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Efficient discrimination and removal of phospholipids during electromembrane extraction from human plasma samples

Aim: For the first time, extracts obtained from human plasma samples by electromembrane extraction (EME) were investigated comprehensively with particular respect to phospholipids using ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS). The purpose was to investigate the potential of EME for phospholipid cleanup in different EME systems. **Results & discussion:** No traces of phospholipids were detected in any of the acceptor solutions, whereas the model analytes were extracted with recoveries up to 50%. Thus, the EME systems tested in this work were found to be highly efficient for providing phospholipid-free extracts. **Conclusion:** Ultra-HPLC–MS/MS analysis of the donor solutions revealed that the phospholipids principally remained in the plasma samples. This proved that the phospholipids did not migrate in the electrical field and they were prevented from penetrating the supported liquid membrane.

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Keywords: electromembrane extraction • phospholipids • plasma samples • supported liquid membrane • ultra-HPLC–MS/MS

Electromembrane extraction (EME) as a novel principle in the field of analytical microextraction was introduced in 2006 [1]. Since then, a substantial amount of work has been conducted to develop different applications and to obtain a fundamental understanding on how different parameters influence the extraction performance. As in liquid-phase microextraction (LPME), basic or acidic compounds are transported from an aqueous sample, across an organic supported liquid membrane (SLM) and into an aqueous acceptor solution [2]. However, in LPME, the driving force for the extraction is a pH gradient, while in EME an electrical field is applied across the SLM, facilitating electrokinetic migration of charged compounds. For extraction of basic analytes, the cathode is located in the acceptor solution and the anode is located in the sample. For efficient electrokinetic

transfer, neutral or acidic conditions are maintained both in the sample and in the acceptor solution. For extraction of acidic analytes, the direction of the electrical field is reversed, and neutral or alkaline conditions are used in the sample and acceptor solution. Several advantages are associated with EME: rapid extraction, high analyte enrichment, efficient sample cleanup, low solvent consumption and high selectivity by tuning the SLM and the electrical field [3]. Also, EME can be performed in the 96-well configuration [4]. This makes it interesting to further develop different applications and to maximize the potential of the technique. To date, basic and acidic drugs have been extracted successfully from biological matrices such as whole blood, plasma and urine [5–12]. In addition, EME has been demonstrated for amino acids [6], peptides [7], metal ions [8–18] and different envi-

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ronmental pollutants [9,10–20]. A substantial number of EME applications have been published, and these have been reviewed recently [11–23].

A significant number of research papers on EME have been devoted to bioanalysis of small-molecule drug substances, which have been extracted from biological fluids. Biological fluids represent a challenge as they contain a highly complex matrix, and with liquid chromatography-mass spectrometry (LC–MS) matrix components can interfere massively by causing matrix effects, namely ion suppression or enhancement [12]. Therefore, extensive sample cleanup is often crucial prior to LC–MS in order to avoid incorrect or misleading results. This is highly applicable when dealing with blood plasma as the biological matrix, due to its high content of phospholipids. Although there are many matrix components in plasma, phospholipids represent a group of zwitterionic lipids that are well known to cause serious matrix effects in the MS and column contamination in the LC [13–31]. Consequently, it is desirable to remove these interfering compounds before introducing the sample to the LC–MS instrument [14]. While several reports have discussed sample cleanup in general by EME [11–23], there are no specific data on EME and phospholipid cleanup. In a related paper published recently [15], phospholipids were shown not to cross SLMs in an LPME type of system, where a pH gradient was served as the driving force for extraction. However, in EME there is a very strong electrical field across the SLM, and there is currently no information about the behavior of phospholipids under such conditions. Therefore, the objective of the current work was to investigate and document the level of phospholipid cleanup using different EME systems. The results are very important for future justification and development of new EME applications related to blood samples and for the future propagation of EME.

Experimental section

Chemicals & solutions

Deionized water was purified with a MilliQ water purification system (Molsheim, France). 2-nitrophenyl octyl ether (NPOE), di-(2-ethylhexyl)-phosphate (DEHP), bis(2-ethylhexyl) phosphite (DEHPi), 1-ethyl-2-nitrobenzene (ENB), 1-isopropyl-4-nitrobenzene (IPNB), 1-heptanol, 1-octanol, venlafaxine, citalopram, benzamidine, ephedrine and trimethoprim were purchased from Sigma-Aldrich (MO, USA). Hydrochloric acid (HCl, 37%), phosphoric acid, acetonitrile (ACN), formic acid (HCOOH) and methanol were all purchased from Merck (Darmstadt, Germany). Sodium hydroxide (NaOH, 99%) was from VWR (Leuven, The Netherlands).

Standard solutions

Stock solutions of each model analyte with a concentration of 1 mg ml⁻¹ were prepared in methanol and stored at -32°C. Standard solutions of the basic and acidic model analytes were prepared by adding the respective stock solutions and diluting with 10 mM HCl and 10 mM NaOH, respectively.

Plasma samples

Drug-free human blood plasma was supplied by Oslo University Hospital (Oslo, Norway) and stored at -32°C. The plasma was thawed prior to extraction and transferred to the sample vials, either concentrated or diluted, 1:6 with deionized water or 50 mM phosphoric acid to a total volume of 900 µl. Finally, the plasma was spiked with 100 µl standard solution containing the model analytes.

Electromembrane extraction

Figure 1 illustrates the experimental setup when performing EME of basic drugs; the cathode was located in the acceptor solution and the anode was located in the plasma sample. In the experiments where EME was performed with acidic drugs, the polarity was reversed. A 2.0-ml 2-SV glass vial (sample vial) from Chromacol® (Welwyn Garden City, UK) was filled with 1000 µl of the plasma sample, detailed in the previous section. Second, a PP Q3/2 hollow fiber of polypropylene with a wall thickness of 200 µm, an internal diameter of 1.2 mm and a pore size of 0.2 µm (Membrana, Wuppertal, Germany) was dipped into the organic solvent serving as SLM. Any excess of organic solvent was gently removed with a medical wipe before connecting the fiber to a screw cap (Chromacol). The lumen of the hollow fiber was then filled with 25 µl of aqueous acceptor solution using a microsyringe. Finally, two platinum wires with a diameter of 0.5 mm (KA Rasmussen, Hamar, Norway) were put in the sample and acceptor solution, serving as electrodes. Prior to extraction, the electrodes were connected to an external DC power supply Model ES 0300–0.45 from Delta Power Supplies (Delta Electronika, Zierikzee, The Netherlands), with programmable voltage in the range of 0–300 V, and with current output from 0 to 450 mA. The voltage from the external power supply was turned on to initiate extraction, and this caused electrokinetic transfer of charged compounds across the SLM. To further promote extraction, the whole setup was placed in an Eppendorf thermomixer comfort (Eppendorf, Hamburg, Germany), providing agitation of 900 × g r.p.m. during the EME process. A Caltek® CM3900A digital multimeter (Caltek Industrial Ltd, Kwai Chung, Hong Kong, China) was connected to monitor the current across the SLM. After a predetermined time, the volt-

age was turned off and the acceptor solution was transferred to ultra-HPLC–MS/MS (UHPLC–MS/MS) analysis for the determination of model analytes and for the investigation of phospholipids.

Protein precipitation & assessment of phospholipids in EME extracts

To assess whether phospholipids were present in the EME extracts or not, the UHPLC–MS/MS phospholipid chromatograms obtained for the EME extracts were compared with that obtained from protein precipitation (PP) experiments. The protein precipitates served to check that the UHPLC–MS/MS system worked properly for phospholipid detection, as human phospholipids are highly abundant in protein precipitates [16]. Second, the protein precipitates served as a reference for semiquantification of trace phospholipids.

ACN was used as the PP reagent. In one set of experiments (comparison with EME system #1), PP was performed by mixing 1000 µl of plasma and 1000 µl of ACN (1:1 v/v). In another set of experiments (comparison with EME system #2 and 3), PP was performed by mixing 1000 µl of diluted plasma (1:6 v/v with deionized water, similar to the corresponding EME systems) and 2000 µl of ACN (1:2 v/v). In both cases, PP was completed with agitation on an IKA® MS3 digital vortex mixer (Staufen, Germany) for 2 min, followed by centrifugation for 5 min in a Centrifuge 5804 from Eppendorf AG (Hamburg, Germany) with a speed of 10,000 × *g* r.p.m. The supernatant was subsequently transferred to microinserts for UHPLC–MS/MS analysis.

Ultra-HPLC–MS/MS

UHPLC–MS/MS analysis was performed on a chromatographic system comprising a Dionex UltiMate 3000 RS Pump, Column Compartment, and Autosampler, followed by a Thermo Scientific LTQ XL Linear Ion Trap Mass Spectrometer from Thermo Scientific (CA, USA). Chromatographic separation was obtained with an Acquity UPLC® HSS T3 column from Waters (Wexford, Ireland) with a length of 100 mm, an inner diameter of 2.1 mm, a particle size of 1.8 µm and a pore size of 100 Å. The column temperature was set to 40°C. Data acquisition was performed with the software Xcalibur (version 2.2. SPI.48) from Thermo Scientific.

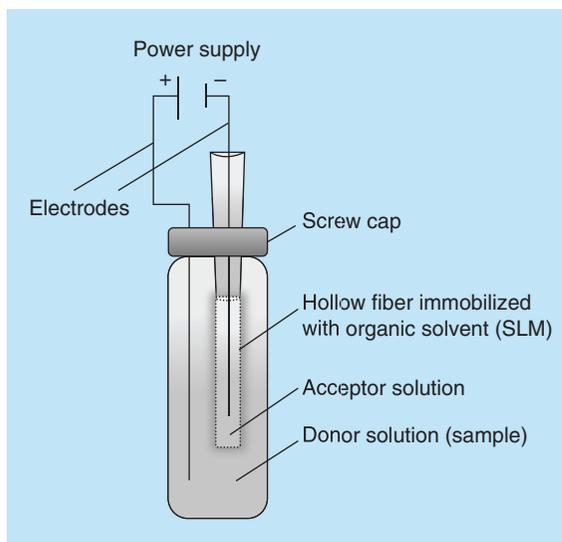


Figure 1. Experimental setup for electromembrane extraction.

SLM: Supported liquid membrane.

Mobile phases A and B comprised 20 mM HCOOH and methanol in the ratios 95:5 and 5:95 v/v, respectively. Throughout the analysis, the mobile phases were pumped with an overall flow rate of 0.3 ml min⁻¹ following a preset gradient: 10% B at 0.0 min, 100% B at 0.3 min, 100% B at 6.0 min, 10% B at 6.1 min and 10% B at 8.5 min. Total run time was 8.5 min, and the injection volume was 2.0 µl.

Detection of phospholipids was acquired in the selected reaction monitoring mode with ESI in the positive mode. The transition *m/z* 184→184 was monitored. Detection of the model drugs was based on the *m/z* transitions, presented in Table 1. The source fragmentation energy was 65 V and the collision energy was 10%.

High-HPLC–UV detection

HPLC–UV analysis was performed using a Dionex Ultimate 3000 system, comprising a degasser (SRD-3200), a pump (HPG-3200M) and an autosampler (WPS-3000SL) from Dionex Corporation (CA, USA). Instrument control, data processing and data collection were conducted using Chromeleon software (v. 6.80 SP2 Build 2212) from Dionex Corporation. All analytes were detected with a wavelength of 214 nm by a VWD-3400 UV/VIS detector (Dionex Corporation) [17].

Table 1. Mass spectrometry parameters.

Analyte	Selected reaction monitoring		Collision energy (%)
	Precursor	Fragment	
Venlafaxine	278.18	260.1	35
Citalopram	325.19	262.1	24

Separation of the polar basic drugs was obtained as reported previously [17] with an YMC-Triart C18 column (150 mm × 2.00 mm, 5 μm) from YMC America, Inc. (PA, USA). The injection volume was 20 μl. Mobile phases A and B were an aqueous phase (20 mM formic acid and 5 mM sodium 1-heptanesulfonate in MilliQ-water) and methanol in the ratios 95:5 and 5:95 (v/v), respectively. In 5.8 min, mobile phase B was increased from 18 to 40%, whereas the flow rate was decreased from 0.4 to 0.2 ml min⁻¹. Further on, mobile phase B was increased to 80% in 0.1 min at a flow rate of 0.4 ml min⁻¹, and this condition was maintained for 1.7 min. Finally, mobile phase B was decreased to 18% at a flow rate of 0.4 ml min⁻¹ in 0.1 min, and this condition was maintained for 2.3 min to achieve equilibration [17].

Calculations

Recovery (R%) was calculated according to the following equation for each analyte:

$$R\% = \frac{n_a \text{ final}}{n_d \text{ initial}} \times 100\% = \frac{V_a}{V_d} \times \frac{C_a \text{ final}}{C_d \text{ initial}} \times 100\%$$

where n_d initial is the initial number of analyte moles present in the donor solution (sample), and n_a final is the final number of analyte moles collected in the acceptor solution. V_d and V_a represent the donor and acceptor volumes, respectively, whereas C_d initial and C_a final

represent the initial and final analyte concentrations in the donor and acceptor solutions, respectively.

Results & discussion

Main EME systems

Initially, different EME applications, previously reported in the literature for the extraction of basic and acidic drugs, were reviewed and categorized into three main EME systems; EME system #1 for nonpolar basic drugs, EME system #2 for polar basic drugs and EME system #3 for nonpolar acidic drugs. As illustrated in Figure 2, each main EME system was tested with two or three different SLMs. The pH conditions in samples and acceptor solutions were according to the literature, and the selected model analytes were stable under these conditions [17,18]. This was also the case with venlafaxine, which was extracted by EME for the first time. In EME systems #1 and #2, the experiments were conducted with the cathode located in the acceptor solution, facilitating extraction of positively charged compounds. For EME system #3, the direction of the electrical field was reversed, and the anode was placed in the acceptor solution for the extraction of negatively charged compounds. After completion of each EME experiment, the acceptor solutions were analyzed twice by UHPLC–MS/MS or HPLC–UV to detect selected model analytes. This was performed solely to verify the extraction performance. However, the primary focus and novelty was on EME

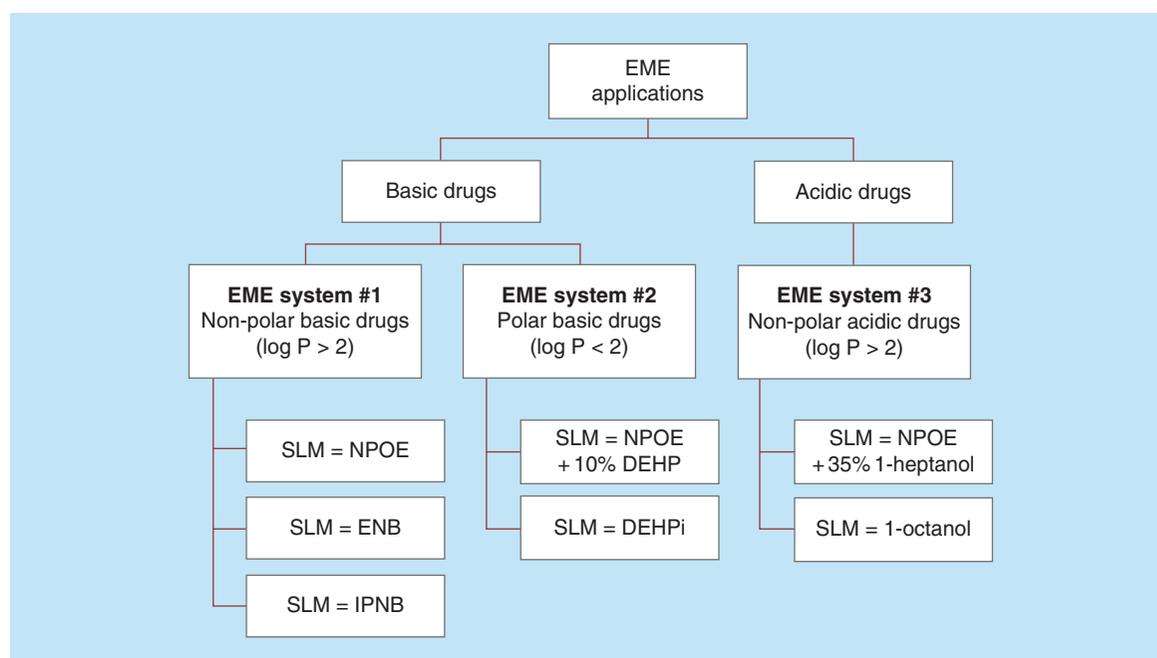


Figure 2. Generic electromembrane extraction systems for (#1) nonpolar basic drugs, (#2) polar basic drugs and (#3) nonpolar acidic drugs.

DEHP: Di-(2-ethylhexyl)-phosphate; DEHPi: Bis(2-ethylhexyl) phosphite; EME: Electromembrane extraction; ENB: 1-ethyl-2-nitrobenzene; IPNB: 1-Isopropyl-4-nitrobenzene; NPOE: 2-Nitrophenyl octyl ether; SLM: Supported liquid membrane.

as an efficient cleanup method for phospholipids, and therefore the acceptor solutions were reanalyzed by UHPLC–MS/MS to examine whether phospholipids from the plasma samples were transferred across the SLM and into the acceptor solutions. EME acceptor solutions containing no traces of phospholipids were considered as successful experiments in this context. The acceptor solutions were analyzed for phospholipids by UHPLC–MS/MS using in-source fragmentation and detection of the m/z 184→184 transition. This mass transition corresponds to the phospholipid backbone of phosphatidylcholines (PCs), lyso-phosphatidylcholines (lyso-PCs) and sphingomyelins (SMs), as presented in **Figure 3**. This LC–MS/MS approach (phospholipid mode) has been developed and applied previously [19], and PCs account for approximately 60–70% of human phospholipids [20].

EME system #1 (nonpolar basic drugs)

In a first set of experiments, EME system #1 for nonpolar basic drugs was addressed. Based on earlier experience, NPOE was selected as solvent for the SLM [21]. Venlafaxine and citalopram were selected as model analytes for the experiments. These compounds are nonpolar basic drug substances, and are frequently measured in bioanalytical laboratories. Because the pKa values of the model analytes were higher than 7.4, they were positively charged in plasma under physiological conditions. Therefore, pH adjustment of the samples was not required, and EME was conducted directly from undiluted plasma [22]. Based on earlier experiences [23], 10 mM of HCl was selected as the acceptor solution, the extraction voltage was set to 300 V and the extraction time was 5 min, without any further optimization.

After EME, the acceptor solutions were first analyzed for the content of model analytes using UHPLC–MS/MS with the specific m/z transitions defined in **Table 1**. A typical chromatogram is shown in **Figure 4A**, and recoveries were ranging from 30 to 40%. EME of citalopram has been reported previously [21,24], whereas venlafaxine was extracted for the first time. Subsequently, the acceptor solutions were reanalyzed with UHPLC–MS/MS in the phospholipid mode. The UHPLC–MS/MS system was now tuned for phospholipids based on in-source induced collision and detection of the m/z 184→184 transition. The experiments were conducted in six replicates, and one of the chromatograms is depicted in **Figure 5B**. Direct injection of a protein precipitate, not subjected to EME, is illustrated for comparison in **Figure 5A**. The latter shows abundant peaks for phospholipids as expected, and this served as a standard for the original content of phospholipids in the plasma sample. In **Figure 5B**, no

peaks were observed in the retention window for phospholipids, and this proved that the EME acceptor solution was free of phospholipids. This was observed for all six replicates. Thus, in the current setup, the EME system extracted the nonpolar drugs, and at the same time the phospholipids were effectively discriminated. Under physiological conditions in plasma (pH 7.4), the phospholipids were present as zwitterions, and therefore their electrokinetic migration was zero.

In two follow-up experiments, extraction of the nonpolar model analytes (EME system #1) was repeated under extended extraction time conditions and acidic conditions in the sample to further investigate the discrimination of phospholipids. First, the extraction time was prolonged from 5 to 30 min to eliminate the possibility of slow transfer of phospholipids. The resulting chromatograms (m/z 184→184) obtained for the acceptor solutions after 30 min of EME ($n = 2$, data not shown) were still free from PCs, lyso-PCs and SMs. Second, it was investigated whether the phospholipids behave differently in the EME system with a net positive charge. Thus, plasma samples were acidified by diluting with 50 mM phosphoric acid (1:6 v/v). The final pH of the samples was now approximately 2 based on a rough inspection using pH paper. NPOE was used as SLM, and EME was performed for 5 min. The resulting chromatograms ($n = 6$, data not shown) revealed that the acceptor solutions were still free from phospholipids, even after acidification. After EME, the donor solutions were also analyzed by UHPLC–MS/MS in the phospholipid mode. The results showed that phospholipids still were in the sample. Thus, although the phospholipids were net positively charged, and consequently influenced by the electrical field, they were still unable to penetrate and distribute into the organic SLM.

Additional experiments with EME system #1 were performed using ENB and IPNB as SLMs, as these organic liquids have previously been used as alternative SLMs for nonpolar basic drugs [25]. The current across the SLM was monitored, and the applied voltage was set to 50 and 20 V for ENB and IPNB, respectively. Additionally,

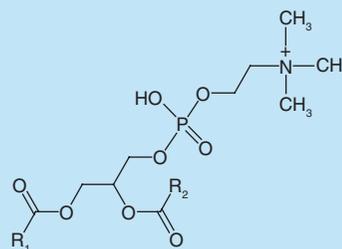


Figure 3. Phospholipid backbone of phosphatidylcholines ($m/z = 184$).

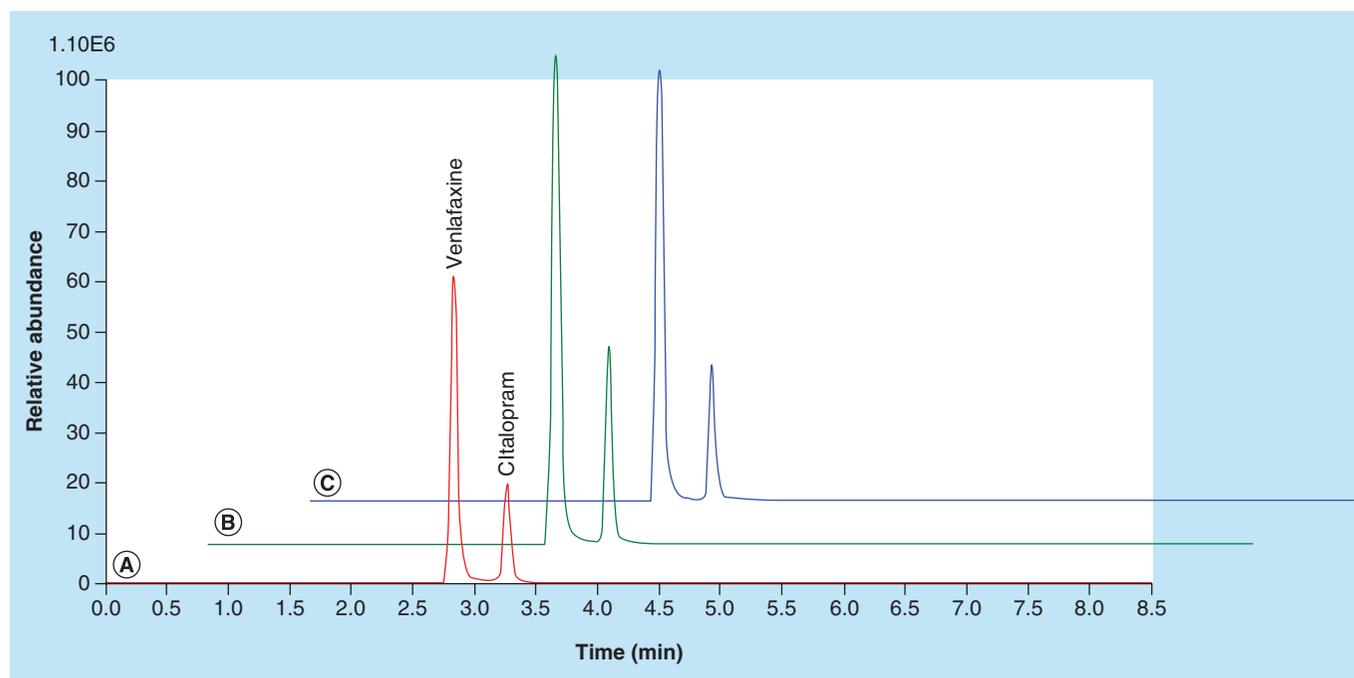


Figure 4. Extraction performance for electromembrane extraction system #1. Ultra-high-performance liquid chromatography tandem mass spectrometry chromatograms of venlafaxine and citalopram after performing electromembrane extraction (system #1) with (A) supported liquid membrane, 2-Nitrophenyl octyl ether; (B) supported liquid membrane, 1-ethyl-2-nitrobenzene; and (C) supported liquid membrane, 1-Isopropyl-4-nitrobenzene.

plasma was diluted 1:6 (v/v) with deionized water when using ENB as SLM. The latter was done to reduce the current in the EME system. Under these conditions, the current was below the recommended limit of 50 μA [26]. Once again, the acceptor solutions were first analyzed for the content of venlafaxine and citalopram by UHPLC–MS/MS. Representative chromatograms are shown in Figure 4B & C, and recoveries ranged between 40 and 50%. Further on, the acceptor solutions were reanalyzed with UHPLC–MS/MS in the phospholipid mode. The resulting phospholipid chromatograms ($n = 3$, data not shown) were similar to the chromatograms obtained in the previous experiments with NPOE. No phospholipids were detected in the acceptor solutions when using pure ENB or IPNB. Thus, although the chemical composition of the SLM was changed, the phospholipids were still discriminated effectively.

EME system #2 (polar basic drugs)

When performing EME of polar basic analytes, a carrier has to be included in the SLM to achieve successful mass transfer. In the literature, DEHP has been used as an effective anionic carrier for this purpose [27]. DEHP acts as a carrier by forming neutral hydrophobic ion pairs with the polar basic drugs, thereby enabling their transition from the aqueous sample and into the organic SLM. Therefore, in a new set of experiments, 10% (w/w) of DEHP was added to NPOE. Benzamidine, ephed-

rine and trimethoprim were selected as model analytes for the experiments. The addition of DEHP increased the current across the SLM extensively and reduced the robustness of the extraction system [28]. To counteract this, and to keep the current below the recommended limit of 50 μA [26], the applied voltage was reduced to 15 V. Additionally, plasma was diluted to 1:6 (v/v) with deionized water. The acceptor solution comprised 25 μl of 10 mM HCl, and EME was carried out for 5 min. The acceptor solutions ($n = 6$) were first analyzed by HPLC–UV, solely to verify the extraction performance. Figure 6A illustrates a typical chromatogram, with recoveries in the range 25–30%. The results verified the capability of the current EME setup for successful extraction of polar basic drugs. Matrix effects are not an issue in HPLC–UV analysis. Therefore, the acceptor solutions were reanalyzed by UHPLC–MS/MS in the phospholipid mode ($n = 6$). One of the resulting chromatograms is presented in Figure 4C. Interestingly, even with an anionic carrier in the SLM, the phospholipids were still efficiently prevented from entering the acceptor solution. This may be explained by the zwitterionic nature of phospholipids, and that complexation with DEHP results in net negatively charged phospholipid complexes, which are migrating in the opposite direction and toward the anode.

In a follow-up experiment, EME was repeated from acidified plasma. Acidification was achieved by dilut-

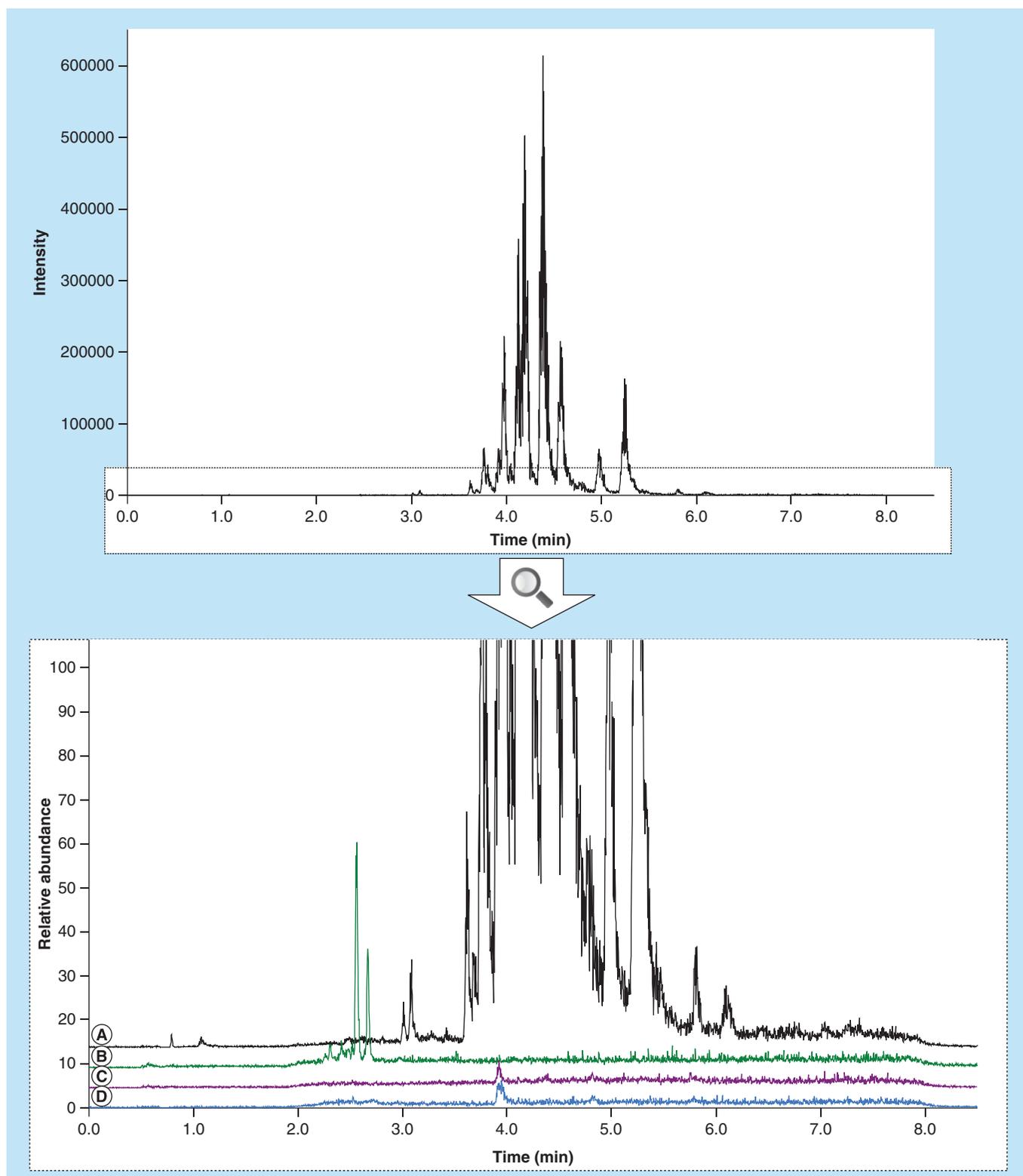


Figure 5. Ultra-high-performance liquid chromatography tandem mass spectrometry phospholipid chromatograms. Upper chromatogram: full scaled chromatogram of phospholipids. Lower chromatograms: **(A)** protein precipitated plasma; **(B)** electromembrane extraction system #1, supported liquid membrane, 2-Nitrophenyl octyl ether (NPOE); **(C)** electromembrane extraction system #2, supported liquid membrane: NPOE +10% Di-(2-ethylhexyl)-phosphate; and **(D)** electromembrane extraction system #3, supported liquid membrane: NPOE +35%1-heptanol.

ing with 50 mM phosphoric acid, providing a final sample pH of approximately 2 (verified by pH paper). The SLM comprised NPOE with 10% DEHP, and EME was performed for 5 min (EME system #2). The resulting UHPLC–MS/MS chromatograms obtained in the phospholipid mode ($n = 6$, data not shown) revealed that the acceptor solutions were still free from phospholipids. The donor solutions were also analyzed, and this revealed that the phospholipids remained in the acidified plasma samples. This observation confirms the discriminative nature of the SLM.

Recently, DEHPi was reported as a new and suitable SLM for EME of polar basic analytes [29]. Therefore, EME extracts obtained with DEHP as SLM were included in the present work, with benzamidine, ephedrine and trimethoprim as model analytes. The electrical potential was 50 V, 25 μl of 10 mM HCl was used as acceptor solution, and the extraction time was 5 min. After EME, the acceptor solutions were analyzed by HPLC–UV to verify the extraction performance. A typical chromatogram is illustrated in Figure 6B, with recoveries ranging from 10 to 25%. After HPLC–UV, the acceptor solutions were reanalyzed by UHPLC–MS/MS in the phospholipid mode. The resulting chromatograms showed no signals for phospholipids ($n = 6$, data not shown). Thus, it was concluded that EME systems using DEHPi as SLM also provided extracts free from phospholipids.

EME system #3 (nonpolar acidic drugs)

Successful EME of nonpolar acidic drugs has recently been reported using a combination of 1-heptanol and NPOE as SLM [30]. Therefore, 35% of 1-heptanol added to NPOE was tested as SLM in the first experiment with the reversed electrical field. 1-heptanol caused

system instability by increasing the current across the SLM. Therefore, reducing the applied voltage to 10 V was necessary in order not to exceed a current of 50 μA . Also, plasma was diluted 1:6 (v/v) with deionized water to achieve a stable extraction system. The acceptor solution comprised 25 μl of 10 mM NaOH. Extractions were carried out for 5 min ($n = 6$). UHPLC–MS/MS was subsequently performed in the phospholipid mode. One of the chromatograms is presented in Figure 4D. No phospholipid peaks were detected for PCs, lysophospholipids or SMs in the acceptor solutions. Thus, even when reversing the electrical field, the phospholipids were still unable to transfer the SLM.

EME extracts obtained with 1-octanol as SLM were investigated in a final set of experiments [9]. Again, plasma was diluted 1:6 (v/v) with deionized water; the potential was adjusted to 10 V; the extraction time was 5 min; and the acceptor solution was comprised of 25 μl of 10 mM NaOH. The chromatograms obtained with UHPLC–MS/MS analysis in the phospholipid mode ($n = 6$, data not shown) revealed once more that no traces of phospholipids were detected in the acceptor solutions.

Conclusion & future perspective

For the first time, EME extracts obtained from human plasma samples have been investigated comprehensively with particular respect to phospholipids. EME was performed with different generic EME systems previously reported in the literature, and extraction performance was verified for appropriate model analytes using UHPLC–MS/MS or HPLC–UV. By performing UHPLC–MS/MS in the phospholipid mode, it was demonstrated that acceptor solutions from all the investigated EME systems were free from phospholipids. This was mainly explained by the zwitterionic

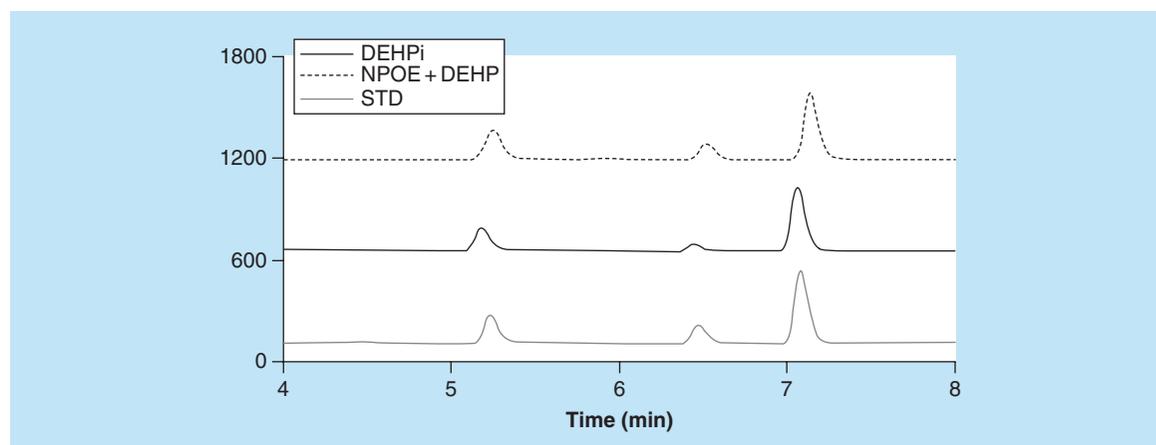


Figure 6. Extraction performance for electromembrane extraction system #2. HPLC–UV chromatograms of benzamidine, ephedrine and trimethoprim after performing electromembrane extraction (system #2) with (A) supported liquid membrane, NPOE +10% DEHP, and (B) supported liquid membrane, DEHPi. DEHP: Di-(2-ethylhexyl)-phosphate; DEHPi: Bis(2-ethylhexyl) phosphite; NPOE: 2-Nitrophenyl octyl ether; STD: Standard; 10 $\mu\text{g}/\text{mL}$ benzamidine, ephedrine, and thrimethoprim in 10 mM HCl.

nature of phospholipids at physiological pH, which prevented their net migration in the electrical field. To challenge this, EME was performed with acidified plasma (pH 2), but the phospholipids were still unable to transfer the SLM. The latter revealed a low solubility or affinity of phospholipids for the organic SLMs tested in this work. Thus, EME can be used highly efficiently to obtain phospholipid-free extracts in the future, for different applications involving human plasma or serum samples.

Financial & competing interests disclosure

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no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Summary points

Electromembrane extraction

- Extraction of charged substances from an aqueous sample across a supported liquid membrane driven by an electrical field.

Phospholipids

- Group of lipids that should be removed from biological samples prior to liquid chromatography-mass spectrometry analysis, as they may cause column contamination and matrix effects (ion suppression).
- Highly present in plasma samples.

Distribution of phospholipids in different electromembrane extraction systems

- Phospholipids are zwitterions at physiological pH conditions (pH 7.4), and net positively charged in acidified samples (pH 2).
- Phospholipids are prevented from entering the organic supported liquid membranes tested in this work under both neutral and acidic conditions in the sample.

Extensive sample cleanup

- Electromembrane extraction extracts from the currently investigated electromembrane extraction systems for nonpolar basic drugs (#1), polar basic drugs (#2) and nonpolar acidic drugs (#3) were all free from phospholipids.

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- of interest; •• of considerable interest

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