Journal of Chromatography A, 1216 (2009) 5160–5163

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

A simple novel configuration for in-vial microporous membrane liquid–liquid extraction

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ARTICLE INFO

Article history:
Received 30 January 2009
Received in revised form 25 April 2009
Accepted 28 April 2009
Available online 3 May 2009

Keywords:
Microporous membrane liquid–liquid extraction
Gas chromatography–mass spectrometry
2-Ethylhexyl 4-(dimethylamino)benzoate
Urine

ABSTRACT

A novel arrangement for microporous membrane liquid–liquid extraction from the aqueous donor phase to the organic acceptor phase within a micro-vial, which is compatible with the chromatograph autosampler is presented. The device consisted of a stoppered glass micro-vial containing the organic solvent where the septum of the screw stopper was replaced by a sized piece of membrane which is hermetically assembled to the volumetric flask containing the aqueous donor solution. The placement of the membrane in alternative contact with the solutions was achieved by orbital agitation. As a preliminary study, 2-ethylhexyl 4-(dimethylamino)benzoate has been determined (limit of quantification 0.11 $\mu g\,L^{-1}$, precision 7.4%). The small quantity of organic solvent used, the achieved sample cleanup, and the minimal handling and risk of cross-contamination are significant operational advantages.

1. Introduction

Microporous membrane liquid–liquid extraction constitutes a well-accepted and promising choice for sample preparation [1–4]. The extraction takes place by diffusive transport through the pores of the organic solvent impregnated membrane. The maximum extraction yield is determined by the partition coefficient and the kinetics to reach the steady state depends on membrane characteristics and agitation conditions.

Several configurations have been proposed using a membrane bag, a hollow fibre membrane or a flat sheet membrane cell.

The arrangement in membrane bag consists of a membrane bag filled with the organic solvent that is placed into a vial containing the sample [5]. The use of a capillary hollow fibre membrane allowed the reduction of the amount of organic solvent [6–8]. In such commented arrangements, sample agitation was accomplished by magnetic stirring while the acceptor phase remains static. A loss of extraction solvent has been observed for high stirring rates [9]. After extraction, the solvent remaining within the lumen of the membrane is analysed which requires a cautious handling [10].

Approaches based on flat sheet membranes are commonly flow cells [11,12], being the on-line coupling of the extraction unit to the measurement instrumentation a noteworthy characteristic of this configuration [13]. Much less development is found in the use of flat sheet membranes in arrangements for off-line extraction [14].

In this paper a new arrangement for microporous membrane liquid–liquid extraction is presented. Preliminary studies have been carried out using 2-ethylhexyl 4-(dimethylamino)benzoate as target analyte [15–17].

2. Experimental

2.1. Reagents

Decane and 2-ethylhexyl 4-(dimethylamino)benzoate (EDP) were purchased from Aldrich (Steinheim, Germany). A 100 $mg\,L^{-1}$ EDP stock standard solution was prepared in liquid chromatography grade ethanol from Scharlau Chemie (Barcelona, Spain). EDP standards in decane were prepared for enrichment factor determination. A 1.0 M phosphate buffer of pH 7.2 was prepared with potassium dihydrogen phosphate and sodium hydroxide. Purified water by a Milli-Q system (Bedford, MA, USA) was used.

2.2. The device for microporous membrane liquid–liquid extraction

Hydrophobic PTFE membranes bonded to a high density polyethylene support (150 μm wall thickness, porosity 85%, 0.22 μm pore size) were purchased from Millipore (Billerica, MA, USA), and the glass micro-vials used for extraction with open top screw caps were bought from Teknokroma (Barcelona, Spain).

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doi:10.1016/j.chroma.2009.04.082

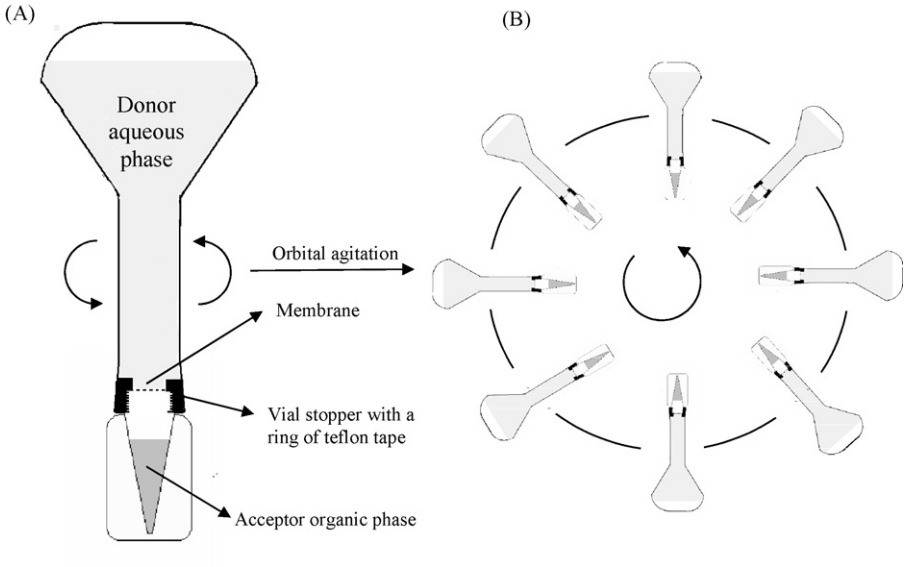

Fig. 1. (A) Device used for in-vial microporous membrane liquid–liquid extraction. (B) A rotary mixer with 8 devices.

A stoppered micro-vial compatible with the GC autosampler was coupled directly to the rim of a volumetric flask. The septum of the screw stopper was replaced by an appropriately sized piece of membrane obtained by portioning the PTFE membrane disc. The exchange surface of the membrane coincides with the area of the opening of the cap (0.79 cm^2).

The stoppered micro-vial, containing the organic solvent and the inserted membrane was assembled to the flask by means of a PTFE ring surrounding the stopper (the ring was made from PTFE tape). Typically, the micro-vial contained 150 μL of decane, and the volumetric flask 40 mL of the aqueous solution. The placement of the membrane in alternating contact with the solutions was achieved by orbital agitation using an Ovan rotatory mixer provided with a platform with clamps for 20 tubes supplied by Scharlab (Barcelona, Spain) (Fig. 1).

2.3. Equipment and conditions for gas chromatography–mass spectrometry

Analyses were performed using a Shimadzu GC2010 gas chromatograph with a mass selective detector Shimadzu GCMS-QP20105, and a SPB-5 fused silica capillary column (30 m, 0.32 mm i.d., 0.25 μm film thickness) purchased from Supelco (Bellefonte, PA, USA). Helium was used as carrier gas at a 43.3 cm s^{-1} linear velocity. The GC oven temperature program was: 60 °C for 1.5 min; 30 °C min^{-1} to 275 °C, and then held for 20 min. 2 μL of each solution were injected in the splitless mode using an auto injector Shimadzu AOC-20i. For quantification area peaks corresponding to *m*/*z* 165 were measured.

2.4. Samples

Urine samples were collected from volunteers 2 h after a self-application of 7 g of the skin cream containing 8% EDP.

2.5. Procedure for membrane assisted extraction

5 mL of phosphate buffer, *x* mL of ethanol and 20 mL of urine were added to a series of 50 mL volumetric flasks. Then, aliquots of (0.8 − *x*) mL of EDP ethanol standard containing 200 μg L^{-1} were added to the flasks. The mixtures were diluted with 14.2 mL of water. Each volumetric flask was then assembled to a stopped micro-vial (with membrane) containing 150 μL of decane (Fig. 1). The assembled system was fixed to a 20-clamp platform, and rotation was run at 30 rpm for 3 h. After extraction, the flask-vial was disassembled, and the top of the micro-vial was dried by simple contact with absorbent paper (homogenisation of the acceptor phase at this point is recommended if a viscous solvent such as decane is used). Finally, the vial was placed at the autosampler for chromatographic analysis.

3. Results and discussion

The device presented in this paper allows the extraction of analytes from a large volume of donor aqueous phase through a reduced membrane surface (0.79 cm^2) into a microlitre volume of an organic solvent. The contact area between the membrane and the solutions is important to ensure exploitable extraction efficiencies in a reasonable extraction time, and the reduced area of this device might constitute a limitation of the device. However, as the arrangement described here allows the easy agitation of both phases (aqueous and organic) the thickness of the stagnant boundary layer formed at both sides of the membrane is minimised, favouring the mass transfer through the membrane. This constitutes a beneficial difference with respect to other devices, where only the aqueous phase is stirred. It is believed that both effects on the extraction kinetics (low mass transfer due to reduced membrane surface and favoured diffusion by agitation) compensate one another, in part, and consequently the extraction time necessary to reach extraction efficiencies useful for chemical analysis is comparable to other devices.

Agitation was carried out by orbital rotation and depending on the number of clamps of the platform, various samples can be extracted simultaneously which reduces the extraction time per sample.

The extraction efficiency will depend on both the contact time of the solutions with the membrane by cycle and the frequency of the rotation. Both variables are determined by the rotary speed of the mixer. When the speed is increased, the number of cycles increases, and thus diminishes the contact time per cycle.

The volume of acceptor and donor phases is a working condition that determines the analytical features. It is known that, the higher acceptor volume the higher extraction efficiency (defined as the ratio of extracted analyte to the total amount of analyte); and that

the lower acceptor volume the higher enrichment factor (defined as the ratio between the concentration in the acceptor phase after extraction and the initial concentration of the analyte in the donor phase) [5,18]. The enrichment factor determines the sensitivity and limit of detection, and for this reason conditions that maximize it are normally recommended. For the presented device, volumes of phases can be adjusted at convenience in a broad range using either vials of 2 mL or 200 μL, and volumetric flasks of 50 or 100 mL. This flexibility is an advantage with respect to other devices for membrane assisted extraction.

3.1. Study of experimental variables on the extraction

3.1.1. Organic acceptor phase

The organic solvent has a determinant role on the enrichment factor, since the partition coefficient of the analyte is the driving force of the extraction. Other important properties are the volatility and the viscosity. Low volatility prevents solvent evaporation, which improved the repeatability of the measurements, while low viscosity is desirable to favour mass transfer and consequently faster extraction. As it is known low volatility and low viscosity are mutually exclusive properties, and consequently the choice usually is based on experimental comparison. Four organic solvents were compared. Extensive loss of hexane was observed due to its high volatility. Higher enrichments were found for decane than for 1-octanol or ethyl acetate. Consequently decane was selected to continue the study.

When working with decane, a loss of organic solvent (about 30%) was observed for long extraction times (12 h).

3.1.2. Orbital agitation

Agitation is essential to obtain good enrichment factors within a reasonable period of time. Considering the configuration of the extraction system, orbital agitation at a controlled angular speed is an alternative to obtain reproducible agitation conditions. 50 mL of donor phase was extracted at several angular rates (from 15 to 50 rpm). A maximum enrichment factor was at 25 rpm. The decrease of the enrichment factor at rotations rates higher than 25 rpm was a consequence of the formation of a motionless aliquot of the donor aqueous solution that remained attached to the membrane due to centrifugal forces. So, the stagnant boundary layer formed at the membrane was not renewed efficiently, and the mass transfer was not favoured. The formation of this motionless portion was delayed when the volume of the donor phase in the 50 mL volumetric flask diminished. Nevertheless, as will be documented later lower volumes of donor solution diminished the enrichment factor. As a compromise, subsequent experiments were carried out with 40 mL of donor solution at a 30 rpm rotation speed.

3.1.3. Extraction time

Long time was required for stationary conditions to be reached due to the reduced surface of the membrane. Nevertheless, the high volume ratio between donor and acceptor solutions used allowed a satisfactory enrichment factor to be obtained even when far from equilibrium conditions. In order to avoid extensive time for measurements, the study of the rest of variables was carried out at 3 h extraction time. This prolonged time is compensated by the minimal handling required after extraction. Besides a number of samples can be extracted simultaneously thus reducing the extraction time for sample.

3.1.4. pH of the donor aqueous phase

The pH value in the aqueous sample was varied from 5.6 to 10.6. Maximum enrichment factor was obtained when working at a pH close to the neutrality. So, samples and standards were buffered at pH 7.2 using a buffer of dihydrogen phosphate/hydrogen phosphate.

3.1.5. Addition of ethanol to the donor phase

As aqueous working standards contained ethanol, its influence on the enrichment factor was studied in the range 0.2–6%. The enrichment factor remained constant for low ethanol concentration, but a significant decrease was noticed for ethanol concentration higher than 3%. Further experiments were carried out in the presence of 2% ethanol.

3.1.6. Addition of sodium chloride to the donor phase

The presence of salts usually enhances the extraction yield as a consequence of the called salting-out effect [19–22]. It was found that the presence of sodium chloride at a concentration level close to the saturation enhanced the enrichment factor of EDP up to double value. It can be taken in consideration as an additional strategy to increase the sensitivity of the analytical procedure, if necessary.

3.1.7. Donor and acceptor phase volume

Enrichment factors and extraction efficiencies depend on the partition coefficient and on the volumes of acceptor and donor phases as well. As the volumes can be selected over a broad range, their effects on the extraction were also studied. The enrichment factor significantly increased with the volume of the donor solution, while an opposite effect was observed from an increase of the acceptor volume. For the 100–150 μL interval, the enrichment factor remained constant. Nevertheless, a significant decrease was noticed when using 700 μL. Experiments have been also carried out with 50 μL of acceptor phase; for such a small volume, the organic solvent used (decane) did not contact efficiently the membrane since most of the solvent was adhered to the micro-vial wall. For this reason, volumes lower than 100 μL are not recommended.

3.2. Validation data

The obtained calibration graph in the range of 0.11–1.5 $\mu g\,L^{-1}$ EDP was:

$$\text{peak area} = 34\,000(\pm 1900)[\text{EDP}] + 1900(\pm 1400)$$

(standard deviation of the slope and the ordinate were calculated from the standard deviation of y-residuals $s_{y/x} = 2250$; $n = 5$; $R^2 = 0.991$).

Limits of detection and quantification were calculated as the concentration corresponding to 3 and 10 times the standard deviation of a EDP standard containing 0.05 $\mu g\,L^{-1}$ EDP (6 replicates). Obtained values were 0.03 and 0.11 $\mu g\,L^{-1}$, respectively. Precision within-day was 7.4%.

Using standards in decane, the enrichment factor and extraction efficiency corresponding to each point of the calibration graph were calculated. The average value for the enrichment factor was 57 (SD = 8), which corresponded to an extraction efficiency of 22% (SD = 3).

3.3. Urine samples

Fig. 2 depicts a typical chromatogram for a sample spiked with 0.20 $\mu g\,L^{-1}$ EDP. Several peaks due to the organic solvent and to epoxide compounds coming from the membrane were eluted before the analyte. So, in order to achieve a separation from such potentially interfering compounds, a temperature program that delayed the analyte elution up to approximately 10 min was selected.

Preliminary experiments have evidenced that the extraction is matrix dependent, and for this urine samples were analysed using

Table 1
Determination of EDP in urine[a] by the method of standard additions.[b].

Sample	Regression line	R^2	[EDP] μg L^{-1} (S_{XE})[c]	Confidence limit[d]
1	Area = 3900 + 20 966 [EDP]	0.993	0.38 (0.04)	±0.13
2	Area = 31 039 + 10 603 [EDP]	0.992	5.8 (0.2)	±0.7
3	Area = 7131 + 31 896 [EDP]	0.993	0.44 (0.04)	±0.14
4	Area = 11 492 + 24 685 [EDP]	0.986	0.92 (0.08)	±0.26
5	Area = 35 926 + 22 855 [EDP]	0.984	3.1 (0.2)	±0.64
6	Area = 12 279 + 18 007 [EDP]	0.97	1.36 (0.15)	±0.48

[a] Samples were diluted at 50% with water.
[b] Four additions were made on each sample.
[c] Standard deviation of the extrapolated value.
[d] 95% confidence limits (3 degrees of freedom).

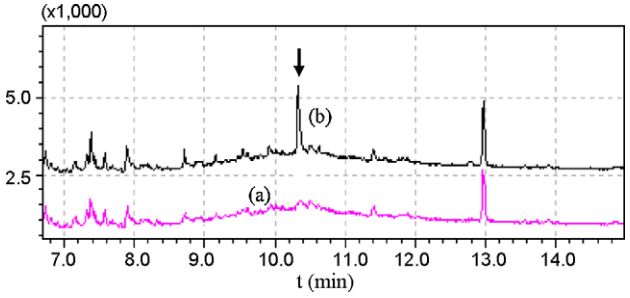

Fig. 2. Typical chromatogram for urine samples. (a) Urine blank and (b) urine spiked with 0.2 μg L^{-1} EDP.

the method of standard additions. The results obtained are summarised in Table 1. In all cases a linear fit was obtained, normally with slopes lower than the slope corresponding to the calibration obtained without urine. Found results were in the interval 0.38–5.8 μg L^{-1} EDP.

4. Conclusions

A novel arrangement for microporous membrane liquid–liquid extraction is presented. Technically the device is simple, flexible, low cost and its home-made assembly is trouble free; for extraction, orbital agitation worked efficiently.

As in other published formats, extensive sample cleanup and analyte enrichment were achieved using small quantities of the organic solvent. The obtained organic extracts from urine can be injected without additional laboratory operation into a GC–MS system. Thus, the required minimal handling for sample preparation is a significant operational advantage.

From the analytical point of view, this device can be classified as precise and robust at the trace level with no risk of cross-contamination. Moreover, it allowed sample preparation with a minimal handling.

In closing, it should be added that an attractive improvement would be future design modifications to allow shorter extraction times.

Acknowledgement

Financial support by “Ministerio de Ciencia e Innovación” (Spain) project CTQ 2006-01851 is acknowledged.

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