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Exhaustive electromembrane extraction of some basic drugs from human plasma followed by liquid chromatography–mass spectrometry

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ABSTRACT

Citalopram, loperamide, methadone, paroxetine, pethidine, and sertraline were extracted exhaustively with electromembrane extraction (EME) by increasing the number of hollow fibers from one to three. Experiments reported recoveries in the range 97–115% from 1000 μl spiked water samples. EME was accomplished with 200 V as extraction voltage, the extraction time was set to 10 min (equilibrium), and the extraction unit was subjected to 1200 revolutions per minute (rpm). The same experiment with different geometry in a stagnant system conducted with 21 μl acceptor solution provided recoveries from 50 μl undiluted human plasma (pH 7.4) in the range of 56–102% for the six basic model substances. In each experiment the acceptor solution was distributed into three separately hollow fibers in the same sample vial. The importance of an electrical field was verified by comparing EME with liquid-phase microextraction (LPME) under optimal conditions and demonstrated that the time needed to reach equilibrium was reduced by EME. EME–LC/MS provided linearity >0.99 (r^2 values) for the six basic model substances, and the repeatability within the low therapeutic range (10 ng/ml) was in the range 5.1–21.4% RSD. LC–MS provided estimated limit of quantification ($S/N = 10$) in the range 0.6–3.2 ng/ml. Eventually, patient samples from a reference laboratory were analyzed and provided reliable results with a relative difference <14% compared to stated values from the reference laboratory.

1. Introduction

The interest for microextraction techniques as an alternative to conventional liquid–liquid extraction (LLE) and solid-phase extraction (SPE) have increased substantially in last two decades. The growing interest has been reflected by the large number of published papers recently. Performing a search in Science Direct with the keyword microextraction displays 1124 papers in 2010; only 5 years ago the same number was 604. The benefit with microextraction techniques is their relatively low consumption of chemicals and sample compared to LLE and SPE. The smaller formats also provide a potential for automation in an auto sampler and further simplify sample preparation.

Different microextraction techniques perform with different extraction recoveries. A drawback with microextraction techniques like electromembrane extraction (EME) and liquid-phase microextraction (LPME) are their limited extraction recovery. Neither EME nor LPME are yet able to extract exhaustively from spiked water- or biological samples [1,2], although it has been reported exhaustive extraction of an acidic drug from spiked water samples in 2007 [3].

Since the introduction of EME in 2006 [4], it has been developed as an alternative to LPME providing faster extractions [5]. Early experiments with EME demonstrated that charged basic substances migrated electro-kinetically through a supported liquid membrane (SLM) immobilized in a hollow fiber and toward the cathode located in the acceptor solution inside the hollow fiber [4]. Further papers have reported extraction of both basic and acidic substances from water, plasma, urine, whole blood, breast milk, and tap water [3,6–14]. Recently a review article stated that typical EME extractions were from 500 to 1000 μl sample within 10 min extraction time, 10–300 V, and with agitation at 1200 rpm [15]. Peptides have also been extracted successfully with EME [16]. In order to simplify the EME setup and reduce the required amount chemicals and sample, some recent papers reported the ability of replacing the adjustable power supply with a 9 V battery and successfully extract from 70 μl untreated human plasma in a stagnant system [12,17]. Another substantial benefit is the enrichment factor obtained without any need for evaporation or reconstitution. The

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theoretical enrichment factor is determined by the ratio between donor- and acceptor phase. The aqueous extracts provided with EME are compatible with high performance liquid chromatography (HPLC) and capillary electrophoresis (CE).

Currently, one of EMEs disadvantage is the limited extraction recovery. Flux across a SLM is a process dependent on several parameters, which are described by the modified Nernst–Planck equation [18]. One of the most decisive parameters is the thickness of the membrane; a thicker membrane results in increased diffusion path and theoretically reduced extraction recovery. Another adjustable parameter is the electrical field and the flux over the SLM increases by increasing the applied voltage over the membrane. A third parameter is the ion balance, which is the total ionic concentration in the acceptor solution to that in the donor solution. A decrease in the ion balance provide a theoretical increase in the flux over the SLM. Eventually, the substances log *P* value and affinity for the SLM determine whether the analyte remain in the donor solution, trapped in the SLM or transported from the SLM and into the acceptor solution [18].

Until now extraction recovery for a given EME setup is in the range of 50–80% [2,15] and exhaustive EME extraction has not been reported. The reason for the limited extraction recovery has not been clarified. However some papers have assumed that electrolysis and subsequent pH-shift in the acceptor solution could have a negative effect regarding the extraction recovery [4,19]. The present work demonstrates for the first time exhaustive extractions with EME from both spiked plasma- and water samples. The practical approach to this challenge was to increase the ratio between organic- and aqueous donor phase by optimization of the surface available for electrokinetic migration.

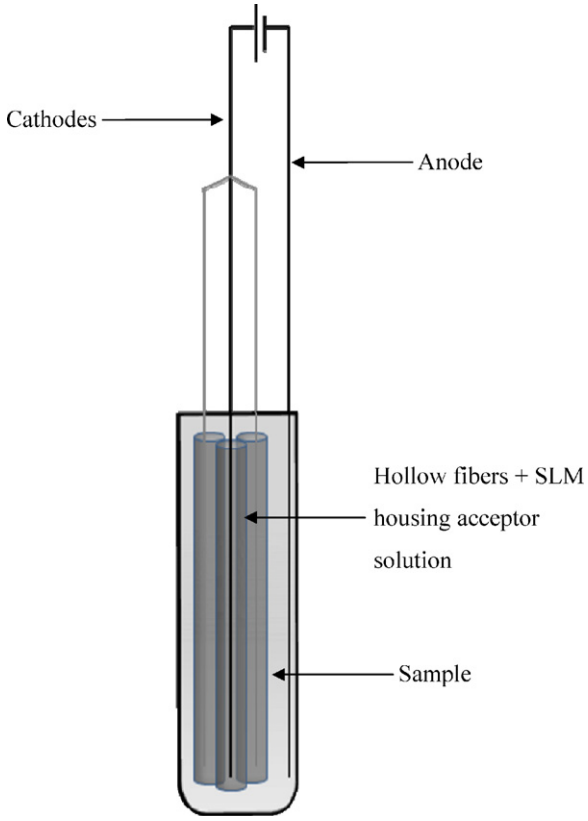

Fig. 1. Illustration of EME-setup.

2. Material and methods

2.1. Chemicals and solutions

Methadone hydrochloride, pethidine hydrochloride, and loperamide hydrochloride were all from Sigma–Aldrich (St. Louis, MO, USA). These substances were dissolved at 1 mg/ml to obtain appropriate standard solutions of methadone, pethidine, and loperamide. In order to obtain standard solutions at 1 mg/ml of paroxetine, sertraline, and citalopram; a paroxetine hydrochloride 20 mg tablet from HEXAL A/S (Hvidovre, Denmark) was extracted with 20 ml ethanol, a sertraline hydrochloride 50 mg tablet from Pfizer Italiana (Latina, Italy) was extracted with 50 ml ethanol, and a citalopram hydrobromide 20 mg tablet from H. Lundbeck (Copenhagen, Denmark) was extracted with 20 ml ethanol. 2-Nitrophenyl octylether was from Sigma–Aldrich (St. Louis, MO, USA). Water was obtained with a Milli-Q water purification system (Molsheim, France). Formic acid, methanol, and acetonitrile were all from Merck (Darmstadt, Germany). Drug-free human plasma was obtained from Ullevaal University Hospital (Oslo, Norway) and stored at −32 °C.

2.2. Electromembrane extraction (EME)

Two different formats have been investigated; a 100 μl vial (500 x 0.1-CVG vials from Chromacol. Trumbull, CT, USA) with an internal diameter of 4 mm and height of 32 mm and a 1500 μl vial (Screw cap vial, Agilent Technologies, Germany) with internal diameter of 10 mm and height of 32 mm have been used as sample compartment for the donor solution. The EME setup is illustrated in Fig. 1. A piece of PP Q3/2 polypropylene hollow fiber (Membrana, Wuppertal, Germany) with pore size 0.2 μm, wall thickness 200 μm, and with a length of 30 mm were closed in the lower end by mechanical pressure. The internal diameter of the hollow fibers being used was 0.6 mm and 1.2 mm for the 100- and 1500 μl compartment, respectively. The supported liquid membrane was made by impregnating the pores of three porous hollow fibers with 2-nitrophenyl octylether (NPOE) for 5 s. The excess NPOE was gently removed with a medical wipe. 10 mM formic acid was filled with an airtight syringe in the lumen of each of the three porous hollow fibers. Depending on the format, 7 or 30 μl acceptor solution was used for the 100- and 1500 μl compartment, respectively. The 100 μl compartment was filled with 50 μl sample and the 1500 μl compartment was filled with 1000 μl sample. Platinum wires with a diameter of 0.2 mm (K.A. Rasmussen, Hamar, Norway) were connected to the power supply and utilized as electrodes. The anode was placed in the sample compartment and the cathodes were placed in the lumen of the porous hollow fibers, one in each of the hollow fibers. A power supply ES 0300–0.45 from Delta Power Supplies (Delta Electronika, Zierikzee, The Netherlands) with adjustable voltage in the range of 0–300 V and current in the range of 0–450 mA, respectively was used as the driving force. The 1500 μl compartment was agitated at 1200 rpm with a Vibramax 100 (Heidolph Instruments, Kelheim, Germany), while the 100 μl compartment was stagnant. The acceptor phases from the hollow fibers were collected in a vial, mixed, and eventually analyzed with HPLC.

2.3. Liquid-phase microextraction (LPME)

The setup used for LPME was the same as discussed in Section 2.2; the only exception was that both power supply and electrodes were removed.

2.4. High performance liquid chromatography (HPLC)

The HPLC analyses were performed with an Ultimate 3000 system, which consisted of a WPS-300TSL auto sampler, a HPGM-3200 pump, a VWD-3400 UV/VIS detector, and Chromeleon software version 6.80 for operation, and data acquisition (all Dionex Corporation, Sunnyvale, CA, USA).

The chromatographic separation was performed with a Gemini 5 μm C18, 150 mm × 2 mm column (Phenomenex, Torrance, CA, USA) and the detection was accomplished at 214 nm.

Mobile phases consisted of A: 20 mM formic acid and acetonitrile (95:5, v/v) and B: 20 mM formic acid and acetonitrile (5:95, v/v). The mobile phase flow rate was 0.300 ml/min and the injection volume was 20 μl. The analyses started with 10% mobile phase B and increased linearly up to 55% after 8 min. There after the concentration of mobile phase B was increased to 100% for 2 min. Subsequently the column was flushed with 10% B for 2 min. Total run time was 12.2 min.

2.5. Liquid chromatography–mass spectrometry (LC–MS)

The chromatographic system consisted of a Shimadzu SIL-10ADvp auto injector, two Shimadzu LC-10ADvp gradient pumps, a Shimadzu DGU-14A degasser, a Shimadzu SCL-10Avp system controller, and a Shimadzu LCMS-2010A single-quadrupole MS detector (all Shimadzu Scientific Instruments, Kyoto, Japan). Data acquisition and processing were carried out using Shimadzu LCMS Solution Software Version 2.04-H3.

Chromatographic separation was carried out on a 50 mm × 1 mm I.D. Biobasic-C_8 column (Thermo Fisher Scientific, Waltham, MA, USA) with average pore size of 300 Å, and particle diameter of 5 μm.

The mobile phases consisted of A: 20 mM formic acid and methanol (95:5, v/v) and B: 20 mM formic acid and methanol (5:95, v/v). The flow rate was 50 μl/min and the injection volume was set to 20 μl.

A linear gradient was run up to 100% mobile phase B within 15 min using 80% mobile phase A/20% mobile phase B as starting point. Subsequently the mobile phase composition was kept constant for 3 min. There after the column was flushed with 80% mobile phase A for 6.1 min before a new injection.

An electro spray ionization (ESI) source operated in the positive ionization mode was used to interface the HPLC and the MS. Analyses were performed with selected ion monitoring (SIM), where the following m/z values were used; 325, 477, 310, 330, 248, and 306 for citalopram, loperamide, methadone, paroxetine, pethidine, and sertraline, respectively.

The MS operating conditions were as follows: flow rate of drying gas 10–20 L/min, flow rate of nebulizing gas 1.5 L/min, temperature of the curved desolvation line (CDL) was 200 °C, block temperature was set to 200 °C and probe voltage of +4.5 kV.

2.6. Calculation of extraction recovery

Extraction recovery (R) for the analytes was calculated with the following equation:

$$R = \frac{n_{a,final}}{n_{s,initial}} \times 100\% = \left(\frac{V_a}{V_s}\right)\left(\frac{C_{a,final}}{C_{s,initial}}\right) \times 100\%$$

where $n_{s,initial}$ is the number of mole of the analyte initially present in the donor solution, and $n_{a,final}$ is the number of mole of analyte finally collected in the acceptor solution. V_a And V_s are the volume of the acceptor solution and the sample solution, respectively. $C_{a,final}$ is the final concentration of the analyte in the acceptor solution, and $C_{s,initial}$ is the concentration of the analyte in the donor solution prior to extraction.

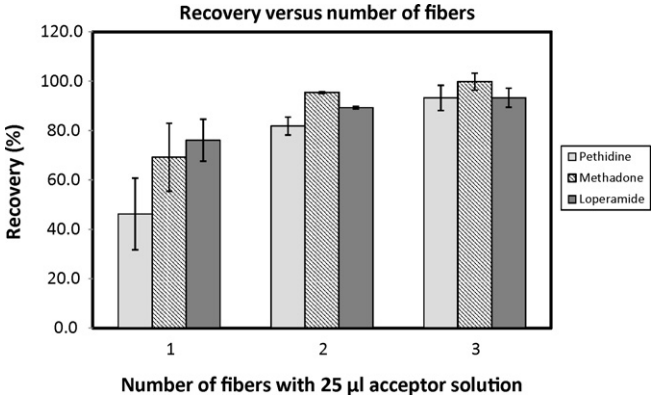

Fig. 2. Extraction recovery versus number of fibers. Sample = 1000 μl spiked water sample (pH 7), SLM = NPOE, acceptor solution = 25, 50, or 75 μl of 10 mM HCOOH, extraction voltage 200 V, agitated at 1200 rpm, and extraction time = 10 min.

Enrichment (E) during the extraction was calculated according to the following equation:

$$E = \frac{C_{a,final}}{C_{s,initial}}$$

3. Results and discussion

The objective of the present paper was to investigate the possibility to extract basic model substances exhaustively (recovery > 95%) with EME by optimizing the geometry of the extraction device. The basic drugs citalopram, loperamide, methadone, paroxetine, pethidine, and sertraline were chosen as model substances based on earlier experiences, where they have shown acceptable extraction recoveries with EME [5,12,17,20–22]. The approach was to increase the surface area of the SLM and the volume of the acceptor phase, by extracting simultaneously with three hollow fibers for the first time in the same sample compartment. The setup with three hollow fibers is illustrated in Fig. 1. On the basis of initial experiments NPOE was chosen as organic phase and the applied electrical field was fixed to 200 V. Because of the success obtained with those initial experiments no further optimization of critical parameters like applied voltage and proton concentration in acceptor phase were carried out.

3.1. Extraction from spiked water samples

Initial experiments were conducted to check if two or three hollow fibers (each containing 25 μl acceptor solution) were more efficient than a single hollow fiber (containing 25 μl acceptor solution). As seen in Fig. 2, the extraction recovery increased by using three hollow fibers instead of a single fiber. Therefore three fibers were used for each extraction in the rest of this work. This experiment demonstrated that extraction recoveries were improved when both the volume of the acceptor phase and the surface area of the SLM and thus the contact area between SLM and the donor phase was increased. The net result of this approach was an increased transport of analytes from the donor solution and into the acceptor solution.

3.1.1. Exhaustive electromembrane extraction

In a next series of experiments two different EME systems were investigated. The first system was agitated and the sample volume was 1000 μl. The second system was totally stagnant and the sample volume was 50 μl. In both cases, three hollow fibers

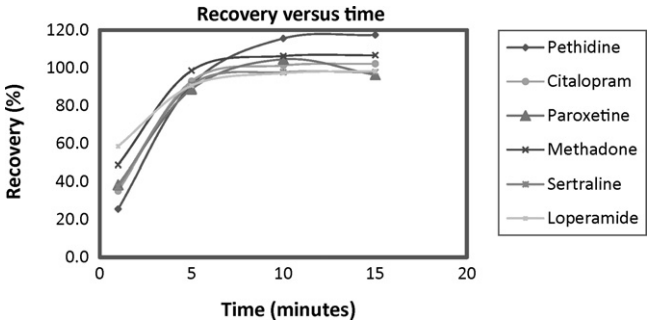

Fig. 3. Extraction recovery versus extraction time for 1000 μl compartment with EME from water. Sample = 50 μl spiked water sample (pH 7), SLM = NPOE, acceptor solution = 90 μl of 10 mM HCOOH, extraction voltage 200 V, agitated at 1200 rpm, and extraction time = 10 min.

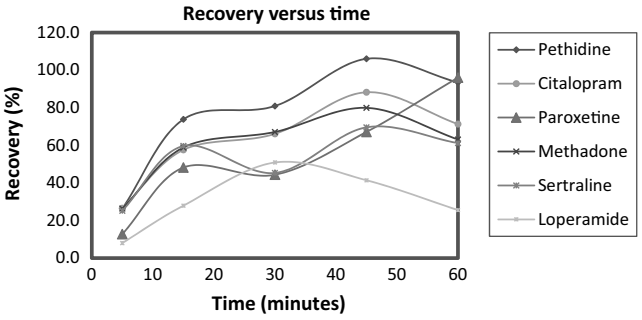

Fig. 5. Extraction recovery versus extraction time for 1000 μl compartment with LPME from water. Sample = 1000 μl spiked water sample (pH 12), SLM = NPOE, acceptor solution = 90 μl of 10 mM HCOOH, agitated at 1200 rpm, and extraction time = 10 min.

were placed in each sample to maximize recovery. As seen in Fig. 3, recoveries were in the range 97–115% from the 1000 μl sample (enrichment between 10.8 and 12.7), and all the model substances were extracted exhaustively after 10 min. This is the first time exhaustive extraction has been demonstrated with EME. In these experiments, the voltage was 200 V, and the extraction time interval was from 1 to 15 min. For the rest of the EME experiments 10 min was chosen as extraction time. The extraction compartment was agitated at 1200 rpm and this agitation promoted mass transfer from the sample and into the SLM. A similar experiment with no agitation gave no detectable peaks and emphasized the importance of agitation in connection with the 1000 μl sample. As illustrated in Fig. 3 the recoveries were highly dependent on extraction time, and after 10 min both donor solution and SLM was totally depleted with regard to the six basic model analytes.

A similar experiment was conducted from 50 μl sample, using 200 V and 10 min extraction time, under totally stagnant conditions. As shown in Fig. 4, recoveries in the range of 81–102% were obtained for the same six basic model analytes in this case (enrichment between 1.9 and 2.4) Agitation was also tested for this EME system, but was found not to improve the extraction recoveries. Probably, agitation was ineffective in this case due to the small dimensions of the sample compartment. Although this system was totally stagnant, it provided exhaustive extraction for methadone, citalopram, and paroxetine after 15 min extraction. The short distance from the sample and to the SLM circumvented the disadvantage of the stagnant conditions.

3.1.2. Comparison with liquid-phase microextraction

To investigate the impact of the electrical field, the results with EME in Figs. 3 and 4 were compared to LPME conducted with a similar setup as described in Section 2.3. In this experiment, the electrical field was replaced with a pH gradient as the driving force. Thus, the sample was alkaline (pH 12) and the acceptor phase was acidic (pH 2). As seen in Fig. 5, the compartment with 1000 μl donor solution performed with recoveries in the range 41–106% for the basic model substances after 45 min. Only one of the model substances was extracted exhaustively in this case, and obviously the system was less efficient than the corresponding EME system. The same six model substances were extracted with the smaller compartment (50 μl) with recoveries in the range 63–121% after 60 min; also here the recoveries were spread over a wider range compared to a similar EME set-up, and only two of the drug substances were extracted exhaustively.

In order to summarize the experiments conducted with spiked water samples as donor solution, it was clear that EME was a more rapid extraction procedure compared to LPME. A comparison between EME and LPME carried out with the large compartment, reported equilibrium time for all the basic model substances close to 10 min for EME. At this point the EME system performed exhaustive extractions as seen in Fig. 3. As seen in Fig. 5 the time needed to reach equilibrium was 45–60 mines for LPME and only a few of the model substances have been extracted exhaustively. In the case of the smaller extraction compartment reported in Figs. 4 and 6, the time needed to reach equilibrium was 10 and 60 min for EME and LPME respectively. In addition, a series of experiments with the 1000 μl compartment conducted under totally stagnant conditions reported no detectable peaks, most probable due to limited contact between the donor solution and the hollow fiber.

3.2. Extraction from spiked plasma samples

3.2.1. Electromembrane extraction

In a next series of experiments, EME was accomplished from untreated human plasma, and the results (recoveries) are

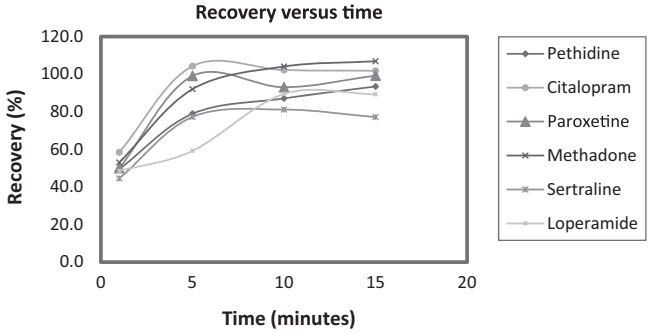

Fig. 4. Extraction recovery versus extraction time for 50 μl compartment with EME from water. Sample = 50 μl spiked water sample (pH 7), SLM = NPOE, acceptor solution = 21 μl of 10 mM HCOOH, extraction voltage 200 V, and extraction time = 10 min.

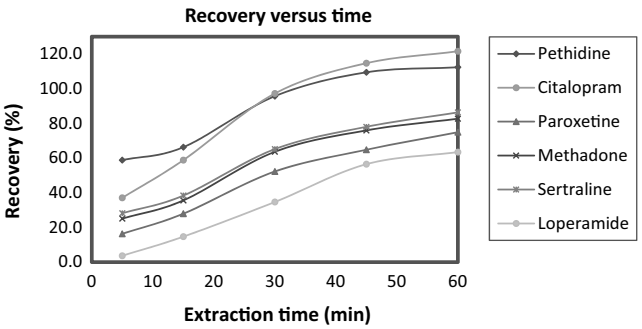

Fig. 6. Extraction recovery versus extraction time for 50 μl compartment with LPME from water. Sample = 50 μl spiked water sample (pH 12), SLM = NPOE, acceptor solution = 21 μl of 10 mM HCOOH, and extraction time = 10 min.

Table 1
Extraction recovery (%) with EME from undiluted human plasma (pH 7.4), with 1000- and 50 μl sample.

Model substance	Extraction recovery (%)		Protein binding[a]
	1000 μl sample	50 μl sample	
Citalopram	93	102	55%
Loperamide	68	73	97%
Methadone	85	93	87%
Paroxetine	63	65	95%
Pethidine	88	99	57–72%
Sertraline	55	56	97%

Sample = 50 or 1000 μl undiluted human plasma (pH 7.4), SLM = NPOE, acceptor solution = 21- or 90 μl of 10 mM HCOOH for 50- and 1000 μl sample, respectively, extraction voltage 200 V, and extraction time = 10 min.
[a] SciFinder.

Table 2
Extraction recovery (%) with LPME from human plasma (pH 12) and 45 min extraction time.

Model substance	Extraction recovery (%)	
	1000 μl	50 μl
Citalopram	86	84
Loperamide	50	27
Methadone	83	72
Paroxetine	48	46
Pethidine	76	85
Sertraline	71	67

Sample = 50 or 1000 μl pH adjusted human plasma (pH 12), SLM = NPOE, acceptor solution = 21- or 90 μl of 10 mM HCOOH for 50- and 1000 μl sample, respectively, and extraction time = 10 min.

summarized in Table 1. Extractions were accomplished both from 1000 and 50 μl samples, and with three fibers in each sample. From 50 μl sample the recoveries for pethidine and citalopram were 99 and 102%. This demonstrated the ability for exhaustive extraction by EME also from untreated human plasma. As shown in Table 1, pethidine and citalopram are both characterized by low protein binding. For sertraline, paroxetine, loperamide, and methadone, which are strongly bound to plasma proteins, the recoveries were in the range of 56–93%. For the latter four compounds, the relative differences in their extraction recoveries where in agreement with their relative differences in protein binding. This experiment supported the assumption that the reason for the lowered extraction recovery achieved from untreated human plasma compared to water samples, was due to binding of the model substances to plasma proteins. All chromatograms from extractions of plasma samples were very clean, with no interfering peaks for the sample matrix. Thus, although extraction recoveries were improved in this paper with the three-fiber configuration, this was found not to sacrifice sample clean-up.

The extractions in Table 1 were also performed with increased voltage (from 200 to 300 V) and with prolonged extraction time (from 10 to 15 min), but the extraction recoveries were the same. The voltage experiment pointed out that the electrical field was unable to totally suppress the drug–protein interactions. It is assumed that the electrical field contributed to break bonds between proteins in plasma and the drugs, and thus contributed to exhaustive extractions for the relatively low protein bounded drugs. In a subsequent experiment, breaking the protein–drug bonds in plasma samples was examined based on chemical displacement. Thus, both trifluoracetic acid 20% (v/v) and acetonitrile 20% (v/v) were added to sample, however the extraction recoveries were unaffected.

3.2.2. Liquid-phase microextraction

Recoveries were in addition investigated with the LPME-setup from pH-adjusted plasma (pH 12) with 45 min extraction time. Extractions were accomplished both from 1000 to 50 μl samples, and with three fibers in each sample. As seen in Table 2, the stagnant compartment housing 50 μl sample resulted in recoveries in the range 27–85%. The 1000 μl compartment extracted with recoveries in the range of 48–86% after 45 min, and with agitation at 1200 rpm. Thus none of the LPME experiments resulted in exhaustive extractions from human plasma.

3.3. Validation

In a next series of experiments, the EME system was combined with LC–MS and validated for the determination of citalopram, loperamide, methadone, paroxetine, pethidine, and sertraline in human plasma. The EME setup was the same as reported in Section 3.2 (50 μl compartment) and the extracts were analyzed by LC–MS as specified in Section 2.6. The validation results are summarized in Table 3. Because recoveries with EME were high, and because EME was combined with highly sensitive LC–MS analysis, the limits of quantification were estimated well below the normal therapeutic range for the six model drugs [23], in spite of the fact that only 50 μl plasma was used for each analysis. This finding emphasized the potential of electromembrane extraction for endogenous drug concentration levels. Linearity was obtained for all the six drugs within the range of 1–1000 ng/ml and with R^2 values exceeding 0.990. The repeatability was examined for 10 and 1000 ng/ml with methadone as internal standard, and the relative standard deviations were all below 21.4% for the remaining five basic model analytes. This was considered as satisfactory, especially taking the sample volume into consideration. A typical chromatogram obtained with EME–LC/MS is illustrated in Fig. 7.

3.4. Patient samples

To demonstrate the feasibility of the validated method reported in Section 3.3, plasma from two patients were obtained and

Table 3
Validation results with EME–LC/MS from plasma spiked with model substances.

	Citalopram	Loperamide	Methadone	Paroxetine	Pethidine	Sertraline
LOQ (ng/ml)						
Signal to noise (ratio = 10)	2.3	1.5	0.6	3.2	2.0	3.0
Linearity						
Range (ng/ml)	1–1000	1–1000	1–1000	1–1000	1–1000	1–1000
R^2	0.9927	0.9978	0.990	0.9999	0.9978	0.9991
Repeatability (%)[a]						
1000 ng/ml (n = 6)	9.5	12.7	–	20.5	7.4	15.2
10 ng/ml (n = 6)	8.6	5.1	–	6.3	13.8	21.4

Sample = 50 μl human plasma (pH 7.4), SLM = NPOE, acceptor solution = 21 μl of 10 mM HCOOH, extraction voltage 200 V, and extraction time = 10 min.
[a] Methadone used as internal standard for citalopram, loperamide, paroxetine, pethidine, and sertraline.

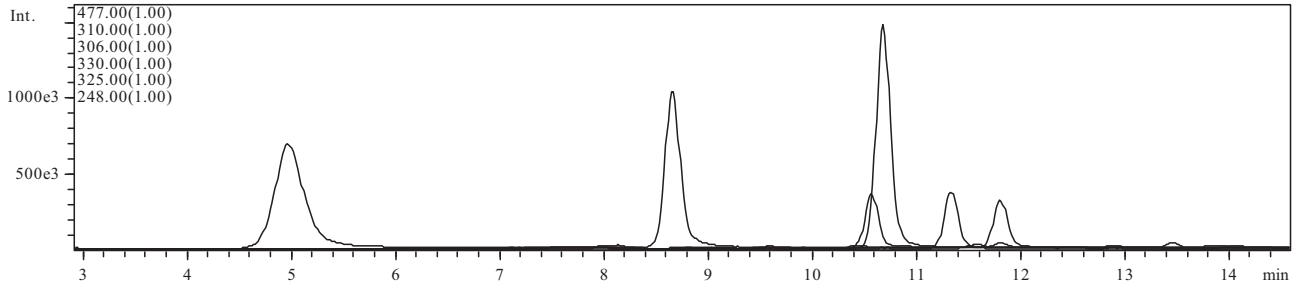

Fig. 7. Typical chromatogram for EME followed by LC/MS for pethidine, citalopram, paroxetine, methadone, loperamide, and sertraline. Sample = 50 μl spiked plasma sample, SLM = NPOE, acceptor solution = 21 μl of 10 mM HCOOH, and extraction time = 10 min.

Table 4
Determination of plasma concentration levels in real patient samples by EME–LC/MS.

	Reference laboratory[a]	EME[b]
Patient 1	86.8 ng/ml	86.5 ng/ml
Patient 2	50.7 ng/ml	58 ng/ml

[a] Results obtained with SPE and LC–MS at Diakonhjemmet Hospital, Oslo, Norway.
[b] $n = 3$, sample = 50 μl human plasma (pH 7.4), SLM = NPOE, acceptor solution = 21 μl of 10 mM HCOOH, extraction voltage 200 V, and extraction time = 10 min.

analyzed with respect to citalopram. The calibration curve was made within the therapeutically relevant concentration range using spiked plasma at 1, 10, 50, and 100 ng/ml. The calibration curve was described by the following linear expression; peak height at m/z 325 = 9409.4 × [plasma concentration in ng/ml] + 61031, $R^2 = 0.999$. As shown in Table 4 the results with EME/LC–MS were satisfactory with a relative difference <14% as compared to the values stated by the reference laboratory for the two samples.

4. Conclusions

Exhaustive extraction has for the first time been investigated with EME from pure water samples and from untreated human plasma. From water samples all the six basic model substances were extracted exhaustively. From plasma samples two of the drugs were extracted exhaustively, whereas the extraction recoveries for the remaining four drugs were somewhat affected by their strong plasma protein binding which prevented them from being exhaustively extracted from spiked human plasma. Exhaustive EME was accomplished by increasing the surface area of the SLM and the volume of acceptor phase, by using three separate hollow fibers in the same sample. Three cathodes were inserted, one in each of the three hollow fibers. Two different compartments have been evaluated; a stagnant compartment with 50 μl sample and an agitated compartment with 1000 μl sample. EME combined with LC–MS provided limit of quantification well below the normal therapeutic range even when only 50 μl sample was used and offered adequate repeatability. The ability to use the validated method in real life was demonstrated by determining concentrations in plasma from patients medicated with citalopram, and the EME experiment provided results comparable with those reported by a reference laboratory utilizing SPE and LC–MS.

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