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Journal of Chromatography A, 975 (2002) 211–217

JOURNAL OF
CHROMATOGRAPHY A

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On-line automated sample preparation for liquid chromatography using parallel supported liquid membrane extraction and microporous membrane liquid–liquid extraction

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Abstract

An automated system was developed for analysis of non-polar and polar ionisable compounds at trace levels in natural water. Sample work-up was performed in a flow system using two parallel membrane extraction units. This system was connected on-line to a reversed-phase HPLC system for final determination. One of the membrane units was used for supported liquid membrane (SLM) extraction, which is suitable for ionisable or permanently charged compounds. The other unit was used for microporous membrane liquid–liquid extraction (MMLLE) suitable for uncharged compounds. The fungicide thiophanate methyl and its polar metabolites carbendazim and 2-aminobenzimidazole were used as model compounds. The whole system was controlled by means of four syringe pumps. While extracting one part of the sample using the SLM technique, the extract from the MMLLE extraction was analysed and vice versa. This gave a total analysis time of 63 min for each sample resulting in a sample throughput of 22 samples per 24 h.

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Keywords: Sample preparation; Supported liquid membranes; Microporous membranes; Fungicides

1. Introduction

Automated sample preparation often results in larger sample throughput, is generally expected to be less labour intensive and has other benefits such as minimised contact with hazardous chemicals, reduced sample contamination and improved overall accuracy and precision [1–4]. The combination of liquid membrane technology for sample preparation and chromatographic methods for final determination has turned out to be relatively simple to accomplish in automated systems. Hence this type of automation has been developed using supported liquid membrane (SLM) extraction combined with GC or LC for determination of ionisable compounds [5], and microporous membrane liquid–liquid extraction (MMLLE) combined with GC for the determination of basic drug compounds [6] or with LC for the determination of cationic tensides [7]. Jönsson and Mathiasson have described the SLM and MMLLE techniques in recent reviews [8,9]. In short, SLM involves a three-phase system (aqueous/organic/aqueous) where a thin film of an organic liquid, immobilised by capillary forces in a hydrophobic porous polymer membrane, is placed between two aqueous liquids (the donor and the acceptor, respectively) in a flow system. Different transport mecha-

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PII: S0021-9673(02)00880-4

nisms can be utilised but all the analytes must pass through the organic membrane liquid as uncharged species by a diffusion process. For example, weak bases can be extracted from a basic sample into the organic membrane, diffuse through the membrane liquid and be trapped as cations in an acidic acceptor solution. This is chemically equivalent to an aqueous/organic liquid–liquid extraction (LLE) followed by an organic/aqueous back extraction. In MMLLE, a two-phase (aqueous/organic) system is used, where the organic liquid fills the pores of a hydrophobic porous polymer membrane as well as the chamber on the acceptor side. Thus, a liquid–liquid extraction is performed in a flow system where the distribution coefficients determine the driving force of the analytes into the organic solvent. These techniques offer a number of advantages compared to classical LLE, such as higher selectivity, higher volume ratios and enrichment factors, less or no consumption of organic solvents and considerably easier automation.

In this work an automated system is outlined, combining for the first time SLM extraction and MMLLE with HPLC. This system offers the possibility of simultaneous determination of polar ionisable compounds and non-polar compounds demonstrated by using a mixture of the fungicide thiophanate methyl (TM) and its polar metabolites carbendazim (MCB) and 2-aminobenzimidazole (2-AB) as a model sample.

2. Experimental

2.1. Chemicals

Thiophanate methyl (KVK Agro A/S, Køge, Denmark), carbendazim (Du Pont de Nemours, Wilmington, USA), and 2-aminobenzimidazole (Jansen Chimica, Geel, Belgium) were used as model analytes.

The membrane liquids used were di-*n*-hexylether with 15% of TOPO (tri-*n*-octylphosphineoxide) (both from Sigma Chemicals, St. Louis, MO, USA), and *n*-octanol (Sigma–Aldrich). For SLM extraction, the acceptor and the donor solutions consisted of 0.015 *M* sulfuric acid (pH 2.5) and of di-sodiumtetraborate decahydrate (pH 9.2), respectively. In the case of MMLLE, *n*-octanol filled the acceptor channel and tri-sodiumcitrate-2-hydrate buffer adjusted to pH 6.2 with citric acid served as the donor solution. All chemicals used in the donor and the acceptor were of p.a. quality from Merck (Darmstadt, Germany).

The mobile phase for HPLC was a mixture (50:50) of methanol (HPLC quality, Merck) and reagent water with 0.6% of ammonia (Lab-scan, Dublin, Ireland). Standard solutions were prepared from stock solutions of the fungicides (100–200 μg/ml) in methanol (HPLC quality, Merck). All working solutions were prepared with reagent water purified with a Milli-Q/RO4 unit (Millipore, Bedford, MA, USA). Natural water samples were collected in the Höje stream at Lund, Sweden.

2.2. Membrane units

The membranes used were porous PTFE membranes. For SLM extraction Fluoropore FG (Millipore) was used with pore size 0.2 μm, porosity 0.70 and total thickness of 175 μm (60 μm of PTFE and 115 μm of a netlike polyethylene backing as a mechanical support. For MMLLE a TE 35 membrane (Schleicher & Schuell, Dassel, Germany) was used with pore size of 0.2 μm, porosity of 0.6–0.8 and membrane thickness of ca. 60 μm backed up with a 180-μm polyester film as support.

The choice of membranes was based on results in previous experiments showing that for SLM extraction the extraction performance was better using Fluoropore FG while for MMLLE the TE 35 membrane was to be preferred [6,10].

The membrane unit itself consists of two blocks of inert material, in this case PTFE or titanium, with the membrane clamped between the blocks (see Fig. 1).

In each block a channel is machined and liquid connections are provided in both ends, forming one flow-through channel on each side of the membrane. For SLM extractions, membrane blocks of PTFE material with total channel volumes of 150 μl (channel dimensions: $0.15 \times 2.0 \times 500$ mm) were used. The PTFE-blocks were further stabilised by two 6-mm-thick aluminium blocks (not shown in Fig. 1) bolted together with five bolts. For MMLLE, membrane blocks of titanium were utilised with

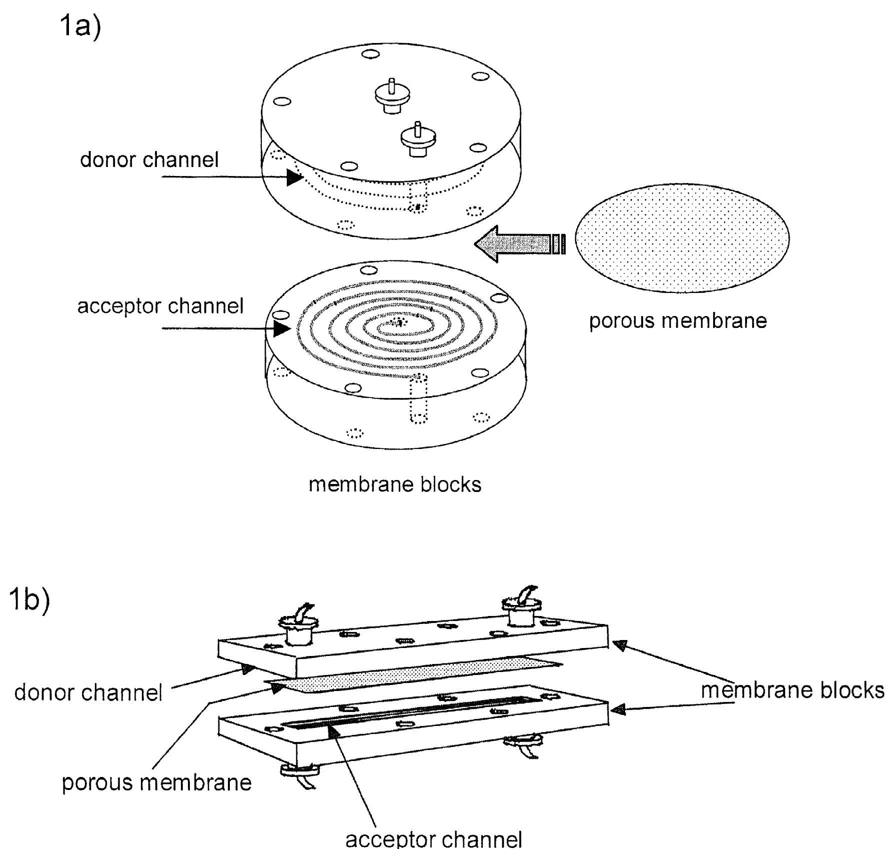

Fig. 1. The membrane units used: a 150 μl membrane unit for SLM extraction (a) and a 12 μl membrane unit for MMLLE extraction (b).

channel volumes of 12 μl (channel dimensions: 0.15×2.0×40 mm).

For SLM extraction, the membrane was impregnated with di-*n*-hexylether containing 15% TOPO before it was sandwiched between the blocks. The impregnation was simply done by soaking the membrane in the liquid during ~15 min. Before the use of the units for extraction, donor and acceptor buffers were pumped through their respective channel during 5 min to remove excess of organic liquid present outside the membrane pores. In the case of MMLLE the membrane was first mounted between the two blocks before organic liquid was pumped through the acceptor channel. Surplus liquid, that passed the hydrophobic membrane and entered the empty donor channel, was removed by pumping reagent water through the donor channel during 10 min before starting the first extraction.

2.3. Configuration of the automated system

The automated system used in this work is shown in Fig. 2.

Four syringe pumps (Kloehn, Las Vegas, NV, USA) were connected to the donor and acceptor channels of the two membrane units. Each syringe pump consisted of a pump module (step resolution of 48,000), a syringe and a six-way distribution valve. For pumps 1, 2, 3, and 4, the size of the syringes were 5 ml, 1 ml, 50 μl, and 5 ml, respectively.

The acceptor channels of the SLM (5) and the MMLLE (6) membrane units were connected to a low-pressure 10-port two-way valve (7) (Cheminert, Valco Instruments, Houston, TX, USA), which in turn was connected to the six-port injection valve (8) (Vici, Valco Instruments) with a 250-μl external injection loop (9). Both valves were pneumatically

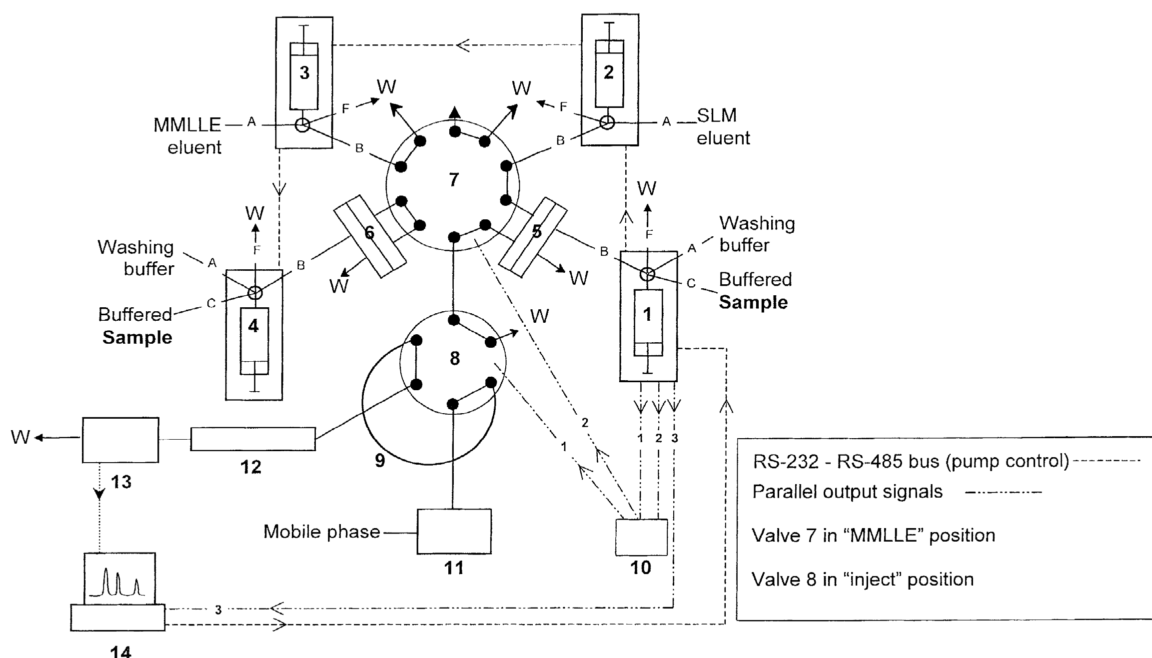

Fig. 2. Set-up of the analytical system for determination of thiophanate methyl (TM), carbendazim (MBC) and 2-aminobenzimidazole (2-AB). 1, Syringe pump (5 ml); 2, syringe pump (1 ml); 3, syringe pump (50 μl); 4, syringe pump (5 ml); 5, SLM extraction unit; 6, MMLLE unit; 7, 10-port, two-way valve; 8, six-port, two-way injection valve; 9, injection loop (250 μl); 10, box for pneumatic actuation; 11, HPLC pump; 12, analytical column; 13, UV-detector; 14, computer.

actuated (10). A high-pressure pump (11), Model SP 8800 (Spectra Physics, San Jose, CA, USA), a stainless steel analytical C_{18} column (12) (Kromasil 100, 5 μm, 2.0×200 mm; MZ analysentechnik, Mainz, Germany) and a UV detector at 270 nm (13) (Spectroflow 783, Kratos Analytical, NJ, USA) were utilised for chromatographic analysis. The entire system was controlled by a computer (14) and the chromatographic data were processed by means of a JCL 6000 Chromatography Data System (Jones Chromatography, Hengoed, Mid-Glamorgan, UK). For the syringe pump control, the Kloehn computer program provided with the pumps, was employed. The Kloehn pumps have each three on/off output signals that can be used to control other equipment units. In this system one of the pumps (1) was used to control valves 7 and 8, and to start chromatographic data acquisition. To minimise the total analysis time, syringe speeds for aspiration were generally as high as possible. During the wash cycles, the choices of dispense flow-rates were based on minimisation of the analysis time while maintaining the performance of the membranes. Suitable wash flow-rates were for the SLM device 1.25 ml/min in both donor and acceptor channel, and for MMLLE 1 ml/min and 0.031 ml/min in the donor and acceptor channel, respectively. During enrichment the optimal donor flow-rates were for SLM 0.45 ml/min and for MMLLE 1 ml/min. In both systems the acceptor phase was stagnant during extraction. Elution flow-rates for the transfer of extracts from the acceptors into the injection loop (9) were for SLM and MMLLE 0.25 ml/min and 0.031 μl/min, respectively.

2.4. Operation

The driving force in MMLLE is the attainment of distribution equilibrium. The magnitude of the distribution coefficient determines the linear range of analyte enrichment factor versus processed sample volume and hence also the maximal enrichment time. In an SLM extraction system the enrichment time possible may well exceed 24 h, when proper conditions in the trapping solution are applied, leading to enrichment factors of thousands of times [9,11].

This is normally larger than the maximum enrichment time suitable in MMLLE.

The optimisation of the chemistry and the physical parameters for achieving good extraction efficiency of thiophanate-methyl and its metabolites was earlier presented [12]. Here it was found that for MMLLE linear conditions were fulfilled with a correlation coefficient of better than 0.99 for up to 30 min of enrichment at a flow-rate of 1 ml/min resulting in an enrichment factor of 76 times. Steady state was reached after 40 min of extraction [12]. The set-up parameters of the automated system illustrated in Fig. 2 and in Table 1, were based on these previous experiences.

The system was started after creating a method for JCL 6000 to run the number of analyses required. Prior to SLM enrichment valves 7 and 8 were set as shown in Fig. 2 and Table 1. The SLM donor was washed using pump 1 and three portions of 400 μl of borax buffer solution pH 9.2. The donor wash was followed by SLM acceptor wash using pump 2 and three portions of 400 μl acceptor eluent (0.015 *M* sulfuric acid, pH 2.5). After washing, the SLM acceptor channel was closed by turning valve 7. For enrichment, 9 ml (in three portions) of sample solution were introduced by means of pump 1 and the analytes were extracted into the stagnant acceptor solution. After SLM extraction the acceptor content was kept stagnant another 6 min to assure that the majority of analyte molecules present in the membrane liquid could diffuse into the acceptor solution [12]. Meanwhile, four portions of *n*-octanol (4×45 μl) were pumped using pump 3 to wash the MMLLE acceptor. To prepare for analysis of the SLM acceptor solution and to start the MMLLE, both valves were turned. Valve 7 is now in the position shown in the figure, and valve 8 in the opposite, ''load'' position. By means of pump 2, 250 μl of acceptor eluent were used to transfer the sample into the injection loop (9). Then valve 8 was switched to the ''inject'' position and the sample was injected into the column (12). For data acquisition JCL 6000 was started with a pulse. While the SLM extract was analysed (15 min), MMLLE was initiated by washing the MMLLE donor side with three portions (100 μl) of 1 *M* citrate buffer pH 6.2 using pump 4. For extraction, 30 ml (10 portions of 3 ml) of the sample solution were pumped through the donor channel by means of pump 4. After enrichment, valve 7 was turned opening the MMLLE acceptor channel. Then 24 μl of acceptor liquid (*n*-octanol), corresponding to the tubing volume between the membrane unit (6) and the injection loop (9), were first pumped to waste by means of pump 3 bypassing the injection loop. For the transfer of the sample into the loop (9), valve 8 was switched to its ''load'' position and 25 μl of sample, i.e. two times the acceptor channel volume were transferred into the loop (9) for final analysis and a new data acquisition was started.

3. Results and discussion

3.1. Performance of the automated system

According to the conditions for the automated process presented in Table 1, the total time for SLM extraction including SLM wash and MMLLE accep-

Table 1
A scheme showing the different operations during an analysis

Sample preparation event		Final analysis		Output signals			Total time (min)
				Valve 7	Valve 8	JCL control	
SLM donor wash	1 (min)	MMLLE final analysis	30 (min)	MMLLE	Inject	Pulse	1
SLM acceptor wash	1.5 (min)						2.5
SLM enrichment	20.5 (min)			SLM			23
SLM stagnant extract/ MMLLE acceptor wash	6.5 (min)						29.5
SLM acceptor transfer	1 (min)			MMLLE	Load		30.5
MMLLE donor wash	0.5 (min)	SLM final analysis	15 (min)		Inject	Pulse	31
MMLLE enrichment	31 (min)						62
MMLLE acceptor transfer 1	0.5 (min)			SLM			62.5
MMLLE acceptor transfer 2	0.5 (min)				Load		63

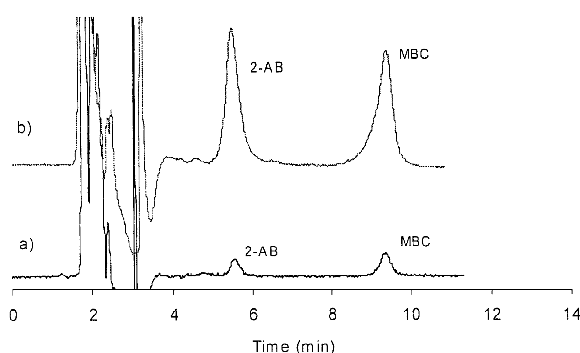

Fig. 3. Chromatograms of extracted natural water samples containing carbendazim (MBC) and 2-aminobenzimidazole (2-AB) at concentrations 0.25 ng/ml (a) and 2 ng/ml (b) in 0.025 *M* borax buffer pH 9.2.

tor wash is 29.5 min. SLM extraction is carried out while the MMLLE extract is analysed (30 min) and vice versa, giving a total analysis time of 63 min per sample and a throughput of 22 samples per 24 h. This system can be used continuously for at least 20 extractions before exchange of the SLM membrane. The limiting factor is the stability of the membrane liquid in the SLM extraction device, in this case 15% TOPO in *n*-di-hexylether. As discussed in previous work [12], the stability depends on the nature of the membrane liquid, the most important factor being its water miscibility. For example, membranes containing liquids such as undecane or mixtures of undecane and dihexylether can be used for several weeks [11,13,14] but these liquids are too non-polar for efficient extraction of the actual compounds. In MMLLE no limitations in long-term membrane stability were observed, as expected. In MMLLE the membrane liquid is replaced before each new analysis in accordance with the procedure described in Section 2.

The automated system was tested by extraction of buffered natural water solutions spiked with 0.25 or 2 ng/ml of both MBC and 2-AB for SLM extraction and with 0.5 or 3 ng/ml TM for MMLLE. Chromatograms obtained in this test are shown in Figs. 3 and 4.

For triplicate analyses the RSD was less than 5% for all analytes with an enrichment time of 30 min. This resulted in an enrichment factor of 76 for TM using MMLLE extraction and of 12 for MBC and 2-AB using SLM extraction. In the latter case the extraction efficiency was for both MBC and 2-AB

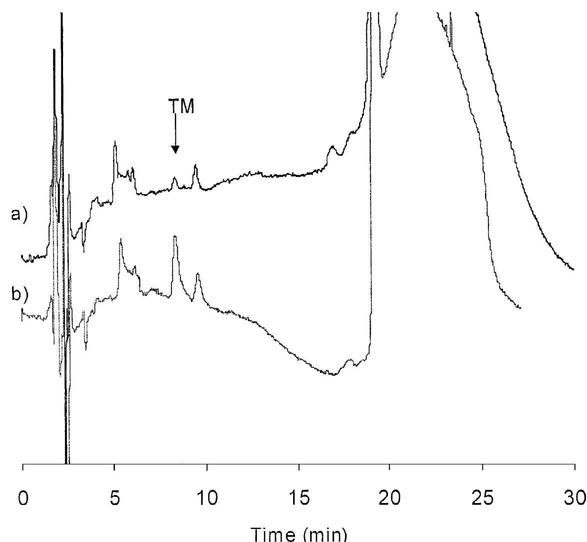

Fig. 4. Chromatograms of extracted natural water samples containing thiophanate methyl (TM) at concentrations 0.5 ng/ml (a) and 3 ng/ml (b) in 1 *M* citrate buffer at pH 6.2.

20%, the sample volume was 9 ml and the acceptor volume was 150 μl. Detection limits achieved were 0.5 ng/ml for TM and 0.25 ng/ml for both MBC and 2-AB determined as three times the baseline noise. These results are similar to results obtained in previous studies of SLM and MMLLE extractions [12] performed in a non-automated system.

The injection of a non-aqueous solvent into a reversed-phase HPLC column is not a common procedure. The topic was discussed earlier [12], considering that the solvent shall both be suitable as an acceptor liquid for MMLLE, and be reasonably well accepted as an injection solvent in terms of baseline disturbance and additional peak broadening. It was found that *n*-octanol could be used with an injection volume up to 30 μl and 25 μl was selected for safety. This aliquot contains 45% of the extracted analytes, and the *n*-octanol is the cause of the large baseline disturbance around 20 min in Fig. 4.

4. Conclusion

Automated SLM systems, especially for environmental applications, have previously mainly been based on peristaltic pumps. Due to the limited flow precision of such pumps, large membrane units (channel volumes of 1 ml) have been used, necessita-

ting analyte focusing, e.g. using solid-phase extraction [15,16]. In this work, a new approach for automatic analysis has been tested utilising syringe pumps with multi-positioning valves combined with low volume membrane units that enable injection of the whole acceptor bulk without further analyte focusing. In comparison with systems built on peristaltic pumps, syringe pump systems result in more precise and accurate measurements due to better flow control. The need for good control is especially important on the acceptor side, where transferring the acceptor bulk for chromatographic analysis is a critical procedure. A minor disadvantage, prolonging the time for an analysis is the need for washing the dead volumes of the syringes a number of times between each sample if very dirty samples such as industrial wastewater are processed. A comparison based on our experiences with non-automated relatively simple peristaltic pump systems, reveals that the precision using the present relatively complicated system is better, RSD<5% compared to RSD 4–8%.

With the developed system utilising both SLM and MMLLE, samples containing substances with large differences in chemical properties (i.e. polarity and/or ionisability) can be handled. This substantially simplifies the use of liquid membrane methodology for sample preparation, since such types of samples previously had to be divided and run on different systems. For ionisable and unstable substances such as thiophanate methyl, it may be impossible to find suitable conditions for efficient SLM extraction. Here the possibility to use MMLLE may be a solution. By adding salt to the sample solution, relatively high organic phase/water partition coefficients can be achieved also for moderately polar compounds, thus further increasing the area of MMLLE applications.

5. Nomenclature

2-AB	2-aminobenzimidazole
MBC	carbendazim
MMLLE	microporous membrane liquid–liquid extraction
LLE	liquid–liquid extraction
SLM	supported liquid membrane
TM	thiophanate methyl
TOPO	tri-*n*-octylphosphineoxide

Acknowledgements

The Swedish Natural Research Council (NFR) is acknowledged for financial support.

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