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Fast Determination of Clenbuterol and Salbutamol in Feed and Meat Products Based on Miniaturized Capillary Electrophoresis with Amperometric Detection

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The fast separation capability of a novel miniaturized capillary electrophoresis with an amperometric detection (μCE-AD) system was demonstrated by determining clenbuterol and salbutamol in real samples. The effects of several factors such as the acidity and concentration of the running buffer, the separation voltage, the applied potential and the injection time on CE-AD were examined and optimized. Under the optimum conditions, the two *β*-agonists could be baseline separated within 60 s at a separation voltage of 2 kV in a 90 mmol/L H_3BO_3-$Na_2B_4O_7$ running buffer (pH 7.4), which was not interfered by ascorbic acid and uric acid. Highly linear response was obtained for above compounds over three orders of magnitude with detection limits ranging from 1.20×10^{-7} to 6.50×10^{-8} mol/L ($S/N=3$). This method was successfully used in the analysis of feed and meat products with relatively simple extraction procedures.

Keywords miniaturized capillary electrophoresis-amperometric detection, *β*-agonist, clenbuterol, salbutamol

Introduction

The synthetic *β*-agonists clenbuterol and salbutamol with molecular structures shown in Figure 1 can accelerate the formation of muscle albumen and inhibit the formation and accumulation of fat, consequently enhancing the rate of muscle in poultry and livestock. Unfortunately both clenbuterol and salbutamol are easily left behind in animal bodies, which is a potential danger to human health.[1,2] To guarantee the rights and interests of consumers, many countries and regions have put a ban on *β*-agonist in stockbreeding. According to the regulations of US Food and Drug Administration and World Health Organization, the maximum amount of clenbuterol in an animal body is 0.2, 0.6, 0.6, 0.2 and 0.05 μg/kg for meat, liver, kidney, fat and milk, respectively. In spite of the strict regulations of *β*-agonist, several recent accidents[3,4] related to clenbuterol drew much attention from the society, and such accidents still happened due to failure of detection. Therefore, fast and effective analysis of *β*-agonist is of prime importance.

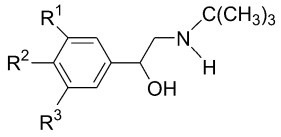

Figure 1 The molecular structures of clenbuterol (R^1=Cl, R^2=NH_2, R^3=Cl) and salbutamol (R^1=CH_2OH, R^2=OH, R^3=H).

So far gas chromatography-mass spectrometry (GC-MS),[5-7] high performance liquid chromatography (HPLC)[8-10] and immunoassay (IA)[11-14] have been employed for this purpose. Compared with above mentioned methods, capillary electrophoresis (CE) has many advantages, such as small sample volume required, short analysis time, high separation efficiency, *etc*. In recent years, conventional capillary electrophoresis has been applied to analyze *β*-agonist and several papers have been reported.[15-17] Amperometric detection (AD) is attractive for both conventional CE and μCE,[18-20] since numerous compounds are detectable without the need for derivatization. Although AD is usually less sensitive than LIF, it is much more versatile, economical, and easier to scale down in the micrometer range.

The aim of the work introduced here has been to develop a laboratory-built novel μCE-AD for the determination of clenbuterol and salbutamol in both feed and meat products, which is dependable, inexpensive, easy to operate, and has no need for a micro-fabrication. The μCE-AD system has been successfully applied to fast analyse of clenbuterol and salbutamol in feed and meat products.

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Experimental

Reagents and materials

All reagents were of analytical grade. Clenbuterol, salbutamol, ascorbic acid and uric acid were all purchased from Sigma (St. Louis, MO, USA). The stock solutions of clenbuterol, salbutamol, ascorbic acid and uric acid were 5×10^{-3}, 5×10^{-3}, 1×10^{-2} and 1×10^{-3} mol/L, respectively. Standard solutions and samples were prepared by diluting to desired concentrations with appropriate amounts of the running buffer, immediately before use.

Apparatus

The laboratory-built CE-ED system has been constructed and described previously.[20] A $\pm$ 30 kV high-voltage dc power supply (Shanghai Institute of Nuclear Research, China) provided a voltage between the ends of the capillary. The inlet of the capillary was held at a positive potential with the capillary outlet in the detection cell effectively grounded. The separations proceeded in an 8.5 cm length fused silica capillary fixed on a plexi-glass plate.

A carbon-disk electrode with 300 μm diameter was employed as the working electrode. Before use, the disk surface of the carbon electrode was polished with emery paper, sonicated, in deionized water, and then carefully positioned opposite the capillary outlet through the guiding metal tube.

The three-electrode cell system employed in all experiments consisted of a carbon disk working electrode, a platinum auxiliary electrode and a reference of saturated calomel electrode (SCE), which were used in combination with a BAS LC-4C amperometric detector (Biochemical System, West Lafayette, IN, USA). The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument Factory, China).

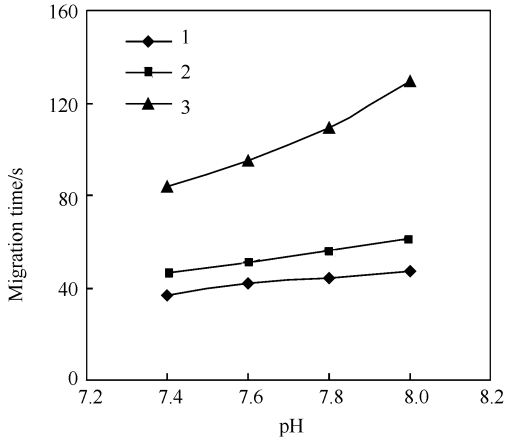
Sample preparation

Four feed samples were obtained from a feed provider, and meat products (meat, liver, stomach, heart and kidney) were purchased from a local supermarket. Feed and meat product samples were prepared according to references 21 and 22, respectively. All sample solutions were stored in a refrigeratory at 4 ℃.

Results and discussion

Optimization of miniaturized CE-AD system

Effects of pH and the buffer concentration The resolution and migration time of analytes were studied in several buffers. Borate buffer was employed as the running buffer in this experiment because it could chelate with analytes to form more soluble complex anions[23]. In the pH range of 7.4—8.0, clenbuterol and salbutamol could be base-lined separated. Ascorbic acid and uric acid did not interfere with the detection. The migration time of all analytes increased with the increase of pH value (Figure 2). On the other hand in the pH range of 6.6—7.2 in a borax-phosphate buffer, clenbuterol and salbutamol could be base-lined separated, but it took longer time, often over 120 s. In the pH range of 5.8—8.0 in a phosphate buffer, clenbuterol and salbutamol could not be separated effectively. Therefore, borate buffer was employed as the running buffer, and pH 7.4 was selected as the optimum pH value for this work.



Fiure 2 The effects of running buffer pH on the migration time of each analyte. Working conditions: Fused-silica capillary: 25 μm i.d.×8.5 cm; working electrode: 300 μm diameter carbon disk electrode; running buffer: 90 mmol/L; separation voltage: 2 kV; injection time: 2 s/2 kV; peak identification and concentration of each analyte: (1) clenbuterol: 5.0×10^{-5} mol/L, (2) salbutamol: 5.0×10^{-5} mol/L, (3) ascorbic and uric acids: 1.0×10^{-4} mol/L, each.

In addition, the concentration of the running buffer was another important factor. So the effect of the running buffer concentration on migration time was also studied, and the optimum running buffer concentration was 90 mmol/L. At this concentration, clenbuterol and salbutamol could be base-lined separated in 60 s.

Effect of the potentials applied to the working electrode There are electroactive functional groups in clenbuterol and salbutamol molecules, therefore, these two compounds are detectable both without the need of derivatization. In an amperometric detection, the potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this method. Hydrodynamic voltametry experiment was investigated to obtain optimum detection. As shown in Figure 3, when the applied potential exceeded +500 mV (vs. SCE), the peak current of the two analytes increased rapidly. However, when the applied potential was greater than +900 mV (vs. SCE), both the baseline noise and the background current increased substantially, which was a big disadvantage for sensitive and stable detection, although the peak current of the analytes still had certain increment. Therefore, the potential applied to the working electrode was maintained at +900 mV (vs. SCE), where the background current was not too high and the signal-to-noise ($S/N=3$) ratio was the highest.

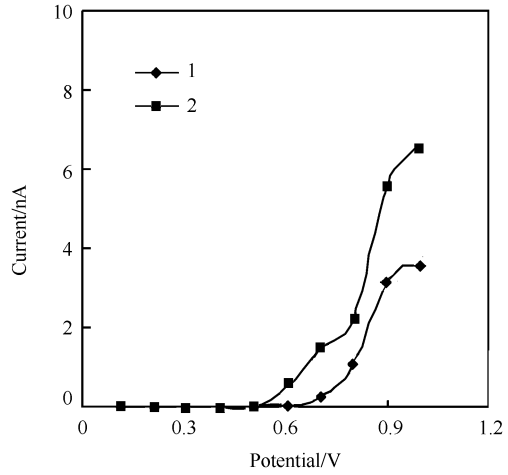


Figure 3 Hydrodynamic voltammograms (HDVs) of clenbuterol and salbutamol. Working electrode potential +900 mV (vs. SCE), running buffer concentration: 90 mmol/L (pH 7.4); other experiment conditions and labels were the same as those in Figure 2.

Effect of separation voltage and injection time For a given capillary length, the separation voltage determines the electric field strength, which affects both the velocity of electroosmotic (EOF) and the migration velocity of the analytes, which in turn determines the migration time of the analytes.

The effect of separation voltage on the migration time of both the analytes was shown in Figure 4. Increasing the voltage gave shorter migration time, but when the separation voltage was over 2.5 kV, back ground noise increased rapidly. Therefore, 2 kV was chosen as the optimum voltage to accomplish a good compromise.

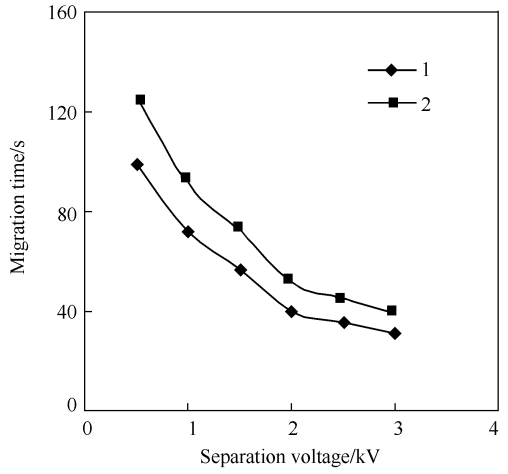


Figure 4 The effect of separation voltage on the migration time of each analyte. The experiment conditions and labels were the same as those in Figure 3.

The injection time determining the amount of sampling affected both peak current and peak shape. The effect of injection time on separation was investigated by varying sampling time (1, 2, 3, 4 s at a voltage of 2 kV, as shown in Figure 5). When the injection time was between 1 and 2 s, the peak current increased with the increase of injection time. When injection time was longer than 3 s, the peak current leveled off and peak broadening became more severe. 2 s (at 2 kV) was, therefore, selected as the optimum injection time.

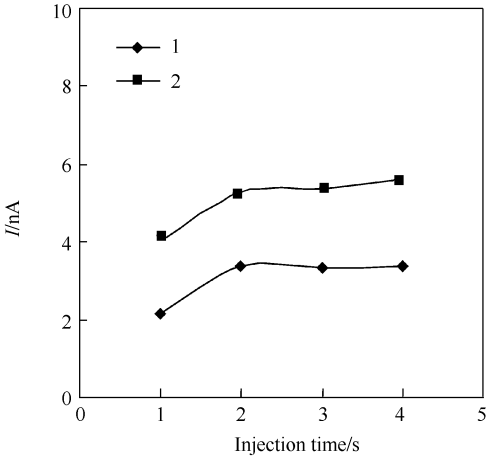


Figure 5 The effect of the injection time on peak current of each analyte. The experimental conditions and labels were the same as those in Figure 3.

Through the experiments above, the optimum conditions for determining clenbuterol and salbutamol have been decided. The typical electropherogram for a standard mixture solution of the two analytes was shown in Figure 6a, from which we could see good separation was achieved within 60 s.

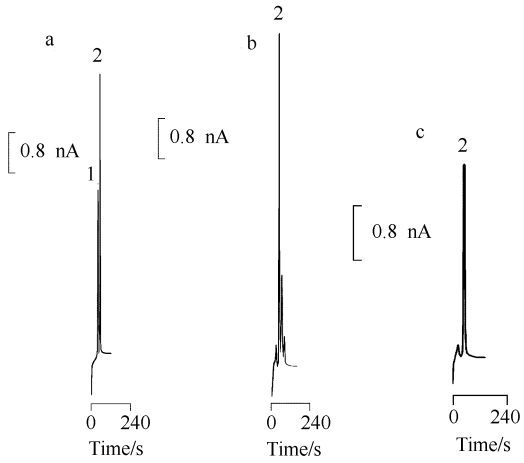


Figure 6 Electropherograms of a standard mixture solution (a), feed (b) and pig heart (c) sample solutions. The experimental conditions and labels were the same as those in Figure 3.

Method validation

Linearity and detection limits To determine the linearity between the peak current and concentrations of clenbuterol and salbutamol, a series of standard solutions from 1.0×10^{-7} to 1.0×10^{-3} mol/L were tested. The results showed that peak current and analyte concentration had good linear relation in the range of 2.0×

10^{-7}—1.0×10^{-4} mol/L. The regression equations of clenbuterol and salbutamol were $Y=8.94\times10^{-4}X-0.07$ ($r=0.9993$) and $Y=1.38\times10^{-4}X-0.02$ ($r=0.9994$) respectively. The detection limit was 1.20×10^{-7} mol/L for clenbuterol and 6.50×10^{-8} mol/L for salbutamol.

Reproducibility: A standard mixture solution of clenbuterol and salbutamol, 5.0×10^{-5} mol/L for each, was analyzed for seven times to determine the reproducibility of peak current and migration time for the two analytes under the optimum conditions. The relative standard deviations (RSDs) of peak current and migration time are 2.3% and 0.8% for clenbuterol, 1.7% and 0.6% for salbutamol, respectively. The above experimental results demonstrated good performance of the novel μCE-AD system. Compared with traditional CE-AD[22] and HPLC method,[24] this method had the advantage of much shorter analysis time (60 s vs. 518 min) while keeping the same detection limit.

Recovery: The recovery and reproducibility experiments under the optimum conditions were also conducted to evaluate the precision and accuracy of the method. Accurate amounts of standards were added to the actual samples, and the recovery values could be obtained by comparing the increase of the peak height before and after the addition of standards. Take feed 2 for example, the recovery ($n=3$) was between 76.6% and 85.8%. Average recovery and relative standard deviation were 84.2% and 1.3% for clenbuterol, 81.2% and 1.7% for salbutamol, respectively.

Applications

Under the optimum conditions, the determination of clenbuterol and salbutamol in real samples was carried out. Typical electropherograms were shown in Figures 6b and 6c. The analyte of clenbuterol was not found in real samples. The contents of salbutamol and relative standard deviation (RSD) were listed in Table 1.

Table 1 The assay results of real samples ($n=3$)[a]

Sample	Salbutamol/(mg•kg^{-1})	RSD/%
Meat	2.07	4.1
Liver	3.44	2.4
Stomach	1.48	1.4
Heart	4.49	3.7
Kidney	8.83	1.46
Feed 1	6.34	1.7
Feed 2	50.58	1.1
Feed 3	31.65	1.8
Feed 4	23.11	1.5

[a] The same condition as Figure 3.

The above experimental results demonstrate the capability and the advantages of the novel μCE-AD system for the determination of clenbuterol and salbutamol in real samples. The advantages of this determination method are its simple design and construction, low cost, high degree of integration, portability, good performance, and speediness. Furthermore, by varying the working electrode and separation mode, this μCE-AD system can be used to determine numerous compounds.

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