**Membrane Extraction versus Liquid-Liquid Extraction**

# Effluent analysis made easy

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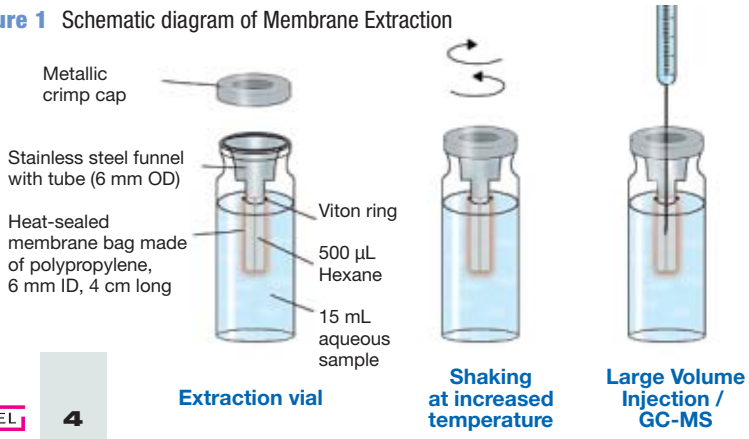
## Introduction

We can do without oil but not without water. The need to investigate water for possible contamination is obvious; not only drinking water, but more particularly effluent and brackish water, in order to identify the causes of the contamination. If the sample has a high content of suspended foreign particles, analysis may be complicated. Membrane Extraction can simplify analysis of difficult samples: a new automatable method for sample preparation developed by the Environmental Research Institute (UFZ) Leipzig-Halle with further development and marketing under licence by GERSTEL.

Numerous sample preparation methods have been developed for chromatography using recognised laboratory procedures for Liquid-Liquid Extraction (LLE), although this technique has several known disadvantages: it is difficult to automate many of the steps, and it results in relatively large volumes of possibly toxic organic solvents. The extract may need several purification steps with volume reduction in order to achieve acceptable detection limits. There are currently no viable alternatives to LLE for the many applications, leaving many users without options. However, Membrane Extraction changes all that:

**Figure 1** Schematic diagram of Membrane Extraction




Membrane-supported solvent extraction leads to small volume LLE using a flat Low-Density Polyethylene membrane (LDPE), which separates the aqueous sample from the organic solvent. Like Liquid Phase Micro extraction (LPME), Membrane Extraction is carried out offline in a vial, from which the organic extract is transferred into a sampler vial: it is followed by a Large Volume Injection (LVI). This technique was recently introduced by Hauser et al. [1]

In this study, the extraction method was adapted for membrane-supported LLE, to allow use of a conventional 20-mL headspace vial. A flat, 0.05 mm thick non-porous polypropylene membrane was heat-sealed into a tube of 6 mm outer diameter to form a membrane bag, which was fastened to a stainless steel funnel and placed in a 20-mL glass vial filled with 15 mL of the aqueous sample. Annotation: GERSTEL now offers membrane bags with a wall thickness of 0.03 mm.

The sampler is a Multi Purpose Sampler MPS 2 from GERSTEL, which is able to fill the membrane bag with 500 µL of an organic solvent, shake it at a defined temperature and injects the extract directly into GC, where LVI is possible.

## Methods

### Preparation of standards

Pure standard substances were dissolved to make solutions of 1 µg/µL, mixed working standards were prepared containing 0.05, 0.5, 5 and 50 ng/µL. The internal standard consisted of methanolic solutions of simetryne (50 ng/µL) and pentachlorobenzene (20 ng/µL); simetryne was used as the internal standard for optimisation of the extraction parameters and added directly to the hexane extraction medium.

Pentachlorobenzene was used for calibration of the membrane-supported LLE and was added to the aqueous sample before extraction. The LVI/GC-MS was calibrated directly with mixed standards: 1 – 500 pg/µL in hexane for 10-µL injection; 0.01 – 100 pg/µL for 100-µL injection.

For membrane-supported LLE, aqueous standards were prepared: suitable aliquots of methanolic mixed standards were diluted with 15 mL water, the methanol content not exceeding 0.2 percent by volume. To each aqueous sample, 5 g sodium chloride was added, in order to promote extraction of triazines.

**Membrane-supported solvent extraction**

For membrane extraction, 8 to 10 membrane bags were conditioned by extracting three times with 50 mL hexane at room temperature. The vial was filled with 15 mL aqueous sample, the membrane bag was fixed to the metal funnel using a Viton ring, and the funnel suspended in the opening of the vial. Finally, the membrane bag was filled with 500 µL hexane to which 1 µL of the simetryne internal standard was added, and the vial was sealed with a metallic crimp cap.

For extraction, the vial was placed in the interval shaker of the MPS and shaken for a defined time at a set temperature. Finally, it was automatically removed from the MPS and transferred to the sample tray. The organic extract was removed manually from the membrane bag using a microlitre syringe and transferred into a 2 mL sampler vial.

Editor's remark: In the time since this study was performed a new PrepStation software for the Multi Purpose Samplers MPS has been released. This software allows automation of all steps of the Membrane Extraction method.

## LLE of river water

In order to compare the quantitative results of membrane-supported LLE with in-vial LLE without a membrane, 15 mL river water was filled in a 20-mL headspace vial with 1 and 5 µg/L of each analyte and 1.3 µg/L pentachlorobenzene as internal standard. After introduction of 1 mL hexane, the vial was sealed with a metal crimp cap and shaken for 30 minutes at 35 °C at a rate of 750 rpm in the MPS 2 interval shaker. The organic layer was removed using a microlitre syringe and transferred to a auto sampler vial. Large-Volume Injection was used for GC/MS analysis with an injection volume of 100 µL. Direct calibration of the LVI/GC-MS was achieved with mixed standards in hexane and an injection volume of 100 µL (1µL/s) for determination of the analytes spiked in river water.

## Results and discussion

**Membrane-supported solvent extraction**

With Membrane Extraction, hydrophobic organic compounds are extracted through a membrane into a small volume of organic solvent. Relatively non-polar solvents are used, since they have low solubility in water, and solvent loss through the membrane is avoided. On the other hand, very polar solvents can be used with minimal loss through the membrane. Water-miscible polar solvents cannot be used for normal LLE. This is different for Membrane Extraction, because they do not get in contact with water phase. In a pilot HPLC study, polyaromatic hydrocarbons were successfully extracted from aqueous samples using acetonitrile inside the membrane bag.

The solvent should also not be too volatile, since it may diffuse through the membrane and into the headspace of the sample and condense there. It needs to be volatile enough, however, to be removed effectively by the split vent during the LVI.

Editor's remark: GERSTEL supplies ready-prepared membrane bags with a wall thickness of 0.03 mm, suitable for the application described here, and tested with cyclohexane as the solvent.

## Optimisation of the extraction parameters

**Effect of matrix constituents**

Table 1 (page 6) shows the optimised parameters affecting membrane-supported solvent extraction: salt, methanol content and pH. If the solution is saturated with NaCl, extraction of triazines is improved; these are relatively polar analytes. By contrast, the recovery rate of the nonpolar constituents α-HCH and phenanthrene slightly fell.

If the methanol content was increased to 6.66 Vol%, it had no significant effect on the extraction of most constituents, with exception of S-triazines, whose recovery rate fell to 10 to 20 %.

The pK values of triazines range from 1.6 (simazine) to 4.3 (prometone).

The pH value of the aqueous sample should therefore by somewhat higher than 6; an even higher pH value does not improve the extraction results. For all extractions, the aqueous samples were saturated with 333 g/L NaCl (5 g NaCl in 15 mL water).

**Instruments and analytical conditions used**

- Gas chromatograph 6890 (Agilent Technologies)

Column: HP5MS, length 30 m, ID 0.25 mm,
Film thickness: 0.25 µm
Carrier gas: Helium
Flow: 1 mL/min (constant flow)
Column pressure: 53 kPa (initial)
Oven temperature profile:
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

- Mass spectrometer 5973 (Agilent Technologies)

Acquisition mode: Scan 30 – 350 amu
Temperatures: Transfer liner 280 °C
MS-Quadrupol 150 °C
MS-Ion source 230 °C

- Cooled Injection System CIS 4 (GERSTEL; $LN_2$ cooling)

Empty and buffled glas liner were used and had been manually deactivated.
Injector temperature: 20 °C (initial)
Column pre-pressure: 5 kPa reduced
Mode: Solvent Venting
Flow: 100 mL/min at split vent, up to 0.08 min, then splitless.
Temperature programm:
20 °C - 0,12 min - 12 °C/s - 250 °C - 1 min - 12 °C/s - 330 °C - 3 min.

- Multi Purpose Sampler MPS 2 (GERSTEL)

**Optimisation of shaking rate**

The sample must be mixed thoroughly and the boundary layers in the membrane bag minimised, in order to improve the transport of the analytes through the membrane into the organic solvent. The shaking rate of the MPS 2 was varied between 250 and 750 rpm. The extraction yields increased for all analytes by 30 – 50 % when agitation speed was increased to 500 rpm; further increases in speed gave only minimal increased yield. Mixing was more important for triazines than for non-polar constituents (α-HCH and phenanthrene); for all further studies, the shaking rate of the MPS 2 was therefore set at 750 rpm.

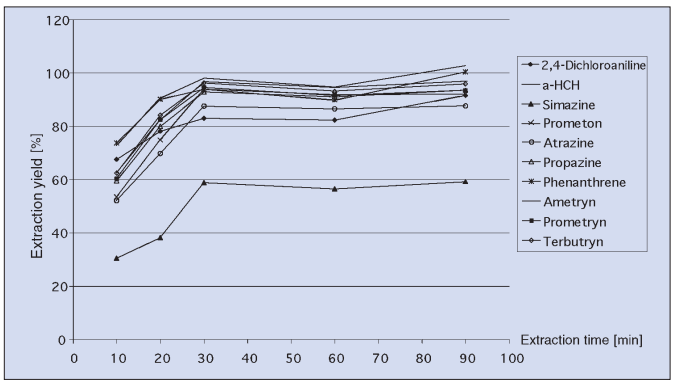



**Figure 2** Optimisation of extraction time (6,7 µg/L each component, 333 g/L NaCl, 55 °C, 750 rpm, injection volume 10 µL)

| Component | Extraction yield [%] | | | |
|---|---|---|---|---|
| | from pure water | plus 333 g/L NaCl | plus 6.6% by 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 |

**Table 1**
Effect of matrix components on extraction recovery for Membrane Extraction (spiking level 6.7 µg/L, extraction time 1 h at 35 °C and 750 rpm; injection volume 10 µL)

| | 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 ($R^2$) | Detection limit [ng/L] | Linear dynamic range [µg/L] | Correlation coefficient ($R^2$) |
| 2,4-Dichloroaniline | 2.1 | 10 | 0.05 – 100 | 0.9971 | 5 | 0.005 - | 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 |

**Table 2**
Validation results for Membrane Extraction: 10-µL injection (a): extraction time 30 min, 333 g/L NaCl, 750 rpm; 6.7 µg/L each component. 100-µL injection (b): extraction duration 1 h, 333 g/L NaCl, 45 °C, 750 rpm.

| | River water, spiked to 1 µg/L | | River water, spiked to 5 µg/L | |
|---|---|---|---|---|
| | Membrane extraction[a] | In-vial LLE[b] | Membrane extraction[a] | In vial LLE[b] |
| Components | [µg/L] | [µg/L] | [µg/L] | [µg/L] |
| 2,4-Dichloroaniline | 0.98 | 1.67 | 4.34 | 6.7 |
| α-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 |

**Table 3**
Analytical results (µg/L) of spiked river water; comparison of Membrane Extraction (a) with in-Vial LLE (b) [a: extraction time 1h, 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]

**Optimisation of temperature**

The interval shaker of the MPS 2 can be set to a defined temperature. Increasing the temperature during the shaking phase from 35 to 55 °C (hexane boils at 69 °C) improved the recovery of all constituents by 10 to 30 %; the effect was particularly marked for water-soluble triazines, and less for 2,4-dichloroaniline, α-HCH and phenanthrene. For determining the validation values, extraction was carried out at 55 °C for subsequent 10-µL injection.

**Optimisation of extraction time**

An extraction time of 30 minutes led to optimal enrichment of all components (Figure 2); increasing the time did not lead to any improvement. After 30 minutes, the recovery was 60 to 100 %, sufficient for validation of the method.

## Validation of method

The performance of membrane-supported solvent extraction was tested under optimised extraction conditions; table 2 shows the validation results. The linear dynamic range was determined by extraction of spiked aqueous samples, and for 10 µL ranged from 0.05 to 100 µg/L; the regression coefficient was 0.9965 or better. After 30 minutes extraction, the detection limit of 10 to 100 ng/L was achieved (see summary).

The detection limits were determined by blank values from co-extracting matrix components; components in the blanks originate from the heat-sealed bag made of polypropylene. The co-extracting components were also recorded in single-ion mode. Figure 3 shows the chromatogram of an extract obtained after membrane-supported solvent extraction of water again spiked with 50 ng/L; this should illustrate the high background, seen especially with the 100-µL injections, reducing the accuracy of peak integration at low concentrations.

The reproducibility of the whole extraction procedure proved to be good. The coefficient of variation of five successive extractions ranged between 2.1 and 14.3 %.

In order to improve the detection limits, 100 µL was injected. This led to detection limits of 1 – 10 ng/L and a linear dynamic range of 0.005 – 10 µg/L; the regression coefficient was 0.9970 or better.

The results of validation showed semi-automated membrane-supported LLE is a reliable sample preparation technique for aqueous samples. Editor's remark: In the time since this study was performed a new PrepStation software for the Multi Purpose Samplers has been released that allows a wider choice of syringe size options for full automation of the Membrane Extraction. For example, using a 250 µL syringe allows transport of up to 1 mL extraction solvent (4x250 µL), while still allowing injection volumes as small as 25 µL. If an injection volume of 100 µL is chosen, the solvent addition in the membrane bag can be integrated in the automated procedure. For the purpose, the sampler can be provided with a 1000-µL syringe; this allows both precise addition of 500 µL hexane and also use of 100 µL aliquot for the LVI after extraction.

## Membrane Extraction versus In-Vial-Extraction

The results of membrane-supported solvent extraction were compared with in-vial extraction of spiked river water (Table 3). The river water samples were taken from the White Elster in Leipzig, and were spiked with 1 and 5 µg/L methanolic mixed standards. The analyte levels in spiked river water extracted using membrane-supported LE were determined by calibration using aqueous standards (0.001 – 10 µg/L) extracted under identical conditions. The in-vial LLE was carried out as described above.

The recovery rate of in-vial LLE was 100 %. It was, however, difficult to remove the organic extract since the organic layer was very shallow and the phase boundary was distorted by particles. Automation of in-vial LLE required use of at least 4 mL hexane in order to ensure a clear phase separation and to enable removal of the extract through the sampler vial – without risking removal and injection of water. Since the Membrane Extraction approach can easily use 500 µL extraction volume, the 4 mL extraction volume needed for LLE results in 8-fold more diluted sample for injection and therefore poorer detection limits.

The analytical accuracy of in-vial extraction was better than that of membrane-supported solvent extraction (Table 3). The mean variation of analytical results from the spiked concentration values was: 12.4 % for in-vial LLE with 5 µg/L spiked river water and 22.9 % for Membrane Extraction, which is, however, easier to perform: If an injection volume of 100 µL was used, the whole procedure, that is extraction and injection, can be fully automated.

## Summary

The Multi Purpose Sampler MPS 2 allows complete automation of membrane-supported solvent extraction, which is a promising enrichment technique for various organic substances, including polar analytes such as triazines: under optimised conditions with an extraction time of 30 minutes, recoveries of 60 to 100 % were obtained. The detection limits were in the low ng/L range. Since the non-porous polypropylene membrane holds back water, salts, particles and macromolecular constituents from the organic extract, Membrane Extraction is especially suitable for complex samples with a high organic content.

Membrane Extraction is especially suitable for applications in food analysis and bioanalysis: by choosing solvents that are miscible with water, such as acetonitrile, the method can be used with reversed phase HPLC. Polar solvents do not dissolve in the membrane material and therefore cannot pass into the aqueous sample.

In combination with GERSTEL MASter software, in sample preparation mode, the MPS 2 allows automation of a sequence of membrane-supported solvent extractions, giving high sample throughput.

## Conclusion

GERSTEL Membrane Extraction, shown here with the example of triazines and other semi-volatile contaminants, with direct coupling to Large Volume Injection and GC-MS detection is an advantageous procedure in terms of cost and time for investigation of effluent and brackish water with high content of suspended material. It fulfils the requirements of the German Drinking Water Ordinance (0.1 µg/L for individual pesticides) [2] and Drinking Water Recommendations of the World Health Organisation WHO (2 µg/L atrazine and simazine) [3].

### Literature


[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.

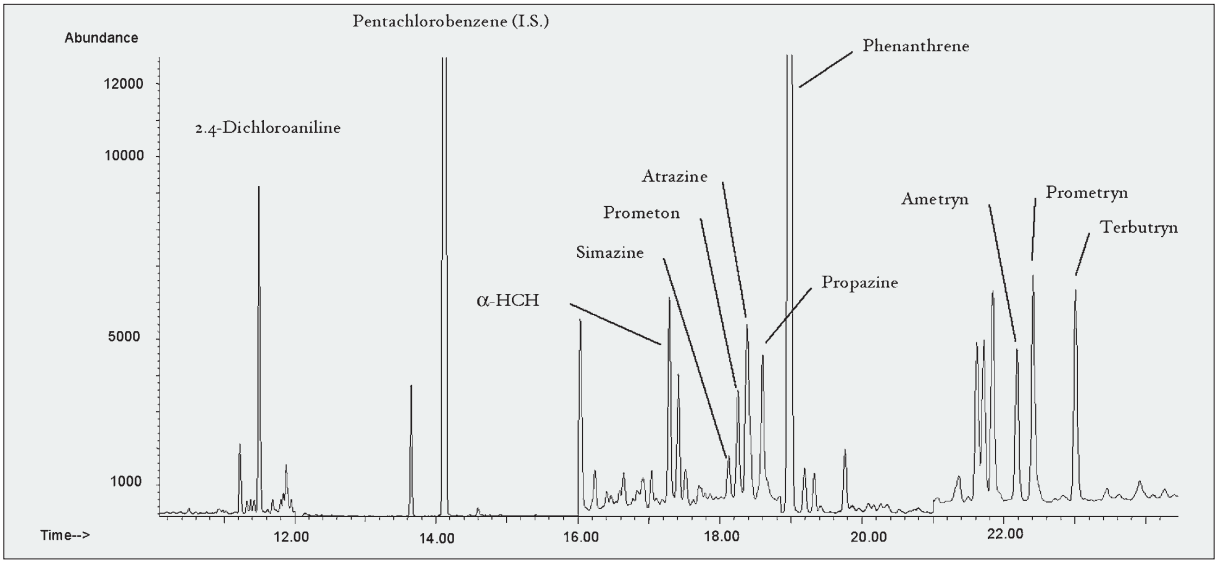




**Figure 3**
LVI/GC-MS-Chromatogram of Single-Ion Monitoring after Membrane Extraction of 15 mL water, spiked with 0.05 µg/L of each component (extraction time: 1 h, 333 g/L NaCl, 45 °C, 750 rpm, injection volume 100 µL)