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On-line double focusing of atenolol and metoprolol in human urine using capillary electrophoresis with the aid of β-cyclodextrin

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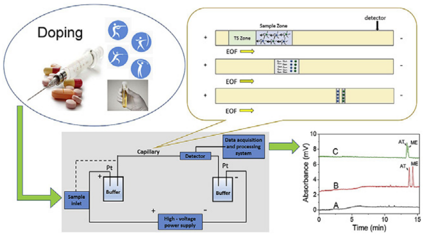
HIGHLIGHTS

- The sensitivity of AT and ME could be improved 197 and 205 times by on-line double focusing technique, respectively.
- The β-CD affords 5.5- and 3.5-fold improvements for AT and ME in sensitivity than that of without of β-CD in sample matrix.

GRAPHICAL ABSTRACT



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ABSTRACT

A maneuverable and sensitive on-line double focusing technique combined field amplified sample stacking (FASS) with micelle to solvent stacking (MSS) with the aid of β-cyelodextrin (β-CD) is developed to detect the contents of AT and ME in human urine by capillary electrophoresis (CE) with UV detector. Small amount of β-CD not only increase the critical micelle concentration (CMC) of SDS, but also strengthen the interaction between SDS and the aimed compound by forming inclusion complexes. The result indicates that the addition of β-CD affords 5.5- and 3.5-fold improvements for atenolol (AT) and metoprolol (ME) in sensitivity than that of in the absence of β-CD in the double focusing system, respectively. Under the optimal conditions, about 200-fold improvement in sensitivity for analytes is achieved compared with conventional CE method. The limits of detection (LODs) at a signal-to-noise of 3 (S/N = 3) of the two β-blockers can be reached 3.3 and 3.7 ng mL^{-1} respectively, which are lower than minimum required performance levels (MRPLs) set by the World Anti Doping Agency. The relative standard deviations (RSDs) of peak areas of intra-day and inter-day are 3.51–3.38% and 2.34–4.28% ($n = 6$), respectively. AT and ME in urine without special pretreatment and additional instrument are analyzed. The recoveries are 82–98% with RSDs less than 2.0%.

1. Introduction

Atenolol (AT) and metoprolol (ME) belong to selective adrenergic receptor antagonists (β-blockers), which can reduce the athlete's anxiety level by controlling muscle tremors and slowing heart rate to improve the stabilization and coordination of actions. AT and ME have been listed as banned drugs by the International Olympic Committee [1], and the World Anti-Doping Agence specifies MRPLs to monitor the content of β-blockers (inferior to 100 ng mL^{-1} in different biological liquid samples [1]). Taking banned drugs in sports competition not only violates the fairness of

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List of abbreviations

FASS	field amplified sample stacking
MSS	micelle to solvent stacking
β-CD	β-cyelodextrin
CE	capillary electrophoresis
AT	atenolol
ME	metoprolol
LODs	limits of detection
S/N	signal-to-noise
MRPLs	minimum required performance levels
RSDs	relative standard deviations
LC-MS	liquid chromatographic - mass spectrometry
LC-GC	liquid chromatographic-gas chromatography
GC-MS	gas chromatography-mass spectrometry
ECL	electrochemiluminescence
AAS	atomic absorption spectrophotometry
DAD	diode array detector
BGE	background electrolyte
TS	trapping solution
EOF	electroosmotic flow
CMC	critical micelle concentration
SEFs	sensitivity enhancement factors

competition, but also damages the athlete's health. Therefore, approaches with high-sensitivity and acceptable resolution are essential for simultaneous analysis of AT and ME with low concentration in biological sample.

As expected, numerous methods based on high performance liquid chromatography [2,3], liquid chromatographic - mass spectrometry (LC - MS) [4], liquid chromatographic - gas chromatography (LC - GC) [5], gas chromatography - mass spectrometry (GC - MS) [6–8], electrochemiluminescence detection (ECL) [9,10], flame atomic absorption spectrophotometry (AAS) [11], diode array detector (DAD) [12], fluorescence [13] and assisted capillary electrophoresis [14,15] were developed for the analysis of β-blockers in biological sample. Although the sensitivity among these methods were up to the requirement, most of them need expensive equipment or costly analytical columns, beyond that, time-consuming and labor-intensive sample pretreatment were emerged. Given all that, a simple, sensitive detection method would be very popular.

Recent studies indicated that on-line concentration techniques could improve the sensitivity of CE with UV detector. Therefore, the CE method coupled with on-line focusing received much attention due to low cost, easy handle, minimal sample and solvent consumption [16–19]. In this study, an on-line stacking method combined FASS with MSS was explored to analysis ME and AT in urine, and the mechanism of focusing was elaborated. Based on the interactional function between β-CD and SDS [20,21], we furtherly improved the sensitivity of the on-line double focusing technique by adding β-CD into sample matrix. The high sensitivity enhancement factors(SEFs) and short analysis time were obtained without expensive instrument and attachment. The analysis of urine sample could be accomplished in 15 min, the sensitivity of analytes was improved about 200-fold. The LODs of CE with UV detector were lower than MRPLs set by the World Anti Doping Agency.

2. Materials and methods

2.1. Chemicals and materials

AT and ME were purchased from National Institute for Food and Drug Control (Beijing, China). Dodecyl sodium sulfate(SDS)and Sodium hydroxide(NaOH)were obtained from Guanghua Sci-Tech Co., LTD. (Guangdong, China). Sodium phosphate monobasic dihydrate ($NaH_2PO_4.2\ H_2O$) was from Tianjin Fuchen Chemical reagents factory (Tianjin China). β-CD was from Beijing arbor star biological technology Co., Ltd. (Beijing, China). Methanol(CH_3OH)and sulfuric acid (H_2SO_4) and chloroform were obtained from Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China). The above mentioned reagents were at least analytical reagent grade and were used without further purification. All solutions in experiment were prepared by distilled water and should be filtered by the 0.45 mL syringe filters before using.

2.2. Apparatus

All experiments were carried out by a high performance capillary electrophoresis apparatus which equipped with a UV detector (CL1030, Beijing Cailu Instrumental Co., Beijing, China) [18] (for details, see Xu, Liu, Jia, & Shu, 2015). The dimension of the capillary with uncoated fused-silica were 50 cm × 75 μm I.D. × 375 μm O.D. The active length of the capillary column, which was used for preconcentration and separation, was 41.5 cm from the injection end to the detector. Gravity injection was adopted by rising 15.5 cm sample tube. The wavelength of the UV detector was chosen as 200 nm [22]. The pH values were adjusted by a PH-3C acidity meter (Shanghai Hongyi Instrument Co., Ltd., Shanghai, China).

2.3. Solution preparation

The standard stock solutions of AT and ME (1 mg mL^{-1}) were prepared by 50% (v/v) aqueous methanol and dissolved by the ultrasonic bath. The background electrolyte (BGE) consisted of 40 mM NaH_2PO_4 was freshly prepared and adjusted to the desired pH ($pH = 4.5$) using 1 M NaOH. For on-line stacking, the sample solutions were manufactured by proper diluting from the stock solution with distilled water and sample matrix which including of 10 mM SDS and 5 mM β-CD. The trapping solution (TS) was composed of 70 mM H_2SO_4 and 55% (v/v) CH_3OH which also was freshly prepared every day. All solutions should be filtered by 0.45 μm syringe filters before introducing into capillary.

2.4. Preparation of sample and standard solutions

The urine sample was collected from the healthy volunteer. The pH value of urine was adjusted to 12.5 with the aid of 1 M NaOH. The spiked solutions were achieved by respectively adding different concentration standard solutions into 10 mL-centrifugal tubes containing 200 μL urine and 4 ml chloroform. The mixture in the tubes were vortexed for 30 s at 50 Hz and centrifuged for 3 min at 10000 rpm. After phase separation occurred in the tube, we reserved the lower organic layer and evaporated it by a rotary vacuum evaporator at room-temperature, and then the residue on the bottle wall was dissolved in 1.0 mL sample matrix including of 10 mM SDS and 5 mM β-CD. These sample solutions should be filtrated by 0.45 μm syringe filter before giving an injection into the separation column of CE.