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Short communication

Determination of aminoheterocycle and azabicycle in gliclazide bulk by capillary zone electrophoresis with amperometric detection

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Abstract

A simple, reliable and reproducible method, based on capillary zone electrophoresis with amperometric detection (CZE-AD), was developed for simultaneous determination of 3-amino-3-azabicyclo[3,3,0]octane (aminoheterocycle) and 3-azabicyclo[3,3,0]octane (azabicycle) in gliclazide bulk drug. The optimal conditions of CZE-AD were 50 mM borate solution (pH 9.0) as running buffer, 14 kV as separation voltage and 0.95 V (versus SCE) as detection potential. Under the selected optimum conditions, the two analytes could be perfectly separated within 9 min. The linearity range of aminoheterocycle was from 1.0×10^{-6} to 1.0×10^{-3} M and that of azabicycle was from 2.0×10^{-6} to 1.0×10^{-3} M. Their detection limits were 5.0×10^{-7} and 1.0×10^{-6} M, respectively, (S/N = 3). This proposed method demonstrated long-term stability and reproducibility with relative standard deviations of less than 2% for both migration time and peak current. It has been successively used for the determination of these two analytes in gliclazide bulk drug, and the assay results were satisfactory.

Keywords: Capillary zone electrophoresis; Amperometric detection; 3-Amino-3-azabicyclo[3,3,0]octane; 3-Azabicyclo[3,3,0]octane; Gliclazide

1. Introduction

Impurities in drugs, which are usually originated from various sources and phases of the synthetic process and preparation of pharmaceutical dosage forms, often possess unwanted pharmacological or toxicological effects [1]. Because safety and efficacy of pharmaceuticals are two fundamental issues of importance in drug therapy, it is necessary to found accurate methods to assure the quality and safety of drugs by monitoring and controlling the impurities effectively in modern pharmaceutical industry.

Gliclazide, chemically named 1-(4-methylbenzenesulphonyl)-3-(3-azabicylco(3,3,0)octyl) urea (I), is one of persral second-generation sulphonylureas. The medicine is commonly used in type II diabetes, previously known as non-insulin-dependent diabetes mellitus (NIDDM). Besides reducing blood sugar, gliclazide can improve the cruor function and further delay the occurring of blood vessel neopathy. It is also suggested that due to its short-term acting, gliclazide may be suitable for use in diabetic patients with renal impairment and elderly patients whose reduced renal function may increase the risk of hypoglycemia following some sulphonylureas [2,3]. For above advantages, gliclazide is widely regarded as one of the best persral medicines for diabetics. Some analytical methods, such as radioimmunassay [4], spectrofluorimetry [5], capillary gas chromatography [6], TLC [7] and HPLC [8–10], have been applied to the quantitative determination of gliclazide alone. 3-Amino-3-azabicyclo[3,3,0]octane (aminoheterocycle) and 3-azabicyclo[3,3,0]octane (azabicycle) are two kinds of impurities in gliclazide bulk drug and strictly restricted by the safety requirement of authorities. However, there are few activities on monitoring of impurities in gliclazide bulk drug.

In recent years, capillary electrophoresis (CE) has been extensively studied and applied as a highly effective analytical method in modern pharmaceutical analysis. CE has many advantages, such as much lower sampling volume and higher separation efficiency. Because amperometric detector (AD) is more sensitive than UV–vis detector and much cheaper

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than LIF detector, it can be coupled with CE to provide high sensitivity and selectivity for the determination of electroactive substances in many medical and pharmaceutical analysis areas [11–13].

In this paper, capillary zone electrophoresis with amperometric detection (CZE-AD) is firstly applied to determine aminoheterocycle and azabicycle in gliclazide bulk drug. The results also show that monitoring and controlling aminoheterocycle and azabicycle in gliclazide bulk drug by this method is of quickness, low-volume sampling, simple instrument and operation, high sensitivity and high reproducibility.

2. Experimental

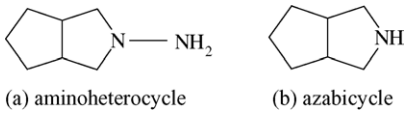
2.1. Apparatus

CZE-AD system was laboratory-built [14,15]. Electrophoresis was driven by a high-voltage supplier (±30 kV, Shanghai Institute of Nuclear Research, Shanghai, China). Separations were performed in a fused silica capillary (Hebei Yongnian Laser-fiber factory, China) with 25 μm i.d., 360 μm o.d. and 75 cm long. Potential control and current output were employed by a CH-2 amperometric detector (Jiangsu Electrochemical Analytical Instrumental Factory, China). Electropherograms were recorded by a chart recorder (Model XWT-204, Shanghai Dahua Instrument Factory, China). Electrochemical experiments were carried out by a CHI 630 electrochemical analyzer (CH Instruments, USA). A three-electrode system, which consisted of a carbon disc working electrode (Ø 300 μm), a saturated calomel reference electrode (SCE) and a platinum wire counter electrode, was used in both electrochemistry and detection experiments.

2.2. Reagents

3-Amino-3-azabicyclo[3,3,0]octane (aminoheterocycle) and 3-azabicyclo[3,3,0]octane (azabicycle) (Fig. 1) were provided by Shanghai Institute for Drug Control. The stock solutions of aminoheterocycle and azabicycle with a concentration of 1.0×10^{-3} M were prepared with doubly distilled water and diluted with running buffer to the needed concentrations in CZE experiments. All reagents were of analytical reagent grade. All solutions were prepared with double distilled water.

Before CZE experiment, all used solutions were filtered through 0.22 μm polypropylene acrodisc syringe filter and sonicated for 5 min to remove bubbles.



(a) aminoheterocycle (b) azabicycle

Fig. 1. Molecular structures of aminoheterocycle and azabicycle.

2.3. Procedure

2.3.1. Preparation of carbon working electrode

A carbon disc working electrode was prepared by using a 300 μm diameter pencil lead. The used carbon electrode was prepared according to the introduced procedure in our former paper [16]. Prior to use, the carbon disc electrode was firstly polished with emery paper and 0.05 μm alumina powders, then ultrasonicated in deionized water, and finally carefully positioned opposing the capillary outlet with the aid of a micromanipulator.

2.3.2. CZE experiments

Before CZE experiments, the three-electrode system was fixed in the corresponding holes of the electrochemical cell and the carbon disc electrode was positioned straightly opposite the capillary outlet as close as possible by a three-dimension positioner.

Before each run in CE experiments, the capillary was sequentially rinsed with 0.5 M hydrochloric acid, doubly distilled water, 0.2 M sodium hydroxide, 3 min for each and with running buffer till the inside current of the capillary reached stability. This was important to get a reproducible electroosmotic flow.

CZE was performed at the separation voltage of 14 kV with 50 mM borate solution (pH 9.0) used as running buffer. The potential applied to the working electrode was 0.95 V (versus SCE). Samples were electrokinetically injected at 14 kV for 8 s.

2.3.3. Sample preparation

Gliclazide bulk drug was provided by Shanghai Institute for Drug Control. An accurate weight of the powder (0.70 g) was dissolved in 20 ml of 50 mM running buffer and sonicated for 30 min. After cooling, the mixture was filtered through a paper filter. This solution was diluted to 100 ml in a volumetric flask with running buffer for analysis. Peak identification was performed by standard addition method.

3. Results and discussion

3.1. Conditions of amperometric detection

Since aminoheterocycle and azabicycle can be electrochemically oxidized at a relatively moderate potential, electrochemical detection was used in this work. Fig. 2 shows the hydrodynamic voltammograms (HDVs) of the above analytes, which were obtained by monitoring the current responses after CZE separations within the applied potential range from 0.4 to 1.2 V. It was found that the current responses of these analytes increased with the applied potential increasing. In order to determine above analytes together and obtain the best signal-to-noise ratio, 0.95 V was selected as the most suitable detection potential in this experiment.

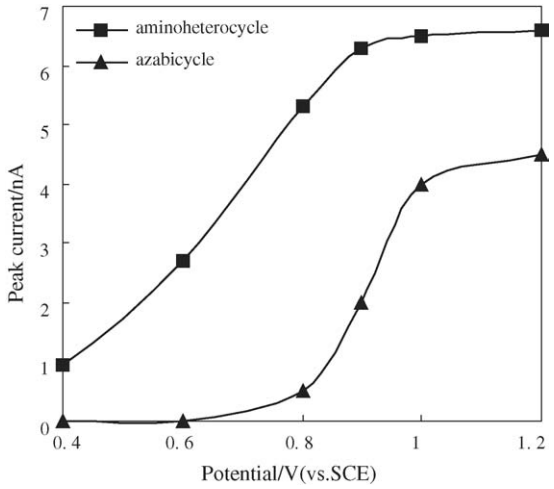


Fig. 2. Hydrodynamic voltammograms for aminoheterocycle (5×10^{-5} M) and azabicycle (5×10^{-5} M); the CZE conditions are as the optimal.

3.2. Effects of pH and concentration of running buffer

In order to improve the resolution and sensitivity, borate, phosphate, borate–phosphate mixture were employed as running buffer, respectively, in this CZE separation, and the best results were got with borate buffer.

The pH effect was investigated within the pH range from 7.0 to 11.0. It was found that azabicycle hardly had current correspondence when pH value was less than 8.0. Within the pH range from 8.0 to 11.0, the base-line separation for two analytes can be achieved, and their current responses increased and the migration time was not effected so much with the increase of the pH value. In this experiment, pH 9.0 was selected as the optimum pH.

In addition, the effect of concentration of borate buffer was examined. The results showed that with the increase of the concentration of buffer, the peak currents of the two analytes increased. The migration time increased with the increase of buffer concentration too, this is because the increase of ionic strength of borate buffer resulted in the decrease of electroosmotic flow in the capillary. The electric current inner the capillary also increased with the increase of the concentration of borate buffer, which caused higher Joule heat and the current peaks exhibited a significant broadening. For a comprehensive thought, 50 mM borate buffer was chosen as the running buffer in this experiment.

3.3. Effect of separation voltage and sampling time

The separation efficiency of CZE was investigated within the separation voltage range from 8 to 16 kV. The migration time of the analytes was significantly shortened and their corresponding current peaks were sharpened when the separation voltage was increased. However, if the separation voltage was too high, more Joule heat was produced because of the higher current inside the capillary, which caused peak broadening and reduced separation efficiency. For a comprehensive thought, 14 kV was selected as the optimum separation voltage in this experiment.

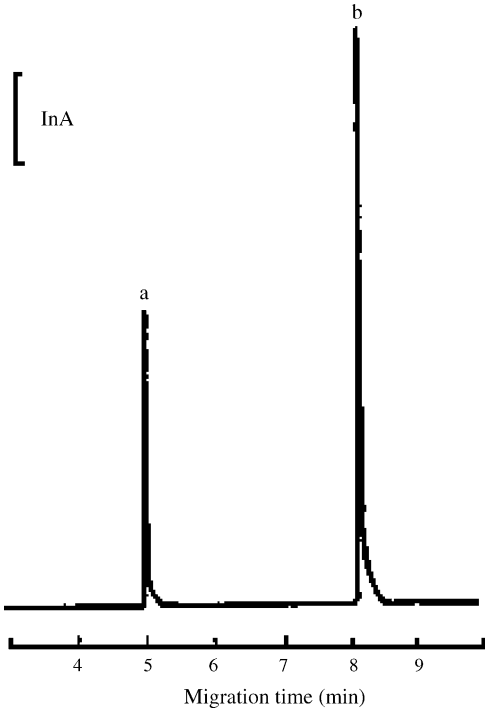


Fig. 3. Electropherograms of a standard solution containing 5×10^{-5} M aminoheterocycle and azabicycle under the optimal CZE-AD conditions: a, azabicycle; b, aminoheterocycle.

Electrokinetic sampling was used in the CZE experiment. The effect of injection time was investigated by selecting different sampling time (2, 4, 6, 8, 10 s at a voltage of 14 kV). It was found that when the sampling time was prolonged, the peak currents increased correspondingly. However, the current response peaks of the analytes were obviously broadened if the sampling time was more than 10 s. So, 8 s was selected as the sampling time in this experiment and satisfactory results were obtained under this condition.

Through the experiments above, the optimum conditions of CZE-AD for determining aminoheterocycle and azabicycle were detection potential 0.95 V (versus SCE), separation voltage 14 kV, electrokinetic sampling time 8 s at 14 kV and 50 mM borate buffer (pH 9.0). The typical electropherograms for a standard aminoheterocycle and azabicycle mixture solution are shown in Fig. 3. A base-lined separation for two analytes can be achieved within 9 min.

3.4. Reproducibility, linearity and detection limits

A standard mixture solution of 1.0×10^{-4} M aminoheterocycle and 1.0×10^{-4} M azabicycle was used to determine the reproducibility of the current response and migration time under the optimum conditions in this experiment. The relative standard deviations (R.S.D.) of peak current and migration time are 1.55% and 1.50% for aminoheterocycle, 1.97% and 1.06% for azabicycle, respectively, when the analysis was

Table 1
The regression equations and the detection limits of aminoheterocycle and azabicycle

Analyts	Regression equation C (M); I (nA)	Correlation coefficient	Linear range (M)	Detection limit[a] (M)
Aminoheterocycle	$I = 1.25 \times 10^5 C + 0.01$	0.9981	1.0×10^{-6}–1.0×10^{-3}	5.0×10^{-7}
Azabicycle	$I = 6.5 \times 10^4 C + 0.01$	0.9985	2.0×10^{-6}–1.0×10^{-3}	1.0×10^{-6}

[a] Detection limit was estimated according to three times of signal-to-noise.

Table 2
The detection results of sample and the ratio of recoveries ($n = 5$)

Analytes	Original amount ($\times 10^{-5}$ M)	Added amount ($\times 10^{-5}$ M)	Found amount ($\times 10^{-5}$ M)	Recovery (%)	R.S.D. (%)
Aminoheterocycle	0.97	1.00	1.95	98.0	2.60
Azabicycle	–	1.00	0.97	97.0	2.02

repeated for six times, which demonstrated that this method was of good reproducibility.

To determine the linearity for aminoheterocycle and azabicycle at the carbon disc electrode in this method, a series of mixed standard solutions of the two analytes were tested. The linear ranges, regression equations, correlation coefficients and detection limits are listed in Table 1. Good linear relationships of the two analytes were obtained in the concentration range from 10^{-6} to 10^{-3} M. Their detection limits were 1.0×10^{-7} M and 2.0×10^{-7} M, respectively, (S/N = 3).

3.5. Sample analysis and recovery

Under the optimum conditions, the determination of aminoheterocycle and azabicycle in gliclazide bulk drug was carried out according to the procedures described above. Typical electropherogram of sample is shown in Fig. 4. The contents of aim analytes were determined by standard addition method. The content of aminoheterocycle in gliclazide bulk drug was calculated as 0.17‰, and that of azabicycle was not found.

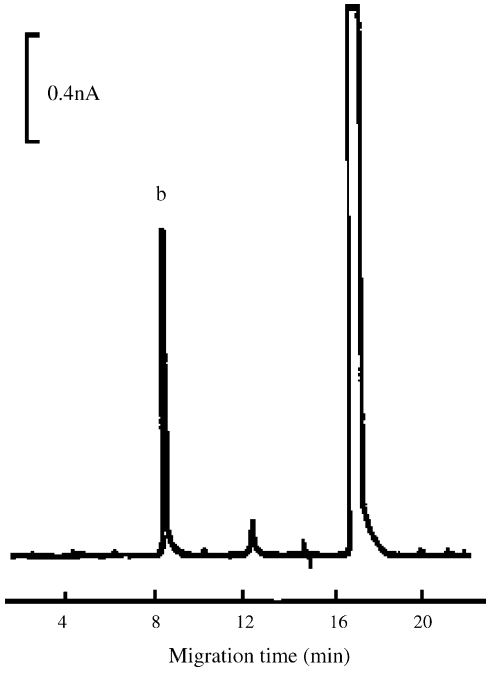


Fig. 4. Electropherograms of gliclazide bulk sample solution under the optimal CZE-AD conditions: b, aminoheterocycle.

Recovery test was performed in this experiment too. Accurate amounts of aminoheterocycle and azabicycle were added into the sample solution. The detection results of sample and the recoveries ($n = 5$) are listed in Table 2. Experimental results indicate that this method is accurate, sensitive, reproducible, and feasible for determining aminoheterocycle and azabicycle in gliclazide bulk drug.

4. Conclusions

Simultaneous determination of aminoheterocycle and azabicycle in gliclazide bulk drug by CZE-AD was performed in this experiment and the results showed that this method was of high separation efficiency, good selectivity, short analysis time, convenience of sample analysis. Under the optimum conditions, aminoheterocycle and azabicycle were separated completely within 9 min, and good linearity, reproducibility and recovery results were obtained. Their detection limits were 1.0×10^{-7} and 5.0×10^{-6} M, respectively. This method was successfully used to determine the aminoheterocycle and azabicycle in gliclazide bulk drug, and satisfactory assay results were obtained.

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