Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 285–289
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Separation and determination of pseudoephedrine, dextromethorphan, diphenhydramine and chlorpheniramine in cold medicines by nonaqueous capillary electrophoresis

Yuming Dong [a,b], Xiaofeng Chen [a], Yonglei Chen [a], Xingguo Chen [a,*], Zhide Hu [a]

[a] *Department of Chemistry, Lanzhou University, Lanzhou 730000, PR China*
[b] *Department of Pharmacy, Lanzhou University, Lanzhou 730000, PR China*

Received 7 January 2005; received in revised form 15 February 2005; accepted 25 February 2005
Available online 2 April 2005

Abstract

An easy, rapid and simple nonaqueous capillary electrophoresis (NACE) method was developed for the identification and determination of four basic nitrogenous compounds, i.e. pseudoephedrine (PE), dextromethorphan (DXM), diphenhydramine (DHM) and chlorpheniramine (CLP). The most suitable running buffer was composed of 40 mM ammonium acetate, 10% acetonitrile (ACN) in methanol with a fused-silica capillary column (47 cm × 75 μm i.d.), 25 kV applied voltage and 25 °C capillary temperature. The calibration curves revealed linear relationships between the peak area for each analyte and its concentration (correlation coefficients: 0.9993 for PE, 0.9971 for DXM, 0.9991 for DHM, and 0.9995 for CLP, respectively). The relative standard deviations of the migration time and peak area of the four compounds were 0.37, 3.90, 0.73 and 0.68, and 2.80, 3.50, 1.60 and 3.70%, respectively. The method was successfully applied to determine the four compounds in five cold medicines, the recoveries of the four constituents ranging between 91 and 109%.

Keywords: Nonaqueous capillary electrophoresis; Pseudoephedrine; Dextromethorphan; Diphenhydramine; Chlorpheniramine

1. Introduction

Pseudoephedrine (PE) is one of the major bioactive components in the Ephedra herb that is a commonly used Chinese traditional medicine intended for diaphoretic purposes [1]. It is an adrenergic agent with the chemical name [*S*-(*R**, *R**)]-α-[1-(methylamino)ethyl]-benzenmethanol. Dextromethorphan (DXM) ((+)-3-methoxy-17-methyl-(9α,13α,14α)-morphinan) is an antitussive drug. Diphenhydramine (DHM) (2-(diphenylmethoxy)-*N*,*N*-dimethylethylamine) is a sedating antihistamine. Chlorpheniramine (CLP) (2-pyridinepropanamine, γ-(4-chlorophenyl)-*N*,*N*-dimethyl, (*Z*)-2-butenedioate) is an antihistamine and is widely used as an ingredient in antitussive preparations [2]. These four compounds are the pharmacologically active constituents found in most cold medicines.

All of the analytes are basic nitrogenous compounds and the separation and determination of these compounds in dosage forms is complicated due to their similarities in some physical and chemical properties [3]. High performance liquid chromatography (HPLC) is the most used technique for the quantification of the four compounds in preparations and plasma [4–12], but owing to the basic properties of these compounds they strongly interact with the stationary phase of the HPLC column. Thus the use of HPLC is restricted by long analysis times, peak asymmetry and poor efficiency [13,14]. Difference spectrophotometric methods [15,16], proton nuclear magnetic resonance (NMR) [17] and gas–liquid chromatography [18] have also been reported for the determi-

Abbreviations: NACE, nonaqueous capillary electrophoresis; PE, pseudoephedrine; DXM, dextromethorphan; DHM, diphenhydramine; CLP, chlorpheniramine

* Corresponding author. Tel.: +86 931 8912763; fax: +86 931 8912582.
E-mail addresses: dongym03@st.lzu.edu.cn (Y. Dong), chenxg@lzu.edu.cn (X. Chen).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.02.032

nation of these analytes. These methods usually require extraction and complicated pretreatment procedures prior to analysis.

PE has been separated by capillary zone electrophoresis (CZE) and detected with laser-induced fluorescence [14]. This method was very sensitive, but the sample needed to be derivatised. Though CLP and DXM have been separated by micellar electrokinetic chromatography (MEKC) [19] and CZE [3], their quantification was not investigated. DHM has also been separated and determined by MEKC and CZE [20]. These methods used aqueous background buffers but the peaks were not very symmetrical and the separation times were long. However, there is no report on a nonaqueous capillary electrophoresis (NACE) separation and determination of this combination.

Here a NACE method was developed for the identification and determination of pseudoephedrine (PE), dextromethorphan (DXM), diphenhydramine (DHM) and chlorpheniramine (CLP) in cold medicines.

2. Materials and methods

2.1. Instruments

The nonaqueous capillary electrophoresis experiments were performed on a P/ACE5510 system (Beckman Coulter Instrument, Fullerton, CA, USA) equipped with a P/ACE diode-array detector. The system was controlled by a P/ACE station software. The separation was carried out on a 47 cm (40 cm to the detector) × 75 μm i.d. fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China) house in a cartridge with a 800 μm × 200 μm detector window. Samples were introduced by applying a pressure of 0.5 psi for 2 s. The capillary was conditioned with 0.1 M NaOH for 10 min and distilled water for 5 min, followed by the electrophoresis buffer for 5 min, prior to use. After each run the capillary was rinsed with 0.1 M NaOH for 2 min, distilled water for 2 min and then the running buffer for 2 min. The buffer was renewed after every two runs to ensure good reproducibility. The capillary was maintained at 25 °C. To avoid buffer and sample evaporation, the buffer and sample reservoir must have lids.

2.2. Reagents

Standard pseudoephedrine hydrochloride, dextromethorphan hydrobromide, diphenhydramine hydrochloride and chlorpheniramine maleate were obtained from the National Institute for the Control of Pharmaceutical and Bio-products of China (Beijing, China). Ammonium acetate and acetic acid were purchased from Tianjin First Chemical Factory. Acetonitrile was purchased from Tianjing Secondary Chemical Factory. Methanol was purchased from Shanghai Zhenxing First Chemical Factory. Cold medicines were purchased from Zhongyou Pharmaceutical Store, Lanzhou, China. All reagents were of analytical grade, unless otherwise specified. Stock solutions of PE (520 mg/L), DXM (160 mg/L), DHM (420 mg/L) and CLP (300 mg/L) were prepared in methanol. Analytical solutions were prepared from them by appropriate dilution with the run buffer. The run buffer was prepared from 5 mL of 0.2 mol/L ammonium acetate (methanol medium) and 2.5 mL of acetonitrile in a 25 mL flask and diluted to 25 mL with methanol. Prior to use, all solutions and run buffers were degassed by ultrasound for 10 min and filtered through a 0.45 μm membrane filter.

2.3. Sample preparation

Ten tablets of Fufang Ganmaoling tablets, black tablets (*Hei Pian*) and white tablets (*Bai Pian*) of compound pseudoephedrine HCl tablets (*Bai Jia Hei*), compound paracetamol and amantadine HCl tablets (*Gankang*) were weighed exactly and the average weights of tablets were calculated, then, powdered respectively, and 0.8000, 0.5000, 0.5000 and 1.000 g powders were weighed exactly and extracted with 10 mL methanol in an ultrasonic bath for 1 h, respectively. Ten capsules of compound pseudoephedrine HCl sustained capsules (*Xin Kangtaike*) were weighed exactly and the average weight of capsules was calculated, then, opened and 0.2000 g powder was weighed and extracted with 10 mL methanol in an ultrasonic bath for 1 h. Prior to analysis, all of the extracts were filtered through a 0.45 μm membrane filter, 2.0 fold diluted with run buffer and the resulting solutions were prepared for analysis.

3. Results and discussion

To achieve good sensitivity and satisfactory separations the optimization of separation conditions was of primary importance. The first investigations were carried out using 40 mM ammonium acetate and 10% (v/v) ACN buffer with methanol as solvents.

3.1. Effect of concentration of ammonium acetate

Buffer concentration markedly affects the separation because it can influence the EOF and the viscosity of electrolyte. In order to obtain the best resolution of the four analytes, the effect of the concentration of ammonium acetate was investigated in the range from 20 to 100 mM with 10% (v/v) ACN and 25 kV applied voltage. The migration time of the four analytes increased with the ammonium acetate concentration increasing. This phenomenon may be attributed to the higher Joule heating caused by conductivity of the buffer increasing and the EOF decreases with increasing ion strength (decrease of zeta-potential). Increases in migration times as well as current were observed when the concentration of buffer increased. Resolution also increased for higher concentration. When the ammonium acetate concentration was 40 mM, their detection sensitivity and resolution were optimum.

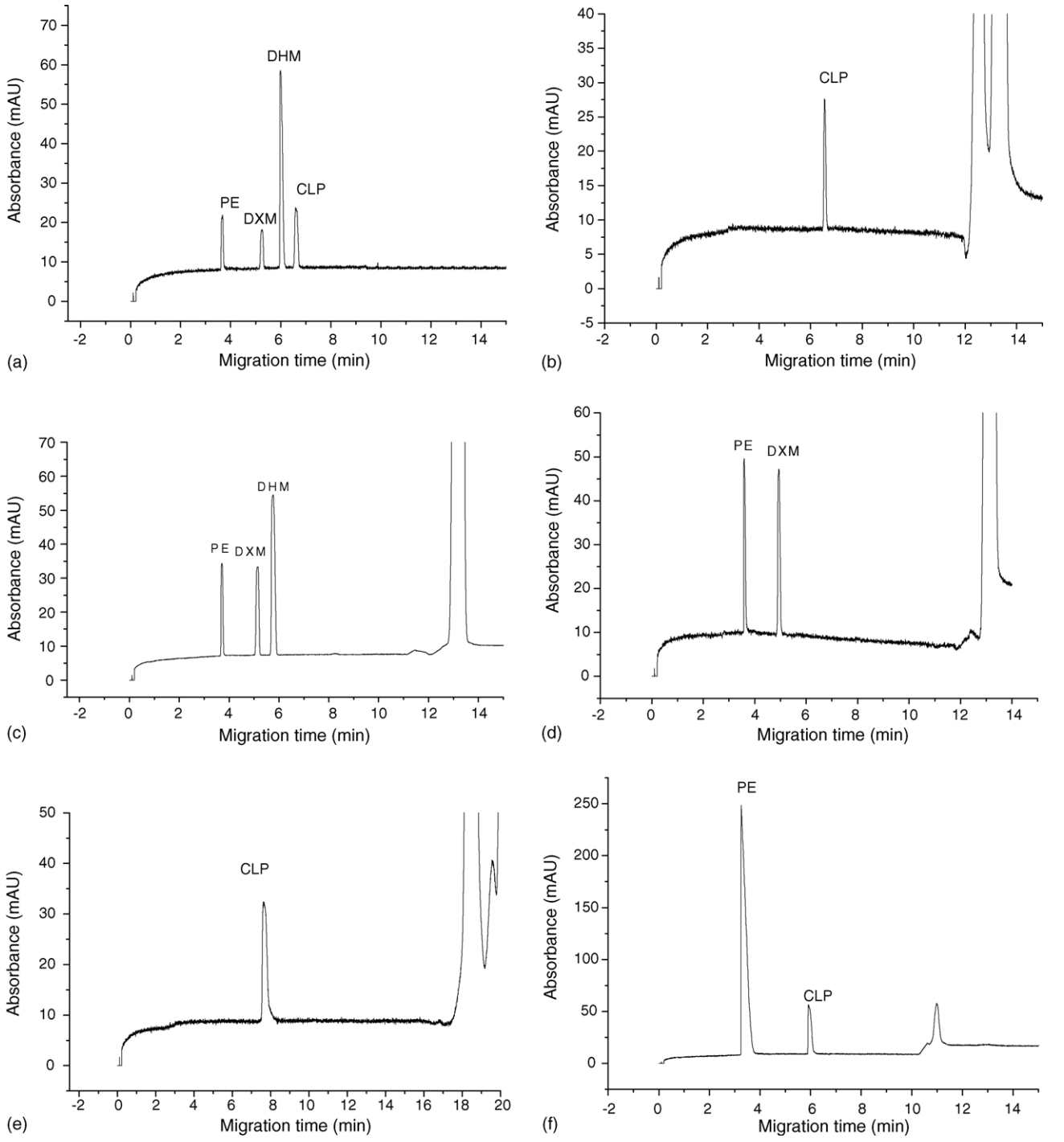

Fig. 1. Electropherograms of the standard mixture solution and the real samples: (a) standard mixture; (b) Fufang Ganmaoling tablets; (c) black tablets (*Hei Pian*) and (d) white tablets (*Bai Pian*) of compound pseudoephedrine HCl tablets (*Bai Jia Hei*); (e) compound paracetamol and amantadine HCl tablets (*Gankang*); (f) capsules of compound pseudoephedrine HCl sustained capsules (*Xin Kangtaike*). Buffer, 40 mM ammonium acetate, 10% ACN; capillary, 47 cm (40 cm to the detector) × 75 μm ID; applied voltage, 25 kV; cartridge temperature, 25 °C; detection, 214 nm.

3.2. Effect of ACN concentration

The migration times of the four analytes considerably decreased when the ACN percentage in buffer was increased. This behavior is mainly due to the modification of the dielectric constant-to-viscosity ratio. When the concentration of ACN was 10% (v/v), the detection sensitivity of the four compounds was highest. Considering the total analysis time and peak area, 10% (v/v) ACN was chosen as the optimum.

Table 1
Results of regression analysis on calibration curves and detection limits

Analyte	Regression equation $Y = a + bc$[a]	Correlation coefficient	Linear range (mg/L)	Detection limit (mg/L)[b]
PE	$Y = 30360.21 + 888.65c$	0.9993	8.0–520	1.94
DXM	$Y = 31216.37 + 2519.95c$	0.9971	2.5–160	0.98
DHM	$Y = 103065.58 + 4609.03c$	0.9991	6.6–420	0.66
CLP	$Y = 55355.31 + 2475.35c$	0.9995	4.5–300	1.19

[a] Y and c stands for the peak area and the concentration (mg/L) of the analytes, respectively.
[b] The detection limit was defined as the concentration where the signal-to-noise ratio is 3.

3.3. Influence of applied voltage and capillary temperature

It was found with the applied voltage ranging from 15 to 27.5 kV that the migration time decreased with increasing applied voltage and the curve reveals higher linear relationships between the run current and applied voltage. The breakdown of electric current occurred sometimes during the experiments when the voltage was high than 27.5 kV. Therefore, 25 kV was selected for relatively good separation of four compounds with shorter analysis time and acceptable electric current. The influence of capillary temperature was also studied with optimized electrophoretic medium between 15 and 27.5 °C. When the temperature was higher than 25 °C,

Table 2
Determination results of the analytes in the samples ($n = 3$)

Samples	Compounds	Found	Labeled	Relative error (%)
Fufang Ganmaoling tablets	CLP	0.59 mg/tablet	–	–
Black tablet of compound pseudoephedrine HCl tablets	PE	29.98 mg/tablet	30.00 mg/tablet	−0.07
	DXM	15.08 mg/tablet	15.00 mg/tablet	0.53
	DHM	23.04 mg/tablet	25.00 mg/tablet	−7.84
White tablet of compound pseudoephedrine HCl tablets	PE	29.46 mg/tablet	30.00 mg/tablet	−1.80
	DXM	14.45 mg/tablet	15.00 mg/tablet	−3.67
Compound paracetamol and amantadine HCl tablets	CLP	1.95 mg/tablet	2.00 mg/tablet	−2.50
Compound pseudoephedrine HCl sustained capsules	PE	93.67 mg/capsule	90.00 mg/capsule	4.08
	CLP	3.82 mg/capsule	4.00 mg/capsule	−4.50

Table 3
Recovery of the four analytes ($n = 3$)

Samples	PE		DXM		DHM		CLP	
	Added (mg/L)	Recovery (%)	Added (mg/L)	Recovery (%)	Added (mg/L)	Recovery (%)	Added (mg/L)	Recovery (%)
Fufang Ganmaoling tablets	–	–	–	–	–	–	15	102
	–	–	–	–	–	–	30	105
	–	–	–	–	–	–	45	104
Black tablet of compound pseudoephedrine HCl tablets	90	95	45	91	60	106	–	–
	180	98	90	96	120	103	–	–
	270	97	135	93	180	105	–	–
White tablet of compound pseudoephedrine HCl tablets	90	92	45	101	–	–	–	–
	180	94	90	104	–	–	–	–
	270	98	135	109	–	–		
Compound paracetamol and amantadine HCl tablets	–	–	–	–	–	–	75	91
	–	–	–	–	–	–	150	96
	–	–	–	–	–	–	225	92
Compound pseudoephedrine HCl sustained capsules	260	107	–	–	–	–	150	97
	520	108	–	–	–	–	300	99
	780	105	–	–	–	–	450	101

breakdown of electric current was sometimes observed. So 25 °C was selected as the optimum.

A typical electropherogram for a standard mixture under the optimum conditions was shown in Fig. 1a. All four analytes were well separated within 10 min.

3.4. Linearity, reproducibility, and detection limits

The linear relationship between the concentration of the four analytes and the corresponding peak area were investigated under the optimum separation conditions. The results are shown in Table 1. The detection limits are also given in Table 1. The R.S.D. ($n = 5$) of the migration time and the peak area were 0.37 and 2.80% for PE, 3.90 and 3.50% for DXM, 0.73 and 1.60% for DHM, 0.68 and 3.70% for CLP, respectively.

3.5. Applications

The method was used for the analysis of PE, DXM, DHM and CLP in five cold medicines. The typical electropherograms for the separation of PE, DXM, DHM and CLP are illustrated in Fig. 1b–f, respectively. The peaks were identified by comparison of the migration times and spectra of the separated compounds and standards, and by spiking the standards to the sample solution. Each sample was extracted as described in Section 2.3 and measured by analyzing three times with the proposed method. The resulting contents of the four compounds in the sample are given in Table 2. The recovery of the method was determined with the standard addition method for PE, DXM, DHM and CLP in the five sample solutions, respectively. The results are shown in Table 3. The percent of the labeled amount of each compound is in the range of 90–110%.

4. Conclusions

The present paper described a highly convenient, rapid and accurate method for the separation and quantification of PE, DXM, DHM and CLP by NACE in five cold medicines for the first time. The results demonstrate that the method is also simple for the identification of analytes. In addition, using NACE, not only was the analysis time shortened, but also a high degree of peak symmetry was obtained. Therefore, the method can be applied to the quality control of cold medicines.

Acknowledgements

The project was supported by the National Natural Science Foundation of China (No. 20275014).

References

[1] New Medical College of Jiangsu, Dictionary of Chinese Traditional Medicine, People's Publisher of Shanghai, 1977, p. 2222.
[2] Z.Y. Zhou, S.X. Wang, New Handbook of Common Use Medicine, Gold Shield Publisher, 1987, p. 317.
[3] L. Suntornsuk, Electrophoresis 22 (2001) 139–143.
[4] V. Galli, C. Barbas, J. Chromatogr. A 1048 (2004) 207–211.
[5] L. Io-Wah, M.C. Shing, J. Chromatogr. A 693 (1995) 45–54.
[6] M.G. Agusti, L.M. Monferrer-Pons, M.C. García-Alvarei-Coque, J.E. Romero, Talanta 54 (2001) 621–630.
[7] O.W. Lau, K. Chan, Y.K. Lau, W.C. Worng, J. Pharm. Biomed. Anal. 7 (1989) 725–736.
[8] C.L. Webb, M.A. Eldon, Pharm. Res. 8 (1991) 1448–1451.
[9] S.B. Mahato, N.P. Sahu, S.K. Maitra, J. Chromatogr. 351 (1986) 580–584.
[10] H. Ge, Z. Zhou, X.J. Zhi, H. Wang, Yao Xue Xue Bao 39 (2004) 281–284.
[11] K. Selinger, J. Prevost, H.M. Hill, J. Chromatogr. 526 (1990) 597–602.
[12] M. Nieder, H. Jaeger, J. Chromatogr. 424 (1988) 73–82.
[13] K.D. Altria, M. Kelly, B.J. Clark, Trends Anal. Chem. 17 (1998) 204–226.
[14] J.Y. Zhang, J.P. Xie, X.G. Chen, Z.D. Hu, Analyst 128 (2003) 369–372.
[15] A.G. Davidson, L.M.M. Mkoji, J. Pharm. Biomed. Anal. 6 (1988) 449–460.
[16] M. Palabiyik, E. Dinc, F. Onur, J. Pharm. Biomed. Anal. 34 (2004) 473–483.
[17] G.M. Hanna, J. AOAC Int. 78 (1995) 946–954.
[18] L.Y. Lo, G. Land, A. Bye, J. Chromatogr. 222 (1981) 297–302.
[19] M.L. Riekkola, Electrophoresis 23 (2002) 3865–3883.
[20] M.R. Gomez, R.A. Olsina, L.D. Martnez, M.F. Silva, J. Pharm. Biomed. Anal. 30 (2002) 791–799.