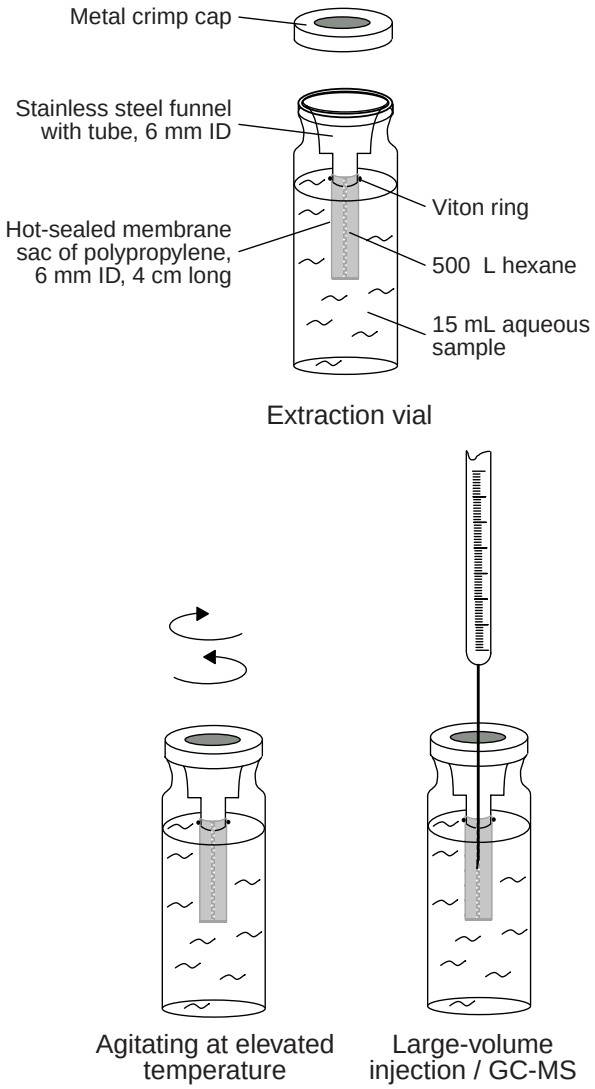

Figure 1. Experimental design for membrane extraction.

Publisher's note: At the time of the investigation the software of the MultiPurposeSampler only permitted single-step operation and not the processing of sequences of automated membrane-assisted LLE of multiple samples. All steps are now carried out automatically.

Apparatus
Gas chromatograph 6890 (Agilent Technologies)
Mass spectrometer 5973 (Agilent Technologies)
MultiPurposeSampler MPS 2 (GERSTEL)
Cold Injection System CIS 4 (GERSTEL)

Analysis conditions

CIS 4:	0.08 min solvent vent (100 mL/min) at 5 kPa, splitless sample transfer 20°C (0.12 min), 12°C/s, 250°C (1 min), 12°C/s, 330°C (3 min)
Column:	30m HP-5 (Agilent), d_i= 0.25mm, d_f= 0.25mm
Pneumatics:	He, P_i= 53 kPa (initial), Constant flow = 1 mL/min
Oven:	50°C (2 min), 10°C/min, 160°C (1 min), 3°C/min, 200°C (1 min), 10°C/min, 250°C (2 min)
MSD:	Scan 30 – 350 amu

LLE of river water. The quantitative results of membrane-assisted LLE were compared with an in-vial LLE without membrane, by spiking 15 mL river water in a 20 mL headspace vial with 1 and 5 µg/L per analyte and 1.3 µg/L pentachlorobenzene as internal standard. After addition of 1 mL hexane the vial was sealed with a metallic crimp cap, agitated for 30 minutes at 35°C and 750 rpm in the agitator of the MPS 2. The organic layer was then removed with a microlitre syringe and transferred to a sample vial. The large-volume injection for GC/MS analysis was made using an injection volume of 100 µL (1 µL/s). Direct calibration of the LVI/GC-MS with mixed standards in hexane and an injection volume of 100 µL was used to calculate the concentrations in the spiked river water.

Results and discussion

Membrane-assisted solvent extraction. During membrane-assisted solvent extraction hydrophobic, organic compounds are extracted via an dense polypropylene membrane into a small volume of organic solvent. Relatively non-polar solvents should be used, since they have a low solubility in water, in order to avoid loss of solvent through the membrane. Alternatively, it is also possible to use very polar solvents with good water solubility: their lipophobic properties prevent their passage through the membrane. For instance, polyaromatic hydrocarbons have been successfully extracted from aqueous samples with acetonitrile in the course of an HPLC investigation.

Again the solvent should not be too volatile, since then it could diffuse through the membrane into the headspace of the sample and condense there. However, it must be volatile enough to be effectively removed via the split outlet during LVI. (Editorial note: for this application cyclohexane has proved to be a very suitable solvent in the case of membrane sacs, commercially available from GERSTEL, having a wall thickness of 0.03 mm.)

Table 1. Influence of the matrix components on extraction yields in membrane extraction (per component spiked at 6.7 µg/L, extraction for 1 hour at 35°C and 750 rpm; injection volume 10 µL)

Component	Extraction yield [%]			
	from pure water	plus 333 g/L NaCl	plus 6.6 vol-%.MeOH	at pH 8
2,4-Dichloroaniline	66.1	90.6	58.5	68.2
α-HCH	107.6	96.2	104.5	69.2
Simazine	1.7	30.3	1.5	1.5
Prometon	6.4	74.5	5.0	4.5
Atrazine	5.0	69.4	3.9	4.5
Propazine	16.7	85.1	10.7	14.1
Phenanthrene	107.1	92.9	103.4	107.6
Ametryn	21.4	94.5	14.8	19.1
Prometryn	54.8	85.9	38.1	48.9
Terbutryn	76.0	89.3	57.9	71.2

Optimization of the extraction parameters- Influence of the matrix components. Table 1 shows the optimized influence of factors on membrane-assisted solvent extraction: salt, methanol concentration and pH.
The extraction yield of triazines is increased if the solutions are saturated with salt (NaCl); these are relatively polar analytes. In contrast the recovery rates for the non-polar components, α-HCH and phenanthrene, were reduced.

Increasing the methanol content to 6.66% by volume had no significant effect on the extraction of most components, with the exception of the S-triazines, whose recovery rates were reduced by up to 20%.

The pKa values of the triazines range from 1.6 (Simazine) to 4.3 (Prometon). The pH of the aqueous sample should, therefore, be a little over 6; raising the pH above this level does not improve the extraction yield. The aqueous samples were saturated with 333 g/L NaCl (5 g NaCl to 15 mL water) for all extractions.

Optimization of the agitating rate. In order to improve the transport of the analytes through the membrane into the organic solvent, the sample must be well mixed and the boundary layers minimized. The agitating rate of the MPS 2 was varied between 250 and 750 rpm; the recovery rate of all analytes was increased by 30-50% up to 500 rpm, while they fell again from 500 to 750 rpm. Mixing was found to be more important for the tiazines than for the non-polar components, α-HCH and phenanthrene; hence, the agitating rate of the MPS was set to 750 rpm for all further investigations.

Optimization of the temperature. It is possible to operate the agitator of the MPS 2 at a defined temperature. The recovery rate of all components improved by 10 to 30% if the temperature of agitating was raised from 35 to 55°C (hexane boils at 69°C), this was particularly marked for the water-soluble triazines, less for 2,4-dichloroaniline, α-HCH and phenanthrene. Extraction for the validation results for the 10 µL injection below was carried out at 55°C.

Optimization of the extraction time. Extraction for 30 minutes led to an optimal enrichment of all components (see Figure 2); prolonging extraction further did not lead to a better result. After 30 minutes the recoveries were between 60 and 100 %; adequate for validation of the method:

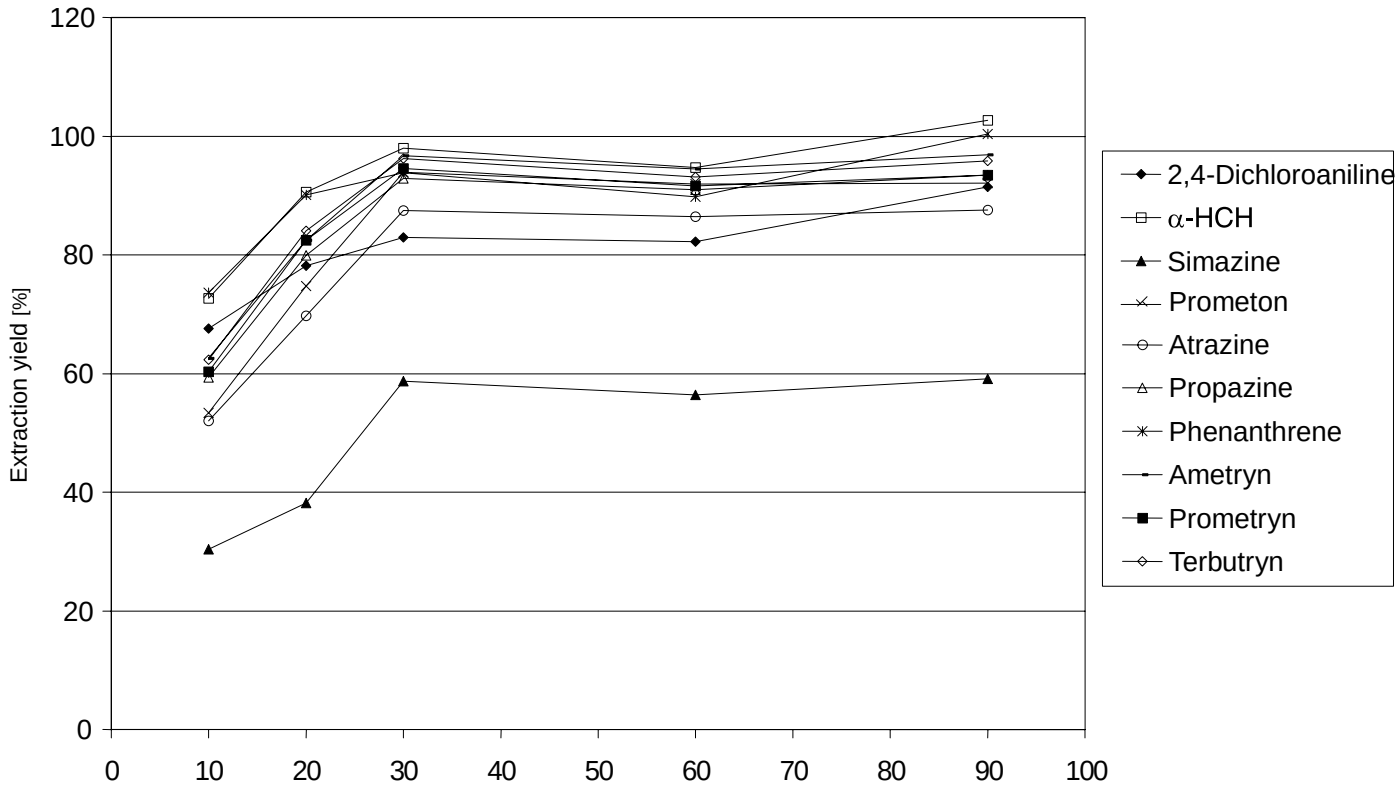

Figure 2. Optimization of the extraction time (6.7 µg/L of each component, 333 g/L NaCl, 55 °C, 750 rpm, injection volume 10 µL)

Validation of the method. The efficiency of the membrane-assisted solvent extraction was determined under optimized extraction conditions; Table 2 shows the results of the validation. The linear dynamic range was determined by extraction of spiked aqueous samples and it is between 0.05 and 100µg/L for 10 µL; the correlation coefficient was 0.9965 or higher. The determination limits, reached after 30 minutes of extraction, were 10 to 100 ng/L.

Table 2. Results of validation of membrane extraction (10 µL injection (a): extraction time 30 min, 333 g/L NaCl, 750 rpm; *6.7 µg/L of each component. 100 µL injection (b): extraction time: 1 hour, 333 g/L NaCl, 45°C, 750 rpm.

	10-µL injection (a)				100 µL injection (b)		
Component	Reproducibility* 30 min extraction RSD [%] (n=5)	Detection limit [ng/L]	Linear dynamic range [µg/L]	Correlation coefficient (R2)	Detection limit [ng/L]	Linear dynamic range [µg/L]	Correlation coefficient (R2)
2,4-Dichloroaniline	2.1	10	0.05 – 100	0.9971	5	0.005 - 5	0.9971
α-HCH	5.2	25	0.05 – 100	0.9987	10	0.01 - 10	0.9990
Simazine	10.4	100	0.1 – 100	0.9999	5	0.005 - 10	0.9942
Prometon	13.3	50	0.1 – 100	0.9965	5	0.005 - 10	0.9987
Atrazine	8.4	50	0.1 – 100	0.9991	1	0.005 - 10	0.9979
Propazine	11.9	50	0.1 – 100	0.9984	5	0.005 - 10	0.9994
Phenanthrene	3.7	10	0.05 – 100	0.9990	1	0.1 - 10	0.9998
Ametryn	10.7	50	0.1 – 100	0.9981	5	0.005 - 10	0.9993
Prometryn	14.3	50	0.1 – 100	0.9998	5	0.005 - 10	0.9970
Terbutryn	13.1	50	0.1 – 100	0.9993	5	0.005 - 10	0.9973

Hence, the method meets the requirements of the German drinking water regulations [2] (0.1 µg/L for individual pesticides) and the drinking water recommendations (2 µg/L atrazine and simazine) of the World Health Organization [3].

The limits of determination depend on blank values for co-extracted matrix components; they came from the hot-sealed polypropylene membrane sacs. The co-extracted components were also determined in the single ion mode. Figure 3 shows the chromatogram of an extract, that was obtained from a membrane-assisted solvent extraction of water, which had been spiked to 50 ng/L, demonstrating the high background resulting from a 100 µL injection, which reduced the accuracy of peak integration at lower concentrations.

The whole extraction procedure was found to be very reproducible. The relative standard deviation for five successive extractions lay between 2.1 and 13.3 %. Then 100 µL extract was injected in order to improve the detection limits. This yielded detection limits of 1 – 10 ng/L and a linear dynamic range of 0.005 – 10 µg/L, with correlation coefficients of 0.9970 or higer.

The results of validation revealed: the semi-automatic membrane-assisted LLE is a reliable sample preparation technique for aqueous samples. If injection volumes of 100 µL are selected, the sample addition step to the membrane sac can be integrated into the automatic procedure. The sampler can be equipped with a 1000 µL syringe, which can be used for the precise addition of 500 µL hexane and for the LVI of 100 µL extract after agitating.

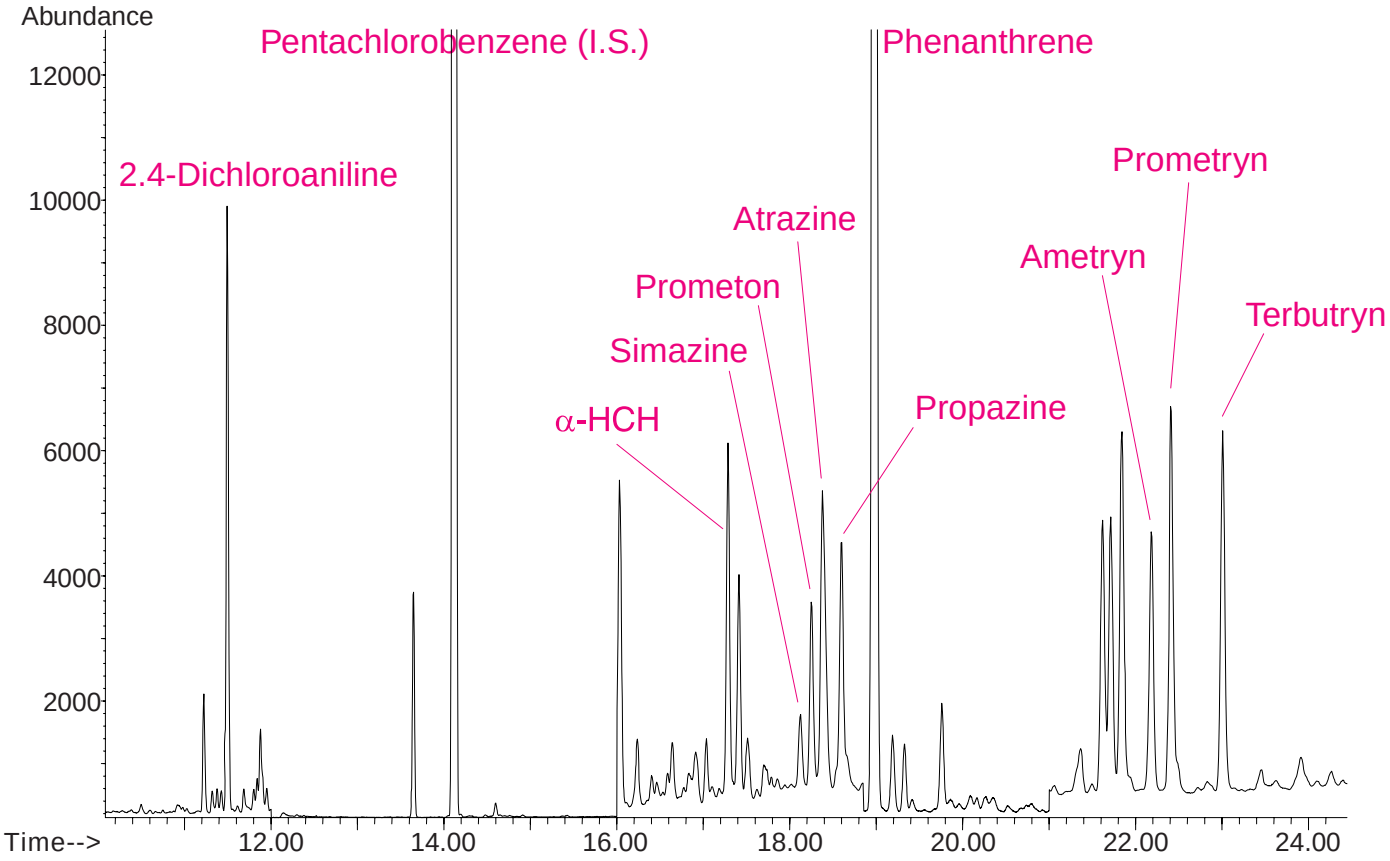

Figure 3. LVI/GC-MS chromatogram of single-ion monitoring after membrane extraction of 15 mL water spiked to 0.05 µg/L of each component (extraction time: 1 hour., 333 g/L NaCl, 45°C, 750 rpm, injection volume 100 µL).

Membrane contra in-vial extraction. The results of membrane-assisted solvent extraction of spiked river water samples were compared with in-vial extraction (Table 3). The river water samples were taken from the Weiße Elster in Leipzig and spiked to 1 and 5 µg/L using methanolic composite standards. The calculation of the quantities in the spiked river water, that was extracted by membrane-assisted LLE, was carried out after calibration with aqueous standards (0.001 – 10 µg/L), that were extracted under identical conditions. The in-vial LLE was carried out as described above.

The recovery rate for in-vial LLE was 100 %. However, difficulty was experienced in taking off the organic extract, because the organic layer was very thin and the phase boundary was disturbed by particles. It is necessary to use at least 4 mL hexane for the automated in vial LLE to permit removal of the extract by the sampler, while obtaining clean phase separation and a clear extract – without the risk of taking up and injecting water. The detection limit is a factor of 8 lower than for the membrane-assisted solvent extraction.

However, the analytical accuracy of the in-vial extraction exceeded that of the membrane-assisted solvent extraction (Table 3). The average deviation of the analytical result from the spiked concentration revealed: with 1 µg/L spiked river water 12.4% for in-vial extraction and 23.9% for membrane-assisted solvent extraction.

On the other hand, the membrane-assisted solvent extraction is simpler to carry out: if an injection volume of 100 µL was selected, the whole procedure, that is extraction and injection, could be completely automated.

Table 3. Analytical results [µg/L] for spiked river water; comparison of membrane extraction[a] with in-vial LLE[b] (a: extraction time 1 hour, 333 g/L NaCl, 45°C, 750 rpm, 1.3 µg/L pentachlorobenzene as internal standard in water. b: extraction time 30 min, 333 g/L NaCl, 45°C, 750 rpm, 1.3 µg/L pentachlorobenzene as internal standard, 1 mL hexane as extraction solvent, injection volume 100 µL).

	River water, spiked to 1 µg/L		River water, spiked to 5 µg/L	
Components	Membrane extractiona [µg/L]	In-vial LLEb [µg/L]	Membrane extractiona [µg/L]	In vial LLEb [µg/L]
2,4-Dichloroaniline	0.98	1.67	4.34	6.7
alpha-HCH	1.18	1.33	6.04	5.68
Simazine	1.28	0.85	5.83	3.49
Prometon	1.30	1.05	6.31	4.51
Atrazine	1.33	1.07	6.10	4.53
Propazine	1.31	1.11	6.22	4.71
Phenanthrene	1.07	1.10	6.29	4.76
Ametryn	1.29	1.12	6.32	4.84
Prometryn	1.36	1.12	6.45	4.70
Terbutryn	1.25	1.10	6.23	4.69
Average deviation from spiked concentration [%]	23.9	18.2	22.9	12.4

Summary

The MultiPurposeSampler MPS 2 makes it possible to automate membrane-assisted solvent extraction, which has been shown to be a promising enrichment technique for various organic components, including polar analytes, such as triazines: optimized conditions involving extraction for 30 minutes give yields of 60 to 100%. The detection limits are in the lower ng/L range.

Since the non-porous polypropylene membranes prevent water, salt, particles and macromolecular components from entering the organic extract, membrane extraction is particularly suitable for complex samples with a high organic content.

Membrane extraction is particularly suitable for applications in food analysis and bio-analysis. Worthy of note: selection of a solvent, that is miscible with water, such as acetonitrile, makes it possible to couple the method with HPLC. Polar solvents do not dissolve in the membrane material and, hence, cannot enter the aqueous sample.

When used with GERSTEL MASter software, mode: sample preparation with the MPS 2 permits automation of a sequence of membrane-assisted solvent extraction, which allows a large sample throughput in a short time.

Result

GERSTEL Membrane Extraction, presented using triazines and other semi-volatile contaminants as an example, with direct coupling to large-volume injection and GC-MS detection has been shown to be a quick and economical procedure for the investigation of surface and waste waters containing a high proportion of sediment. At the same time, it fulfils the requirements of the German drinking water regulations and the drinking water requirements of the World Health Organization (WHO).

References

[1] B. Hauser and P. Popp, J. Sep. Sci., 24 (2001), 551.

[2] TVO-BRD, Appendix 2/I, 01.04.1998.

[3] WHO: Guidelines for drinking water quality, 2nd edition, volume 1, Geneva 1993.

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