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Combination of liquid-phase microextraction and on-column stacking for trace analysis of amino alcohols by capillary electrophoresis

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Abstract

We described a new method for the enrichment of basic drugs present in water samples via liquid-phase microextraction (LPME) combined with on-column stacking in capillary electrophoresis. Two steps were employed to enhance the detection sensitivity of four amino alcohols. The analytes were first extracted from aqueous sample (donor solution) that were adjusted to basic through a thin layer of 1-octanol entrapped within the pores of a polypropylene hollow fiber, and then into a 5-μl acidic acceptor solution inside the hollow fiber. The extract was then further enriched through on-column stacking in capillary electrophoresis. With this two-step enrichment procedure, the method provided 72–110-fold preconcentration of the target amino alcohols. The limits of detection were 0.08–0.5 μg/ml. Relative standard deviation ($n=6$) ranged between 4.3 and 6.9% for the studied drugs utilizing 2-amino-1-phenylethanol as internal standard. The extraction of amino alcohols in spiked urine samples was evaluated using the developed procedure.

Keywords: Liquid-phase microextraction; Extraction methods; Field-enhanced sample stacking; Beta-Blockers; Amino alcohols

1. Introduction

During the last 10 years, capillary electrophoresis (CE) has developed into a highly attractive separation technique [1], which can be applied in several modes of separation. The technique has many advantages such as high separation efficiencies, fast separation and minimal reagent consumption. It has been applied to the analysis of pharmaceuticals, agrochemicals, raw materials, organic pollutants and DNA, etc. [2]. However, the applicability of CE suffers from its relatively high concentration detection limits. In addition, in most cases sample cleanup is required for complicated biological and environmental samples prior to their analysis by CE. These are also problems that are applicable to the analysis of drugs in various types of samples.

Amino alcohols (β-blockers) are a class of drugs that can be determined by CE [3]. They are used for the treatment of various cardiovascular disorders such as hypertension, angina pectoris, and cardioarrhythmia [4–6]. Usually, amino alcohols are present at low concentrations in aqueous matrices. Therefore, sample preparation must be carried out on these samples before the drugs can be determined by CE.

Liquid–liquid extraction (LLE) and solid-phase extraction (SPE) are the most commonly used tech-

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niques for preconcentration and cleanup of drugs prior to CE analysis [7]. However, these methods are time-consuming, tedious and particularly for LLE, require large volumes of samples and solvents. SPE is also relatively expensive. Solid-phase microextraction (SPME) is a more recent procedure and has been developed for pretreatment of amino alcohols [8–12]. It is fast and solvent-free. However, SPME also suffers from some problems such as sample carry-over, relatively high cost and fiber fragility.

Recently, sample workup of drugs has been reported with liquid-phase microextraction (LPME) for CE utilizing polypropylene hollow fibers [13–15]. This technique is simple and inexpensive with the added benefit of the fiber being disposable after use. The deionised analytes (the analytes were contained in a basic donor solution) were extracted from the sample solution into the organic solvent impregnated in the pores of the hollow fiber, and further into the inside of the hollow fiber holding a small volume of an aqueous acidic solution providing high solubility for the analytes (acceptor phase). Due to the acidic nature of the acceptor phase, the analytes were ionized, and were prevented from re-entering the organic solvent. Hence, the three-phase LPME system provided simultaneous extraction and back-extraction. It also served as a method for sample clean-up and provided very clean extracts. Only several kinds of drugs have been investigated by this method hitherto. In the present work, we simplified this method by only making use of one syringe so that the device is easier to be controlled than previously [13–15]. Additionally, the LPME device was investigated for preconcentration of amino alcohols for the first time, in combination with CE. On-column stacking in CE was combined with LPME to further improve detection sensitivity. Our method has three advantages: (1) it can be used for a large number of different samples providing a high sample capacity due to the low cost of each extraction unit (the hollow fiber); (2) sample carry-over is avoided due to the extraction unit being disposable and the use of fresh sample and fiber for each extraction; (3) high partition coefficients of the analytes and high volume ratio between the sample and the acceptor solution (sample, 3.5 ml; acceptor phase, 5 μl) make possible high enrichment factors in the extraction. The compounds were first extracted from spiked pure water samples to gain basic understanding of the process and for optimization of the extraction conditions. Different aspects of the extraction procedure such as the kinds of organic solvent suitable for the immobilization, composition of the acceptor and donor phase and the extraction time was investigated. Also, the injection time of on-line stacking in CE was optimized. In addition, this method was evaluated by application to human urine sample analysis by CE.

2. Materials and methods

2.1. Apparatus

Separation was carried out on a Prince (Prince Technologies, Emmen, The Netherlands) CE system equipped with a UV detector, with detection at 195 nm. A 60-cm (effective length 47 cm)×50-μm I.D. bare fused-silica capillary tube was used for CE. The support buffer was 30 m*M* Tris–H_3PO_4 adjusted to pH 2.5 with concentrated phosphoric acid. The voltage during separations was 20 kV. Samples were injected by pressure (100 mbar).

2.2. Materials

Hydrochloric acid (HCl) (37%) and sodium hydroxide (NaOH) of analytical grades were obtained from Merck (Darmstadt, Germany). Phosphoric acid was purchased from Carlo Erba (Milan, Italy). Tris(hydroxymethyl)aminomethane (Tris) was purchased from J.T. Baker (Phillipsburg, NJ, USA). 1-Octanol (>99.5%), isooctane, *n*-hexane and di-*n*-hexyl ether were obtained from Merck. Ultrapure water was produced on a Nanopure system (Barnstead, Dubuque, IA, USA). Atenolol was purchased from Sigma (St. Louis, MO, USA). Norephedrine, pindolol and 2-amino-1-phenylethanol were purchased from Aldrich (Milwaukee, WI, USA). Amino alcohols were dissolved in methanol to make stock solutions at concentrations of 1 mg/ml. Mixtures containing each amino alcohol at different concentrations in 1 *M* NaOH were prepared from the stock solutions and used as working solutions. All solutions were stored at 4 °C.

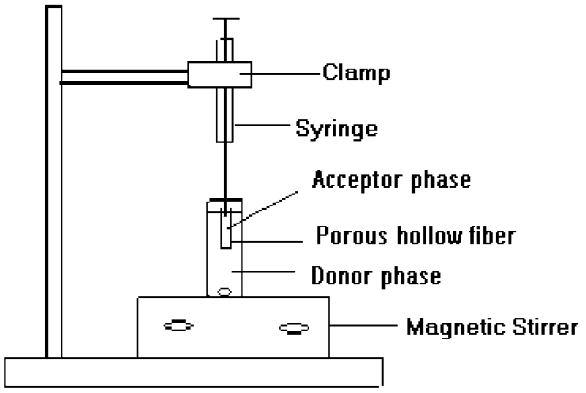

Fig. 1. Schematic of the LPME extraction system (not to scale).

2.3. Extraction of water samples

The LPME extraction device for LPME is illustrated in Fig. 1. The sample solution was filled into a 4-ml vial. One conventional 10-μl HPLC syringe (Hamilton, Australia) of 0.8 mm O.D. was used to introduce the acceptor solution into the hollow fiber prior to extraction, support the hollow fiber and also utilized for collection and injection of the acceptor solution after extraction. Because the extraction units should be compatible with both aqueous solutions and organic solvents, polypropylene hollow fiber (Membrana, Wuppertal, Germany) was selected. The inner diameter of the hollow fiber was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.64 μm. A sample solution (prepared in 0.1 or 1 *M* NaOH solution) of volume 3.5 ml was placed in a 4-ml sample vial. Five μl of acceptor solution (either 0.1 *M* HCl or 0.05 *M* HCl, etc., as discussed below) was injected into a 2-cm length of hollow fiber (the other end of the hollow fiber was flame-sealed) with the 10-μl syringe. The hollow fiber, affixed on the needle, was subsequently dipped for 5 s in the organic solvent (typically 1-octanol) used for impregnation; the latter procedure served to fill the pores of the hollow fiber with the organic solvent. The hollow fiber was then placed in the sample solution. During the extraction, the sample solution was agitated at a stirring rate of 1000 rpm. After extraction, the acceptor solution was withdrawn back into the syringe and injected into a 200-μl vial. The solution (5 μl) was reconstituted with 35 μl aqueous solution containing 30 m*M* Tris–H_3PO_4, prior to CE analysis. Fig. 2 shows a typical LPME-CE chromatogram of four amino alcohols.

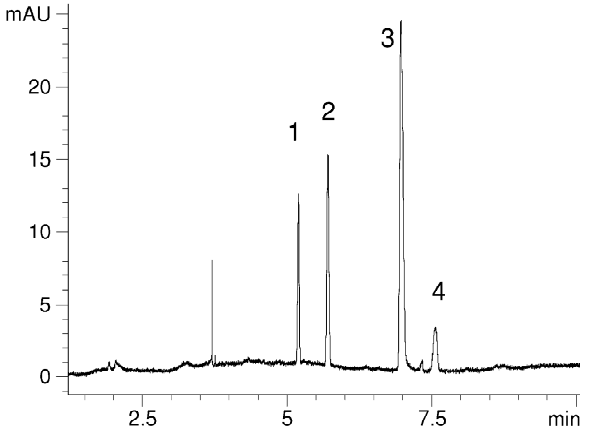

Fig. 2. Electropherogram of a spiked water sample (2 μg/ml) obtained by LPME–CE. Capillary: 60-cm (effective length, 47 cm)×50-μm I.D.; buffer, 30 m*M* Tris–H_3PO_4 (pH 2.5); detection, UV 195 nm; voltage, 20 kV; injection, 100 mbar·s; injection time, 0.1 min. Peaks: (1) 2-amino-1-phenylethanol; (2) norephedrine; (3) pindolol; (4) atenolol.

3. Results and discussion

3.1. Determination of amino alcohols by capillary zone electrophoresis

To evaluate the effectiveness of the preconcentration approaches under investigation, the potential of capillary zone electrophoresis (CZE) alone was first examined. The amino alcohols are weak bases. Similar compounds have been reported to be separated as positive ions at acidic pH values using CZE, or separated as almost neutral molecules at basic pH values using micellar electrokinetic chromatography (MEKC) [16,17]. In this work, amino alcohols were separated under the former mode. The amino alcohols were well separated within 10 min. However, detection limits were unsatisfactorily high in the range of 5–10 μg/ml, implying that preconcentration was needed in order to improve the concentration sensitivity.

3.2. Determination of amino alcohols by CZE with field-amplified concentration

In this work, we chose normal polarity field-amplified concentration (FAC) for sample stacking. Burgi and Chien [18] have suggested that the optional condition for sample stacking is to prepare the sample in a buffer concentration that is about 10 times less than that used for the electrophoretic separation and a sample plug length up to 10 times the diffusion-limited peak width. We followed a similar procedure and prepared samples in the separation buffer diluted 10-fold. The pH of the diluted sample buffer (3 m*M* Tris–H_3PO_4) was ca. 2.5, at which the basic amino alcohols were partially protonated, forming cations which were to be stacked at the front of the sample matrix band by FAC using the normal injection mode. Pressure injection was used, and the pressure was maintained at 100 mbar in all runs with injection times varied in steps of 10 s. The peak area of pindolol (most intense) was used to show the influence of injection time to the detection signal (Fig. 3). It is seen the detection signal increased as the injection time increased. However, a further increase in injection time after 1 min brought only a slight enhancement in detection, while leading to serious interference of early-eluting amino alcohols by solvent. Based on this, 1 min was selected as the optimum injection time.

3.3. Determination of amino alcohols by CZE with LPME–FAC-CE

From the above experiments, it was found that FAC could enrich amino alcohols rapidly and easily, but the preconcentration factors from FAC were insufficient. For any meaningful real analysis, however, interferences could also be concentrated. LPME by itself was useful for sample cleanup, was very easy to control and gave relatively high preconcentration factors. However, detection sensitivity was reduced due to the dilution of the extract in order to obtain the minimum volume (40 μl) for CE detection. Thus, the combination of FAC with LPME was considered to achieve both sample cleanup and satisfactory to pre-concentration. We diluted the acceptor solution (5 μl) in LPME to 40 μl, with running buffer (3 m*M* Tris–H_3PO_4) prior to further concentration by FAC.

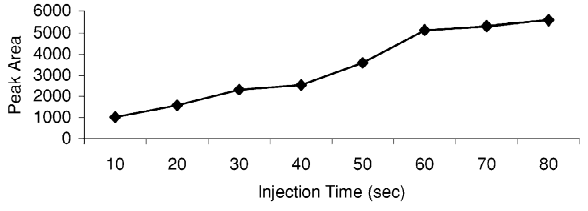

Fig. 3. Plot of the peak area of pindolol versus injection time. Conditions as in Fig. 2.

3.3.1. Selection of organic solvent for impregnation of the hollow fiber

It was one of the critical steps in LPME to select an organic solvent for pretreatment (immobilization) of the porous polypropylene hollow fiber. Based on earlier LPME experience [13,15], 1-octanol and di-*n*-hexyl ether were evaluated as immobilization solvents. Both easily immobilized on the polypropylene hollow fiber; furthermore, they were immiscible with water and their volatility were low. *n*-Hexane and isooctane were also investigated as immobilization solvents, but no enrichment of the analytes was observed. This may be related to the relative incompatibility of polarity between these solvents and the amino alcohols. With 0.1 *M* HCl as the acceptor phase, 1 *M* NaOH in the 3.5 ml donor phase and 40 min extraction of all the amino alcohols, 1-octanol was found to provide higher preconcentration of the four amino alcohols than di-*n*-hexyl ether (Table 1). This is probably due to the relatively

Table 1
Efficiencies of 1-octanol and di-*n*-hexyl ether as impregnation solvent[a]

Compounds	Enrichment (-fold)	
	1-Octanol	Di-*n*-hexyl ether
2-Amino-1-phenylethanol	50±4.7%	36±4.2%
Norephedrine	50±8.9%	33±7.3%
Pindolol	79±8.6%	55±6.9%
Atenolol	34±7.5%	19±7.8%

$n=3$.

[a] Water samples at a concentration of 1 μg/ml of each compound. Data were obtained from mean values of three determinations.

Table 2
Enrichment of pindolol utilizing different donor and acceptor solutions

Donor, NaOH (*M*)	Acceptor, HCl (*M*)	Enrichment factor (-fold)
1	0.1	70±8.3%
1	0.05	40±6.9%
1	0.5	–[a]
0.1	0.1	64±8.8%
0.1	0.05	28±8.5%
0.01	0.1	62±7.2%
0.001	0.1	55±5.6%

[a] Problems related to high ionic strength of sample during capillary electrophoresis (peak of pindolol could not be identified).

higher polarity of 1-octanol and its greater affinity for the amino alcohols. On the basis of the above experiments, 1-octanol was selected as immobilization solvent for the rest of this study.

3.3.2. Composition of the acceptor phase and donor phase

The composition (pH) of both the donor and acceptor solutions was another important parameter in LPME. Basically, the acceptor phase should be strongly acidic in order to promote dissolution of the alkaline analytes while the donor phase should be strongly alkaline in order to deionize the analytes and consequently reduce their solubility within the sample. In this way, a high partition coefficient results leading to high preconcentration by LPME. In this study, we chose HCl at different concentrations as acceptor solution and NaOH at different concentrations as donor solution. Table 2 gives the results of the experiments of optimizing the composition (based on pH) of both the donor and acceptor solutions. All the experiments were conducted over 40 min with 1-octanol as the solvent for impregnation of the hollow fiber. From Table 2, we see that the preconcentration factors were not significantly affected by the NaOH concentrations (donor phase) while the preconcentration factors were sensitive to the HCl concentrations (acceptor phase). On the basis of the above experiments, 0.1 *M* HCl and 1 *M* NaOH were selected as the acceptor solution and donor solution, respectively, for the rest of the work.

3.3.3. Extraction time

In this study, the preconcentration was studied as a function of extraction time (Fig. 4). All the experiments were performed with the hollow fiber impregnated with 1-octanol, 1 *M* NaOH in the donor solution and 0.1 *M* HCl as the acceptor solution. For all drugs, the preconcentration factors increased with extraction time up to 50 min. Since there was no significant change (increase or decrease) in preconcentration after 50 min of extraction, this time was selected. Although the extraction time was relatively long, simultaneous extracting a large number of different samples could result in a very high sample capacity to compensate for this disadvantage.

3.4. Quantitative analysis

Quantitative data (LPME and FAC–CE) are

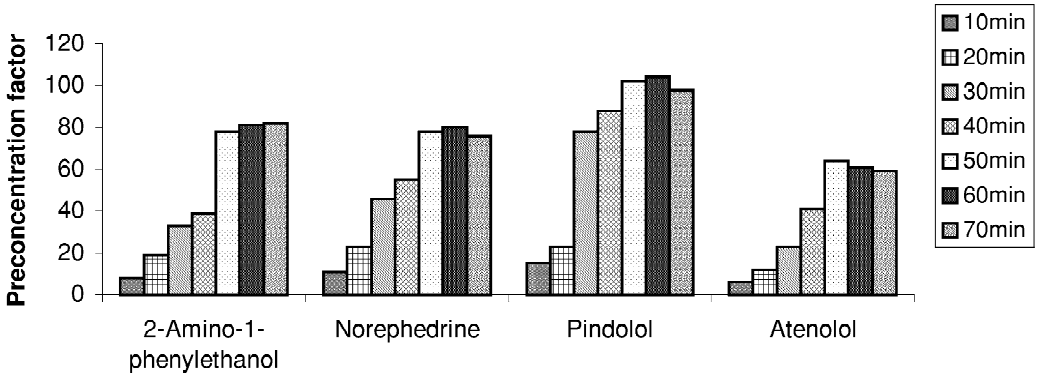

Fig. 4. Plot of preconcentration factors for amino alcohols versus extraction time. Injection time, 1 min; other conditions as in Fig. 2.

Table 3
Quantitative results of LPME–FAC-CE from water samples

Compounds	Enrichment (-fold)	LOD (μg/ml)	RSD (%)[a]	
			No correction with I.S.	Correction with I.S.
2-Amino-1-phenylethanol	84	0.3	5.1	I.S.
Norephedrine	89	0.3	10.8	6.9
Pindolol	110	0.08	9.7	5.9
Atenolol	72	0.5	8.6	4.3

[a] $n=6$.

shown in Table 3. The extraction and determination of amino alcohols was performed with the optimal LPME conditions and FAC. Under these optimum conditions, the LPME acceptor phase was directly compatible to CE. The four amino alcohols could be preconcentrated up to 110-fold, as shown in Table 3. Linearity was observed over the range of 0.5–10 μg/ml for the analytes except for atenolol (1.0–10 μg/ml). Coefficients of correlation (r^2) were all above 0.9936. Six replicate experiments of amino alcohols (1 μg/ml) were investigated under the optimized conditions to give 5.1–10.8% RSD without use of the internal standard, while the results were improved to 4.3–6.9% RSD when correlations were applied based on 2-amino-1-phenylethanol (1 μg/ml) as internal standard. The repeatability was acceptable and comparable with other microextraction techniques reported in the literature [19]. The limits of detection ($S/N=3$) ranged from 0.08 to 0.5 μg/ml.

3.5. Human urine sample analysis

Amino alcohols were preconcentrated from human urine in order to investigate the influence of biological fluid on our method. Urine sample (4 ml) was spiked with 1.5 μg/ml amino alcohols except for 2.5 μg/ml atenolol, and subsequently 200 μl of 2 *M* NaOH was added to the sample to provide an approximately 0.1 *M* concentration in the donor solution. The blank urine sample and spiked urine sample were investigated under the same conditions. LPME was performed for 50 min by utilizing a 0.1 *M* solution of HCl as acceptor solution. Initially, injection time was fixed at 1 min for CE analysis. In this case, the peaks of amino alcohols turned very broad. Obviously, the matrix of the urine sample quenched the injection time adjustment. So, injection time was fixed at 40 s for analysis of extract from urine samples. Repetitive extractions from human urine varied within 6.3–12.4% RSD without use of the internal standard, while the results were improved to 5.7–7.4% RSD when correlations were applied based on 2-amino-1-phenylethanol (1.5 μg/ml) as internal standard (Table 4). As illustrated in Fig. 5a, amino alcohols were effectively preconcentrated from the biological samples. The detection limits ($S/N=3$) were around 1 μg/ml for most amino alcohols except for atenolol (2 μg/ml). In addition to enrichment, significance sample cleanup was observed with the LPME procedure. For drug-free urine sample (Fig. 5b), only one peak emerged in the electropherogram, which enabled very rapid CE runs in 10 min.

4. Conclusion

The present work has demonstrated the potential

Table 4
Within-day repeatability for LPME–FAC-CE of amino alcohols from urine samples

Compounds	RSD (%)[a]	
	No correction with I.S.	Correction with I.S.
2-Amino-1-phenylethanol	6.3	I.S.
Norephedrine	12.4	7.4
Pindolol	9.9	6.2
Atenolol	9.6	5.7

[a] $n=6$.

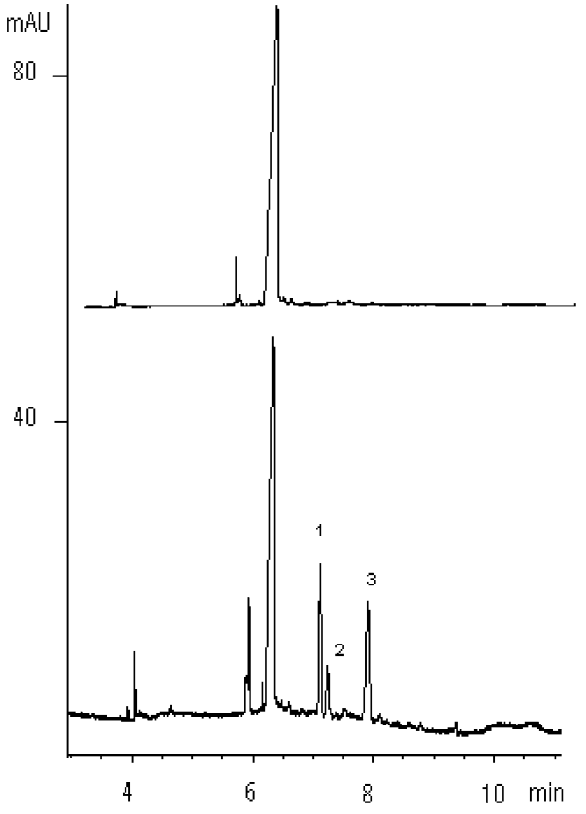

Fig. 5. LPME–FAC-CE of urine sample. (a) Urine sample spiked with amino alcohols at 1.5 μg/ml; (b)drug-free urine; injection time, 40 s. Other conditions as in Fig. 2. Peaks: (1) 2-amino-1-phenylethanol; (2) norephedrine; (3) pindolol.

of LPME using for of amino alcohols followed by CE analysis with field-amplified concentration. Based on disposable extraction devices, the extraction is simple, inexpensive and easy to use. The amounts of materials used (acceptor phase, 5 μl; donor phase, 40 μl, the minimum needed for one CE system) were relatively low. By combining LPME and FAC–CE, up to 110-fold enrichment and effective sample clean-up were achieved. The LPME combined with the on-line stacking method has been demonstrated to be precise, reproducible and linear over a wide range. We have shown this technique to be effective for the analysis of amino alcohols from aqueous samples.

Acknowledgement

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