Determination of thiophanate-methyl and its metabolites at trace level in spiked natural water using the supported liquid membrane extraction and the microporous membrane liquid–liquid extraction techniques combined on-line with high-performance liquid chromatography

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Abstract

On-line supported liquid membrane (SLM) extraction and microporous membrane liquid–liquid extraction (MMLLE) techniques for sample preparation of natural water samples have been developed for the determination of thiophanate-methyl (TM), carbendazim (MBC) and 2-aminobenzimidazole (2-AB) using reversed-phase HPLC. The combination of SLM extraction and MMLLE offers extraction conditions that makes it possible to determine a wide variety of compounds, i.e., permanently charged, ionisable and non-polar at sub ppb level. The detection limits obtained after extraction are about 0.1 μg/l for MBC and 2-AB using SLM, and 0.5 μg/l for TM using MMLLE and the precision is better than 5% for both systems. Typical enrichment rates are 0.6 and 2.7 times/min using SLM and MMLLE, respectively. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Several commonly used pesticides are unstable in nature and a large part of the pesticide activity comes from one or several of their degradation products. This is the case for thiophanate-methyl and benomyl; two worldwide used agents for fungi control. In order to estimate the remaining activity of such a formulation in the field it is thus necessary to be able to measure both the original active ingredient as well as the most important active metabolites in complex matrices such as soil and surface water. For estimation of the environmental impact, e.g., concerning quality of drinking water, residues of the original active substance and its metabolites need to be measured at very low concentration levels. Thus an analytical method capable of handling both these aspects requires both high selectivity and low limit of detection.

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Capillary gas chromatography is commonly used for hydrophobic pesticides while high-performance liquid chromatography (HPLC) is preferred for the determination of polar pesticides including their polar degradation products [1].

Liquid membrane techniques have been demonstrated to be efficient for sample preparation in flow systems [2–4]. Two different approaches have been utilised, supported liquid membrane (SLM) extraction and microporous membrane liquid–liquid extraction (MMLLE). In short, MMLLE is a two-phase aqueous–organic solvent system and SLM a three phase aqueous–organic solvent–aqueous system, which leads to more selective extraction. MMLLE is best suitable for non-polar compounds but can also be used for charged species, e.g., by utilising ion-pair formation. In addition MMLLE is an attractive approach for compounds that are not stable in aqueous solutions. The pH of the aqueous solution is often a critical parameter and with a flow MMLLE system the residence time of an analyte at an unsuitable pH can be kept very short (below 30 s).

The combination of liquid membrane extraction techniques for sample preparation, with their enrichment capability, and chromatography offers both high selectivity and low detection limits.

The fungicide thiophanate-methyl is difficult to handle due to its non-stability in aqueous solutions at pH values suitable for sample preparation with SLM technique. In this paper we outline the possibilities to use MMLLE for the determination of thiophanate-methyl and SLM extraction for its polar metabolites, carbendazim and 2-aminobenzimidazole in environmental aqueous samples. Carbendazim is also as a major product in the degradation pathway of benomyl.

2. Experimental

2.1. Chemicals

The structures of thiophanate-methyl (TM; KVK Agro, Köge, Denmark), carbendazim (MBC; DuPont de Nemours, Wilmington, DE, USA) and 2-amino-benzimidazole (2-AB; Jansen, Geel, Belgium) are shown in Fig. 1. TM has both weakly acidic and alkaline properties. pK_a values, calculated by the program ACD/pH (Advanced Chemistry Development, Toronto, Canada), for its deprotonation is about 7 (7.28 according to Ref. [6]) while for its protonation it is 2.8. Both MBC and 2-AB are bases with pK_a values around 4.5 and 7.5, respectively.

The membrane liquids were mixtures of di-*n*-hexyl ether and TOPO (tri-*n*-octylphosphineoxide) (both from Sigma, St. Louis, MO, USA), 6-unde-canone (Sigma–Aldrich, Steinheim, Germany), and *n*-octanol (Sigma–Aldrich). In the case of SLM the acceptor solutions consisted either of sulfuric acid or citric acid buffer. For the donor a 0.025 *M* borate buffer at pH 9 was used. In the case of MMLLE the acceptor was *n*-octanol (Sigma–Aldrich) and citrate buffers at different ionic strengths and pH. All chemicals used in donor and acceptor were of analytical-reagent grade from Merck (Darmstadt, Germany).

For liquid chromatography, methanol (HPLC-grade, Merck) and ammonia (Lab-Scan, Dublin, Ireland) diluted with reagent water, purified by a Milli-Q/RO4 unit (Millipore, Bedford, MA, USA), were used. Stock solutions of the fungicides in methanol (HPLC-grade, Merck), 100–200 μg/ml, were utilised for preparation of standard solutions. TM dissolved in methanol is reported to be stable at
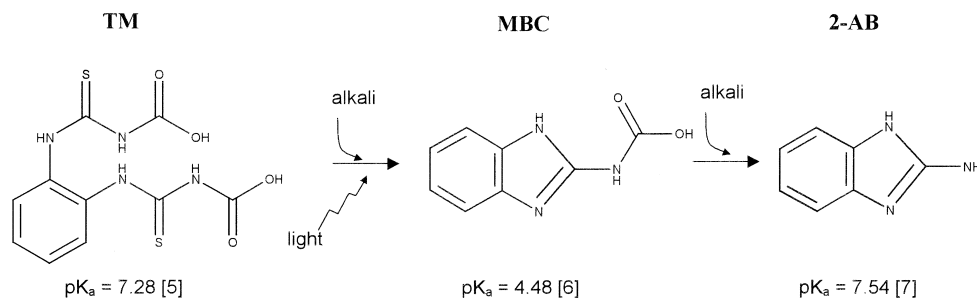

Fig. 1. Thiophanate-methyl with its major metabolite carbendazim, which is further metabolised to 2-aminobenzimidazole.

24°C for at least 50 days [7]. Stock solutions were stored up to 1 month at 4°C. All standard solutions were made with reagent water. Natural water samples were collected in the Höje stream at Lund, Sweden.

2.2. Membrane units

Membrane units with total channel volumes on each side of the membrane of 150 μl with spiral channels [2] and 12 μl with straight channels [3] were used for SLM extraction and MMLLE, respectively.

For SLM extraction a porous PTFE membrane, Fluoropore FG (Millipore) [8] was impregnated during at least 20 min in the membrane liquid and clamped between two blocks of PTFE.

For MMLLE a dry PTFE membrane, TE35 (Schleicher & Schuell, Dassel, Germany) [9] was mounted between two blocks of titanium.

2.3. The analytical system

On-line sample preparation was performed utilising two syringe pumps (Kloehn, Las Vegas, NV, USA) connected to the donor and acceptor channels of the membrane unit, respectively, as shown in Fig. 2. To assure stagnant acceptor solution during enrichment, the pump for the acceptor and the six-port injection valve (Vici; Valco Instruments, Houston, TX, USA) in the LC system were connected via a four-port low-pressure valve (Cheminert; Valco In-

Fig. 2. Set-up of the analytical system.

struments). Membrane units of different sizes were utilised for SLM extraction and MMLLE, respectively.

The HPLC system consisted of a ternary high-pressure pump, Model SP 8800 (Spectra Physics, San Jose, CA, USA), a pneumatically controlled six-port injection valve with a 250-μl external injection loop, a stainless steel analytical C_{18} column (Kromasil 100, 5 μm, 200×2.0 mm; MZ Analysentechnik, Mainz, Germany) and a UV detector (Spectroflow 783; Kratos Analytical, Chestnut Ridge, NY, USA). The chromatographic data were processed with a computer using a JCL 6000 Chromatography Data System (Jones Chromatography, Hengoed, UK). The mobile phase, methanol–water (50:50) with 0.6% ammonia, was pumped at a flow-rate of 0.2 ml/min and the analytes were detected at 270 nm.

2.4. Operation

2.4.1. Determination of TM

Two approaches were investigated for the determination of TM: (1) after degradation to MBC and 2-AB, which were determined using SLM extraction and (2) direct determination using MMLLE.

2.4.1.1. Thermal degradation to MBC and 2-AB

TM is stable in acidic medium (pH 2–5) but fairly unstable in alkaline medium [5,7,10,11] and sensitive to light [5]. Its major metabolite is MBC. The half-life of TM in aqueous solutions at pH 7 and 22°C is 46 days, and at pH 9 and 65°C it is 3.9 min [10]. MBC can be further hydrolysed, especially under alkaline conditions, to 2-AB [11] with a half-life of 54 days at pH 9 and 25°C [5]. In this study TM was thermally degraded at 65°C and determined as the increase in the amount of MBC and 2-AB. To optimise the degradation time, solutions of TM in 0.025 M borate buffer (pH 9) were kept at 65°C for different times. Prior to determination of the MBC and 2-AB formed, the solutions were chilled to room temperature before further treatment.

2.4.1.2. Direct determination by MMLLE

The membrane was conditioned by pumping *n*-octanol in the acceptor channel and surplus liquid
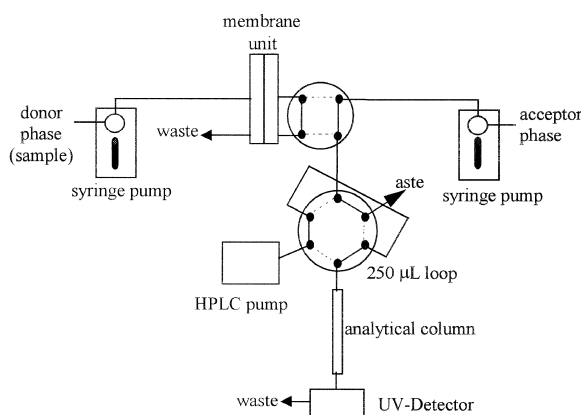
was removed by pumping reagent water through the donor channel. Reagent and natural water solutions were spiked with TM and buffered with citrate at different pH values. The unprotonated TM was extracted at a typical flow-rate of 1 ml/min. After extraction, the acceptor volume of 12 μl and additionally 13 μl were transferred into the 250-μl loop by displacement with *n*-octanol. The liquid filling the connection tubes between the membrane and the loop (24 μl), was pumped to waste before the injection valve was switched to its load position. Prior to the next extraction, the acceptor was washed with 200 μl of *n*-octanol.

2.4.2. Determination of MBC and 2-AB by SLM extraction

Buffered sample solutions (0.025 *M* borate buffer, pH 9) of reagent water or natural water spiked with analyte standards were pumped at 0.45 ml/min through the donor channel, while the acceptor channel contained 0.015 *M* sulfuric acid. To prevent memory effects the acceptor was kept stagnant for 5 more min after enrichment. The acceptor solution was transferred at a flow-rate of 0.45 ml/min to fill the 250-μl injection loop used for final analysis.

3. Results and discussion

3.1. Determination of TM

3.1.1. Thermal degradation followed by SLM extraction

3.1.1.1. Degradation

For ionisable compounds SLM is normally the extraction method of choice in terms of selectivity. However, TM (which is ionisable) is not stable in alkaline and acidic environments, restricting the

available pH range for the SLM process. Preliminary experiments in this range showed practically no extraction. Thus direct determination could not be performed using SLM extraction, and thermal degradation to MBC and 2-AB, as well as direct determination using MMLLE was instead investigated.

An increase of the temperature to 65°C has a large impact on the degradation rate for TM but also in its degradation pattern [5]. At 65°C the degradation pathway leading to the desired products MBC and 2-AB is favoured. From Table 1 follows that after 10 min, about 80% of TM has been degraded and the concentration of the two interesting degradation products MBC and 2-AB are now in total around 79%, compared to 25% at 22°C. A further increase of the degradation time to 20 min decreased the TM concentration with only about 5%. Accordingly, a degradation time of 10 min was used in further experiments. The degradation rate also seems to be concentration independent, as is also the ratio of 2-AB and MBC formed (Table 1). It should be noted that degradation results may be different at trace level concentrations. In the determination of TM using thermal degradation at 65°C it is possible to measure either the concentration of MBC, which is the dominating species, or to use the sum of MBC and 2-AB. Fig. 3 shows that MBC and 2-AB appear as two well resolved peaks in the HPLC system. After determination of the sum of MBC and 2-AB before and after off-line degradation. TM is calculated as the difference.

3.1.1.2. Optimisation of the SLM procedure

The SLM performance is evaluated by the extraction efficiency $E = n_A / n_I$, where n_A and n_I are the number of moles found in the acceptor phase and the number of moles in the extracted sample, respectively. Another way to express performance is by the evaluation of the enrichment factor $E_e = c_A / c_I$, which

Table 1
Degradation of 100, 500 and 1000 μg/l TM in 0.025 *M* borax buffer (pH 9) at 65°C during 10 min ($n=2$)

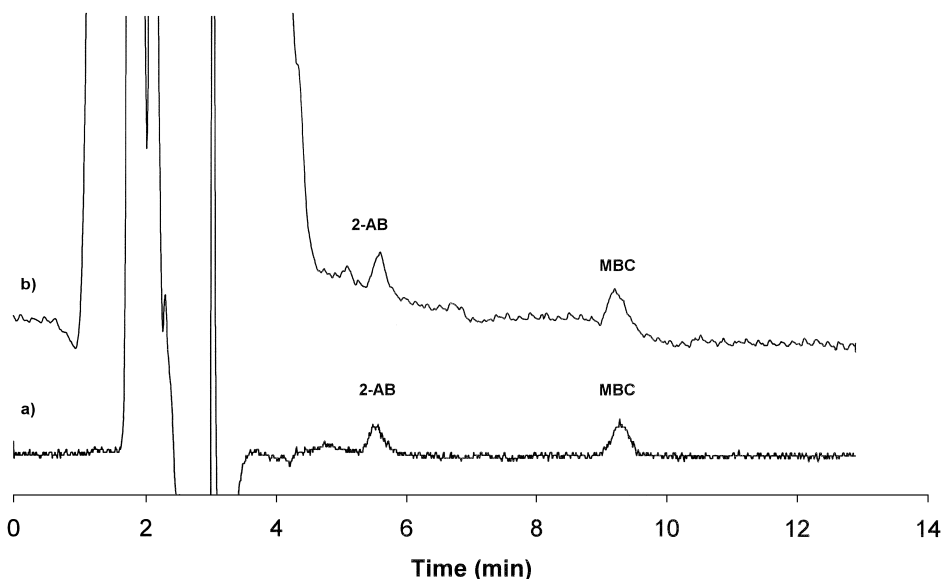| Initial amount of TM (ng/ml)	Remained amount of TM (%)	Formed amount of 2-AB (%)	Formed amount of MBC (%)	Sum of formed MBC and 2-AB (%)
1000	19	13	68	81
500	18	13	68	81
100	21	12	62	74

Fig. 3. On-line SLM–HPLC chromatograms (a) after 20 min of extraction in reagent water (0.25 ng/ml of both analytes using 0.015 *M* sulfuric acid pH 2.5 as acceptor solution) and (b) after 50 min of extraction in natural water (0.15 ng/ml of both 0.1 *M* citric acid pH 2.5 as acceptor solution). Water blanks contained no detectable levels of analytes.

is the concentration quote of the analyte concentration found in the acceptor phase and analyte concentration in the extracted sample [3].

Important parameters controlling the extraction efficiency (E) are the donor and acceptor pH and the membrane liquid [2–4]. Optimal pH values in donor and acceptor were found to be pH 9 and pH 2, respectively and pH 2.5 was chosen in the acceptor to protect the analytical column.

Two membrane liquids, 6-undecanone and di-*n*-hexylether (DHE) containing different amounts of TOPO (0–15%) were tested at a donor flow-rate of 0.75 ml/min. On the basis of the results presented in Table 2 a TOPO concentration of 15% was chosen for further experiments.

According to the theory [3], the extraction efficiency is donor flow dependent. In the range 0.1 to 0.55 ml/min, for a channel volume of 150 μl, rather flat curves were obtained for MBC and 2-AB. Consequently, it is possible to operate under higher flow-rates which increases the sample throughput per time unit [3].

With 15% of TOPO in DHE, 0.025 *M* borate buffered sample at pH 9 as donor solution pumped at 0.45 ml/min during 20 min and 0.015 *M* H$_2$SO$_4$ as

acceptor solution, E was 20% (RSD 5%, $n=5$) for both analytes. As expected, E is lower than for the corresponding TOPO concentration in Table 2, since the residence time now is 20 s rather than 1 min. No memory effects were observed, if the acceptor was kept stagnant during 5 min after enrichment prior to final analysis.

3.1.2. Direct determination by MMLLE

A simplified theory related to the SLM theory

Table 2
Extraction efficiencies achieved for MBC and 2-AB with different membrane liquids

Membrane liquid	Extraction efficiency (%)[a]	
	2-AB	MBC
Undecanone	15	67
DHE	0	26
5% TOPO in DHE	19	66
10% TOPO in DHE	35	67
15% TOPO in DHE	53	67

[a] RSD values were less than 5% in all cases and $n=3$. Donor parameters: 1.0 μg/ml of each analyte in borax buffer (pH 9), flow-rate of 0.75 ml/min. Acceptor parameters: H$_2$SO$_4$ (pH 2). Membrane unit: channel volumes of 1 ml.

describes the influence of different experimental parameters on the extraction efficiency [2,3,9]. On the assumption that the flows in the channels are parallel and that the partition is in equilibrium at the outlet of the channel the following equation is derived, $E = 1 - F_D/(F_D + F_A K)$. Here F_D and F_A are the flow-rates in the donor and acceptor channels, respectively and K the distribution coefficient. This equation shows that compounds with large distribution coefficients are more readily extracted resulting in increased extraction efficiencies with acceptor flow-rate. However, the enrichment factor $[E_e = 1/(1/K + F_A/F_D)]$ will be smaller with increased acceptor flow-rate due to dilution. The last equation shows also that at small acceptor flow-rates the enrichment factors approaches the maximum value, which is K.

The organic liquid must be chosen so that the distribution coefficient is large for efficient enrichment in MMLLE, but also with respect to its compatibility with the mobile phase in the HPLC system. In this case *n*-octanol turned out to be a good compromise. The influence of donor pH on the extraction of ionisable compounds in MMLLE and SLM is similar [3]. Acceptable conditions were found for pH 6.0–8.5. At lower pH TM is protonated

and thus charged and at higher pH it is deprotonated and also degraded into MBC.

An important donor parameter controlling the extraction is the salting out effect, which seriously affects the magnitude of the distribution coefficient. Fig. 4 shows the changes of the enrichment factor for TM in citrate buffer of different ionic strengths. In 1 *M* citrate buffer at pH 6.2 a linear range was obtained for extraction volumes up to 30 ml, which gives an enrichment of 76 times with a RSD of less than 5% ($n = 3$). This gives an enrichment factor per minute of 2.7 times/min, while typical values obtained in SLM extraction are 0.5–0.7 times/min. Fig. 4 also shows that by increasing the ionic strength from 0.27 to 4.43 the distribution coefficient increases about four-fold. Consequently, by increasing the ionic strength the distribution coefficient can be considerably improved increasing the achievable enrichment factors and decreasing the detection limit. This is especially important for compounds with relatively low distribution coefficients, as is the case for TM ($K = 31.4$, *n*-octanol–water) [10].

The influence of the donor flow-rate on the enrichment per time unit was studied in the interval 0.25–1.5 ml/min. For values above 0.8 ml/min a
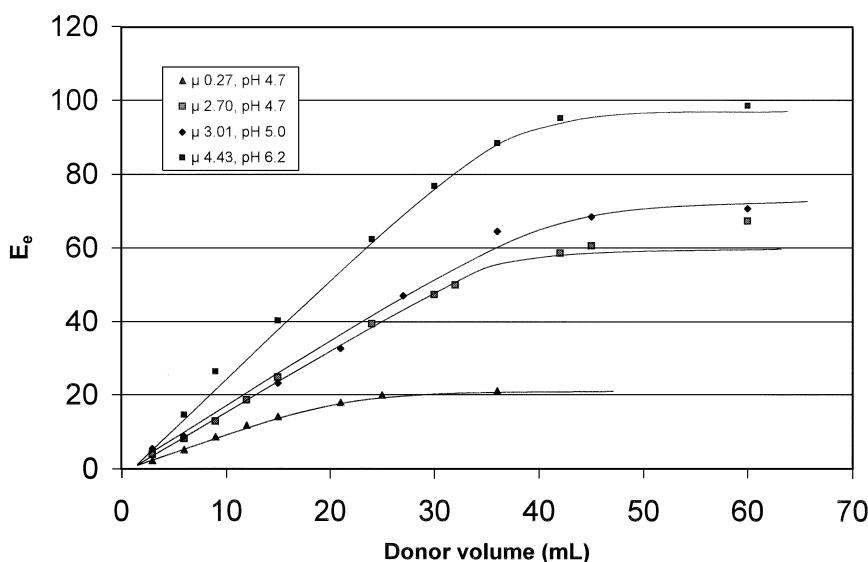

Fig. 4. MMLLE enrichment factors of TM (0.4 µg/ml) at different processed sample sizes and different ionic strengths (μ), using donor solutions of 0.1 or 1 *M* citrate buffer at a flow-rate of 1 ml/min.

plateau was reached. Accordingly, a flow-rate of 1 ml/min was used in further experiments.

3.2. Memory effects

The mass transfer in MMLLE differs from that in SLM [3]. In MMLLE there is no concentration gradient over the membrane–acceptor solution interface. This means that a comparatively larger volume of acceptor liquid is required to wash out the analyte from the channel as well as from the membrane pores [9]. This was studied at TM concentrations of 0.05, 0.15 and 0.4 ng/ml in 1 M citrate buffer at pH 6.2 and a flow-rate of 1 ml/min. To completely remove the analyte from the acceptor channel a volume of about 150 µl was needed or about 12 times the channel volume. After washing with 10 times the channel volume the remaining portion which is about 2% is concentration independent at concentrations studied. This can be compared with values obtained for MBC and 2-AB in SLM extraction where 1.7 channel volumes were sufficient.

Acceptor solutions of 25 µl were injected, corresponding to 45% of the total amount of enriched analyte. The precision, when transferring a 25-µl aliquot of the whole sample, was better than 5% and the relative percentage used for the final analysis was concentration independent. The acceptor channel was washed with 200 µl of *n*-octanol between each run to eliminate any memory effect.

3.3. Liquid chromatography

The same LC system was used for both membrane systems. After SLM enrichment with the extracted analyte dissolved in 0.015 M sulfuric acid, injection volumes of 250 µl could be used without zone broadening due to analyte focusing. After MMLLE, the injected organic solvent gives a zone broadening the magnitude of which depends on the choice of solvent. Testing solvents as *n*-octanol, ethylmethyl ketone, diethyl ether, cyclohexanone and ethyl acetate suitable for extraction it was found that octanol caused least broadening. Volumes up to 30 µl of octanol could be injected without significant zone broadening. In further experiments 25 µl were utilised giving RSD<2% for five injections of TM

injected in intervals of 30 min. The column stability was about 6 months.

3.4. Quantitative measurements

3.4.1. Determination of MBC and 2-AB using SLM extraction

The performance of the MBC and 2-AB analysis was studied at optimal conditions extracted from reagent and natural water. Calibration curves ranging from 0.25 to 10 µg/l showed no evidence of nonlinearity with correlation coefficients larger than 0.99 and intercepts not significantly different from zero at a 95% confidence level. The detection limit calculated as 3 times the baseline noise was about 0.25 µg/l both in reagent water (see Fig. 3a) and in natural water with an enrichment time of 20 min. With an increase of the enrichment time to 50 min, the limit of detection decreased to below 0.1 µg/l (see Fig. 3b). This meets the limit of pesticide concentration in drinking water set by the European Union [12].

3.4.2. Determination of TM using MMLLE

A calibration curve was constructed for five evenly spread concentrations in the range 1–100 µg/l in reagent and natural water. Also here, linear conditions were found with a correlation coefficient larger than 0.99 and intercept not significantly different from zero at 95% confidence level. The enrichment factor after 30 min extraction was 76 (RSD< 5%, *n*=3). Since the sample was diluted two-fold when transferred to the injection loop, the effective enrichment before the final analysis was 38. The limit of detection in natural water calculated as 3 times the baseline noise was 0.5 µg/l (see Fig. 5).

3.5. Stability of the membranes

The stability of the SLM membrane is dependent on the nature of the membrane liquid. Increased polarity, for example increased amount of TOPO, tends to decrease the membrane stability. With 15% TOPO in DHE, the membrane stability is around 1 week and longer at lower concentrations. If MBC is to be determined, which might be the case for natural water samples, 5% TOPO in DHE is a better choice as it gives the same extraction efficiency (see Table
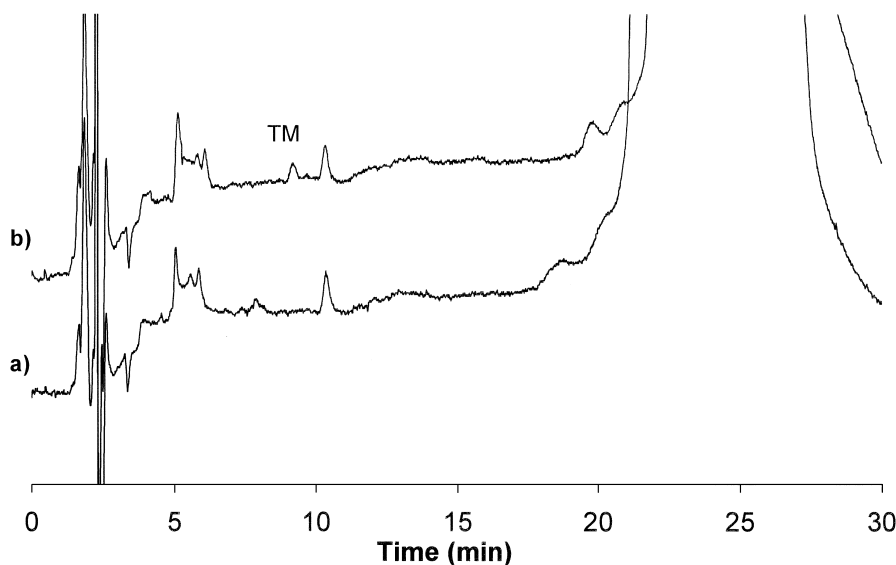
Fig. 5. On-line MMLLE–HPLC chromatograms extracting (a) blank natural water and (b) spiked (0.3 ng/ml TM) natural water during 30 min using 1 *M* citrate buffer, pH 6.2 in the donor solution and *n*-octanol (t_R around 20 min) as acceptor bulk. Water blanks contained no detectable levels of analyte.

2) and better stability. For the undecanone membrane, the stability is only about 1 day, which makes it less suitable for the present application.

As expected, in MMLLE no limitation in the long-term membrane stability is observed, since the organic membrane liquid is exchanged after each analysis.

4. Conclusions

The combination of SLM extraction and MMLLE offers extraction conditions applicable to both permanently charged, ionisable and non-polar compounds and makes it possible to handle also chemically unstable species. Simple tuning of physical and chemical parameters provide sufficient flexibility of the system to achieve selective enrichment and/or clean-up offering low detection limits at a reasonable time (less than 0.5 μg/ml within 30 min for the substances studied).

In comparison with SLM extraction, MMLLE requires larger volumes (about three times) for the transfer of the enriched sample to HPLC analysis. However, since the enrichment rate in MMLLE is

higher (about three times), these two effects practically cancel.

Acknowledgements

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