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Solid-phase microextraction coupled with capillary electrophoresis for the determination of propranolol enantiomers in urine using a sol–gel derived calix[4]arene fiber

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Abstract

A new type of diglycidyloxy-calix[4]arene coated fiber made by sol–gel method was initially prepared for capillary electrophoresis (CE) sample pretreatment. By using headspace solid-phase microextraction (SPME) combined with a novel back-extraction facility coupled off-line to capillary zone electrophoresis (CZE), the simultaneous determination of propranolol enantiomers in human urine was achieved. The clean up effect and preconcentration effect were realized for the first time without derivatization during the SPME process in terms of these strong polarity and thermal stable compounds. Ultrasonic back-extraction and field amplified sample injection (FASI) technologies were employed. Extraction and back-extraction parameters were optimized. Preconcentration of the sample by calix[4]arene fiber based SPME and FASI increased the sensitivity, yielding a limit of detection (LOD) of 0.01 μg/ml by CZE–diode array detection (DAD). Method repeatability (RSD < 6.5%) and fiber reusability (>150 extraction procedures) were observed over a linear range (0.05–10 μg/ml) in urine samples. Based on the superior thermal stability, high alkali- and solvent-resistant ability, marvelous repeatability and long lifetime of the novel fiber, this SPME–FASI–CZE procedure could meet the demand of minimum required performance limit (MRPL) set by the World Anti-doping Agency (WADA) for the detection of propranolol in urine samples.

Keywords: Calix[4]arene; Sol–gel; Solid-phase microextraction; Capillary electrophoresis; Propranolol enantiomers; Doping analysis

1. Introduction

Capillary electrophoresis (CE) has experienced an explosive growth in the last years because of its high separation efficiency, small sample volume requirement, low reagent consumption and high speed of analysis. It has been widely applied for the chiral separation of a large number of compounds in body fluids [1–3]. However, the analysis of body fluids such as urine, plasma and serum presents a variety of problems: a large number of individual compounds in the mixture, the presence of proteins and low concentration of metabolites. Thus, the majority of bioanalytical methods do not use just one simple electrophoretic separation step, but rather include one or more sample pretreatment steps. The conventional sample preparation techniques such as precipitation, liquid–liquid extraction (LLE) or solid-phase extraction (SPE) feature widely in the bioanalytical CE literatures [4]. Although most sample preparation can be accomplished by these conventional procedures, these methods suffer from the disadvantages of using toxic organic solvent, being relatively expensive and in some procedures of being time consuming. Therefore, many efforts have been made to develop sample preparation methods that can overcome these disadvantages. Solid-phase microextraction (SPME), developed by Pawliszyn and co-worker [5], is an ideal alternative technique that has the advantage of simplicity and integrates sampling, extraction, concentration and sample introduction into a single solvent-free (or minute amounts of solvent during the back-extraction procedure when necessary) step [6]. In recent years, SPME combined with GC or HPLC have been widely used for the determination of volatile organic compounds, semi-volatile chemicals in different fields, including the environment, food, natural products, pharmaceuticals, biology, toxicology, forensics, etc. [7].

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Owning to the advantages of SPME, off-line or on-line SPME coupled to CE have been constructed in recent years. An off-line SPME–CE method for the determination of barbiturates was presented initially by Li and Weber [8]. Other cases such as the determination of multiple pesticides in foods, phenol compounds in river were also reported by off-line SPME–CE method [9–11]. On-column interfaces for coupling SPME to CE have been constructed and evaluated for polycyclic aromatic hydrocarbons (PAHS) and antidepressant drugs [12–14]. The fibers employed in the above mentioned literatures for SPME were either poly(vinyl chloride) (PVC), poly(dimethylsiloxane) (PDMS), or poly(acrylate) (PA) coated, and the target analytes were limited to volatile or semi-volatile substances.

Calixarenes are regarded as the third generation of supermolecules next to crown ethers and cyclodextrins, their applications as HPLC stationary phase [15] and GC open-tubular capillary coating [16] have interpreted special selectivity, and relative work about calix[4]arene fiber for SPME–GC have been done previously in our laboratory [17]. Owing to the inherent multifunctional properties of calixarenes and the features of sol–gel chemistry, these coatings exhibit good thermal stability, solvent resistance and high extraction efficiency as well as long lifetime, and thus have the potential to be explored in the CE sample pretreatment procedures.

Propranolol [1-isopropylamino-3-(1-naphthoxy)-2-propanol] is a type of β-blockers which is widely used in therapeutics for its antihypertensive, antiangorous, and antiarrhythmic properties. Pharmacological studies have shown that *S*-(−)enantiomer of propranolol is about 100 times more active than the *R*-(+)enantiomer [18]. Propranolol is prohibited by the World Anti-doping Agency (WADA) in some specific sports such as gymnastics, shooting, football, etc. The minimum required performance limit (MRPL) of propranolol is 0.5 μg/ml for those doping analysis laboratories [19]. Since the chiral separation of enantiomers can provide helpful information in results interpretation, it is always encouraged in doping control [20]. Traditionally, β-blockers are separated and detected by HPLC technique [21,22]. GC–MS has also been used to analyse a mixture of β-blockers containing propranolol from urine, although separation of the stereoisomers was not investigated and the propranolol was derivatised before the LLE or SPE procedures [23]. Marriott reported the enantiomeric separation of propranolol and selected metabolites in aqueous solution by using CE with hydroxypropyl-β-CD as the chiral selector [24]. Other technique such as near-infrared spectrometry was employed to determine the enantiomeric compositions of propranolol, and the sample used was the spiked water [25].

The objective of this study was to develop a SPME–FASI–CZE mode for the determination of propranolol enantiomers in urine. A novel fiber based on diglycidyloxy-calix[4]arene coating was prepared by sol–gel method. A simple and inexpensive device was designed for the back-extraction procedure. The experimental conditions were optimized and the extraction efficiency of this new coating was investigated. Linearity, sensitivity, recovery, repeatability, and urine interference were determined to validate these procedures.

2. Experimental

2.1. Instrumentation

CE was carried out on a P/ACE MDQ capillary electrophoresis instrument equipped with diode array detector (DAD) (Beckman Coulter, Fullerton, CA, USA). The separations were carried out in 50 μm I.D. bare fused silica capillary (Yongnian, China). The total length of the capillary was 60 cm and the effective length from the injection end to the detection window was 50 cm.

Conditioning of the fiber was performed using a SP-6800A capillary GC system (Shandong, China) equipped with a capillary split injector system. Nitrogen was used as a carrier gas at linear velocity of 12–15 cm/s. An Ultrasonator model SY-1200 (Shanghai Ultrasonic Instrument Factory) was employed for the back-extraction of propanolol enantiomers from the fiber, and this ultrasonator was also used to mix various solution ingredients thoroughly. A homemade SPME with diglycidyloxy-C[4] fiber (85 μm O.D.) syringe was used to extract sample for clean up and preconcentration effect. The commercially available PDMS (100 μm O.D.), PA (85 μm O.D.) and PDMS/divinylbenzene (DVB) (65 μm O.D.) coated fibers for comparison were obtained from Supelco (Bellefonte, PA, USA).

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Mercury VX300 instrument at ambient temperature. Tetramethylsilane (TMS) was used as an internal standard for NMR. Infrared (IR) spectra were done on IR instrument model FTIR-8201PC (Shimadzu).

2.2. Reagents

5, 11, 17, 23-Tetra-*tert*-butyl-25, 27-dihydroxy-26, 28-diglycidyloxycalix[4]arene (diglycidyloxy-C[4]) was synthesized by referring to the reported methods [26,27]. Hydroxy-terminated silicone oil (OH-TSO) was purchased from Chengdu Center for Applied Research of Silicone (Chengdu, China). 3-Aminopropyltriethoxysilane (KH-550), tetraethoxysilane (TEOS), and poly(methylhydrosiloxane) (PMHS) were obtained from the Chemical Plant of Wuhan University. Trimethylchlorosilane were purchased from Shanghai Chemical Factory, China.

Propranolol hydrochloride, and hydroxypropyl-β-CD (HP-β-CD) were purchased from Sigma (St. Louis, MO, USA); Sodium chloride (NaCl), Sodium hydroxide (NaOH), triethanolamine, acetonitrile, phosphoric acid, methanol, and other regents were obtained from Shanghai Chemical Reagent Co. (Shanghai, China). All solvents used in this study were of analytical-regent grade.

A standard solution was prepared by transferring 10 mg of propranolol hydrochloride in a volumetric flask and diluting with doubly distilled water to 10 ml at room temperature.

2.3. Preparation of the fiber

Preparation of the sol–gel SPME fiber involves the following steps: (1) pretreatment of the fused-silica fiber; (2) preparation