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Electromembrane extraction of trace amounts of naltrexone and nalmefene from untreated biological fluids

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ABSTRACT

Nalmefene and naltrexone are used to block the effects of narcotics and alcohol. In the present work, for the first time a microextraction technique was presented to reduce matrix interferences and improve detection limits of the drugs in urine and plasma samples. Electromembrane extraction (EME) followed by high performance liquid chromatography (HPLC) coupled with ultraviolet (UV) detection was optimized and validated for quantification of nalmefene and naltrexone from biological fluids. The membrane consists 85% of 2-nitrophenyl octyl ether (NPOE) and 15% di-(2-ethylhexyl) phosphate (DEHP) immobilized in the pores of a hollow fiber. A 100 V electrical field was applied to make the analytes migrate from sample solution with pH 2.0, through the supported liquid membrane (SLM) into an acidic acceptor solution with pH 1.0 which was located inside the lumen of hollow fiber. Extraction recoveries in the range of 54% and 75% were obtained in different biological matrices which resulted in preconcentration factors in the range of 109–149 and satisfactory repeatability ($2.0 < RSD\% < 8.3$). The method offers good linearity with estimation of coefficient higher than 0.9946. Finally, it was applied to determination and quantification of drugs in human plasma and urine samples and satisfactory results were yielded.

1. Introduction

Nalmefene (Nalm) and naltrexone (Nalt) are two opioid antagonist drugs. The chemical structure and physico-chemical properties of the drugs are tabulated in Table 1. Nalm (Revex) and Nalt (Re Via) have both been used against alcohol dependence and other opioids addictions. They are also used for the treatment of opioids overdose and postoperative depression or some respiratory depression which may happen after use of long-acting opioids agonists such as methadone [1,2]. One solution for improving patient compliance is the development of sustained release dosage forms to alleviate the need for taking frequent medication. To calculate the correct dosage of drug for each patient, concentration measurement of drug in urine and plasma is required to estimate the amount of absorbed medicine. Several methods such as radioimmunoassay [3], high performance liquid chromatography (HPLC)/electrochemical detection [4], gas chromatography/mass spectrometry [5], HPLC/tandem mass spectrometry [6], thin layer chromatography [7], gas chromatography with conventional detectors [8–11], HPLC/UV detection [12–17], HPLC/mass spectrometry [18–20], chemiluminescence [21], flow-injection analysis/amperometric detection [22], spectrofluorimetry [23], voltammetry on carbon paste electrode [24], and electrochemical determination with modified glassy carbon [25] were reported for determination of Nalm concentration in different samples. All of these methods require time-consuming sample cleanup steps and in some cases protein precipitation and derivatization are necessary for determination of the drugs in biological samples. With no sample preparation, it is hard to obtain low detection limits and these pretreatment steps make the method expensive and time-consuming. To best of our knowledge, no microextraction technique has been reported for extraction of Nalm and Nalt from biological fluids. In this work, for the first time electromembrane extraction (EME) as a new microextraction technique followed by HPLC with UV detection was optimized and validated for quantification of Nalm and Nalt in biological samples.

EME was introduced for the first time by Pedersen-Bjergaard and Rasmussen [26]. In this technique, an electrical driving force is used for extraction of analytes across an organic liquid membrane. This organic liquid is supported by a hollow fiber. It has been shown that ionizable compounds can migrate across the membrane in an electrical field and this method is more efficient than passive hollow fiber liquid-phase microextraction [27]. In comparison with techniques so far applied to extraction of Nalt and Nalm, simplicity and high sample cleanup are the most important advantages of EME. Also, EME can be applied to extract analytes from plasma samples and other complicated biological matrices requiring no protein precipitation [28]. In the present work, the effects of dif-

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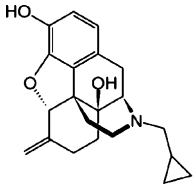
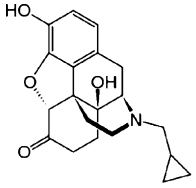
Table 1
Chemical structures, pK_a, log P and protein binding percent (P.B.%) of nalmefen and naltrexone.

Chemical structure	IUPAC name	Abbreviation	pK_a[a]	Log P[b]	P.B.%[c]
	17-(Cyclopropylmethyl)-4,5-α-epoxy-6-methylenemorphinan-3,14-diol	Nalm	7.63	2.66	45
	17-(Cyclopropylmethyl)-4,5-α-epoxy-3,14-dihydroxymorphinan-6-one	Nalt	8.13	1.90	21

[a] Ref. [1].
[b] Ref. [1].
[c] Ref. [1].

ferent variables on EME efficiency were studied and optimized. After optimization, the method followed by HPLC–UV was applied for extraction and determination of Nalt and Nalm in urine and untreated human plasma samples.

2. Experimental

2.1. Equipment for electromembrane extraction

The equipments used for the extraction procedure are shown in Fig. 1. A 3 mL vial with internal diameter of 10 mm and height of 8 cm was used. The electrodes used in this work were platinum wires with diameters of 0.2 mm and 0.5 mm for cathode and anode, respectively, which were obtained from Pars Pelatine (Tehran, Iran). These electrodes were coupled to a power supply model 8760T3 with a programmable voltage in the range of 0–600 V and with a current output in the range of 0–500 mA from Paya Pajoohesh Pars (Tehran, Iran). During the extraction, the EME unit was stirred with a stirring rate in the range of 100–1250 rpm by a heater-magnetic stirrer model 301 from Heidolph Company (Kelheim, Germany) using a 50 mm × 2 mm magnetic bar.

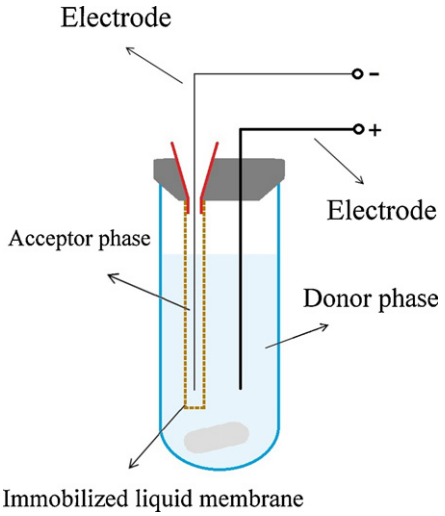


Fig. 1. Schematic diagram of EME setup.

2.2. Chemicals and materials

Nalm and Nalt were obtained from Parand Darou (Tehran, Iran). 2-Nitrophenyl octyl ether (NPOE), 2-nitrophenyl phenyl ether (NPPE), tris-(2-ethylhexyl) phosphate (TEHP) and di-(2-ethylhexyl) phosphate (DEHP) were purchased from Fluka (Buchs, Switzerland). All of chemicals used were of analytical-reagent grades. The porous hollow fiber used for the SLM was a PPQ3/2 polypropylene hollow fiber from Membrana (Wuppertal, Germany) with the inner diameter of 0.6 mm, wall thickness of 200 μm, and pore size of 0.2 μm. Ultrapure water was obtained from a Milli-Q water purification system from Millipore (Madrid, Spain).

2.3. Biological and standard solutions

Drug-free human plasma (blood group A^+) was obtained from Iranian Blood Transfusion Organization (Tehran, Iran). Urine samples were collected from three young addicted persons who were under treatment and one person who had not consumed opiates at all (as match matrix for drawing the calibration curves). The samples were stored at −4 °C, thawed and shaken before extraction. A stock solution containing 1 mg mL^{-1} of each analyte was prepared in acetonitrile and stored at −4 °C protected from light. Working standard solutions were prepared by dilution of the stock solution in acetonitrile.

2.4. HPLC conditions

Separation and detection of the target analytes were performed by a Varian HPLC (Walnut Creek, CA, USA) containing a 9012 HPLC pump (USA), a six-port Cheminert HPLC valve from Valco Instruments (Houston, TX, USA) with a 15 μL sample loop and a Varian 9050 UV–Vis detector. Chromatographic data were recorded and analyzed using Chromana software (version 3.6.4). The separations were carried out on an ODS-3 column (250 mm × 4.6 mm, with particle size of 5 μm) from MZ-Analysentechnik (Mainz, Germany). The mobile phase consisted of 10 mM phosphate buffer, pH 9.0, and acetonitrile (45:55). The flow rate of mobile phase was set at 1.0 mL min^{-1}. Total analysis time was 12 min. The injection volume was 15 μL for all of the samples and detection was performed at wavelength of 210 nm.

2.5. Procedure for EME

Three milliliters of sample solution containing the analytes in 10 mM HCl was transferred into the sample vial. To impregnate the organic solution in the pores of hollow fiber wall, 5.6 cm piece of hollow fiber was cut out and dipped in the solution for 5 s and then the excess of organic solution was gently wiped away by blowing with a medical syringe. The upper end of hollow fiber was connected to a medical needle tip as a guiding tube which was inserted through the rubber cap of the vial. Fifteen microliters of 100 mM HCl (acceptor solution) was introduced into the lumen of the hollow fiber by a microsyringe and the lower end of hollow fiber was sealed with a small piece of aluminum foil. One of the electrodes, the cathode, was introduced into lumen of the fiber. The fiber containing the cathode, SLM and the acceptor solution was afterward directed into the sample solution. The other electrode, the anode, was put directly into the sample solution. The electrodes were subsequently coupled to the power supply and the extraction unit was placed on a stirrer with stirring rate of 1250 rpm. The predetermined voltage was turned on and extraction was performed for 20 min. After the extraction was completed, the acceptor solution was collected by a microsyringe and injected into HPLC instrument for further analysis.

2.6. Calculation of preconcentration factor, extraction recovery and relative recovery

The preconcentration factor (PF) was defined as the ratio of the final analyte concentration in the acceptor phase ($C_{f,a}$) and the initial concentration of analyte ($C_{i,s}$) in the sample solution:

$$\mathrm{PF} = \frac{C_{f,a}}{C_{i,s}} \quad (1)$$

where $C_{f,a}$ was calculated from a calibration graph obtained by direct injection of analytes standard solutions (0.2–200 mg L^{-1}) in 10 mM HCl. Extraction recovery (ER) was defined as the percentage of the number of moles of analyte which was extracted to the acceptor phase ($n_{f,a}$) divided by the number of moles of analyte originally presented in the sample solution ($n_{i,s}$).

$$\mathrm{ER} = \frac{n_{f,a}}{n_{i,s}} \times 100 = \frac{C_{f,a} \times V_{f,a}}{C_{i,s} \times V_{i,s}} \times 100 \quad (2)$$

$$\mathrm{ER} = \left(\frac{V_{f,a}}{V_{i,s}}\right) \mathrm{PF} \times 100 \quad (3)$$

where $V_{f,a}$ and $V_{i,s}$ are the volumes of acceptor phase and sample solution, respectively. Relative recovery (RR) was calculated by the following equation:

$$\mathrm{RR} = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (4)$$

where C_{found}, C_{real}, and C_{added} are the concentrations (μg L^{-1}) of analyte after addition of known amount of standard into the real sample, the concentration of analyte in real sample, and the concentration of known amount of standard which was spiked into the real sample, respectively.

3. Results and discussion

To obtain the maximum extraction recoveries for determination of Nalt and Nalm, the effective parameters of EME including, membrane composition, applied voltage, extraction time, pH in donor and acceptor phases were optimized. All optimizations were done in ultra pure water.

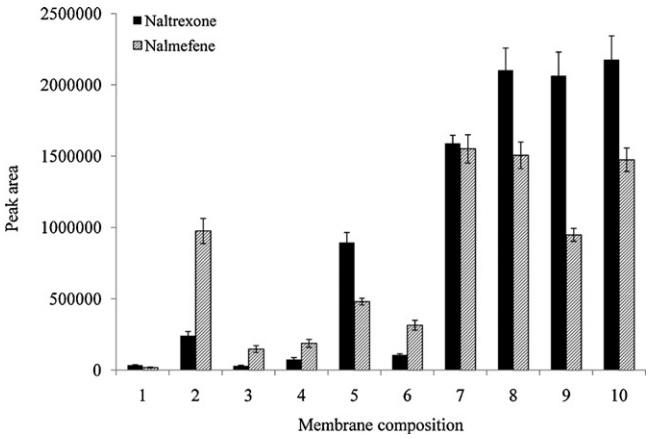


Fig. 2. Optimization of organic liquid membrane. Extraction was performed with a 200 V electrical potential difference and stirring rate of 1000 rpm for 15 min, pH values in donor and acceptor phases were 2.0 and 1.0, respectively. 1: silicon oil, 2: NPPE, 3: NPOE, 4: NPPE + 25%TEHP, 5: NPPE + 25%DEHP, 6: NPOE + 25%TEHP, 7: NPOE + 5%DEHP, 8: NPOE + 15%DEHP, 9: NPOE + 25%DEHP, 10: NPOE + 40%DEHP.

3.1. Selection of organic solvent (membrane composition)

To obtain an ideal SLM, considerations of characteristics of analytes are required. The composition of membrane affects the diffusion coefficient of analytes and also determines the range of applied voltage. To extract relatively hydrophilic drugs, addition of an ion-pair reagent is required. Thus NPOE, silicon oil and NPPE were tested as SLM, and then 25% of DEHP and TEHP were added to NPOE and NPPE as carriers. The obtained results are shown in Fig. 2. One can see that DEHP plays an important role in transferring of the drugs. Thus, presence of DEHP as an ion-pair reagent in the SLM is highly beneficial for the extraction of relatively hydrophilic analytes. In another experiment, different percentages of DEHP (5%, 15%, 25% and 40%) in NPOE were tested as artificial liquid membrane. As seen from Fig. 2, increasing the amount of DEHP from 5% to 15% increases the extraction efficiency. Presence of more than 15% of this carrier in the membrane makes no significant change in extractability but may be followed by decrease of the electrical resistance of SLM and consequently increase of the current level that creates bubbles around the fiber. Bubble formation makes the system unstable and changes the pH in both phases. The pH of acceptor phase may increase due to electrolysis and this occurrence decreases the efficiency of analyte/proton exchange in SLM/acceptor interface and extraction efficiency can be decreased. So, NPOE with 15% DEHP was chosen as SLM for further experiments.

3.2. Applied voltage and extraction time

The main driving force for migration of the analytes across liquid membrane is provided by the electrical field. Strength of the electrical field is dependent on the applied voltage, and the voltage in turn affects the flux of analytes as described in a recent work [29]. Therefore, applied voltage is one of the most important parameters that should be regarded. Voltage and time are two parameters that act in parallel ways. Extraction of drugs was studied in different EME durations ranging from 5 to 20 min and electrical potentials were applied in the range of 100 to 300 V. The results demonstrate that, extractability of both Nalt and Nalm decreased as the voltage increased because of bubble formation and the instability which was discussed in Section 3.1. By applying relatively low voltages, EME can be protracted. Thus the extraction efficiency is improved by decreasing the applied electrical potential and increasing the

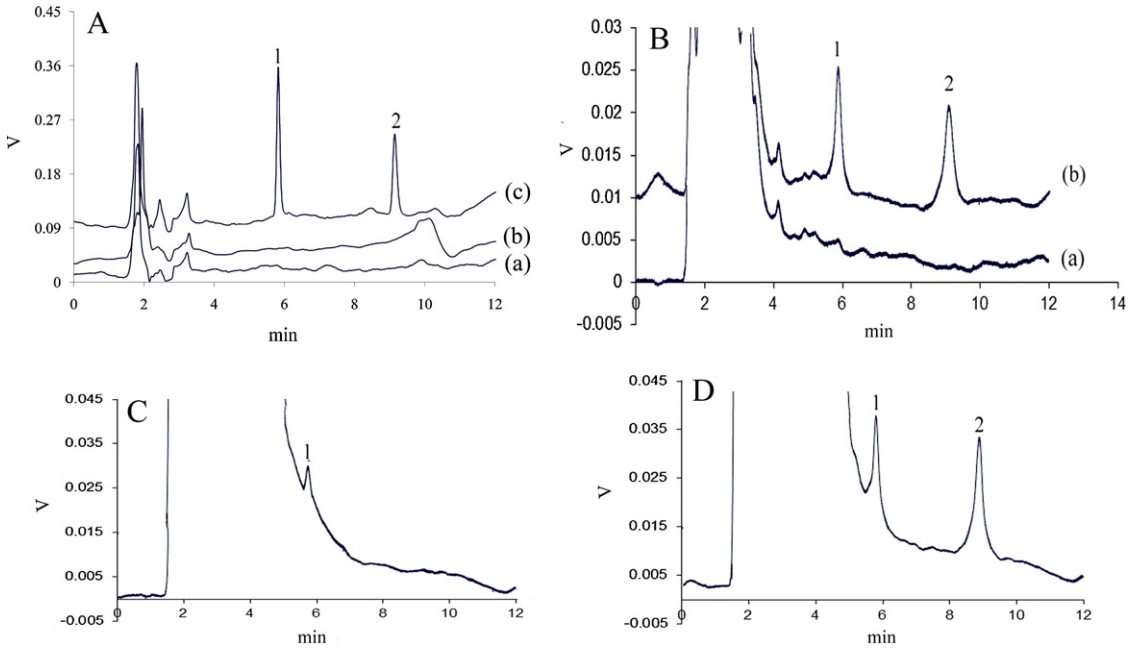


Fig. 3. Chromatograms which were obtained after: (A) extraction of 500 ng mL^{-1} of SB and TB (a) with conventional LPME based on gradient of pH (b) with the extraction conditions same to EME method but in the absence of electrical field (c) with a 100 V electrical potential difference, (B) EME of (a) nonspiked plasma sample, (b) plasma sample spiked at a concentration level of 80 ng mL^{-1} of the drugs. (C) EME of nonspiked urine sample, (D) EME of urine sample spiked at a concentration level of 60 ng mL^{-1} of the drugs. 1: naltrexone, 2: nalmefene.

extraction time. Thus to pass the analytes from sample solution across SLM and into acceptor phase, voltage of 100 V was applied for a duration of 20 min to obtain the best extraction recoveries.

3.3. The pH of sample solution and acceptor phase

The pH values of donor and acceptor phases can determine the ion balance in the system. It was shown that the total ionic concentration on the donor phase to that on the acceptor phase impresses the flux over the membrane [29]. The flux may decrease by increasing this ratio as described by theoretical models [29]. To investigate the effect of this parameter, pH of donor phase was changed in the range of 1.0–3.0, and pH in acceptor phase was varied in the same range too. Maximum amounts of drugs were extracted when pH of the sample solution was adjusted at 2.0 and pH of the acceptor phase was adjusted at 1.0. As it is expected, the extractability of the analytes was increased by deceasing pH of acceptor solution. Decreasing of the ion balance in these conditions is an important reason for increasing of extractability of the analytes. On the other hand, changing of the pH in donor solution may influence the extraction efficiency in two different directions. To pass the analytes through the electrical field, it is necessary to change the drugs to their ionizable forms. So the donor phase must be acidic to the extent that the ionization of both Nalt and Nalm occurs. The results indicate that by decreasing the pH in sample solution from 3.0 to 2.0 the concentration of analytes in acceptor increases due to completion of the ionization. But decreasing the pH from 2.0 to 1.0 diminishes the extraction efficiency due to increase in the ratio of total ionic concentration on the sample side to that on the acceptor side. As a consequence, the optimal condition was obtained by adjusting the pH of donor and acceptor phases at 2.0 and 1.0, respectively and by applying electrical potential of 100 V for duration of 20 min together with stirring of the solution at 1250 rpm. Also, 15% DEHP in NPOE was selected as SLM for the rest of this work.

3.4. Comparison of EME with liquid phase microextraction (LPME)

To test the benefit of applied voltage, a three-phase LPME was carried out under the extraction conditions similar to EME method but electrical field was not applied. The results in Fig. 3A(b) demonstrate that no drugs were extracted in the absence of voltage. Also, in order to compare EME with conventional LPME based on gradient of pH, another experiment was performed. The sample solution was adjusted to pH 12.0 to prevent the analytes from ionization and to promote the distribution of both drugs into the organic liquid membrane. With the same SLM, extraction time, stirring rate and pH in acceptor phase, LPME was performed to extract Nalm and Nalt from sample solution that contains 500 ng mL^{-1} of drugs. The obtained chromatograms, Fig. 3A(a) and (c) proved the ability of EME to extract Nalm and Nalt rather than passive diffusion within the same time.

3.5. Extraction from biological samples

Quantification of Nalm and Nalt in human biological fluids is required to calculate the pharmacokinetic parameters of these drugs. In addition, EME is a powerful method for isolation and cleanup of these analytes from untreated biological fluids. Therefore, the optimal conditions of EME were used for extraction of the drugs from human plasma and urine samples. To reduce matrix effects calibration curves were plotted in drug free urine and plasma samples. Therefore, due to high similarity of media which have been used for plotting calibration curves with media of real samples, obtaining high relative recoveries is expected.

3.5.1. Extraction from human plasma

Plasma samples were diluted with water (1:3) and adjusted to pH 2.0 by addition of proper amount of HCl solution. The drugs were spiked into the human plasma and their quantitative analysis

Table 2
Figures of merit of EME in drug-free plasma and urine samples.

Samples	Analyte	LOD (ng mL^{-1})	Linearity (ng mL^{-1})	R^2	PF[a]	ER%	RSD%[b]	
							Intra day	Inter day
Plasma	Nalt	20	40–1000	0.9956	123	62	5.0	10.6
	Nalm	20	40–1000	0.9946	149	75	2.0	12.4
Urine	Nalt	10	20–1000	0.9989	109	54	8.3	12.7
	Nalm	20	30–1000	0.9977	140	70	3.4	9.6

[a] Drugs were present at 80 ng mL^{-1} and 100 ng mL^{-1} in plasma and urine samples, respectively.
[b] Intra day and inter day RSDs% were obtained by five and three replicate measurements, respectively.

Table 3
Determination of Nalt and Nalm in different urine and plasma samples.

Sample	Analyte	C_{real} (ng mL^{-1})	C_{added} (ng mL^{-1})	C_{found} (ng mL^{-1})	RSD% (n = 3)	RR%	Error%
Plasma 1	Nalt	nd[a]	80.0	81.6	5.1	102	+2
	Nalm	nd	80.0	76.3	2.6	95	−5
Plasma 2	Nalt	nd	80.0	84.0	6.2	105	+5
	Nalm	nd	80.0	77.3	3.4	97	−3
Urine 1	Nalt	22.8	60.0	80.4	7.0	96	−4
	Nalm	nd	60.0	57.5	5.3	96	−4
Urine 2	Nalt	nd	60.0	62.4	4.7	104	+4
	Nalm	nd	60.0	61.7	3.1	103	+3
Urine 3	Nalt	20.0	60.0	77.2	6.5	95	−5
	Nalm	nd	60.0	59.0	4.5	98	−2

[a] Not detected.

was evaluated under optimized conditions. As provided in Table 2, an acceptable linear range (40–1000 ng mL^{-1}) and linearity (0.9946 and 0.9956 for Nalm and Nalt, respectively) were obtained. Precision of the method was determined by five-replicate extraction of the drugs from samples at a concentration level of 50 ng mL^{-1}. The Intraday and inter day RSDs% were found to be 2.0% and 5.0% and 12.6 and 10.6 for Nalm and Nalt, respectively. Limits of detection (LOD) and limits of quantification (LOQ) were 20 and 40 ng mL^{-1}, respectively for the two drugs. Extraction recoveries were 75% for Nalm and 62% for Nalt (corresponding preconcentration factors of 149 and 123, respectively). To evaluate the applicability of EME for human plasma, two plasma samples were analyzed with the proposed method. Since no Nalm and Nalt were found in samples, both plasma samples were spiked with the drugs at a concentration level of 80 ng mL^{-1} and EME was carried out. Chromatograms are shown in Fig. 3B. Plasma samples from different individuals showed no significant differences according to calculated relative recoveries (Table 3) and the method could be performed directly on diluted plasma samples with no need to time-consuming sample preparation steps.

3.5.2. Extraction from human urine

Drug-free human urine was spiked with the two drugs and extraction was accomplished after dilution of urine samples (1:1) and addition of proper amount of HCl solution to achieve pH 2.0. The results are summarized in Table 2. Linear ranges of 30–1000 ng mL^{-1} for Nalm and 20–1000 ng mL^{-1} for Nalt were obtained. Admissible LODs (20 ng mL^{-1} for Nalm and 10 ng mL^{-1}

Table 4
Comparison of the proposed method with other analytical techniques for determination of nalmefene and naltrexone.

Analytical method[a]	Analyte	Sample	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	Linearity (ng mL^{-1})	R^2	RSD%		Reference
							Within day	Between day	
LLE–GC–MS	Nalt	Plasma	0.2	1	1–50	0.9988	6.6	3.7	[30]
SPE–HPLC–DAD	Nalt	Plasma	8	10	10–500	0.9985	–	–	[31]
LLE–LC–MS–MS	Nalt	Plasma	0.0002	0.0002	0.0002–0.1	0.998	12	–	[32]
LLE–LC–MS–MS	Nalt	Urine	0.0002	0.0002	0.0002–0.1	0.998	5	–	[32]
GCE	Nalt	Plasma	0.1 (μM)	10 (μM)	10–100 (μM)	0.996	3.7	6.4	[25]
LLE–LC–MS–MS	Nalt	Plasma	0.1	2	2–50	0.997	11.5	9.76	[33]
LLE–LC–MS	Nalt	Plasma	0.75	1.25	1.25–500	0.999	5.8	6.0	[18]
SPE–LC–MS–MS	Nalt	Plasma	0.2	0.5	0.5–100	–	–	–	[34]
OCE–LC–MS–MS	Nalt	Plasma	0.005	0.005	0.005–100	0.99	9.1	10.1	[35]
SPE–GC–MS	Nalt	Plasma	0.1	0.1	0.1–60	0.999	6.2	24.3	[36]
SFL	Nalt	Water	12	300	300–2000	0.991	3.3	–	[37]
LLE–LC–MS	Nalt	Plasma	0.21	0.25	0.25–150	0.9907	9.4	–	[38]
SPE–GC–MS	Nalt	Plasma	1	2	2–60	0.99	7–14	7.3–9.3	[39]
SPE–GC–MS	Nalt	Milk	1	2	2–20	0.99	4–18	4.3–5.9	[39]
LLE–GC–MS	Nalm	Plasma	–	0.5	0.5–200	0.9956	0.3–7.2	2.2–5.2	[40]
LLE–LC–MS–MS	Nalm	Plasma	–	0.01	0.01–50	0.9972	5.4–6.0	0.9–10.1	[41]
EME–HPLC–UV	Nalt	Plasma	20	40	40–1000	0.9956	5.0	10.6	This work
EME–HPLC–UV	Nalt	Urine	10	20	20–1000	0.9989	8.3	12.7	This work
EME–HPLC–UV	Nalm	Plasma	20	40	40–1000	0.9946	2.0	12.4	This work
EME–HPLC–UV	Nalm	Urine	20	30	30–1000	0.9977	3.4	9.6	This work

[a] Liquid–liquid extraction (LLE), gas chromatography (GC), mass spectroscopy (MS), solid phase extraction (SPE), diode array detector (DAD), liquid chromatography (LC), glassy carbon electrode (GCE), online column extraction (OCE), spectrofluorimetric determination (SFL).

for Nalt) were obtained and LOQ values were 30 and 20 ng mL^{-1} for Nalm and Nalt, respectively. The method offers good linearity as demonstrated in Table 2 (0.9977 for Nalm and 0.9989 for Nalt) and extraction recoveries were 70% and 54% for Nalm and Nalt, respectively. RSDs% values less than 8.3% for intraday and less than 12.7% for inter day confirm the acceptable precision of proposed EME. To investigate matrix effects and ability of the technique to analyze real samples, three urine samples of patients under treatment with Nalt were collected and after dilution (1:1) their pH values were adjusted at 2.0. The prepared solutions were analyzed and the amount of Nalt in each sample was determined (Table 3). In another experiment, all samples were spiked with the analytes at a concentration level of 60 ng mL^{-1} and after dilution (1:1) with proper amount of water and adjusting the pH at 2.0, EME was carried out for 20 min. Chromatograms of the urine samples before and after addition of the drugs are provided in Fig. 3C and D, respectively. The relative recoveries from different urine samples were not found to differ significantly ($95 < RR\% < 105$) and EME appeared as a powerful technique for determination and quantification of the analytes from human urine samples.

3.5.3. Comparison of the proposed method with other techniques

Comparison of the proposed method with different existing methods for extraction and determination of Nalt and Nalm is provided in Table 4. It is shown that along with its simplicity, this technique demonstrated wide linear range, high sensitivity, and an acceptable repeatability and reproducibility. In spite of LLE and SPE, consumption of organic solvents in this technique reaches the minimum amount. Also, in comparison with SPE, EME eliminate possible carry-over problems because of the hollow fiber not expensive and can be discarded after each extraction. Selecting an appropriate organic solvent, EME can create a high sensitivity as well as high cleanup whereas SPE has not selectivity and causes crowded chromatograms after extraction in complex matrices. Although, LC–MS–MS can create high sensitivity but this instrument is very expensive and not accessible as a routine instrument. Determination of most of the drugs using GC–MS needs to a derivatization step which increase cost and time of analyze. Therefore, LC–UV coupling with EME can provide good and sensitive results for determination of these analytes in biological fluids. These characteristics are keys of interest for laboratories doing routine trace analysis of Nalt and Nalm by EME.

4. Conclusions

An efficient EME was developed and validated for determination and quantification of Nalm and Nalt in human plasma and urine samples. Addition of an anionic ion-pair reagent was necessary to extract relatively polar substances and thus a mixture of NPOE and DEHP was selected as organic membrane. Other parameters that influence the process, including applied voltage, extraction time, pH in the donor and acceptor solution were optimized. Satisfactory LODs and RSDs, high preconcentration factor and extraction recovery, and good linearity ranges were obtained. The method was successfully applied to analysis of these drugs in real samples. In addition, no extra cleanup steps were required in complicated biological fluids which can reduce risk of contaminations due to work with these types of samples.

References

[1] A.C. Moffat, M.D. Osselton, B. Widdop, L.Y. Galichet, Clarke's Analysis of Drugs and Poisons in Pharmatceuticals, Body Fluids and Postmortem Material, 3rd ed., Pharmaceutical Press, London, 2004.
[2] C.S. Yuan, Handbook of Opioid Bowel Syndrome, Haworth press, New York, 2005.
[3] R. Dixon, J. Hsiao, W. Taaffe, E. Hahn, R. Tuttle, J. Pharm. Sci. 73 (1984) 1645.
[4] J.Z. Chou, H. Albeck, M.J. Kreek, J. Chromatogr. 613 (1993) 359.
[5] S. Xie, R.F. Suckow, B.J. Mason, D. Allen, T.B. Cooper, J. Chromatogr. B 773 (2002) 143.
[6] P. Li, X. Chen, X. Dai, A. Wen, Y. Zhang, D. Zhonga, J. Chromatogr. B 852 (2007) 479.
[7] K. Verebey, M.J. Kogan, A. DePace, J.M. Salvatore, J. Chromatogr. 118 (1976) 331.
[8] C.C. Nelson, M.D. Fraser, J.K. Wilfahrt, R.L. Foltz, Ther. Drug Monit. 15 (1993) 557.
[9] W. Huang, D.E. Moody, R.L. Foltz, S.L. Walsh, J. Anal. Toxicol. 21 (1997) 252.
[10] C.F. Chan, G.M. Chiswell, R. Bencini, L.P. Hackett, L.J. Dusci, K.F. Ilett, J. Chromatogr. B 761 (2001) 85.
[11] S.W. Toennes, G.F. Kauert, S.M. Grusser, W. Jakel, G. Partecke, J. Pharm. Biomed. Anal. 35 (2004) 169.
[12] A.F. Davidson, T.A. Emm, H.J. Pieniaszek, J. Pharm. Biomed. Anal. 14 (1996) 1717.
[13] W.J. Hurst, I.S. Zagon, H.Y. Aboul-Enein, Pharmazie 54 (1999) 595.
[14] K. Kambia, S. Bah, T. Dine, R. Azar, P. Odou, B. Gressier, M. Luyckx, C. Brunet, L. Ballester, M. Cazin, J.C. Cazin, Biomed. Chromatogr. 14 (2000) 151.
[15] E.F. O'Connor, S.W.T. Cheng, W.G. North, J. Chromatogr. Biomed. Appl. 491 (1989) 240.
[16] K.K. Peh, N. Billa, K.H. Yuen, J. Chromatogr. B: Biomed. Appl. 701 (1997) 140.
[17] L. Olsen, A.S. Christopherson, G. Fropsahl, H. Waal, J. Morland, Br. J. Clin. Pharmacol. 58 (2004) 219.
[18] S. Valiveti, B.N. Nalluri, D.C. Hammell, K.S. Paudel, A.L. Stinchcomb, J. Chromatogr. B 810 (2004) 259.
[19] S.S. Iyer, G.E. Kellogg, H.T. Karnes, J. Chromatogr. Sci. 45 (2007) 694.
[20] M.H. Slawson, M. Chen, D.E. Moody, S.D. Comer, E.S. Nuwayser, W.B. Fang, R.L. Foltz, J. Anal. Toxicol. 31 (2007) 453.
[21] A. Campiglio, Analyst 123 (1998) 1053.
[22] Z.R. Fernandez-Abedul, A. Costa-Garcia, J. Pharm. Biomed. Anal. 16 (1997) 15.
[23] J.A. Murillo Pulgarin, L.F. Garcia Bermejo, J.L. Garrido Lara, Anal. Chim. Acta 495 (2003) 249.
[24] Z.R. Fernandez-Abedu, R. Barreira, G. Costa, Anal. Lett. 30 (1997) 1491.
[25] F. Ghorbani-Bidkorbeh, S. Shahrokhian, A. Mohammadi, R. Dinarvand, J. Electroanal. Chem. 638 (2010) 212.
[26] S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 1109 (2006) 183.
[27] T. Grønhaug Halvorsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 909 (2001) 87.
[28] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Bioanal. Chem. 393 (2009) 921.
[29] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1174 (2007) 104.
[30] M. Rezaei, K. Abdi, R. Dinarvand, S. Vosough-Ghanabri, M. Amini, Chromatographia 70 (2009) 1491.
[31] N. Kambiai, T. Dinei, P. Odou, S. Bah, R. Azar, B. Gressiere, T. Dupin-Sprieti, M. Luyckxi, C. Bruneti, Eur. J. Drug Metab. Pharmacokinet. 29 (2004) 225.
[32] M. Gergov, P. Nokua, E. Vuori, I. Ojanpera, Forensic Sci. Int. 186 (2009) 36.
[33] H.-Y. Yun, S.-C. Banga, K.-C. Lee, I.-H. Baeka, S.-P. Lee, W. Kangb, K.-I. Kwon, Talanta 71 (2007) 1553.
[34] W. Naidong, H. Bu, Y.-L. Chen, W.Z. Shou, X. Jiang, T.D.J. Halls, J. Pharm. Biomed. Anal. 28 (2002) 1115.
[35] C. Clavijo, J. Bendrick-Pearta, Y. Ling Zhanga, G. Johnsona, A. Gasparicc, U. Christians, J. Chromatogr. B 874 (2008) 33.
[36] S.W. Toennes, G.F. Kauert, S.M. Grüsser, W. Jäkel, G. Partecke, J. Pharm. Biomed. Anal. 35 (2004) 169.
[37] J.A. Murillo Pulgarĭin, L.F. Garcĭia Bermejo, J.L. Garrido Lara, Anal. Chim. Acta 495 (2003) 249.
[38] R.P. Huntera, D.E. Kocha, A. Mutlowb, R. Isaza, J. Chromatogr. B 793 (2003) 351.
[39] C. Fen Chan, G.M. Chiswell, R. Bencini, L.P. Hackett, L.J. Duscib, K.F. Ilett, J. Chromatogr. B 761 (2001) 85.
[40] S. Xiea, R.F. Suckowb, B.J. Masonc, D. Allena, T.B. Cooper, J. Chromatogr. B 773 (2002) 143.
[41] P. Li, X. Chena, X. Dai, A. Wen, Y. Zhang, D. Zhong, J. Chromatogr. B 852 (2007) 479.