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Development of a needle device for on-line electroextraction–liquid chromatography

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Abstract

The development of a needle device enabling on-line electroextraction coupled to high-performance liquid chromatography in an autosampler is described. This electroextraction needle meets all the demands set to automated liquid handling and can fully replace the conventional autosampler needle. In addition, the system has been fully automated, and can be used as an interfacing technique between solid-phase extraction and liquid chromatography. A detailed description of the electroextraction needle hardware, as well as some typical examples of its use in on-line electroextraction–liquid chromatography are presented.

Keywords: Electroextraction; Needle device; Extraction methods

1. Introduction

Recently, we presented liquid–liquid electroextraction (EE) as an on-line focusing technique in combination with capillary isotachophoresis–zone electrophoresis (ITP–CZE) [1,2]. EE is a form of liquid–liquid extraction in which the mass transfer from the organic donor-phase to the aqueous acceptor-phase is accelerated by the application of an electric field without the need of mechanical stirring of the two phases. The use of EE allows the rapid extraction of charged compounds from an organic solvent with a volume up to several hundreds of microlitres to a small volume of an aqueous buffer in a 75-μm fused-silica capillary. As an example, the complete EE of a 300-μl organic sample took less than 10 min [1]. Owing to the extremely high local electric field strength in the organic phase, analyte ions migrate very fast in the direction of the liquid–liquid interface and enter the aqueous acceptor-phase. From this point of view, the process of EE is comparable to electrophoretic sample stacking techniques [3–5]. However, the liquid–liquid interface is a physical border at which point the electric field strength drops to a negligible value. It prevents mixing of the donor and the acceptor based on diffusion. In contrast to sample stacking in a continuous liquid system, in EE a distinct separation of the low conductive donor and the highly conducting acceptor is maintained. Therefore, band broadening during EE is limited resulting in a high analyte concentration. We demonstrated that on-line capillary EE–ITP–CZE coupled to electrospray-mass spectrometry allows the detection of a concentration of $5 \cdot 10^{-9}$ mol/l of several β-agonists [2].

This paper describes the application of EE which

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can be used as an interfacing technique between solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) to bypass the evaporation of organic solvents. The conventional HPLC method of analyzing an organic extract derived after e.g. SPE often comprises the evaporation of the organic solvent and subsequent reconstitution of the residue in the HPLC mobile phase. However, these steps are rather time-consuming, complicate the automation of the overall procedure and may lead to analyte loss. This is certainly true in the case of volatile analytes, or sub-quantitative reconstitution, e.g. caused by adsorption losses. EE can overcome off-line solvent evaporation and reconstitution of the residue for ionic or ionizable analytes, while the overall selectivity of the analysis by discriminating between cations, anions and neutrals is enhanced.

The implementation of EE in an interface between automated SPE and HPLC is presented. For on-line SPE–EE–HPLC, a device has been developed to replace the injection needle of the applied autosampler. The system has been modified to meet the requirements set to both the performance of automated liquid handling procedures and the process of EE. The EE needle design preserves all characteristics and liquid handling functions of the autosampler while providing optimum EE performance. Examples of on-line EE–HPLC for the determination of 4-nitrophenol, salicylic acid, some leukotrienes, catecholamines and β-agonists using the EE needle are presented.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical grade. All aqueous solutions were prepared using water purified with a Milli-Q system (Millipore, Bedford, MA, USA). Ethanol (EtOH), ethyl acetate (EtAc), acetic acid, phosphoric acid, citric acid, sodium hydroxide (NaOH) and potassium hydroxide were obtained from Merck (Darmstadt, Germany), 4-nitrophenol, clenbuterol hydrochloride, noradrenaline hydrochloride, adrenaline bitartrate, salicylic acid and octane sulphonic acid from Sigma (St. Louis, MO, USA), leukotriene C_4 and leukotriene E_4 from SPI-Bio (Gif-sur-Yvette, France), dopamine hydrochloride and crystal violet from Janssen Chimica (Beerse, Belgium), acetonitrile and methanol from Rathburn (Walkerburn, Scotland, UK), β-alanine (βAla) from Aldrich (Steinheim, Germany), ammonium hydroxide (NH_4OH) from Brocacef (Maarssen, Netherlands). Mabuterol hemisulphate was kindly donated by TNO Nutrition and Food Research Institute (Zeist, Netherlands).

A standard solution of 4-nitrophenol at a concentration of 10^{-2} mol/l in acetonitrile was stored at 4°C. Standard solutions of the leukotrienes at a concentration of 1 mg/ml were made in ethanol and stored at −20°C. Standard solutions of clenbuterol, mabuterol and salicylic acid at concentrations of 10^{-3} mol/l in methanol were stored at 4°C. Standard solutions of noradrenaline, adrenaline and dopamine at concentrations of 10^{-3} mol/l in acetonitrile were prepared daily and stored at 4°C.

2.2. Apparatus

2.2.1. Autosampler and HPLC system

The automated HPLC system consisted of a Gilson (Villiers-le-Bel, France) ASPEC XL autosampler equipped with two Rheodyne (Berkeley, CA, USA) six-port injection valves of which only one was used. Apart from an 80-μl injection loop, a laboratory-made injection port was connected to the six-port injection valve via a PTFE transfer tubing with a total volume of 16 μl. The laboratory-made injection port (2.3 mm I.D.) necessary to accomodate the outer diameter of the EE needle (2.0 mm O.D.) was equipped with a septum to avoid band-broadening during the transfer of liquid from the EE needle into the injection loop. Furthermore, the automated HPLC system consisted of a Gilson 401C dilutor equipped with a 1-ml syringe, a Gilson 306 HPLC pump, a Gilson 805 manometric module and a Gilson 117 UV–Vis absorbance detector. The system was controlled using Gilson 719 Sampler Controller software. Gilson 715 HPLC software was used for data acquisition. Separation of 4-nitrophenol, the leukotrienes, clenbuterol, mabuterol and salicylic acid were carried out on a Nucleosil C_{18} analytical column (250×4 mm I.D., 5 μm d_p, Macherey-Nagel, Düren, Germany). The HPLC mobile phase for 4-nitrophenol consisted of acetic acid solution

(pH 3)–acetonitrile (62:38, v/v), pumped at a flow-rate of 1.2 ml/min. The mobile phase for the separation of the leukotrienes consisted of 33 mmol/l acetic acid–acetonitrile (50:50, v/v), pumped at a flow-rate of 1.2 ml/min. The mobile phase for the analysis of clenbuterol and mabuterol and salicylic acid consisted of acetic acid (pH 3)–acetonitrile (74:26, v/v), and (91:9, v/v), respectively, all pumped at a flow-rate of 1.2 ml/min. Separation of noradrenaline, adrenaline and dopamine was obtained by ion-pair reversed-phase HPLC using a Varian (Palo Alto, CA, USA) Nucleosil C_{18} analytical column (100×4.6 mm I.D., 5 μm d_p) and a mobile phase consisting of 50 mmol/l phosphoric acid and 50 mmol/l citric acid adjusted to pH 3.1 with potassium hydroxide–methanol (97:3, v/v) containing 100 mg/ml octane sulphonic acid. The flow-rate was 1.0 ml/min. UV absorbance detection of noradrenaline, adrenaline, dopamine, clenbuterol and salbutamol was performed at 254 nm, 4-nitrophenol, leukotriene C_4 and leukotriene E_4 at 280 nm and salicylic acid at 240 nm. EE was performed in an in-house-developed EE needle, using a Brandenburg Alpha Series II (Thornton Heath, UK) high voltage power supply.

2.2.2. Electroextraction needle

Fig. 1 is a detailed representation of the EE needle and Fig. 2A and B are schematic representations of the EE needle in neutral position and in EE position, respectively. The EE needle is mounted onto the autosampler arm, replacing the original needle and connected to the dilutor. The needle reservoir (5 mm I.D.), the lining of the needle tip (500 μm I.D.) and the conical-shaped connection piece between needle reservoir and needle tip are made of PTFE. A stainless steel capillary (2.0 mm O.D.) is placed over the PTFE tip lining to make the needle tip rigid. This stainless steel capillary does not participate in the electrical circuit (Fig. 1). To obtain maximum mass transfer during EE the liquid–liquid interface is positioned at the point indicated as 'I' in Fig. 2B. Thus, the interface area equals the cross-section of the donor-phase liquid column in the needle reservoir (19.6 mm^2), whereas the height of the liquid column equals the maximum migration path length. The stainless steel rod electrode in the needle reservoir, which is kept at ground potential

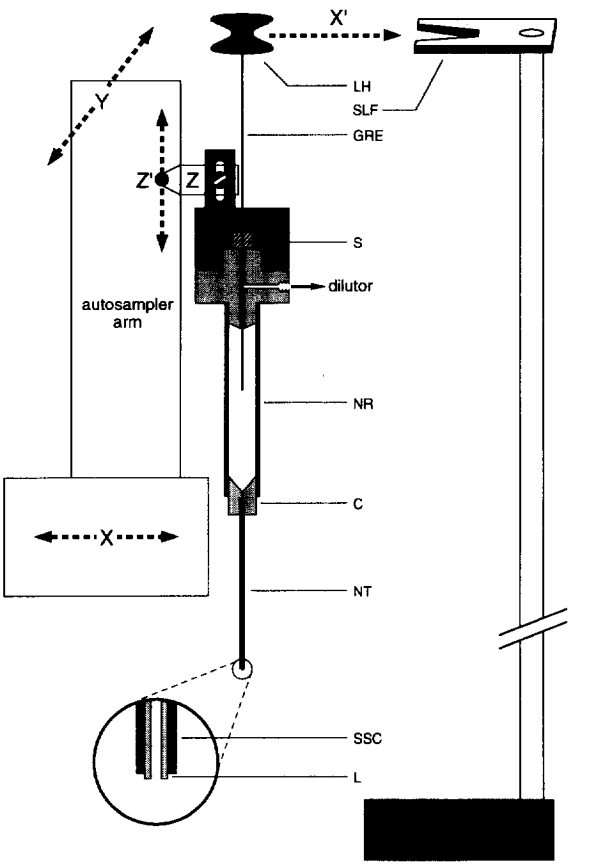


Fig. 1. Detailed representation of the electroextraction needle mounted on the autosampler arm. LH=lift handle, SLF=static lift fork, GRE=grounded rod electrode, S=silicone septum, NR=needle reservoir, C=connector, NT=needle tip, SSC=stainless steel capillary and L=PTFE liner. A dilutor is connected to the needle via a PTFE tubing for drawing and dispensing liquid in and out of the needle. See Section 2.2.2 for an explanation about the movement of the needle and the positioning of the rod electrode.

for safety reasons, can be adjusted in height. For this purpose, the lift handle on the rod electrode above the needle reservoir is placed in the static plexiglass lift fork, according to movement X' in Fig. 1. In this way the grounded rod electrode is fixed. The EE needle, which is attached to the Z-part of the needle arm of the autosampler, can then be moved along the grounded rod electrode by subsequent upward or downward movement of the Z-part of the needle arm, according to movement Z'. The grounded rod electrode does not make physical contact with the Z-part of the needle arm. The upward and downward

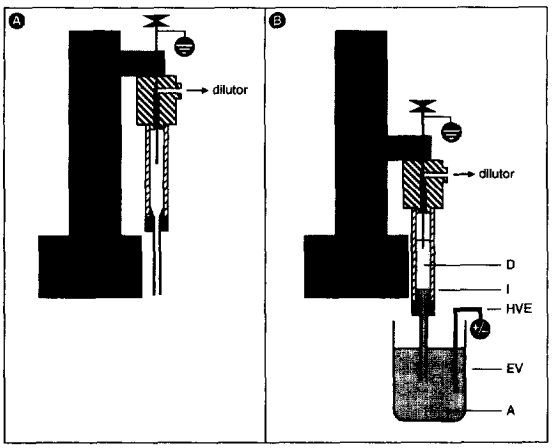


Fig. 2. Representation of the electroextraction needle in neutral position (A) and in electroextraction position (B). D=organic donor phase, I=liquid–liquid interface, HVE=high voltage electrode, EV=electrode vial and A=aqueous acceptor phase.

sliding of the EE needle along the rod electrode is made possible by a septum in the EE needle that clasps the rod electrode. The position of the tip of the grounded rod electrode with respect to the liquid interface of the donor-phase can be adjusted accordingly. This enables the use of any donor-phase volume up to 1 ml. EE of 400-μl donor-phase volumes takes only 2 min.

2.3. Electroextraction–high-performance liquid chromatography procedure

An aliquot of 400 μl of the organic donor solution is introduced into the EE needle followed by 60 μl of the aqueous acceptor solution. Next, the EE needle is positioned in the electrode vial containing the aqueous acceptor solution and a high voltage is applied. For the EE of cations the electrode in the electrode vial is negatively charged, whereas positively charged for anions. After a 2-min EE, the EE needle is positioned into the injection port through the septum inside the injection port. Next, 80 μl (60 μl acceptor solution and 20 μl organic donor solution) are transferred to the injection loop via the PTFE transfer tubing using the Gilson 401C dilutor. Thus, by transferring a total volume of 80 μl, the injection loop will eventually be filled with the whole acceptor solution (60 μl) and an additional 4 μl of the donor solution, as the volume of the PTFE-transfer tubing measures 16 μl. A few microlitres of the organic donor are injected onto the HPLC system to ensure that all analyte ions extracted are analyzed.

3. Results and discussion

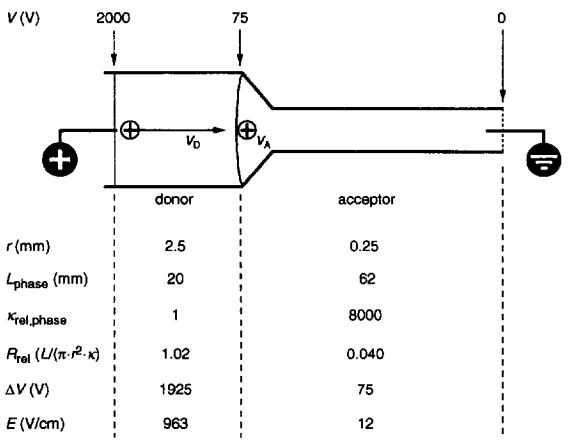
3.1. Automation of electroextraction

Electroextraction is performed in an in-house-developed EE needle. To perform automated EE, a flexible and programmable liquid handling system is required. Besides the accurate positioning of the EE needle in a three-dimensional XYZ-area, accurate liquid handling is required. After aspirating a 400-μl volume of the organic donor-phase, a reproducible amount of the acceptor-phase has to be introduced into the EE needle in order to establish a defined position of the liquid–liquid interface in relation to the electrode tip in the EE needle. A deviation in acceptor volume results in a deviation of the electric field strength in the organic donor-phase or in a deviation of the liquid–liquid interface area. As the number of ions extracted is proportional to the electric field strength and to the interface area [1,5], the recovery will be affected, accordingly. Moreover, when the electric field strength is too high, this may lead to disturbances and instability of the liquid–liquid interface. An intact liquid–liquid interface is required to obtain maximum and reproducible recoveries. The voltage should be as high as possible (typically $\geq$1.0 kV) while maintaining a stable liquid–liquid interface to obtain maximum mass transfer. The EE needle described in this paper is designed to use organic solvents with a density lower than the aqueous acceptor solution.

3.2. Electroextraction needle design

The EE needle (Fig. 1 and Fig. 2) is designed to deal with donor-phase volumes up to 1 ml which makes it compatible with conventionally dimensioned SPE. At the same time, by replacing the original autosampler needle by the EE needle no limitations with respect to the overall performance of the autosampler are met. The configuration of the EE

needle offers full access to the advantages and characteristics of EE mentioned above. The power of EE is based on the large difference of the electric field strengths in the organic donor-phase and in the aqueous acceptor-phase (Fig. 3). Owing to the high electric field strength in the donor-phase the migration rate of ions is high. The migration rate of the same ions in the acceptor-phase is limited as the electric field strength in the acceptor-phase is low (Fig. 3). Therefore, the use of low-conductive donor solvents and high-conductive acceptor solutions is required. A needle tip liner I.D. of 0.5 mm appeared to be an optimum value with respect to overall conductivity of the acceptor-phase on the one hand and total acceptor-phase volume on the other hand (Fig. 4). Diameters lower than 0.5 mm decrease the electric field strength in the donor phase while increasing the electric field strength in the acceptor phase. However, diameters up to 1 mm do not significantly improve the electric field strength in the donor-phase (990 V/cm vs. 963 V/cm with 0.5 mm I.D.). Accordingly, a decrease of the electric field strength from 12 V/cm (0.5 mm I.D.) to 3 V/cm in the acceptor-phase will not contribute to a more efficient electroextraction either. Thus, optimal EE conditions using a needle tip liner I.D. of 0.5 mm are obtained while allowing a considerable EE concentration factor owing to the acceptor-phase volume of 60 μl.



	donor	acceptor
r (mm)	2.5	0.25
L_{phase} (mm)	20	62
$\kappa_{rel,phase}$	1	8000
R_{rel} ($L/(\pi \cdot r^2 \cdot \kappa)$)	1.02	0.040
ΔV (V)	1925	75
E (V/cm)	963	12

Fig. 3. Schematic view (90° rotated) of the EE needle filled with the donor-phase and the acceptor phase, separated by the liquid–liquid interface. The velocity vector v_D indicates the migration rate of a cation in the donor phase, whereas v_A indicates the migration rate of the same cation in the acceptor phase; r represents the radius of the phase, L_{phase} the length of the phase, $\kappa_{rel,\ phase}$ the relative conductivity of the phase, R_{rel} the relative resistance, ΔV the voltage drop over the phase and E the electric field strength in the phase. In the calculations the whole acceptor phase has been regarded as a tube.

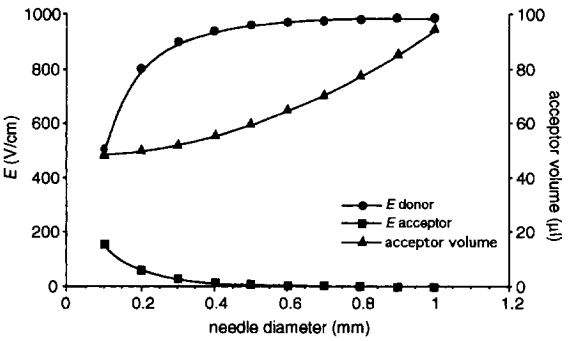


Fig. 4. Relation between the diameter of the needle tip liner and the calculated electric field strengths in the donor-phase (E donor) and in the acceptor-phase (E acceptor) and the total acceptor-phase volume.

A high electric field strength in the organic phase forms the basis of EE as a rapid extraction technique. Considering relative conductivities for the donor-phase and the acceptor-phase of 1:8 000 [1], the application of a voltage of 2.0 kV over the two-phase system yields an electric field strength of 963 V/cm in the organic donor-phase and 12 V/cm in the acceptor-phase (Fig. 3). Electric field strengths in the EE needle are not as high as in the capillary set-up [1,2]. This is due to a larger migration path length (20 mm vs. 2 mm in the capillary set-up) and a lower applied voltage (1–2 kV vs. 10 kV in the capillary set-up). However, the design of the EE needle holds features that contribute to the speed of the EE process. Those features include the relatively large area of the liquid–liquid interface, the short migration path length and the relatively large volume of the donor-phase that is exposed to the electric field. The liquid–liquid interface area of 19.6 mm^2 equals the cross section of the donor-phase liquid column. This large interface area enables a high rate of mass transfer per unit of time, as the number of ions migrating through the interface per unit of time is directly proportional to the interface area [1,5]. In addition to this, the maximum migration path length of an ion to the interface in a 400-μl volume of donor-phase yields 20 mm only. The volume of the

donor-phase that is not exposed to the electric field in the EE needle is relatively small, owing to the conical-shaped PTFE connection piece (Fig. 1) which forms a gradual physical transition to the acceptor-phase in the needle tip. The grounded rod electrode is positioned just beneath the upper liquid level of the donor-phase. Moreover, considering a high-conductivity acceptor solution, the liquid–liquid interface may be regarded as a disk electrode. Combined with accurate positioning of the rod electrode in the donor-phase, this contributes to an efficient distribution of the electric field lines through the donor-phase as schematically represented in Fig. 5. In order to maintain this optimum electric field line pattern for any volume of donor-phase, the grounded rod electrode in the needle reservoir is adjustable in height (Fig. 1). This offers full flexibility with respect to donor-phase volume up to a maximum of the total contents of the needle reservoir (approximately 1.0 ml). The adjustment of the position of the rod electrode is fully automated and controlled by custom-programmed Pascal routines using the autosampler software. By placing the lift handle in the static lift fork, the position of the rod electrode in the needle reservoir can be adapted by moving the EE needle upwards or downwards (Fig. 1).

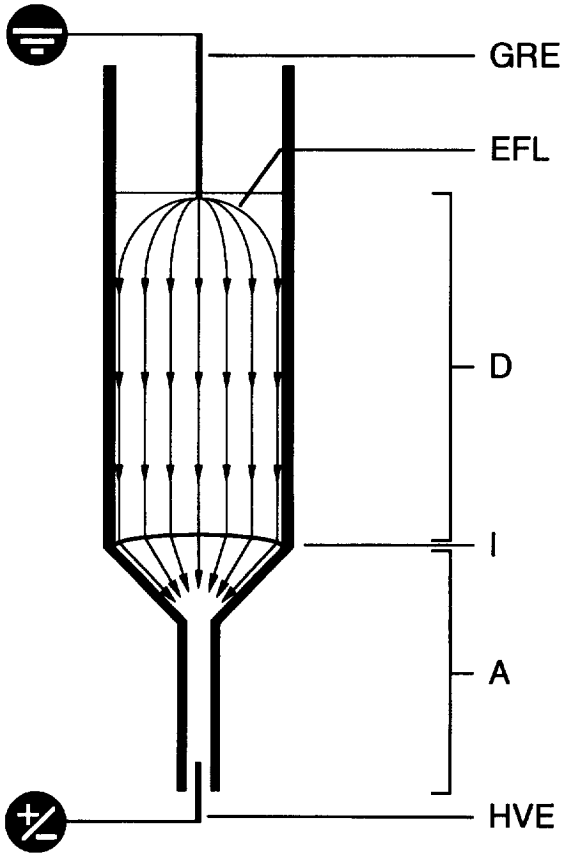


Fig. 5. Graphic representation of the distribution of the electric field lines (EFL) in the electroextraction needle through the donor phase (D), via the liquid–liquid interface (I) into the acceptor phase (A). GRE=grounded rod electrode and HVE=high voltage electrode.

To visualize the process of EE, the EE of the blue cationic dye crystal violet was performed in the EE needle after removing the stainless steel capillary (Fig. 1). This experiment clearly showed that the crystal violet cations concentrated just beneath the liquid–liquid interface and did not migrate into the PTFE liner within the 2-min time period of EE. For this reason injection of a few microlitres of the organic donor is necessary to obtain reproducible recoveries. The injection of the total volume of aqueous acceptor together with a few microlitres of the organic donor minimizes peak broadening. Preliminary results showed that the use of an isotachophoretic buffer system as the acceptor-phase allows the focusing of the extracted ions between the two aqueous buffers, away from the liquid–liquid interface. This has been reported earlier for the capillary set-up [1,2]. In this way the injection of a small amount of the organic donor solution can be prevented. Research will proceed on this topic.

3.3. Electroextraction conditions

Transfer of analytes from the organic donor-phase to the aqueous acceptor-phase only occurs when the analytes are charged. Therefore, the donor phase is saturated with an aqueous solution at an appropriate pH, creating the required conditions to ensure ionization of the analytes of interest. In contrast to the other test compounds, the ionization of the weak acid 4-nitrophenol required more extreme conditions in the donor-phase. The addition of sodium hydroxide-saturated ethanol to the donor yielded the aimed effect. It has to be realized that good solubility of the extracted analytes in the aqueous acceptor solution is required for maximum EE recovery. Therefore, the

Table 1
Experimental data on the EE performance of a number of test compounds using on-line EE–HPLC

Compound*	pK_a[a]	Conc.[b] (mol/l)	Donor[c]	Acceptor[d]	EE V (kV)[e]	Rec[f]. (%)
4-Nitrophenol	7.1	$5 \cdot 10^{-6}$	EtAc–EtOH sat. with NaOH (100:1)	0.1 mol/l NaOH	+1.3	8
Salicylic acid	3.0	$5 \cdot 10^{-6}$	EtAc sat. with 0.1 mol/l NaOH	0.1 mol/l NaOH	+1.1	82
Leukotriene C_4	±5	100 ng/ml	EtAc sat. with 10 mmol/l βAla–NH_4OH pH 10.3	10 mmol/l βAla–NH_4OH pH 10.3	+2.5	54
Leukotriene E_4	±5	100 ng/ml	EtAc sat. with 10 mmol/l βAla–NH_4OH pH 10.3	10 mmol/l βAla–NH_4OH pH 10.3	+2.5	75
Clenbuterol	9.0	10^{-6}	EtAc sat. with 10 mmol/l βAla–HAc pH 4.9	HAc pH 3	−1.2	80
Mabuterol	9.3	10^{-6}	EtAc sat. with 10 mmol/l βAla–HAc pH 4.9	HAc pH 3	−1.2	84
Noradrenaline	8.7	10^{-6}	EtAc sat. with HAc pH 3	HAc pH 3	−1.0	88
Adrenaline	8.7	10^{-6}	EtAc sat. with HAc pH 3	HAc pH 3	−1.0	89
Dopamine	8.8	10^{-6}	EtAc sat. with HAc pH 3	HAc pH 3	−1.0	85

* The names of the acidic test compounds are in plain text whereas the names of the basic test compounds are italicized.
[a] pK_a values in aqueous solution.
[b] Conc., concentration in the donor phase.
[c] The donor phase composition.
[d] The acceptor-phase composition.
[e] The applied voltage.
[f] Rec., recovery.

pH of the acceptor solution was set to a value at which the extracted analytes maintain their charge when entering the acceptor phase (Table 1).

The time needed for maximum EE recovery has been determined experimentally. When performing EE longer than 2 min, no enhancement of the recovery of the compounds tested was achieved. The extraction of the analyte ions proceeds as long as the conditions in the donor-phase are such that the analytes are ionized. However, the ionizing donor-phase additives (acids or bases) are also exposed to an electric field. Dependent on their charge sign they will migrate either in the direction of the liquid–liquid interface and enter the acceptor or in the direction of the electrode in the EE needle where they may be electrolyzed. In both cases, the ionizing properties of the organic donor-phase gradually decline during EE. It has been determined that the analyte fraction that is not recovered in the acceptor-phase remains in the donor phase, probably in uncharged form as a result of changed conditions in the donor phase during EE.

The applied voltage determines the migration velocity of charged species, and accordingly, the extraction time. At voltages of 1 kV or higher, maximum recovery of the tested compounds was achieved during the 2-min EE time.

3.4. On-line electroextraction–high-performance liquid chromatography with the electroextraction needle

The applicability of EE–HPLC has been demonstrated for a number of test compounds. Table 1 shows all experimental data related to the EE–HPLC of each of the compounds used. Fig. 6 and Fig. 7 show chromatograms after EE–HPLC of two leukotrienes and some catecholamines, respectively. Quantitative aspects of EE–HPLC were examined by generating calibration plots of 4-nitrophenol in the concentration range $0–7.2 \cdot 10^{-6}$ mol/l and of clenbuterol in the concentration range $0–10^{-6}$ mol/l. EE of 4-nitrophenol was performed at 2.0 kV with EtAc–NaOH saturated EtOH as the donor-phase, whereas EE for clenbuterol was performed at −1.2 kV. A typical calibration plot for 4-nitrophenol is characterized by $y = 1.16 \cdot 10^{11} x + 2444$ ($r^2 = 0.9994$, $n = 3$), whereas a typical calibration plot for clen-

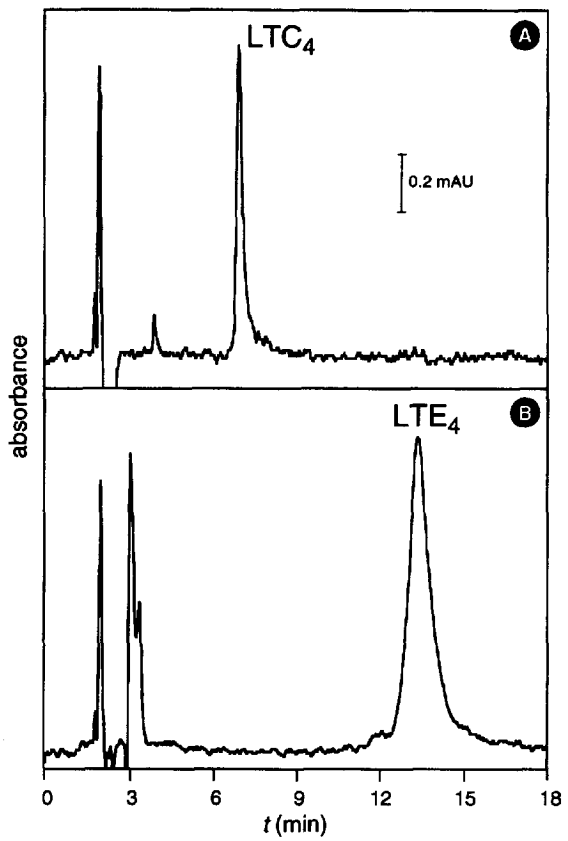


Fig. 6. Chromatograms obtained after EE–HPLC of leukotriene C_4 (LTC_4) (chromatogram A) and leukotriene E_4 (LTE_4) (chromatogram B) from 400 μl of EtAc saturated with 10 mmol/l βAla–NH_4OH, pH 10.3. The analyte concentrations were 100 ng/ml in both cases. See Table 1 for experimental details and EE recoveries.

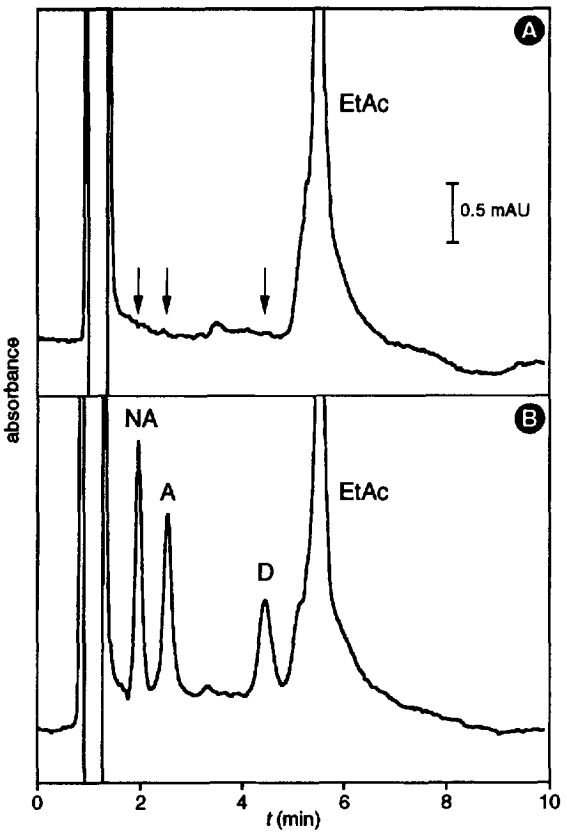


Fig. 7. Chromatograms obtained after EE–HPLC of 400 μl of plain EtAc saturated with acetic acid, pH 3 (chromatogram A) and spiked with noradrenaline (NA), adrenaline (A) and dopamine (D) to a concentration of 10^{-6} mol/l (chromatogram B). The injection of a few microlitres of EtAc resulted in a peak at t=5.6 min. See Table 1 for experimental details and EE recoveries.

buterol is characterized by $y=1.48\cdot10^{12}x+8289$ ($r^2=0.9993$, $n=3$) where y represents the peak area (arbitrary units) and x the analyte concentration in mol/l. Relative standard deviations (R.S.D. values) were determined after repetitive EE–HPLC analyses of 10^{-8} mol/l and 10^{-7} mol/l standard solutions. The R.S.D. values were 3.5 and 4.3%, 3.8 and 2.4% for 4-nitrophenol and clenbuterol, respectively (n= 3).

4. Conclusions

A design of an injection needle for automated EE is presented. The developed EE needle is multifunctional, holding all EE features while preserving the original properties of the standard autosampler needle. Moreover, the design of the EE needle makes the electroextraction of charged compounds from an organic donor solvent to an aqueous acceptor solution a rapid and simple extraction technique. According to the quantitative data, EE shows good linearity and high reproducibility. The process of electroextraction is a very promising technique that can be used to overcome the off-line evaporation and subsequent reconstitution of an organic extract after SPE, containing charged analytes. Compared to the conventional method of solvent evaporation of a column extract and subsequent reconstitution, electroextraction discriminates between charge, takes

only a fraction of the time needed for evaporation and can be fully automated. Future research will be devoted to fundamental aspects of electroextraction, as well as to its application as an interfacing technique between SPE and HPLC for the analysis of biological samples.

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