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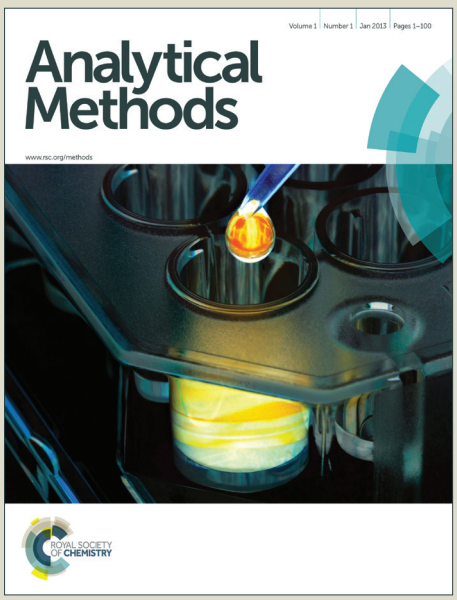

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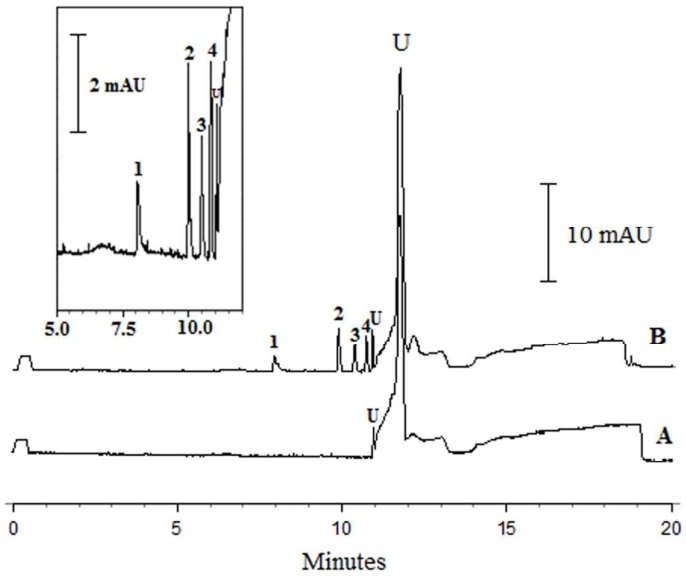

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Abstract

A new on-line concentration method of micelle to solvent stacking in capillary electrophoresis was developed for the determination of three antihistamine drugs (chlorpheniramine, diphenhydramine and promethazine) in human plasma. Palmatine was used as an internal standard. The main parameters that affect the separation and detection sensitivity of the method were investigated and optimized. Under the optimized conditions, the sensitivity enhancement factors obtained by the developed method for diphenhydramine, chlorpheniramine and promethazine were 63, 43 and 111, respectively. The method showed a good linearity in the range of 0.6-60.0 μg mL^{-1} for the three antihistamines with the correlation coefficients varying from 0.9971 to 0.9996. The limits of detection (S/N = 3) were 0.1-0.2 μg mL^{-1}. The relative standard deviations for intra-day (n = 8) and inter-day (n = 5) analysis were found to be less than 9.1%. The recoveries of the method for the analytes were in the range from 89.7% to 108.0%. The method is feasible for fast screening of the antihistamine drugs in plasma.

Keywords: Capillary electrophoresis; Micelle to solvent stacking; Antihistamine; Plasma

Introduction

The antihistamine drugs are mainly used to treat seasonal allergic rhinitis, urticaria, pruritus, and insect bites and stings caused by excessive release of histamine.[1] Diphenhydramine, chlorpheniramine and promethazine are widely used antihistamines, which act by physically blocking the H_1 receptors and stopping histamine from reaching its target. Therefore, they could help to reduce the troublesome symptoms associated with allergy.[2] However, the improper use of these antihistamines may cause severe sedative effects including low blood pressure and decompensation.[3] The intravenous use of promethazine can cause tissue necrosis and it is not recommended. Several cases of overdose with diphenhydramine have also been reported; its effects include seizures and Brugada syndrome, in both cases due to the blockade of type Ia sodium channels.[4] To ensure their safe use and for the study of their metabolism and pharmacokinetics, it is necessary to establish simple and efficient approaches for the determination of the antihistamine drugs in biological samples.

To date, many methods have been developed for the determination of antihistamines in biological samples such as gas chromatography,[5] liquid chromatography[6-11] and capillary electrophoresis (CE).[2,13,14] Among these techniques, CE is the simplest and it requires much smaller amount of both samples and organic solvents. However, unfortunately, due to the small inner diameter of the capillary and the small sample injection amount, the detection sensitivity of CE sometimes is not good enough for trace analysis especially for biological samples when the common UV detection was used. To overcome this disadvantage, the most facile way to improve the sensitivity of CE is on-line preconcentration.

Micelle to solvent stacking (MSS), which was first introduced by Quirino in 2009,[12] is a novel

on-line preconcentration technique for CE. The analyte focusing was based on the reversal of the direction of the effective electrophoretic mobility of the analytes at micelle to solvent stacking boundary (MSSB) formed between the sample solution and background solution (BGS). MSS is not only efficient, rapid and easy to operate, but also can be used in different separation modes including capillary zone electrophoresis (CZE), micellar electrokinetic chromatography and non-aqueous capillary electrophoresis.[13] Moreover, it can be applied for the concentration of some analytes from complex matrix samples.[14-18]

In this study, a simple, efficient and sensitive method for the quantitative determination of the antihistamines (chlorpheniramine, diphenhydramine and promethazine) in human plasma sample was developed via on-line concentration of the analytes by MSS in CZE.

Experimental

Apparatus

All CE experiments were performed on a Beckman P/ACE MDQ CE System (Fullerton, CA, USA) equipped with an auto sampler and a diode array detector. An uncoated fused-silica capillary (Yongnian Ruifeng Optical Fiber Factory, Hebei, China) of 50 cm (effective length, 41.5 cm) × 50 μm i.d. was used throughout the experiments. Data acquisition and instrument control were carried out using Beckman P/ACE MDQ 32 Karat software. The separation was performed at 25 ℃ with a voltage of 20 kV being applied. Unless otherwise stated, the detection wavelength was set at 200 nm. The conductivities were measured using a Delta 326 conductivity meter (Mettler Toledo, Shanghai, China).

Reagents, chemicals and materials

Chlorpheniramine maleate, diphenhydramine hydrochloride, promethazine hydrochloride and palmatine chloride (Internal standard, IS) (all >99%) were purchased from Chinese National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Sodium dodecyl sulfate (SDS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium dihydrogen phosphate (NaH_2PO_4), sodium hydroxide (NaOH), hydrochloric acid (HCl), methanol (MeOH) and acetonitrile were the products of Kaitong Chemical Reagent Co., Ltd. (Tianjin, China). MeOH and acetonitrile were of HPLC grade and other reagents were of analytical grade. All the reagents were used without further purification. All the solvents were filtered through a 0.45 μm Micro Science membrane filter (Tianjin Automatic Science Instrument Co., Ltd. Tianjin, China). The water used throughout the work was double-distilled using SZ-93 automatic double-distiller (Shanghai Yarong Biochemistry Instrumental Factory, Shanghai, China). Blank human plasma was obtained from the Baoding Blood Donor Service (Baoding, China).

A mixture stock solution containing each of chlorpheniramine maleate, diphenhydramine hydrochloride, promethazine hydrochloride at 1.0 mg mL^{-1} and a stock solution of palmatine chloride at 1.0 mg mL^{-1} were prepared in acetonitrile and stored in refrigerator at 4 ℃. The BGS was prepared fresh every day and sonicated for 10 min prior to use.

Preparation of samples

For spiked plasma sample, a 100 μL aliquot of blank human plasma sample was mixed with 20 μL of an appropriate concentration of the mixture solution and 20 μL of 20 μg mL^{-1} IS solution in a 1.5 mL centrifugal tube. Then, 160 μL acetonitrile was added and the mixture was vortexed for 1

min. After the sample was centrifuged at 10 000 rpm for 10 min, the acetonitrile phase was separated, and then 100 μL of the supernatant was transferred to a 0.5 mL vial and evaporated to dryness under a nitrogen stream. The residue was reconstituted in 100 μL of sample matrix (10 mM NaH_2PO_4 containing 10 mM SDS, pH 7.5) for CE analysis.

For human plasma samples, a 100 μL aliquot of human plasma sample was transferred to a 1.5 mL centrifugal tube, to which 20 μL of 20 μg mL^{-1} IS solution and 180 μL acetonitrile was added. Then, the sample was handled in the same way as described above for the spiked plasma sample.

General electrophoresis procedure

Prior to first use, the new capillary was flushed at 20 psi with MeOH for 20 min, H_2O for 10 min, 1.0 M NaOH for 20 min, H_2O for 10 min and finally with BGS for 10 min. At the beginning of each working day, the capillary was conditioned at 20 psi with 0.1 M HCl for 5 min, H_2O for 5 min and then with BGS for 5 min. Between consecutive runs, the capillary was flushed with 1.0 M HCl for 5 min, then with H_2O for 3 min and finally with the BGS for 3 min.

For MSS, the analytes were prepared in sample matrix (10 mM NaH_2PO_4 containing 10 mM SDS at pH 7.5). The BGS was 30 mM NaH_2PO_4 (pH 7.5) containing 55% MeOH (v/v). The conductivities of the solutions were measured and the ratio of the conductivity between the BGS and sample matrix was 1.2 : 1. The sample solution was introduced into the capillary by hydrodynamic injection at 0.5 psi for 60 s. The ratios of the corrected peak areas (peak area/migration time) between the analytes and the IS were used as quantification signals.

Results and discussion

Effect of the pH of BGS

The electroosmotic flow (EOF) is proportional to the ζ (zeta) potential of the capillary wall. It has been known that the EOF increases with the increase of the BGS pH due to the increase in the ζ potential in CZE.[19] Therefore, pH will influence the migration time and separation efficiency of the analytes. In this study, the effect of the pH of BGS was evaluated in the range between 7.0 and 9.0. As a result, a complete separation of all the compounds was achieved at pH 7.5. Therefore, the pH at 7.5 for the BGS was chosen.

Effect of organic solvent content

The concentration of MeOH in BGS is critical for MSS process. A sufficient MeOH in BGS will decrease the affinity between micelles and analytes, and then cause the reversal of effective electrophoretic mobility of the analytes in MSS. Otherwise, MSS will not occur. In this study, the 30 mM NaH_2PO_4 buffers containing 40%, 45%, 50%, 55% or 60% (v/v) of MeOH, respectively, were tested to evaluate the influence of the organic solvent content on the separation. As a result, the maximum peak height was found when the concentration of MeOH was 55% in the BGS. In the experiments, the BGS containing different concentrations of acetonitrile was also investigated and the results indicated that the analytes could not be separated completely in such cases. Therefore, 55% MeOH was adopted.

Effect of the concentration of NaH_2PO_4 in BGS

The effect of the concentration of NaH_2PO_4 in BGS was investigated by changing its concentration in the range from 10 to 50 mM (pH 7.5) while the MeOH concentration was kept

constant at 55%. Consequently, as the concentration of NaH_2PO_4 was increased from 10 to 30 mM, the resolution for all the analytes was improved. However, when the concentration of NaH_2PO_4 was further increased to 40 mM or 50 mM, the peaks for the analytes became broadening with peak heights being decreased slightly. Based on the above results, 30 mM NaH_2PO_4 in BGS was selected.

Effect of the concentration of SDS in sample matrix

For MSS to occur, the concentration of SDS should be higher than its critical micelle concentration to form micelles. However, the concentration should not be too high. Otherwise, the affinity between analytes and micelles would be too strong to reverse the effective electrophoretic mobility of the analytes at MSSB, which should result in the failure of the MSS. To explore the effect of the SDS concentration in sample matrix on the resolution and focusing efficiency of the analytes, different concentrations of SDS (2.0 - 20 mM) were prepared in 10 mM NaH_2PO_4 (pH 7.5) and used as the sample matrix for evaluation. As a result, both the peak heights and peak areas for all the three analytes were increased with increased concentration of SDS from 2.0 to 10 mM. And then, when the concentration of SDS was changed from 10 to 20 mM, the peak height and peak area for promethazine decreased slightly while the peak heights and peak areas for the other two analytes remained almost unchanged. Based on the above results, 10 mM SDS was chosen for further studies.

Injection of sample solution

The effect of sample injection was studied by hydrodynamic injection at 0.5 psi with varying injection times (15, 30, 45, 60 and 75 s). The BGS consisted of 30 mM NaH_2PO_4 (pH 7.5) containing 55% MeOH. The sample matrix was 10 mM NaH_2PO_4 containing 10 mM SDS. When

the injection time was increased from 15 s to 60 s, the peak heights for the analytes were increased; however, when the sample was injected at 0.5 psi for 75 s, the peaks became broader and the separation between chlorpheniramine and promethazine became incomplete. Therefore, the injection of sample at 0.5 psi for 60 s was selected.

Method validation

In order to validate the applicability of the developed method for the determination of the antihistamines in plasma samples, the features of this method in terms of linear range, repeatability and limits of detection (LODs) were evaluated. Calibration curves for antihistamines were investigated over the concentration range of 0.6-60 μg mL^{-1} in human plasma samples with the analyte concentration points at 0.6, 1.2, 3.0, 6.0, 12.0, 30.0 and 60.0 μg mL^{-1} using 4.0 μg mL^{-1} palmatine as the IS. For each concentration level, five replicate determinations were performed under the optimum experimental conditions. The ratios of corrected peak area (peak area/migration time) of the analytes to that of the IS were used as quantification signals. As a result, within the concentration range investigated, a good linearity existed for all the analytes with the correlation coefficients (r) between 0.9971 and 0.9996. The LODs were calculated as the concentrations corresponding to the signal three times of the baseline noise and the LODs of the antihistamines in human plasma samples were estimated to be in the range of 0.1-0.2 μg mL^{-1}.

The precisions of the developed method were evaluated in terms of intra-day and inter-day variations by the analysis of the spiked samples at the concentration of 0.6 μg mL^{-1} in human plasma sample in the same day and on the five consecutive days, respectively. As shown in Table 1, the intra- and inter-day RSDs were lower than 7.5% and 9.1%, respectively, indicating a good precision of the method.

The focusing efficiency of the method was assessed by the comparison of its performance with that of normal CZE, as illustrated in Fig. 1. The mixture solution containing three antihistamines at 150.0 μg mL^{-1} and IS at 40.0 μg mL^{-1} was prepared in BGS. Then, it was injected into the capillary at 0.5 psi for 3 s in normal CZE. For the current method, mixture solution containing each of the analytes at 2.0 μg mL^{-1} and the IS at 4.0 μg mL^{-1} prepared in sample matrix was determined under the optimum conditions. The sensitivity enhancement factors (SEFs) in terms of peak height were calculated according to the following equation:[20]

$$\text{SEF} = \frac{\text{quantification signal in the current method}}{\text{quantification signal in normal CZE}} \times \text{dilution ratio} \quad (1)$$

According to the above equation (1), compared with the conventional CZE injection procedure, the SEFs in terms of peak height were 63, 43 and 111 for diphenhydramine, chlorpheniramine and promethazine, respectively.

The recoveries of the method were evaluated by analyzing the spiked human plasma samples at 2.0, 7.5 and 15.0 μg mL^{-1} each of the three antihistamines and at 4.0 μg mL^{-1} of the IS. Fig. 2 shows the electropherograms obtained by MSS-CZE for both the blank human plasma sample and the spiked human plasma sample with each antihistamine at 2.0 μg mL^{-1}. The results showed that no significant interferences from the matrix were observed for the quantification of the analytes. As listed in Table 2, the recoveries of the method for diphenhydramine, chlorpheniramine and promethazine in human plasma samples were 92.5-107.2%, 89.7-102.7% and 100.7-108.0%, respectively. The precisions expressed as RSDs (n = 5) for the determination of the three antihistamines were less than 7.6%.

Comparison of the current method with other techniques

To evaluate the performance of the current method, a comparison of the current method with

other reported relevant methods in respect of linearity, LODs and RSDs was made. As listed in Table 3, in comparison with the reported methods used for the determination of antihistamines, the sensitivity of the present method was comparable or even better than LC[6,10] and CE[21] methods,but was inferior to GC-MS[5] and LC-MS[10] methods. However, more expensive MS detectors were required in those cases.

Conclusions

In this work, a simple new method has been developed for the analysis of three antihistamines by using the novel CE on-line preconcentration technique MSS in CZE mode. The results demonstrated that the current method has high enrichment factor, good precision and sufficient sensitivity with a short analysis time for the analysis of the antihistamines in human plasma sample.

Acknowledgements

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Table Captions

Table 1 The performance characteristics of the method

Table 2 Recoveries of the method for the determination of the three antihistamines in spiked human plasma samples

Table 3. Comparison of the current method with other reported methods for the determination of antihistamines

Figure Captions

Fig. 1. Comparison of the electropherograms obtained by normal CZE (A) and MSS-CZE (B). (A) Normal CZE: sample was 150 μg mL^{-1} each of the analytes and 40 μg mL^{-1} IS prepared in BGS; injection at 0.5 psi for 3 s. (B) MSS-CZE: sample was 2.0 μg mL^{-1} each of the antihistamines and 4.0 μg mL^{-1} IS. Detection: 200 nm; Voltage: 20.0 kV; Peak identification: 1 – palmatine (IS), 2 – diphenhydramine, 3 – chlorpheniramine, 4 – promethazine, U – unknown.

Fig. 2. Electropherograms of blank human plasma (A) and human plasma spiked at 2.0 μg mL^{-1} each of the antihistamines and 4.0 μg mL^{-1} IS (B). The insert was the zoomed electropherogram of the main part of the electropherogram B. Peak identifications are the same as those in Fig. 1.

Table 1

The performance characteristics of the method

	Diphenhydramine	Chlorpheniramine	Promethazine
Range (μg mL^{-1})	0.6-60.0	0.6-60.0	0.6-60.0
r	0.9996	0.9996	0.9971
LOD (μg mL^{-1})	0.1	0.2	0.2
Intra-day RSD[a] (*n* = 8) (%)	6.8	7.1	7.5
Inter-day RSD[a] (*n* = 5) (%)	8.2	8.1	9.1
SEF	63	43	111

[a] The concentration of three antihistamines were 0.6 μg mL^{-1}.

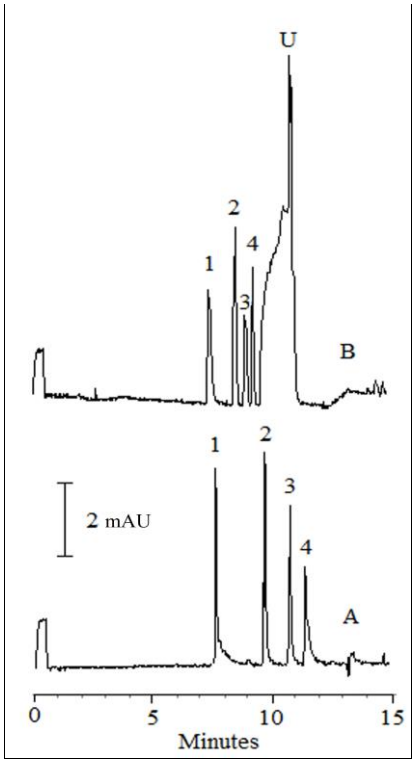
Table 2

Recoveries of the method for the determination of the three antihistamines in spiked human plasma samples

	Spiked (μg mL^{-1})	Found (μg mL^{-1})	Recovery (%)	RSD (%)
Diphenhydramine	15.0	15.6	104.0	2.3
	7.5	8.04	107.2	4.3
	2.0	1.85	92.5	7.6
Chlorpheniramine	15.0	15.4	102.7	2.8
	7.5	7.95	101.2	3.8
	2.0	1.44	89.7	4.6
Promethazine	15.0	15.1	100.7	3.2
	7.5	8.10	108.0	4.2
	2.0	2.08	104.0	5.3

Table 3. Comparison of the current method with other reported methods for the determination of antihistamines

Method	Sample	Linearity ($\mu g\ mL^{-1}$)	LODs ($\mu g\ mL^{-1}$)	RSDs (%)	Ref
GC-MS	Human plasma		0.0002-0.05	9.9	5
HPLC	Pharmaceutical preparation	2-160	0.12		6
HPLC-MS	Human plasma	0.0005-0.2	0.0001	< 12.92	10
CE	Serum		3-28	< 3	21
Sweeping-MSS-CE	Human plasma	0.6-60.0	0.1-0.2	2.3-7.6	This work



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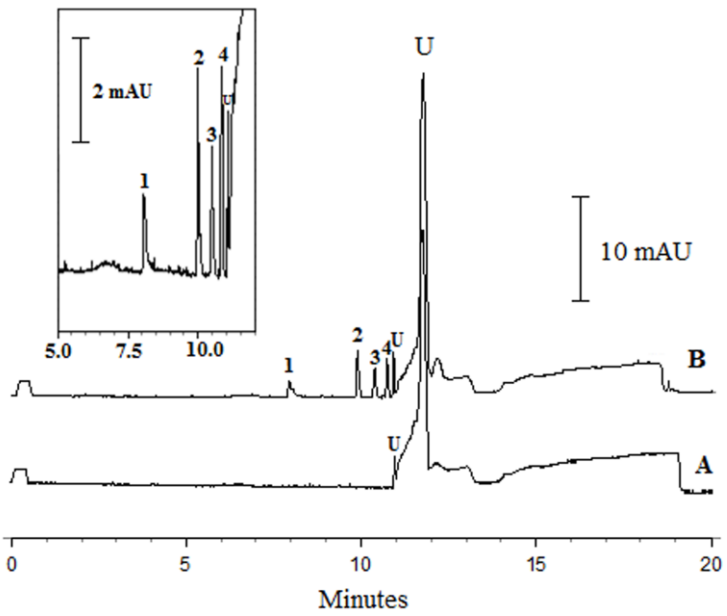

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