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A new method for the analysis of β_2-agonists in human urine by pressure-assisted capillary electrochromatography coupled with electrospray ionization-mass spectrometry using a silica-based monolithic column

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ABSTRACT

A new pressure-assisted capillary electrochromatography coupled with electrospray ionization-mass spectrometry method using a silica-based monolithic column as separation media was developed for the analysis of β_2-agonists in human urine. Experimental conditions including the mobile phase, separation voltage, assisted pressure, and sheath liquid were optimized for the analysis: mobile phase composed of 82% (v/v) ACN and 18% (v/v) 20 mmol/L ammonium acetate (pH 6.0); separation voltage 25 kV; assisted pressure 2 bar; and the sheath liquid consisting of 7.5 mmol/L acetic acid in isopropanol/water 50/50% (v/v) that was delivered at a flow rate of 3.0 μL/min. Six β_2-agonists were separated within 12.5 min with LODs (defined as S/N = 3) in the range of 0.25–2.0 ng/mL. The absolute LODs of the developed method for analyzing six β_2-agonists ranged from 5.75 to 46.0 fg. Method repeatability of run-to-run and column-to-column was satisfactory. The recovery obtained from the analysis of spiked urine samples was between 88.2% and 106% with RSDs lower than 6.68%. The method was successfully applied to the analysis of real urine sample from volunteers.

1. Introduction

β_2-Agonists are a class of drugs normally employed for the treatment of pulmonary disorders and asthma or for the prevention of exercise-induced asthma, owing to their bronchodilator activity [1]. The compounds, especially clenbuterol, salbutamol and terbutaline, are often used as growth promoters in animal feed due to the repartitioning of carcass composition to decrease fat deposition and to increase muscle mass [2]. However, the residues of these compounds in edible tissues are potentially toxic. Several cases of β_2-agonists poisoning have been reported in recent years [3,4]. Therefore, these compounds have been banned as growth promoters in many counties including China and European Communities [5]. The use of most β_2-agonists has been prohibited in sports by the International Olympic Committee (IOC) and World Anti-Doping Agency (WADA) [6] because stimulation and anabolic effects were observed when the intake level of β_2-agonists was higher than therapeutically indicated level.

The conventional methods for the analysis of β_2-agonists include gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS). GC–MS is a commonly used method not only for the screening and confirmation for β_2-agonists in human urine sample [7–10], but also for the multi-residual analysis in animal tissues [11,12]. However, because β_2-agonists have high polarity and low volatility, a time-consuming, tedious and expensive derivatization is required prior to the GC–MS analysis [13]. In the last decades, LC coupled with atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) or electrospray ionization-mass spectrometry (ESI-MS) has attracted significant attention in the analysis of β_2-agonists in human urine and multi-residual samples because no derivatization procedure was required [14–17]. Capillary electrophoresis (CE) has also been applied for the separation and determination of β_2-agonists due to its simplicity, efficiency and low sample consumption [18,19].

Capillary electrochromatography (CEC) coupled with MS is an emerging microanalysis technique and a supplemental method to LC–MS. This microanalysis technique combines the excellent features of both CE and HPLC such as high separation efficiency and low sample consumption of CE as well as high selectivity and large sample loading capacity of HPLC [20,21]. However, CEC–MS has not been widely accepted as a routine analytical technique due to the difficulty in instrumental operation and poor analytical

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repeatability, which may be attributed to the following several reasons. Firstly, a longer analysis time is usually required for CEC–MS than that of CEC with ultraviolet (UV) detection because UV detection is generally performed in-column or on-column, whereas MS detection normally required an additional column connected to MS interface [22]. Secondly, air bubbles are more easily formed in CEC–MS system than that of CEC–UV system, because one end of column must be inserted to the interface. To suppress bubble formation, an assisted pressure or a special column (e.g., an internally tapered column) is usually needed [23]. Thirdly, the requirement on column is more crucial for CEC–MS technique. Therefore, much effort has recently been concentrated on CEC–MS column technology [24]. However, up to now, only limited columns are available for CEC–MS compared to those for LC–MS.

In recent years, monolithic columns have attracted considerable attention and are regarded as a new generation of chromatographic separation media due to their good permeability, fast mass transfer property, high stability and easy modification [25–27]. Compared to the packed column, a significant merit of monolithic column is that the formation of bubble can be reduced or eliminated because no frits are necessary to keep the stationary phase in columns [28]. Based on different materials, monolithic columns can be classified into two categories, namely silica-based and organic polymer-based monoliths [29]. Silica-based beds have the advantages of high mechanical strength, heat stability and resistance to organic solvents [30,31].

To the best of our knowledge, the analysis of β_2-agonists using CEC or CEC–MS has not been reported. The goal of present work was to develop a new, simple and sensitive pressure-assisted CEC–ESI-MS (pCEC–ESI-MS) method for the determination of β_2-agonists (Fig. 1). The method using self-prepared silica-based monolithic column as separation media was applied for the analysis of the real urine sample from volunteers.

2. Materials and methods

2.1. Chemicals and reagents

Clenbuterol, terbutaline, salbutamol, formoterol, procaterol, and salmeterol were purchased from the Chinese Institute of Biological Products Control (Beijing, China). Fused-silica capillary of 100 μm id and 375 μm od was obtained from Yongnian Optic Fiber Plant (Hebei, China). Tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMS), and PEG (M_r = 10 000) were supplied by Alfa Aesar (Tianjin, China). Urea was donated by Cxbio Biotechnology (Shanghai, China). Salbutamol sulfate tablets were provided by Pingguang Pharmaceuticals (Jiangsu, China).

Acetonitrile, methanol, and isopropanol (HPLC grade) were obtained from Sinopharm Chemical Reagents (Shanghai, China). Acetic acid glacial, ammonium acetate, and ammonium hydroxide were analytical reagent grade and purchased from Sinopharm Chemical Reagents. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Column preparation

Silica-based monolithic columns were prepared according to the procedure as previously described [32]. Briefly, a rehydroxylation process was performed to maximize the number of silanol groups on the silica surface before preparation of monolithic columns. The capillary was flushed with water, 1.0 mol/L sodium hydroxide, water, 0.1 mol/L hydrochloric acid, water and acetone for 30 min, 3 h, 30 min, 3 h, 30 min and 30 min in order, respectively, and then purged with nitrogen at 180 °C for 3 h prior to use. 0.44 g PEG and 0.45 g urea were dissolved in 5.0 mL acetic acid solution (10 mmol/L), and then 1.8 mL TMOS, 0.2 mL MTMS were added. The mixed solution was stirred for 45 min in ice bath. The resultant transparent sol was introduced into the pretreated capillary, and both ends were sealed with silicon rubbers. Then the polymerization was carried out at 40 °C in water bath for 20 h. The wet gel was treated for 3 h at 120 °C, and followed by a washing with water and methanol. After drying, heat-treatment was carried out at 330 °C for 25 h.

2.3. pCEC–ESI-MS Instrumentation

All pCEC–ESI-MS experiments were performed on an Agilent ^{3D}CE (Agilent Technologies, Waldbronn, Germany) system coupled with an Agilent 1100 series single quadrupole mass

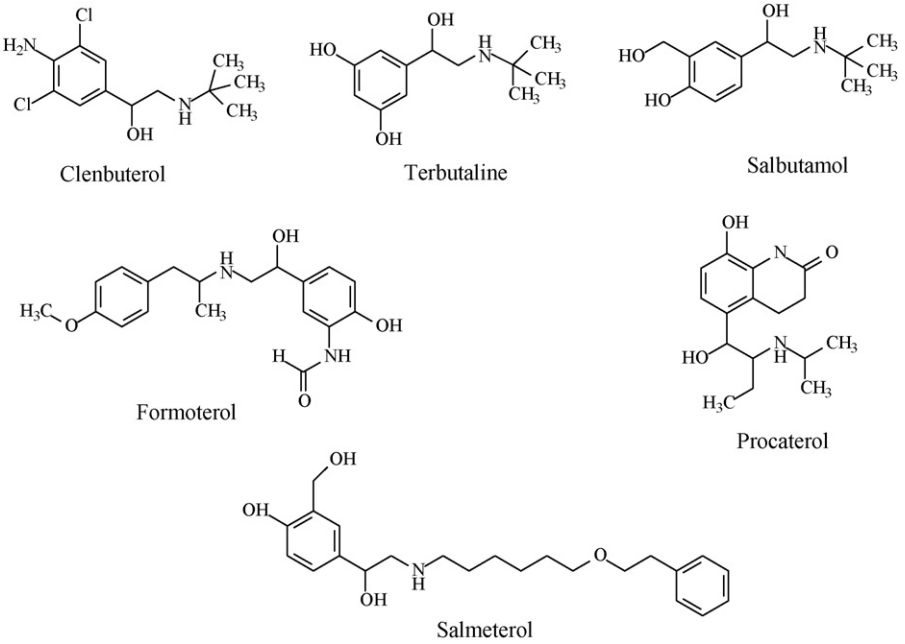

Fig. 1. Chemical structures of six β_2-agonsits.