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Effect of counter-ions on electromembrane extraction of non- steroidal antiinflammatory drugs.

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Abstract

The paper describes a comprehensive study of the importance of cations to improve the efficiency and mass transfer in the electromembrane extraction for the determination of non-steroidal antiinflammatory. The procedure has been demonstrated to be adequate for the analysis of the selected non-steroidal anti-inflammatory drugs in human urine samples. Effects of donor and acceptor solution composition were examined studying the presence of different counter-ions in both aqueous solutions demonstrating to be an important parameter in the transference of the analytes across the membrane. Other parameters that are also critical for electromembrane, such as the organic solvent, the voltage, the extraction time and the donor solution volume were also optimized. The extracts were analyzed by high performance liquid chromatography with diode array and fluorescence detection. The highest enrichment factor was achieved using a pH 7 donor composition adjusted with KOH and an acceptor composition at pH 12 adjusted with NaOH. The enrichment factors were within the range of 85 and 133 for all compounds after 20 minutes extraction, using a voltage of 60V, a stirring speed of 600 rpm and 1-octanol as support liquid membrane. Detection and quantitation limits were within 0.1-1.5 ng mL^{-1} and 0.3-3.0 ng mL^{-1}, respectively. The presence of K^+ and Na^+ as counter ions in the donor and the acceptor solution, respectively, demonstrated a higher enrichment compared to previous reported methods for the determination of non-steroidal antiinflammatory by electromembrane extraction using NaOH/NaOH as counter-ions.

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Keywords: sample preparation; electromembrane extraction; non-steroidal antiinflammatory; urine samples

1. Introduction

The development of new sample preparation techniques has been one of the main challenges of analytical chemistry in the last years in order to achieve the determination of a wide variety of compounds in different matrices, especially in environmental and biological samples. Non-steroidal antiinflammatory drugs (NSAIDs) are one of the most consumed drugs around the world due to their antipyretic, analgesic and antiinflammatory properties. For this reason, they are commonly employed in human therapy [1] being the main reason of its popularity that NSAIDs do not produce sedation or addiction in contrast to other drugs [2].

NSAIDs determination has been traditionally tackled using chromatographic methods such us gas chromatography (GC) or high performance liquid chromatography (HPLC) using UV, fluorescence and mass spectrometry detectors [3,4].

Electromembrane extraction (EME) is an extraction technique based on the selective transfer of analytes from an aqueous donor solution to an aqueous acceptor solution across an organic solvent immobilized as supported liquid membrane (SLM) that uses an electric field as driving force to accelerate the extraction process. It has been widely used for the extraction of different analytes from biological and environmental samples and it has demonstrated to have several advantages in clinical and pharmaceutical analysis. The main advantages that EME presents respect to other sample extraction techniques are: simplicity, low volumes of sample and organic solvents required, high extraction efficiency and selectivity, high compatibility with raw complex matrices and great reduction in time of analysis. It has also provided excellent sample clean-up and analyte preconcentration from complex matrices [5-12].

The selected analytes are generally extracted using a polypropylene hollow fiber that acts as a support of the liquid membrane and contains the acceptor solution. The ionized analytes migrate from the agitated aqueous donor solution to the organic phase (SLM), generally a thin organic solvent layer deposited in the pores of the polymeric support. Once the analytes reach the interphase donor solution/SLM, they migrate into the organic phase mainly due to distribution coefficients of analytes in the two solvents and to the action of an electric potential. Extracted analytes migrate across the SLM and are released into aqueous acceptor solution. Normally, this is achieved by acidification,

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in the case of basic analytes, and alkalization, for acid ones, of acceptor solution [13]. The electric field is generated applying an electrical potential difference between two platinum electrodes, placed in donor and acceptor solutions. In the case of acid analytes the negative electrode is placed in the donor solution and the positive in the acceptor one whereas for basic analytes is the opposite [7].

In order to ensure an efficient electrokinetic mobility, this technique requires the optimization of several operational parameters, such as applied voltage, donor and acceptor composition, extraction time and supported liquid membrane composition.

This work has been focused on the evaluation and study of operational parameters affecting the electromembrane extraction of five widely used non-steroidal anti-inflammatory drugs: salicylic acid (SAC), ketoprofen (KTP) naproxen (NAX), diclofenac (DIC) and ibuprofen (IBU). Once the NSAIDs were extracted, their determination was carried out by high performance liquid chromatography (HPLC) using diode array (DAD) and fluorescence detection (FLD). Previous papers have been published on electromembrane extraction of NSAIDs in several matrices studying several operational variables, however the effect of counter-ions composition on acceptor and donor solutions have never been exhaustively studied [14-16]. Šlampová et al. performed a systematic quantitative study of the effect on EME by several alkaline counter-ions for the extraction of chlorophenols [17]. The authors reported for basic analytes that counter-cations migrate toward the positive electrode depending on their electrophoretic motilities and SLM selectivity. For acid analytes, such as NSAIDs, when different inorganic cations are used in the acceptor solution and a voltage is applied these cations migrate towards the donor solution, were negative electrode is immersed and the transfer of the negatively charged NSAIDs into the acceptor solution occurs in the opposite direction to mantein the ions balance between both sides of SLM in the extraction system. Slampová et al. also proved that the selection of the proper combination of counter-ions could highly influence the transfer of the analytes trough the SLM.

In this work, an exhaustive evaluation of all operational parameters (pH of acceptor and donor solutions, SLM nature, stirring speed, voltage and extraction times) and the effect of counter-ions affecting the EME of the five selected NSAIDs were evaluated finding some discrepancies with previous reported conditions for the optimum EME of NSAIDs in human urine samples. The proposed procedure was satisfactory applied to the determination of some selected analytes in human urine.

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2. Experimental

2.1. Chemicals and reagents

Salicylic acid, naproxen, ketoprofen, diclofenac and ibuprofen were purchased from Fluka-Sigma-Aldrich (Madrid, Spain). The alkaline hydroxides (LiOH, NaOH, KOH, CsOH) and all the organic solvents tested as supported liquid membranes (benzene, 1-butanol, chloroform, cyclohexane, diclhoromethane, dihexyl ether, n-hexane, 2-methyl propan-1-ol, nitrophenyl octyl ether, 1-octanol, 1-pentanol, toluene and xylene) were purchased from Merck (Darmstadt, Germany). All chemicals and reagents were of analytical grade.

All solutions and dilutions were prepared using ultrapure water from Milli-Q Plus water purification system (Millipore, Billerica, MA, USA). Aqueous working solutions of NSAIDs were daily prepared by adequate dilutions from methanolic stock solutions of SAC, KTP, NAX, DIC and IBU (400 mg L^{-1}).

Tablets of NAX and IBU were obtanied from Cinfa® and Normon® laboratories respectively.

S6/2 Accurel® polypropylene (PP) hollow fiber (1800 µm i.d., 450 µm wall thickness and 0,2 µm pore size) was purchased from Membrana (Wuppertal, Germany).

Platinum wire, 0,5 mm, Premion®, 99,997% (metal basis)≈4,21g/m were purchased from Alfa Aesar® (Karlsruhe, Germany).

2.2. Electromembrane extraction conditions

Hollow fibers were cut in 30 mm pieces, washed with acetone in an ultrasonic bath and air dried. The fiber was closed in the lower end by mechanical and thermal pressure using a hot soldering tool. Before the extraction, the fiber was dipped in an organic solvent (1-octanol) to impregnate the pores and the excess of organic solvent was removed with a medical wipe. The lumen of the hollow fiber was filled with 50 µL of pH 12 aqueous solution (10 mM NaOH) as acceptor solution using a 50 µL syringe. The upper end of the fiber was connected to a pipette tip that acted as a guide for the electrode. Finally, the hollow fiber was introduced into the donor solution compartment (pH 7 aqueous solution).

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Two platinum wires with a diameter of 0.5 mm were used as electrodes and placed in donor and acceptor solutions. A voltage of 60 volts was applied between both electrodes using a power source 300 V DC Power Supply (VWR International, West Chest, Pennsylvania, USA) with programmable voltage within the range of 2-300 V. Electrode in donor solution was connected to the negative pole and the electrode in the acceptor solution to the positive one. The extraction was completed after 20 minutes using a stirring speed of 600 rpm. The current of the system was continuously registered during the extraction time (every 0,5 seconds) using a digital multimeter (Peack Tech® model 3430, Ahrensburg, Germany) connected to a computer through an integrate USB connector.

Once the EME was finished the acceptor solution was collected from the lumen of the hollow fiber using an HPLC syringe and injected (20 µL) into the HPLC system.

2.3. Chromatographic conditions

All measurements were carried out using a High Performance Liquid Chromatography system consisting of an Agilent-Technologies 1100 series liquid chromatograph (Palo Alto, CA, USA) equipped with a quaternary pump, a vacuum degasser, a thermostated column compartment, a diode array detector and a programmable fluorescence detector. The injector was a Rheodyne manual injection valve Model 7725i, fitted with a 20 µL sample loop.

Chormatographic conditions were carried out based on the method describes by Ramos Payán et al. [14] with slight modifications. Separations were performed at 26ºC using an Hibar® 100-4,6 Purosphere® STAR RP-18e 3 µm particle size (100mm×4,6mm i.d.) (VWR, Darmstadt, Germany) chromatographic column preceded by a guard column Kromasil® 100 Å pore size, C18, 5 µm particle size (15mm×4,6mm i.d.) (Scharlab S.L., Barcelona, Spain).

The mobile phase consisted of 0.1% formic acid (component A) and acetonitrile (component B) at a flow rate of 0.8 $mL \cdot min^{-1}$. An initial 55% component B was used in isocratic mode for 5 minutes and then a linear elution gradient was programmed from 55% to 100% for component B for 5 minutes more. Finally, 2 minutes at 100% B was applied. Four minutes were waited between injections to allow the re-equilibration of the column to the initial conditions. The chromatographic separation was completed in 12 minutes. KTP and DIC were measured with DAD at 255 and 280 nm, respectively.

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For FLD detection an excitation wavelength of 230 nm was used for SAC, NAX and IBU being their corresponding emission wavelengths 450 nm for SAC and 280 nm for NAX and IBU.

2.4. Preparation of human urine samples analysis using EME

Urine samples were collected from healthy volunteers and patients under medical treatment with some of the studied NSAIDs and stored at 4ºC. All samples were filtered through 0.22 μm PTFE syringe filters (Pall Corporation, Ann Arbor, Michigan, USA) prior to extraction procedure. The samples were diluted with ultrapure water (1:1000, v/v) and adjusted to pH 7 with KOH.

2.5. Calculation of enrichment factor

The enrichment factor of the EME procedure was calculated according to Eq. (1):

$$EF_i = \frac{C_{f,a}}{C_{i,d}}$$

The enrichment factor (EF) for the analyte i is define as the ratio between the final concentration of the analyte in the acceptor solution ($C_{f,a}$) and the initial concentration of the analyte ($Ci_{,d}$) in the donor solution, where ($C_{f,a}$) was determined by HPLC using an external calibration.

3. Results and discussion

3.1. Optimization of EME operational parameters

Figure 1 shows the set-up used for electromembrane extraction. For operational parameters optimization, preliminary experimental conditions were fixed as follows: pH 12 (adjusted with NaOH 10 mM) as acceptor and donor solution composition, 1-octanol as SLM, a voltage of 30 volts, an extraction time of 20 minutes, a stirring speed of 600 rpm and a donor and acceptor solution volume of 10 mL and 50 μL, respectively based on previous assays. The experiments were carried out using an aqueous solution containing 0.5 mg L^{-1} of the NSAIDs.

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3.1.1. Supported liquid membrane selection

First, the nature of the supported liquid membrane was investigated maintaining fixed the rest of parameters.

Benzene, 1-butanol, chloroform, cyclohexane, dichloromethane, dihexyl ether, n-hexane, 1-heptanol, isobutanol, 2-nitrophenyl octyl ether, 1-octanol, 1-pentanol, toluene and xylene were tested as SLM. These solvents were selected trying to approach several functional groups.

Some of the most widely used solvents as supported liquid membranes in liquid phase microextraction showed low or null conductivity. Thus, benzene, cyclohexane, dihexyl ether, hexane, nitrophenyl octyl ether, toluene and xylene could not be used as SLM in EME owing the interruption of electric current. 1-butanol, chloroform, dichloromethane, isobutanol and 1-pentanol produced high electric currents and subsequently, the enrichment factors were very low or null while 1-heptanol and 1-octanol showed lower electric currents (84 μA and 41 μA, respectively) and higher enrichment factors between 11 and 66. As can be seen in table 1 the EFs were higher when 1-octanol was used. A higher electric current stability was observed with 1-octanol and consequently, the %RSD observed were lower, so 1-octanol was selected as SLM for further optimization.

3.1.2. Donor and acceptor solutions pH

To ensure efficient electrokinetic mobility, pH must be adjusted to provide total ionization of the analytes in the two aqueous solutions [13, 18].

Donor solution pH was tested within the range 3-13 using NaOH and HCl aqueous solutions, while the acceptor solution was kept fixed at pH 12 adjusted with NaOH 10 mM. The highest enrichment factors were obtained at pH 7 for all compounds and decreased significantly over pH 12. Figure 2 shows that the higher enrichment factors were obtained at pH 7 for all compounds. Taking into account that all pKa values of all the analyzed compounds are lower than 5, at pH 7 the analytes would be mostly ionized which ensure an efficient electrokinetic mobility.

Once donor solution pH was selected (pH 7), acceptor solution was studied within the pH range 9-13. As shown in figure 3, high pH dependence was observed on the extraction efficiency. The highest enrichments were obtained at pH 12, while under pH

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11.5 and over pH 12.5 compounds were neither extracted or a dramatically decrease in the extraction was observed. At pH values higher than 10, as previously explained by Jin Mun Kim et al. [16], the analytes are partially protonated in the SLM and they can migrate easily through the SLM-acceptor solution interface.

Thus, a pH of 12 as acceptor solution and a pH of 7 as donor solution were fixed for further optimization.

3.1.3. Donor and acceptor solutions volumes

The effect of volume ratio between donor and acceptor solutions was evaluated. Different donor volumes were assayed (5, 10, 15, 20, 25, 50 and 100 mL) keeping the acceptor solution fixed at 50 µL. As can be seen in figure 4, higher enrichment factors within 76-102 were obtained with a 25 mL volume. On the other hand, the volume of the acceptor solution was studied by decreasing the initial volume by half. When a volume of 25 microliters was used compared to 50 microliters, the enrichments decreased by 41, 40, 27, 16 and 34%, for SAC, KTP, NAX, DIC and IBU, respectively. Consequently, a donor volume of 25 mL and an acceptor volume of 50 µL were fixed for the rest of the study.

3.1.4. Voltage and extraction time optimization

The applied voltage and the extraction time are critical parameters to be considered simultaneously because they have a direct influence in the amount of analytes transferred from the donor to the acceptor solution across the SLM. It is necessary to reach a compromise in order to avoid problems such as electrolysis, SLM loss or back diffusion of the analytes [19-23]. The former is important because the electric field generated between the two electrodes is the main driving force for the electrokinetic migration of the analytes across the SLM towards the acceptor solution. As a consequence, the mass transfer through the SLM is highly dependent on the magnitude of this parameter [24-26].

The influence of voltage was tested within 10-100 V (fig. 4) (10, 20, 30, 60, 80 and 100 V). As seen in figure 5, the enrichment factors increased when increasing the voltage from 10 to 60 V. A slight decrease was observed at 80 V, while a significantly decreased was obtained applying 100 V, resulting in an approximately 60% lower than

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the maximum obtained at 60 V. Therefore, 60 V was selected as the optimal voltage for the extraction of all NSAIDs, obtaining enrichment factors within the range 77-106.

Additionally, the current was measured during the extractions at the different applied voltages. The average values obtained were in the range 12-98 μA. The decrease in enrichment factors obtained over 60 V could be due to the higher values obtained for average current which would produce electrolysis phenomena in the solution affecting negatively to the extraction.

Next, the extraction time was optimized keeping the previous optimal conditions fixed (pH 7 as donor solution, pH 12 as acceptor solution, 60 V and a continuous stirring speed of 600 rpm). As shown in figure 6, the highest enrichment factors were obtained after 20 minutes extraction for all compounds and decrease considerably for longer and shorter extraction times in all cases. Thus, 20 minutes was the selected time for EME procedure.

3.1.5. Effect of donor and acceptor composition

To evaluate the effect of counter-cations on the extraction efficiency, the following alkaline hydroxides were used: NaOH, KOH, LiOH and CsOH. The rest of operational parameters were fixed at their optimal values. A total of 16 combinations were performed within donor/acceptor solution in order to evaluate the effect of the different counter-cations in both solutions.

To do so, 500 ng mL^{-1} donor aqueous solution containing the five studied NSAIDs was adjusted to pH 7 with the corresponding alkaline hydroxide and the acceptor solution was prepared by direct dilution of the corresponding hydroxide at 10 mM. The enrichment factors obtained for each combination of acceptor and donor solutions are summarized in table 2.

Taking into account the cation mobilities, that follows the order Li^+, Na^+, K^+ and Cs^+, the higher enrichments should be obtained by the pair KOH/CsOH in donor solution and acceptor solution [17], respectively. Nevertheless, the results obtained in this study showed that the optimal combination was KOH in the donor solution and NaOH in the acceptor solution for most of the analytes. Analyzing each compound individually (fig. 7), the trends show that in general, there is not a great effect on the enrichment factors when most of the combinations were used, except when the pair NaOH/CsOH was used in the acceptor and donor solution, respectively. In this case, the

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enrichment factors decreased below 60 for all analytes. The effect of the presence of a counter-ion was more pronounced in the DIC, observing a clear dependence with the presence of potassium in both donor and acceptor solution, and consequently the enrichment factor was more affected. Previously reported studies about EME of NSAIDs [14-16] (table 3) selected NaOH for both acceptor and donor solutions leading to lower extraction efficiency than the ones obtained in this work, where, the enrichment factors were within 86 and 134, meaning 4 times higher than the better enrichment factors previously reported [14].

As it can be observed in table 2, the average currents obtained were under 50 μA observing, in general, an inverse relationship between current intensity and enrichment factors obtained. SAC, KTP and IBU seemed to be less influenced by the counter-cations selected, except when Cs^+ was used in acceptor solution, obtaining lower enrichment factors and consequently higher intensity current values. NAX and remarkably DIC were significantly affected by the improper counter-cations selection, reaching the lower EFs with Cs^+ in donor and acceptor solutions.

3.2 Method validation

The methodology described in this paper was validated according to EURACHEM (European Analytical Chemistry) guide [27] in terms of linearity, sensitivity, precision, and accuracy.

The validation data of the proposed method are depicted in table 4. To evaluate the linearity of the proposed method a twelve-point (in triplicate) calibration curve was constructed by submitting standard mixtures at different concentrations of the studied analytes to the EME procedure previously described in section 2.2. It can be seen that good linearity was obtained for each analyte over the range 0.5-750 ng mL^{-1} for SAC, 1-500 ng mL^{-1} for NAX and 0.5-500 ng mL^{-1} for IBU (using fluorescence detector) and 3-750 ng mL^{-1} for KTP and DIC (using DAD detector) with correlation coefficients r^2>0.997. Detection and quantification limits were calculated as the minimum concentration of analyte giving peaks whose signal noise ratio are 3 and 10, respectively. Table 4 shows LODs and LOQs obtained using DAD detector for KTP and DIC and FLD detector for SAC, NAX and IBU. The determination of low concentration levels of each analyte is possible due to the high enrichment factor values obtained and the LODs range between 0.1-1.5 ng mL^{-1}.

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To evaluate the precision, repeatability and intermediate precision were evaluated. Standard solutions at three concentration levels (each compound at 5, 50, and 500 ng mL^{-1}) were submitted (in triplicate) to the entire analytical procedure and measured on a single day and one day per week during two months, respectively. Repeatability was in the range 1-8% and intermediate precision was in the range 2-9%, both expressed as relative standard deviation of calculated enrichment factors.

The accuracy was evaluated by means of recovery assays on spiked samples. The recoveries were calculated as percentage of extracted compound at low, medium and high concentration levels (5, 50 and 500 ng mL^{-1}). The samples were submitted to the entire analytical procedure described in sections above. As can be seen in table 5, recoveries were above 80% and no significant differences between the three concentrations spiked levels were found.

4. Application to human urine samples

Urine samples from two patients, who informed consent, under treatment with NAX and IBU respectively were analyzed. Samples were collected after 3 hours of oral NAX 550 mg dose (Cinfa®) and after 7 hours of a 600 mg oral dose in the case of IBU (Normon®). According to the literature approximately 95% of NAX from any dose is excreted in urine. Between 66-92% is excreted as conjugates and the rest percentage is excreted as unchanged naproxen and others of its metabolic forms [28,29]. Approximately a 10% IBU is excreted in urine between 6 and 8 hours after the ingestion as the parent form and the rest is excreted as metabolites or conjugates [30,31]. The concentrations found in the urine samples analyzed (in triplicate %RSD<5) were 164±7 ng mL^{-1} for NAX and 114±4 ng mL^{-1} in the case of IBU. These results are in agreement with the usual excreted levels in the period of time corresponding from the ingestion to the collection (3 and 7 hours respectively).

Figure 8 represented the typical HPLC chromatograms for a blank human urine sample, human urine sample spiked at 300 μg mL^{-1}, human urine sample collected after 3 hours of the ingestion of 550 mg of NAX and human urine sample collected after 7 hours of the ingestion of 600 mg of IBU. As can be seen, the chromatograms present excellent base lines as well as peaks with good resolutions for all the analytes, demonstrating a good clean-up of the samples.

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5. Conclusions

A complete and comprehensive study of the influence of all parameters affecting EME of NSAIDs was carried out. The results obtained in this work indicate that applications describe in previous papers were not the best in terms of enrichment factors. The compounds were only extracted when a long chain alcohol was used as SLM, 1-octanol was selected because it offers the best results in terms of electric current stability and enrichment factors. On the other hand, the highest enrichment factors were obtained for the following EME conditions: donor solution pH 7 (adjusted with KOH), acceptor solution pH 12 (adjusted with NaOH 10 mM), 25 mL donor solution volume, extraction time of 20 minutes and applied voltage of 60 volts.

In addition, the results showed that the nature of the inorganic cations introduce into the donor and the acceptor solution in their alkalinization using different alkaline hydroxides, affect the transfer of the analytes across the SLM. This showed better enrichment factors when the donor solution was alkalinized with KOH and the acceptor solution with NaOH. The enrichment factors were within 86 and 134, four times higher than the better enrichment factors previously reported in bibliography for the determination of non-steroidal antiinflammatory by EME using NaOH in donor and acceptor solutions.

The proposed method has been demonstrated to be adequate for the analysis of the selected NSAIDs in human urine samples, resulting in an excellent clean-up, good base lines, low LODs (0.1-1.5 ng mL^{-1}) and LOQs (0.3-3.0 ng mL^{-1}) and recoveries over 80 % for all the analytes. Finally, the method has been successfully applied to determine the presence of NAX and IBU in urine samples from patients under medical treatment.

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Figure Captions

Figure 1. Ilustration of the EME system.

Figure 2. Effect of pH of the donor solution on EME. %RSD < 10.8%

Figure 3. Effect of pH of the acceptor solution on EME. %RSD < 8.0%

Figure 4. Effect of volume of donor phase on EME. %RSD < 6.9%

Figure 5. Effect of the applied voltage on EME. %RSD < 10.6%

Figure 6. Effect of the extraction time on EME. %RSD < 9.2%.

Figure 7. Effect of alkaline hydroxides on EME. %RSD < 10.6%.

Figure 8. HPLC chromatograms for: a. blank human urine sample, b. human urine sample spiked at 300 µg mL^{-1}, c. sample collected after 3 hours of the ingestion of 550 mg NAX, d. sample collected after 7 hours of the ingestion of 600 mg IBU.

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Table 1. Enrichment factors of the NSAIDs and electric currents for 1-heptanol and 1-octanol using as SLM

Enrichment factors[a]					
SLM	**Analyte**				
	SAC[b]	**KTP**[c]	**NAX**[b]	**DIC**[c]	**IBU**[b]
Heptanol	11	20	11	16	21
Octanol	25	37	31	35	66

[a]Data calculated as average of triplicate determinations. %RSD for heptanol: 10-15 %. %RSD for octanol: 1.5-8%.

[b]FLD detection

[c]DAD detection

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Table 2. Results of the combination of LiOH. NaOH. KOH and CsOH in the donor (pH 7) and acceptor (pH 12) solutions

		Enrichment factors[a]					Electric current (μA)
Donor phase	**Aceptor phase**	**SAC[b]**	**KTP[c]**	**NAX[b]**	**DIC[c]**	**IBU[b]**	
LiOH	**LiOH**	112.2 ± 4.9	106.0 ± 8.8	80.2 ± 2.8	53.2 ± 5.1	90.4 ± 1.5	44 ± 1.8
	NaOH	110.4 ± 6.8	97.7 ± 4.2	75.1 ± 6.3	57.8 ± 3.8	89.7 ± 6.7	39 ± 0.8
	KOH	106.3 ± 0.5	102.9 ± 6.2	78.8 ± 2.7	84.6 ± 4.8	98.2 ± 4.9	32 ± 1.5
	CsOH	79.9 ± 6.7	100.9 ± 4.1	68.8 ± 0.7	54.3 ± 5.4	88.3 ± 2.3	38 ± 3.6
NaOH	**LiOH**	104.1 ± 9.2	99.6 ± 3.2	75.1 ± 6.6	52.2 ± 6.6	90.1 ± 5.3	45 ± 2.9
	NaOH	106.1 ± 4.3	100.5 ± 4.8	92.5 ± 5.6	77.1 ± 3.8	96.7 ± 2.1	37 ± 3.5
	KOH	113.4 ± 11.5	110.2 ± 0.9	73.7 ± 0.4	89.2 ± 0.5	103.4 ± 1.7	36 ± 3.6
	CsOH	63.8 ± 6.8	64.2 ± 5.4	39.5 ± 2.3	25.3 ± 0.6	38.1 ± 4.6	47 ± 3.7
KOH	**LiOH**	110.5 ± 5.3	103.7 ± 3.6	71.2 ± 1.7	52.6 ± 0.9	93.1 ± 7.6	28 ± 2.3
	NaOH	134.3 ± 6.7	121.9 ± 4.1	106.1 ± 7.9	85.6 ± 3.7	115.9 ± 6.7	30 ± 4.5
	KOH	118.5 ± 9.3	106.8 ± 4.6	73.6 ± 3.7	89.4 ± 7.2	101.3 ± 6.0	28 ± 3.5
	CsOH	112.8 ± 5.9	104.9 ± 5.4	73.5 ± 3.7	59.0 ±5.9	104.9 ± 7.1	33 ± 3.1
CsOH	**LiOH**	88.3 ± 2.0	92.7 ± 2.3	70.5 ± 2.7	55.0 ± 5.1	99.5 ± 1.8	37 ± 0.9
	NaOH	101.6 ± 6.0	102.5 ± 2.3	84.4 ± 1.12	58.6 ± 4.9	95.1 ± 1.1	37 ± 1.8
	KOH	100.4 ± 8.0	107.5 ± 5.0	75.7 ± 2.9	72.0 ± 6.1	102.6 ± 5.2	35 ± 2.2
	CsOH	91.3 ± 3.4	91.3 ± 5.9	67.5 ± 4.5	49.6 ± 5.3	85.6 ± 6.1	42 ± 1.3

[a]Average of three determinations ± standard devation (% RSD < 11%).

[b]FLD detection

[c]DAD detection

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Table 3. Comparison with previous studies in bibliography.

EME conditions				
Operational Parameters	**M. Balchen et al. [15]**	**M. Ramos Payán et al. [14]**	**J.M. Kim et al. [16]**	**Proposed method**
Fibber length	31mm	30 mm	32 mm	30 mm
Model of Fibber	Accurel® PP Q3/2	Accurel® S6/2	Accurel® PP/300/1200	Accurel® S6/2
Cleaning	Acetone	Acetone	Acetonitrile	Acetone
SLM	1-heptanol	1-octanol	1-octanol	1-octanol
Donor Phase pH	12 (NaOH)	12 (NaOH)	7 (NaOH)	7 (KOH)
Acceptor Phase pH	12 (NaOH)	12 (NaOH)	11 (5 mM NH_4AcO)	12 (NaOH)
Donor Phase Volume	0.3 mL	10 mL	1 mL	25 mL
Acceptor Phase Volume	30 µL	50 µL	unknown	50 µL
Distance of electrodes	unknown	3 mm	unknown	3 mm
Time (min)	5	10	10	20

Enrichment factors					
	SAC	**KTP**	**NAX**	**DIC**	**IBU**
M. Balchen et al. [15]	-	8.2	2.5	7.4	6.1
M. Ramos Payán et al. [14]	40	43	32	49	28
J.M. Kim et al. [16]	-	8	6	-	12
Proposed method	134	122	106	85	116

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Table 4. Linear ranges. Linearity, detection limits (LODs), quantification limits (LOQs) and enrichment factors (EF) for the proposed EME procedure.

Analyte	Linear range ($ng\ mL^{-1}$)	Linearity		LOD ($ng\ mL^{-1}$)	LOQ ($ng\ mL^{-1}$)	EF
		(r^2)	(%)			
SAC[a]	0.3-500	0.998	98.62	0.1	0.3	134
KTP[b]	3-750	0.9982	98.72	1.5	3	122
NAX[a]	0.5-500	0.9973	98.43	0.2	0.5	106
DIC[b]	3-750	0.9987	98.92	1	3	86
IBU[a]	0.5-500	0.9977	98.51	0.2	0.5	116

[a]FLD detection

[b]DAD detection

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Table 5. Recoveries from a thousand times diluted urine sample spiked at three levels (average of three determinations ± standard deviation). %RSD < 7

Spiked level (ng mL^{-1})	SAC[a]	KTP[b]	NAX[a]	DIC[a]	IBU[b]
5	99.9 ± 4.8	99.9 ± 2.9	85.6 ± 8.3	85.1 ± 4.0	95.8 ± 3.4
50	97.3 ± 7.7	101.5 ± 5.4	82.1 ± 6.8	91.4 ± 2.2	95.4 ± 2.3
500	99.7 ± 1.9	100.3 ± 3.1	88.2 ± 4.9	81.2 ± 1.9	99.5 ± 2.2

[a]FLD detection

[b]DAD detection

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Highlights

- Complete study of the influence of all parameters affecting EME of NSAIDs, focused in the effect of counter-ions.
- EF four times higher than previously reported.
- LODs between 0.1 and 1.5 ng mL^{-1} for salicylic acid and ketoprofen respectively.
- Method was successfully applied to determine NAX and IBU in human urine samples.

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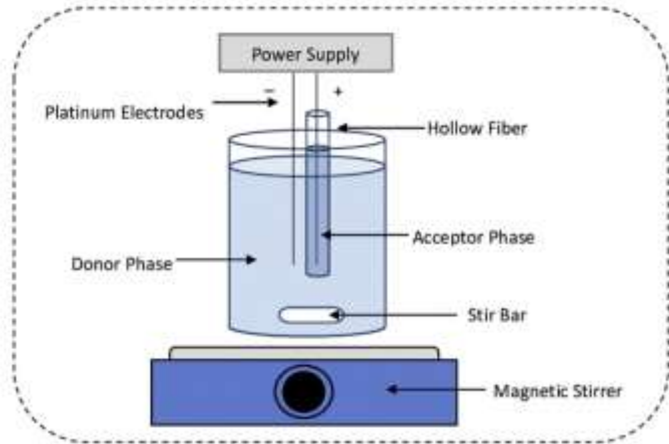

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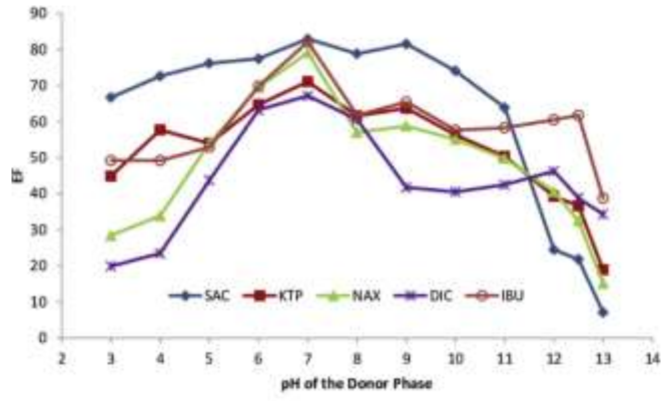

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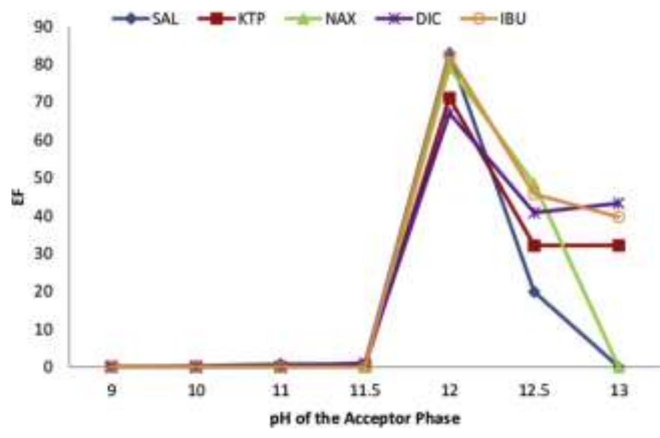

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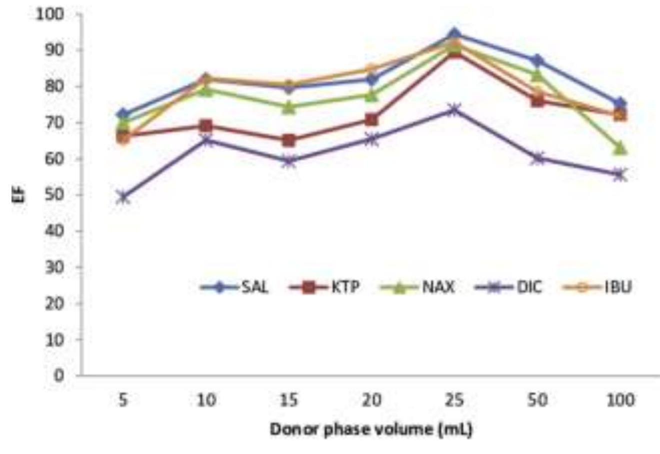

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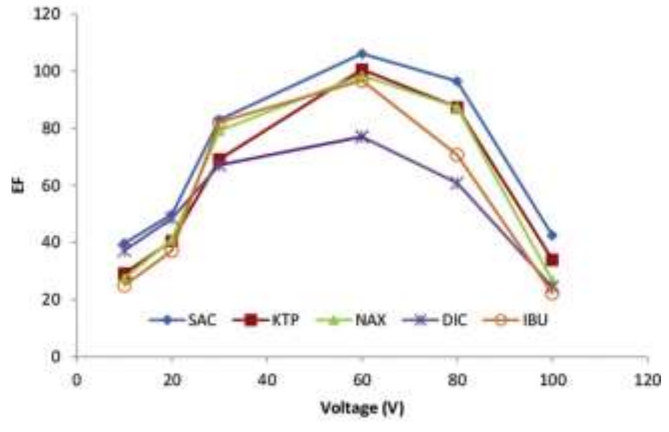

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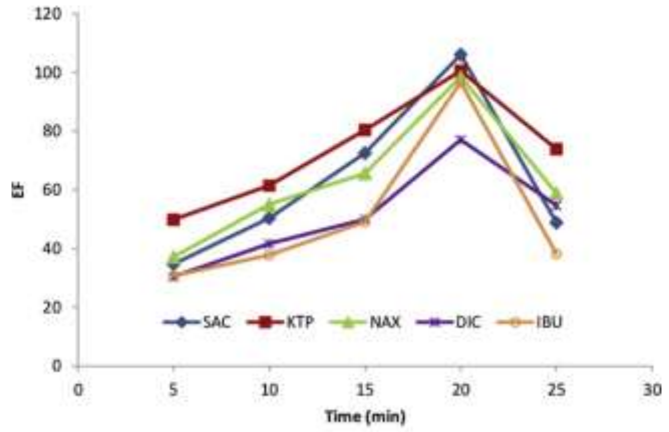

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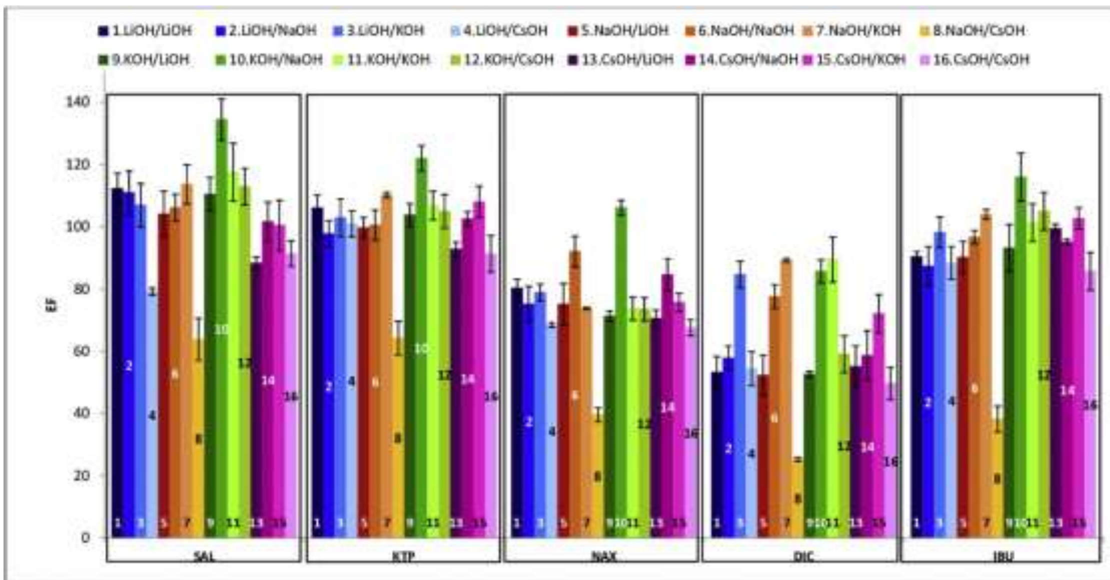

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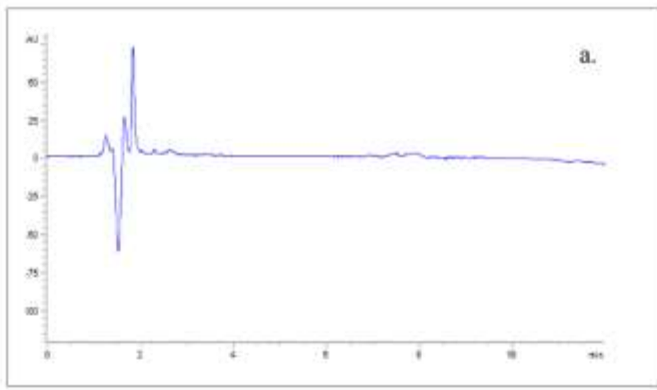

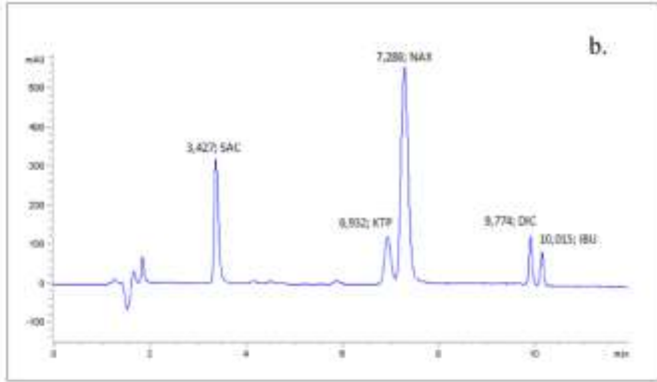

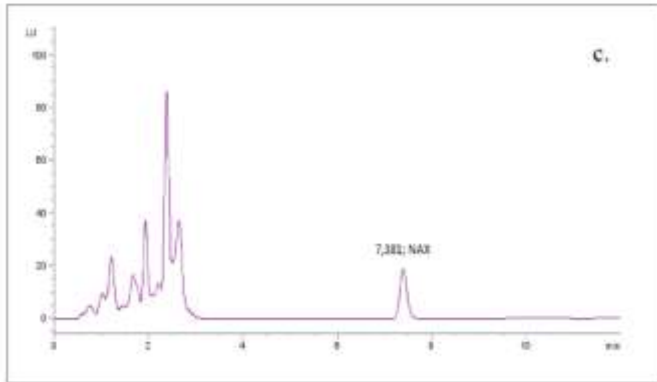

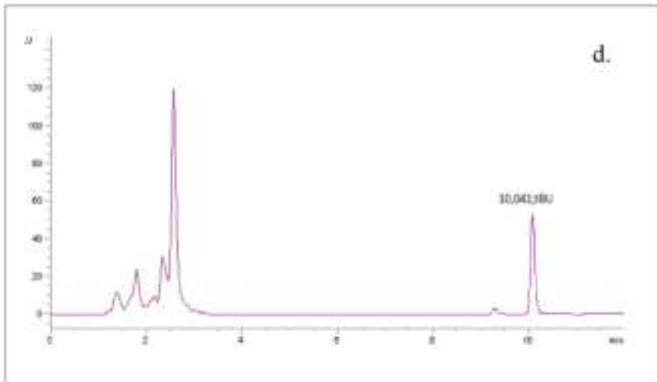

Figure 8