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Determination of ephedrine, theophylline and phenobarbital in a tablet dosage form by capillary electrophoresis

Ahsanul Haque, Xiaohui Xu, James T. Stewart *

Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, GA 30602-2352, USA

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Abstract

A capillary electrophoresis method was developed to separate and quantitate ephedrine (ED), theophylline (TP) and phenobarbital (PB) in a tablet dosage form. Tablets were ground and extracted with methanol using ultrasonication. Aliquots of standard stock solution were hydrodynamically injected for 5 s at the anodic end. Separation was performed on a fused silica capillary (72 cm × 50 μm i.d.; 50 cm to detector) at an applied voltage of 20 kV with a phosphate run buffer (pH 8.0, 50 mM). Analysis was performed at ambient temperature (23 ± 1°C) and the total run time was 9 min with detection at 220 nm. Calibration curves were prepared for ED, TP and PB with methyl *p*-hydroxy benzoate as internal standard. For each analyte, the correlation coefficients were >0.999 ($n=4$). The RSD% of ten replicate injections for each analyte were $<1\%$. The method was applied to the quantitation of ED, TP and PB in a commercial tablet dosage form.

Keywords: Capillary electrophoresis; Tablet dosage form; Theophylline; Ephedrine; Phenobarbital

1. Introduction

A resolution passed by the United States Pharmacopeia Convention in Spring 1995 suggested that the Committee of Revision investigate reducing the amount of reagents and materials used in pharmaceutical tests and assays that have the potential to cause harm to human health and environment, such as a reduction in organic modifier usage in USP 23 assays. Currently, many USP 23 monographs include separate tests for drug substances and related compounds based on classic reversed phase HPLC methods where mobile phases contain significant amounts of organic solvents, and flow rates of 1–2 ml/min with retention times of 5–20 min are common. Capillary electrophoresis (CE) methods offer advantages over HPLC methods. CE is a highly efficient separation system which requires little or no organic modifier for a separation and, in most cases, CE sample preparation and analysis times are faster than HPLC. Investigators have shown that CE methods can generate comparable accuracy and precision to HPLC for pharmaceuticals [1–7].

* Corresponding author. Tel.: +1-706-5425770; fax: +1-706-5425358.

E-mail address: jstewart@rx.uga.edu (J.T. Stewart)

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The official USP 23 assay for a mixture of ephedrine (ED), theophylline (TP) and phenobarbital (PB) in a tablet dosage form uses an HPLC method in which sodium metaperiodate is used to oxidize ephedrine to benzaldehyde and chloroform is used as an aid in the preparation of the standard and assay preparations [8]. A MEKC method for mixtures of TP and selected medications which included ED and PB has also been reported [9]. Separations were achieved using a run buffer of 20 mM borate-phosphate buffer pH 9.2 containing 50 mM sodium dodecyl sulfate (SDS) at 10 kV. The various analyses were baseline resolved within 8 min, but the method was not applied to dosage forms.

There was an interest in these laboratories to develop a CE method for the ED-TP-TB mixture that could be used to determine drug content in a tablet dosage form and that would be comparable to the official HPLC method. The CE method would require little or no organic solvent either in standard or sample preparation, eliminate the ephedrine oxidation step and the need for organic modifier in the run buffer and yet possess acceptable accuracy and precision.

In this paper, a CE method is described for the analysis of a ED-TP-PB mixture using a fused silica capillary. The method uses no organic solvent in the run buffer and gives baseline separation of the three analytes within a 9 min run time. The method was applied to the analysis of ED-TP-PB in a commercial tablet dosage form.

2. Experimental

2.1. Instrumentation

Capillary electrophoresis was performed with an Applied Biosystems CE unit (ABI, Model 270A, San Jose, CA) equipped with a Hewlett Packard integrator (Model HP 3395, Palo alto, CA). The polyimide coating on the capillary was partially removed by burning at the point of detection and the uncovered portion of the capillary was aligned on the detector block. Separation of analytes was performed with a phosphate run buffer (0.05 M, pH 8) on a fused silica (72 cm × 50 mm i.d., 50 cm to detector) capillary with an applied voltage of 20 kV and detection at 220 nm. Separation was performed at ambient using 5 s hydrodynamic injections of analytes at the anodic end.

2.2. Regeneration of capillary and its maintenance

The fused silica capillary was regenerated with a wash solution of 1 M NaOH solution for 20 min. Then the capillary was washed with water for 20 min before been used for analysis. The procedure in Table 1 describes capillary care and wash cycles during analysis.

2.3. Reagents and chemicals

ED, TP, PB and methyl *p*-hydroxy benzoate (IS) were purchased from Sigma Chemical Company (St. Louis, MO). Tedral Tablets® (containing TP, ED hydrochloride and PB, Lot No. VBCEJA) manufactured by Warner Lambert (Morris Plains, NJ) were used in the study. Sodium hydroxide (electrophoresis grade) was purchased from Sigma Chemical for regeneration and washing of silica capillary. Disodium hydrogen phosphate was obtained from J T Baker (Philipsburgh, NJ). Fused silica capillaries were

Table 1
Washing cycles of capillaries

Wash cycles	NaOH (0.1 M), min	Water (min)	Run buffer (min)
Daily wash cycles before starting experiment	10	10	
Wash cycles before each injection	2		2
Daily wash cycles at the end of experiment	10	10	

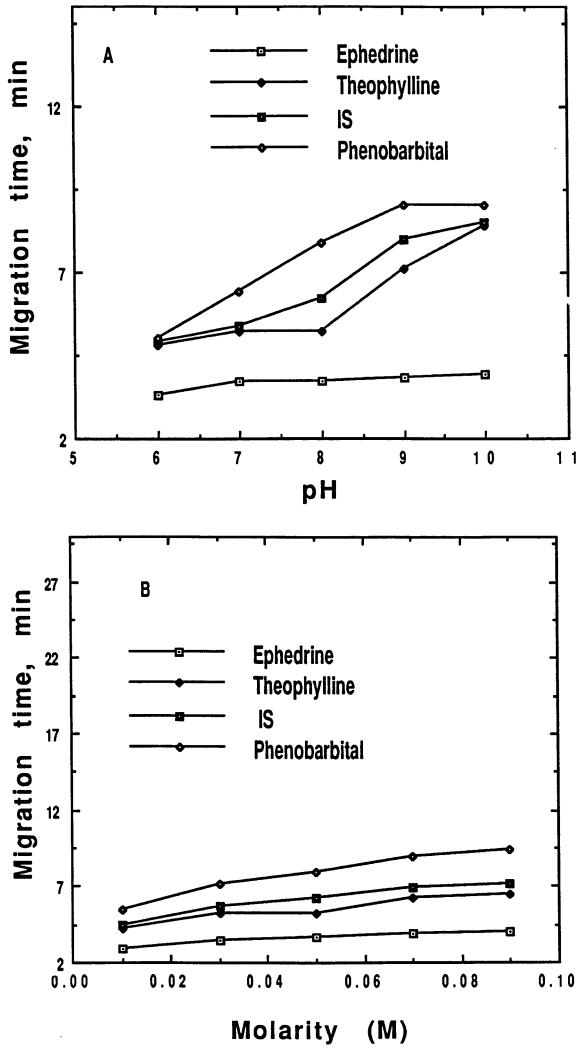

Fig. 1. (A) Plot showing the effect of run buffer pH on migration times of the analytes; (B) plot showing the effect of run buffer concentration on migration times of analytes; Conditions; see Section 2.

purchased from Polymicro Technologies (Phoenix, AZ). Filters (0.2 μm, Nylon) and syringe were purchased from Alltech Associates (Deerfield, IL) and Becton Dickinson (Franklin-Lake, NJ), respectively. All chemicals were of highest chemical grade obtainable in the market place. Fresh double distilled water was used for solution preparation.

2.4. Preparation of the standard stock and spiked sample solutions

A standard stock solution containing ED (960 μg/ml),TP (3.6 mg/ml) and PB (500 μg/ml) was prepared in absolute methanol with ultrasonication for 30 min. A stock solution of methyl *p*-hydroxy benzoate (1 mg/ml) internal standard was prepared in distilled water and stored in a refrigerator at 4°C. Various aliquots of the standard stock solution were taken, the internal standard added, and then diluted to 1 ml with distilled water to provide calibration ranges for ephedrine (38.4–76.8 μg/ml), theophylline (144 288 μg/ml), and phenobarbital (20–40 μg/ml). Linear regression analysis of peak area versus analyte concentration was performed to obtain slope, intercept and correlation coefficient for each analyte. All solutions were filtered (0.22 μm) prior to injection into the CE system. Spiked analyse samples at high and low concentrations within each calibration range were also prepared and were used to calculate accuracy and precision data for each analyte in the mixture.

2.5. Assay of tablet

Ten tablets were ground in a mortar with a pestle and the powder equivalent to one average tablet weight (450 mg) was taken and dissolved in 50 ml of absolute methanol with ultrasonication for 30 min and stirring. An aliquot (60 μl) of the tablet extract was added to 100 μl of internal standard and diluted to 1 ml with distilled water before injecting into the CE system. Peak areas of each analyte were used to determine the amount of each analyte in the tablet using the slope and intercept data generated from the linear regression analysis of calibration data.

3. Results and discussion

3.1. Method development

In a CE separation, pH and buffer concentrations of the run buffer play important roles. Initially, the effect of pH on the separation of ED,

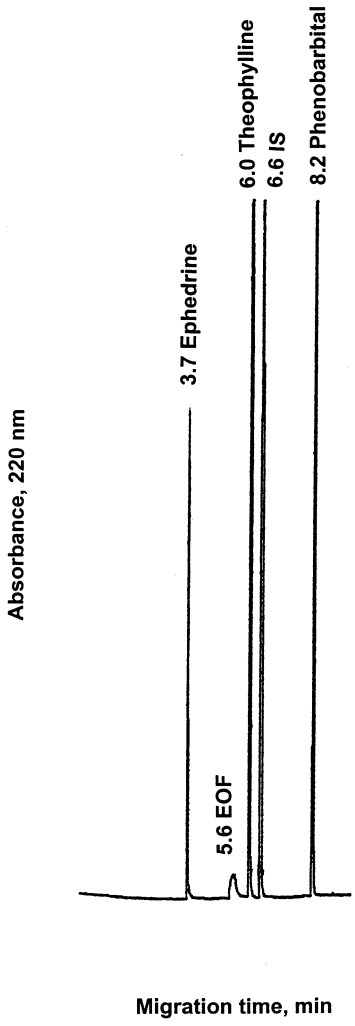

Fig. 2. Typical electropherogram showing the separation of a mixture containing ephedrine, theophylline, phenobarbital and methyl *p*-hydroxy benzoate IS; Conditions: Fused silica capillary (72 cm × 50 μm i.d.); 20 kV; detection − 220 nm; phosphate run buffer (pH 8.0, 50 mM); hydrodynamic injection-5 s, ambient temperature (23 ± 1°C).

TP, PB and IS was studied. Fig. 1(A) shows the plot of migration times of the analytes versus pH. It was observed that at pH 7 or below, overlapping of the analyte peaks occurred. At pH 9 or above, although analytes remained separated, band broadening of the theophylline peak was observed. A run buffer of pH 8 was found to be most suitable for baseline resolution of components. At pH 8, the effect of buffer concentration (0.01–0.09 M) on the separation was also studied (Fig. 1B). The run buffer with a concentration > 0.05 M provided sharper peak shapes and resolution whereas lower buffer concentrations caused peak tailing and overlapping. Finally, a phosphate run buffer of pH 8 (0.05 M) was selected for the analysis of the drug mixture. The separation was performed on a fused silica capillary with an applied potential of 20 kV and detection at 220 nm. Potentials lower than 20 kV increased migration times but maintained resolution. Hydrodynamic injections were made at 5 s. Fig. 2 shows a typical electropherogram of the analytes at ambient temperature. Methyl *p*-hydroxy benzoate, PB and TP remained as anions at the pH of the run buffer and hence, showed longer migration times than ED. ED moved as a cation at the pH of the run buffer and thus migrated before the EOF (See Fig. 2).

3.2. Quantification

Development of precise and repeatable CE assays requires control of a number of factors which include buffer composition and molarity, pH, applied voltage, temperature, and sample concentration [1–5]. The washing cycle of capillaries and their care also contribute to the repeatability of results (See Table 1).

Table 2
Typical linear regression data for the analysis of ED, TP and PB in a spiked mixture

Analyte	Concentration range (μg/ml)	r^2 ($n = 4$)	Slope	Intercept	LOD ($s/n > 3$) (μg/ml)
Ephedrine	38.4–76.8	0.999	0.002	−0.025	2
Theophylline	144–288	0.999	0.019	−0.387	5
Phenobarbital	20–40	0.999	0.018	−0.082	0.5

Table 3
Intra- and inter-day accuracy and precision

Analyte	Concentration added (μg/ml)	Concentration found[a] (μg/ml)	RSD (%)	Error (%)
Ephedrine				
Intra-day[b]	43.2	44.3 ± 1.38	3.11	2.54
	67.2	68.0 ± 2.11	3.10	1.19
Inter-day[c]	43.2	43.8 + 1.42	3.24	1.38
	67.2	67.7 ± 2.17	3.12	0.74
Theophylline				
Intra-day[b]	162	158.8 ± 0.84	0.53	1.98
	252	253.1 ± 5.05	2.00	0.44
Inter-day[c]	162	160.4 ± 2.07	1.29	0.99
	252	250.5 ± 4.48	1.79	0.60
Phenobarbital				
Intra-day[b]	22.5	22.0 ± 0.38	1.72	2.22
	35	35.3 ± 0.36	1.02	0.86
Inter-day[c]	22.5	22.1 ± 0.59	2.67	1.78
	35	35.1 ± 0.66	1.89	0.28

[a] Mean ± SD; deviation based on $n = 3$.
[b] $n = 3$.
[c] $n = 9$.

Calibration curves were prepared for ED, TP and PB with methyl *p*-hydroxy benzoate as the internal standard. Table 2 lists the concentration ranges for calibration curves of each analyte, regression parameters and limits of detection. Each calibration run was performed with replenished run buffer in the reservoir. The RSD% of analyte migration times during a calibration curve run were $< 1\%$. The intra- and interday accuracy (expressed as percent error) of the method was in the range of 0.28–2.54% and intra- and interday precision (expressed as %RSD) was in the range of 0.53–3.11%. The data is shown in Table 3.

3.3. Assay of a commercial tablet

Once the conditions for separation and quantitation were established, the CE method was applied to the determination of a commercial tablet labeled to contain ED hydrochloride (48 mg), TP(180 mg) and PB (25 mg). Calibration curves were prepared for each drug and the tablet extract was injected into the CE system. Assays of ED, TP and PB in the tablet dosage form expressed as percentage of the labeled amount were calculated to be 104, 105 and 96%, respectively.

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