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Short communication

Determination of β_2-agonists by ion chromatography with direct conductivity detection

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Abstract

A simple method for the simultaneous detection of four β_2-agonists (salbutamol, fenoterol, clorprenaline, and clenbuterol) using ion chromatography (IC) with direct conductivity detection (CD) based on their ionization in acidic medium without chemical suppression is presented. The mixture of 1.8 mM HNO_3 and 2% (v/v) acetonitrile was used as eluent. The method could be applied to the determination of the β_2-agonists in pharmaceutical preparations. The recovery of salbutamol and clenbuterol in tablets was more than 97% ($n = 3$) and the relative standard deviation ($n = 11$) less than 2.8%. With the proposed method, salbutamol could also be successfully detected in human plasma. In a single chromatographic run, the four β_2-agonists can be separated and determined in less than 8 min. The linear ranges were of 7.0–1.4 $\times 10^3$ ng/ml for salbutamol, 34–7.8 $\times 10^3$ ng/ml for fenoterol, 8.0–1.6 $\times 10^3$ ng/ml for clorprenaline, and 25–7.5 $\times 10^3$ ng/ml for clenbuterol. The detection limits were 2.0 ng/ml for salbutamol, 10 ng/ml for fenoterol, 3.0 ng/ml for clorprenaline, and 10 ng/ml for clenbuterol.

Keywords: β_2-Agonists; Salbutamol; Fenoterol; Clorprenaline; Clenbuterol; Ion chromatography; Conductivity detection

1. Introduction

Ion chromatography (IC) has developed into the method of choice for the simultaneous determination of mixtures of inorganic anions or cations. The technique has also often been extended to the determination of low molecular mass organic ionic species, such as C_1–C_5 carboxylic acids, sulfonic acids, amines, etc. The application of IC to larger organic ions is much less common. However, theoretically, this technique could also be used for the separation of organic compounds with relatively high molecular weights. There are broad opportunities to use IC to detect larger organic ions [1].

β_2-Agonists such as salbutamol, fenoterol, clorprenaline and clenbuterol can effectively prevent and reverse bronchoconstriction; they are the most powerful bronchodilators currently available and can be used to treat pulmonary diseases of humans and animals [2–5]. Furthermore, β_2-agonist compounds are used in animal rearing as growth promoters [6]. However, the danger from the residues resulting from the abuse of these agents has been underscored by several human poisoning incidences where the consumption of animal food products containing clenbuterol residues was implicated. It is, therefore, necessary to develop rapid and sensitive methods for the separation and detection of β_2-agonists.

Various analytical methods for the determination of β_2-agonists have been described [7–16]. Gas chromatography–mass spectrometry (GC–MS) [7–9] is a common method to detect these bronchodilators. However, a derivatisation step is required prior to injection, which is complicated and time-consuming [13]. Furthermore, the derivatisation procedures proposed for β_2-agonist are not entirely satisfactory

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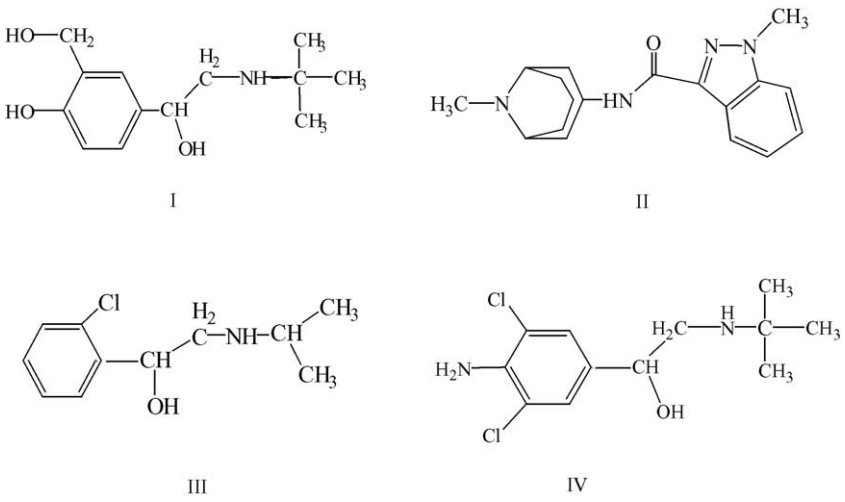

Fig. 1. Structure of four β_2-agonists: I salbutamol; II fenoterol; III clorprenaline; IV clenbuterol.

owing to the low specifity of the resulting electron impact (EI) mass spectra or to the restricted range of applicability. HPLC offers advantages over GC to detect β_2-agonists [13]. The HPLC methods reported all used columns with reserved-phase sorbents with buffered mobile phase, either with or without an ion-pair reagent [17]. UV detection is the most popular detection method applied in HPLC. However, different β_2-agonists have different UV characteristics, the β_2-agonist such as salbutamol and fenoterol do not sufficiently absorb UV light as a result of which HPLC methods with UV detection cannot offer enough sensitivity for the simultaneous detection of a group of β_2-agonists sensitively at a single wavelength UV detection [13,18]. UV detection for β_2-agonists gives good sensitivity only after a post-column derivatisation procedure [19], but it is complicated and time-consuming. Moreover, UV detection cannot offer sufficient selectivity for the determination of β_2-agonist [13] because the complicated matrix would also absorb UV light, causing interference for β_2-agonist detection. In recent years, fluorescence detection has been extensively applied as the detection method in liquid chromatography, as low detection limit can be achieved. However, this includes the need for applying a variety of sample clean-up procedures, so as to decrease background interference from the sample matrix. This usually is the slowest step of the analysis. Solid phase extraction (SPE) has become one of the most popular techniques for sample clean-up procedure in recent years. SPE offers good recoveries and can be automated; however, higher investments are needed. Liquid chromatographic–mass spectrometry (LC–MS) detection methods have been developed for the determination of β_2-agonist intensely [19,20]. However, the powerful and expensive LC–MS equipment does not belong to the facilities of average investigator.

Our preliminary [21] experiments demonstrated that organic compounds with relatively high molecular weights could be ionized in aqueous solution at relatively low pH values. The structure of the four β_2-agonists were shown in Fig. 1; it can be observed that they can ionize in aqueous solution at relatively low pH values. These analytes therefore could be separated on the stationary phase mainly based on ion exchange. They could be detected online by a conductivity detector available in the commercial ion-chromatographic instrument sensitively. To avoid interferences, β_2-agonists could be first extracted from plasma by toluene, and then reversibly extracted by using the diluted HNO_3 solution. The latter solution was then utilized as mobile phase for the ion chromatographic separation. The determination of salbutamol in plasma is important for clinical treatment. Limited data have been published on the pharmacokinetics of salbutamol. Moreover, there are no data on the extent to which inhaled salbutamol undergoes first-pass metabolism. This lack of information is most likely due to the very low plasma concentrations reached after inhalation of therapeutic doses of salbutamol, and the problems in developing an analytical method that is sensitive enough to determine these concentrations [22]. Using this method, we successfully determined salbutamol in plasma. This sample extraction procedure is time saving, cheap and may improve selectivity of analysis. Moreover, the proposed composition of the mobile phase is quite simple, only diluted acid is used for the separation and the analysis time is short (all analytes can be detected within 8 min).

2. Experimental

2.1. Materials

Salbutamol, fenoterol, clenbuterol and clorprenaline were purchased from the National Institute for the Control of Pharmaceutical and Biological Products of the People's Republic of China. Nitric acid, toluene and acetonitrile were obtained from Beijng Yili Chemical Ltd. Company. All other