Talanta 180 (2018) 90–97

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# Rapid and sensitive tapered-capillary microextraction combined to on-line sample stacking-capillary electrophoresis for extraction and quantification of two beta-blockers in human urine

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ARTICLE INFO

*Keywords:*
Microextraction
Packed sorbent
Field amplified sample injection
Capillary electrophoresis
β-blockers

ABSTRACT

A tapered-capillary microextraction (*t*Cap-μEx) combining with field-amplified stacking (FASI) method for CE analysis was developed. The *t*Cap-*μ*Ex method is based on the construction of a micro solid phase extraction (SPE) column by narrowing the end of a silica capillary from 530 μm (inner diameter) to 20 μm, enabling the packing of 45 μm sorbent particles without a frit. Various parameters that may affect the microextraction and FASI-CE analysis have been investigated and optimized. This study shows that microextraction exhibits advantages of small sample and sorbent volumes (less than 200 μL sample and 2 μL sorbent) and fast extraction time of 6 min. The method was successfully applied for efficient determination of atenolol and metoprolol in human urine samples, with recovery of 93.7–105.5% and RSD (n = 3) lower than 8.5%. Twenty-one-fold and nineteen-fold average enhancement of detection sensitivity was achieved for atenolol and metoprolol, respectively, versus the CE method without *t*Cap-*μ*Ex and FASI. The method is environmentally friendly and allows reuse of the sorbent at least 8 times without an obvious loss in performance. The results indicate that the proposed method could be potentially applied in a wide range of doping control, clinical, forensic toxicology, food analysis and environmental analyses.

## 1. Introduction

Because of its unique features such as high separation efficiency, low sample and reagent consumption and relatively short analysis time, capillary electrophoresis (CE) is characterized as an alternative and complementary technique to HPLC for routine analysis in a variety of research areas [1,2]. On the other hand, CE often suffers from poor concentration sensitivity and low tolerance for matrix components. This hampers the application of CE for analysis of complicated biological samples. To overcome this shortcoming, it is essential to develop efficient sample processing techniques to couple with CE, which is expected to improve the concentration detection limit greatly and has received continuous and intense research interest during the past decades [3–6].

Most of the efforts to integrate sample processing with CE can be classified as either electrophoretic [4] or chromatographic based [5]. In electrophoretic based methods, also known as in-capillary sample preconcentration methods, which utilize a stacking technique, preconcentration is performed in the separation capillary by injecting a large volume or amount of analyte, which is then focused into a minimum volume inside the capillary, thus avoiding the band broadening of the analyte [7]. For chromatographic based method, preconcentration occurs in a short chromatographic column, usually a solid phase extraction (SPE) column. Sample eluted from the SPE column can be transferred to a separation capillary by off-line, at-line, in-line, or on-line methods [8]. Various extraction methods have been developed for CE analysis, for example, fiber solid-phase microextraction (in-fiber SPME) [9,10], stir-bar sorptive extraction (SBSE) [11,12], capillary microextraction [13,14] etc. Among them, capillary microextraction, also known as in-tube solid-phase microextraction (in-tube SPME), has gained particular interest because it meets the requirement of recent trends in sample preparation including miniaturization, automation, high-throughput performance, and reduction in solvent consumption and operation time. In addition, capillary microextraction is cost effective and has advantages for analysis of trace- and ultratrace-amount analytes in complex matrices due to the less sample preparation steps [15].

Recent advances have shown that it is very promising by combining

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https://doi.org/10.1016/j.talanta.2017.12.035
Received 10 August 2017; Received in revised form 6 December 2017; Accepted 12 December 2017
Available online 15 December 2017

the electrophoretic- and chromatographic-based sample-processing methods for CE analysis of complicated biological samples. For example, in-fiber SPME was combined with CE sample enrichment technique (acetonitrile stacking method) [16] or in-column field-amplified sample injection [17], for efficient CE analysis of acidic drugs in water samples or capecitabine and its active metabolite (5-Fluorouracil (5-FU)) in human plasma samples; The dispersive liquid-liquid microextraction (DLLME) combined with field-amplified sample stacking-CE was successfully applied for the sensitive determination of steroid hormones from urine samples with the LOD as low as 15 ng/mL [18]; A preparation method of linear polyacrylamide coating and strong cationic exchange hybrid monolith in a single capillary was reported recently, which was applied for bottom-up proteomics by coupling with dynamic pH junction stacking-CE-MS [19]. All these elaborate studies clearly indicate the significant improvement in the concentration detection limit of CE, with combination of the two different types of sample processing methods.

Here, we propose a novel tapered-capillary microextraction (*t*Cap-μEx) combining with field-amplified stacking (FASI) method for CE analysis. Among various electrophoretic preconcentration methods, FASI is one of the simplest and most commonly used, which is based on the change of the analyte migration velocity at the boundary between the sample and running buffer zones via changing the field strength [20,21]. Nevertheless, extraction and clean-up from biological samples prior to CE analysis is an important issue for FASI since this strategy can enrich only low-ionic strength samples. In our approach, we use a fused silica capillary column with one end tapered as the microextraction column for packing PCX sorbent materials. This microextraction approach miniaturizes the conventional packed bed of SPE devices from milli-liter bed volumes to micro-liter volumes. Moreover, the tapered tip of the capillary serves as a frit to retain the packing materials, which allows for easy operation and clean-up of the microextraction column. After *t*Cap-μEx, the samples are injected for FASI-CE. To the best of our knowledge, this is the first time combining in-tube microextraction method with FASI-CE technique. Two β-blockers, atenolol and metoprolol, were employed as the test samples for evaluation of the proposed method. It is well known that trace level quantification of these drugs in biological fluids is an essential concern in therapeutic drug monitoring, doping control and clinical toxicology [22–25]. Our results show that the proposed method is an easy and efficient approach for CE analysis of trace-level β-blockers in real human urine samples with high sensitivity. It is also indicated that our method could be potentially used for the routine analysis of other target compounds in complicated biological samples.

## 2. Experimental

### 2.1. Reagents and materials

Atenolol (99.0% purity), metoprolol tartrate (99%) and formic acid were purchased from Aladdin (Shanghai, China). HPLC grade methanol, ammonium hydroxide, hydrochloric acid, sodium dihydrogen phosphate and disodium hydrogen phosphate were obtained from Beijing Chemical Works (Beijing, China). Deionized water (Milli-Q Water System, Millipore, Bedford, MA, USA) was used for the preparation of the samples and buffer solutions. The PCX stationary phase (45 μm, 100 Å, strong cation exchanger with mixed mode characteristics, hydrophilic styrene Divinylbenzene particles) was supplied by Agilent (Bond Elut Plexa cation exchange).

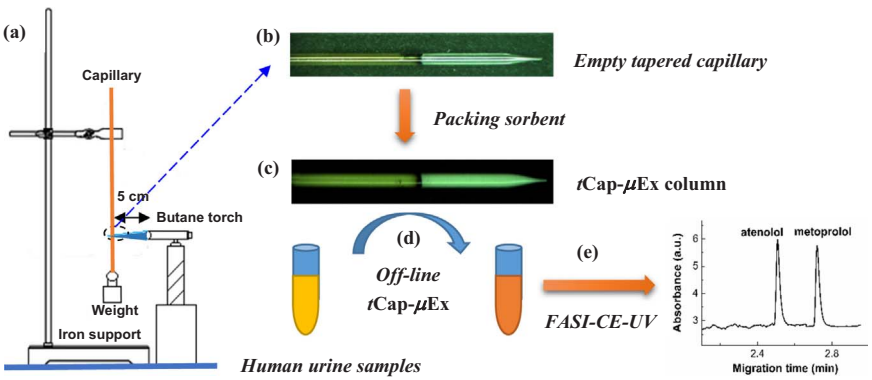
Human urine samples were collected from five healthy human volunteers. The collected samples were immediately frozen at −20 °C. In each case, before the extraction procedure, the urine samples were thawed, equilibrated to room temperature. The urine sample solutions were then spiked with the desired concentration of analytes and prepared by adding the appropriate amount of the stock standard β-blockers (atenolol and metoprolol) to blank urine. After centrifuging at 4000 rpm for 10 min, the supernatant was collected. Prior to processing by microextraction, the urine sample was diluted 1:3 v/v with 2% formic acid (pH 3.0). All solutions were filtered with 0.2 μm syringe hydrophilic polyethersulfone filters.

### 2.2. tCap-μEx-FASI-CE analysis process

As shown in Scheme 1, the analysis process of tCap-μEx approach coupled with FASI-CE for quantification of two β-blockers, atenolol and metoprolol, in human urine involves four steps: 1) fabrication of the tapered end of the capillary (a and b); 2) tCap-μEx column preparation by packing sorbent (c); 3) off-line tCap-μEx preconcentration and clean-up of urine samples using a manually-operated semiautomatic syringe (d); 4) quantification analysis of the extracted samples with CE-UV combining with FASI preconcentration (e). The analysis procedure will be described in detail in the following sections.

#### 2.2.1. tCap-μEx column preparation

A ~5-cm long fused-silica capillary with 530 μm i.d. and 800 μm o.d. (Hebei Yongnian Factory, Hebei, China) was used for the preparation of the *t*Cap-μEx column. Fabrication of the tapered end of the capillary was schematically shown in Scheme 1 (a). A similar approach has proposed in our previous work, in which it was used to improve the separation efficiency in short-capillary electrophoresis [26]. Briefly, the capillary was drawn manually using a vertically suspended section of capillary to which a balancing weight of selected mass was attached. Upon heating with a butane torch, the capillary was stretched and finally broken with a conical tapered tip. The tapered capillary end was then cut using a ceramic cutter under a microscope. The diameter of tip end was kept at about 20 μm for all the tapered capillaries used in this study. The tapered capillary column was then ready to serve as a packing reservoir. A slurry of 2 mg 45 μm PCX sorbent in 10 mL methanol was sonicated for 10 min to prevent aggregation of particles and was subsequently transferred into the reservoir by a syringe pumping




**Scheme 1.** Analysis process of *t*Cap-μEx approach coupled with FASI-CE for quantification of two β-blockers in human urine samples.