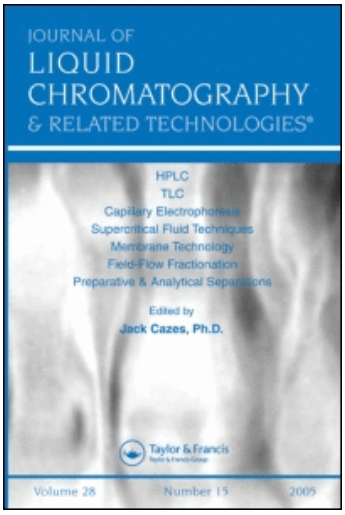

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SIMULTANEOUS DETERMINATION OF CODEINE, CAFFEINE, BUTALBITAL, AND ASPIRIN BY FREE SOLUTION CAPILLARY ELECTROPHORESIS

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SIMULTANEOUS DETERMINATION OF CODEINE, CAFFEINE, BUTALBITAL, AND ASPIRIN BY FREE SOLUTION CAPILLARY ELECTROPHORESIS

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ABSTRACT

A free solution capillary electrophoresis method was developed to separate and quantitate codeine (CD), caffeine (CF), butalbital (BB), and aspirin (AP) in a mixture. The mixture was injected hydrodynamically for 5s at the anodic end and separation was performed on a fused silica capillary (72 cm x 50 μm i.d; 50 cm to detector) at an applied voltage of 20 kV with 0.05 M phosphate run buffer pH 8. Separation was performed at ambient temperature and the total run time for analysis was 14 min. Detection was set at 220 nm. Calibration curves were prepared for CD, CF, BB, and AP with methyl p-hydroxy benzoate as internal standard. For each analyte, the correlation coefficients was greater than 0.999 (n=4). The RSDs % of ten replicate injections for each analyte were less than 1.4 %. The method was applied to the quantitation of CF, BB, and AP in a commercial tablet. Recoveries of the drug components were in the 96-104 % range.

INTRODUCTION

Capillary electrophoresis (CE) is gaining increasing popularity as an analytical tool for the separation of small and large molecules such as drugs,[1] peptides,[2,3,4] proteins,[5] and nucleic acids[6,7] because of its high separation efficiency, selectivity, flexibility, and low operational cost. The development of various types of electrophoresis techniques[8,9] such as capillary zone electrophoresis, isoelectric focusing, isotachophoresis and capillary electro-chromatography has added increased versatility to capillary electrophoresis as an analytical technique. Recent developments in the addition of various additives and selectors[10] to the run buffer have increased the potential of capillary electrophoresis for the analysis of pharmaceutical mixtures.

Fotsing et al.[11] determined six water soluble vitamins in a pharmaceutical formulation by capillary zone electrophoresis on a fused silica capillary (48.5 cm x 50 μm i.d) using borate buffer. Separation was performed at 25 kV and ambient temperature. Boonkerd et al.[1] separated and quantitated the components in an analgesic tablet by micellar electrokinetic chromatography. A mixture of bile salts in pH 9 borate buffer was used for separation. Analysis was performed at 20 kV potential and ambient temperature with detection at 214 nm. Aboul- Enein et al.[12] determined caffeine and ergotamine in a tablet by CE on a fused silica capillary (60 cm x 75 μm i.d.) at an applied voltage of 20 kV and detection at 214 nm.

In another study, free zone capillary electrophoresis was applied to separate and quantitate fourteen basic drugs in illicit drug samples.[13] Separation was achieved on a fused silica capillary (67 cm x 50 μm i.d.) with a phosphate buffer and detection at 230 or 210 nm. Capillary electrophoresis methods were also used to determine a drug and its impurities in the bulk drug substance. Shafaati et al[14] developed and validated a capillary zone electrophoresis method for the determination of atenolol in the presence of its related impurities in bulk and tablet dosage form. Separation was performed on a fused silica capillary (72 cm x 50 μm i.d.) at an applied voltage of 15 kV with a phosphate-borate run buffer and detection at 226 nm.

Reproducibility of analysis by capillary electrophoresis has been studied by many investigators.[15,16] Accuracy and precision of CE methods were found to be as good as HPLC in the analysis of either small or macromolecules. A number of experimental parameters were found to influence the accuracy and precision data of CE analysis. Altria listed[16] the parameters which effect the reproducibility by CE analysis and discussed the measures which can be taken to improve accuracy of the results. Most of the official compendial methods in USP 23 use either TLC or HPLC to determine main drug components in a formulation. Until now CE methods were not included as a compendial assay

method for the determination of drugs or impurities in the bulk and dosage forms. Compared to HPLC, CE methods are more environmentally friendly as they require little or no organic solvents for analysis. Also, in most cases, CE analysis requires less analysis time than HPLC.

In this paper, a method is described for the simultaneous separation of codeine (CD), caffeine (CF), butalbital (BB), and aspirin (AP) by CE in a mixture and quantitation of CF, BB, and AP in a commercial tablet dosage form using the internal standard method. Salicylic acid (SA) was also included in this study since it is the major degradation product of AP. Previously, CE methods were described for the determination of CF and AP in a formulation but no method has been reported for the simultaneous determination of CD, CF, BB, and AP in a mixture. Presently in USP 23, CD, CF, BB, and AP in a dosage form are determined by HPLC with detection set at 277 nm for caffeine and aspirin and at 210 nm for codeine and butalbital.[17]

EXPERIMENTAL

Instrumentation

The CE instrument of Applied Biosystems (ABI, Model 270A) equipped with a Hewlett Packard integrator (Model HP 3395) was used for electrophoresis. The polyimide coating on the capillary was partly removed by burning at the point of detection and the uncovered portion of the tube was aligned on the detector block. Separation of the analytes was performed with phosphate buffer (0.05 M, pH 8) on a fused silica (72 cm x 50 mm i.d., 50 cm to detector) capillary with an applied voltage of 20 kV and detection at 220 nm. Separations were performed at ambient temperature using 5s hydrodynamic injections of analytes at the anodic end.

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 using for analysis. The procedure in Table 1 describes capillary care and wash cycles during analysis.

Reagents and Chemicals

Codeine (CD) phosphate, caffeine (CF), butalbital (BB), aspirin (AP), salicylic acid (SA), and methyl p-hydroxy benzoate were purchased from Sigma

Table 1

Capillary Care and Wash Procedure

Wash Cycles	NaOH (0.1M) Min.	Water, Min.	Run Buffer Min.
Daily wash cycles before starting of experiment.	10	10	
Wash cycles before each injection.	2		2
Daily wash cycles at the end of experiment.	10	10	

Chemical Company (St Louis, MO). Disodium hydrogen phosphate was obtained from J T Baker Inc (Phillipsburg, NJ). Sodium hydroxide (electrophoresis grade) was purchased from Sigma Chemical (St Louis, MO) for regeneration and washing of silica capillary. Tablets (Fiorinal, Lot no 779 W 6330) manufactured by Novartis (East Hanover, NJ) were used in the experiment. Fused silica capillary was purchased from Polymicrotechnologies (Phoenix, AZ). Filters (0.2 μm, Nylon) and syringes were purchased from Alltech Associates Inc (Deerfield, IL) and Becton Dickinson (Franklin Lake, NJ), respectively. All chemicals were of highest chemical grade obtainable in the market. Fresh double distilled water was used for solution preparation.

Standard and Sample Preparations

Buffer for standards preparation and extraction of the tablet

A 0.01M phosphate buffer was prepared and the pH was adjusted to 2.5 with concentrated phosphoric acid. The buffer solution was used for preparing standard solutions and extraction of the commercial tablet.

Preparation of the standard stock solution

A standard stock solution containing CD (250 μg/mL), CF (200 μg/mL), BB (250 μg/mL) and AP (1.625 mg/mL) was prepared in phosphate buffer (0.01 M, pH 2.5) with ultrasonication for 30 min. A stock solution of methyl p-hydroxybenzoate (500 μg/mL) internal standard was prepared in distilled water and stored in a refrigerator at 4°C.

Preparation of the calibration curves

Various aliquots of standard stock solutions were taken, internal standard added and then diluted to 1mL with distilled water to provide calibration ranges for codeine (20-100 μg/mL), caffeine (20-80 μg/mL), aspirin (162.5-650 μg/mL) and butalbital (25-100 μg/mL). Linear regression analysis of peak area vs analyte concentration was performed to obtain slope, intercept, and correlation coefficient for each analyte.

Assay of the tablet

Extraction of the commercial tablet containing caffeine, butalbital, and aspirin was performed similar to the extraction procedure described in the official USP 23 monograph of the tablet. Ten tablets were ground in a mortar with a pestle and the powder equivalent to one average tablet weight (530 mg) was taken and dissolved in 200 mL of phosphate buffer (0.01 M, pH 2.5) with ultrasonication for 30 min and stirring. The final concentrations of the analytes in extraction buffer were approximately CF (200 μg/mL), butalbital (250 μg/mL), and aspirin (1.625 mg/mL). An aliquot (200 μL) of the tablet extract was added to 200 μL of internal standard and diluted to 1 mL with distilled water before injecting into the CE system. Peak area responses 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.

Recovery studies

Aliquots (200 μL) of the standard stock solution and tablet extract were placed in two separate glass tubes, internal standard (200μL) was added, followed by dilution to 1 mL with distilled water and injection into the CE system. Peak area responses of the analytes in the standard solution were compared with that of the tablet extract to calculate recoveries of each analyte. All solutions were filtered prior to injection into the CE system.

RESULTS AND DISCUSSION

Optimization of the Separation

Initially, the effect of pH on the separation of CD, CF, BB, AP, and SA was studied. In Figure 1A migration times of the analytes were plotted against pH. It was observed that a run buffer of pH 8 would be suitable for baseline resolution of all components. Buffer concentrations were varied in the

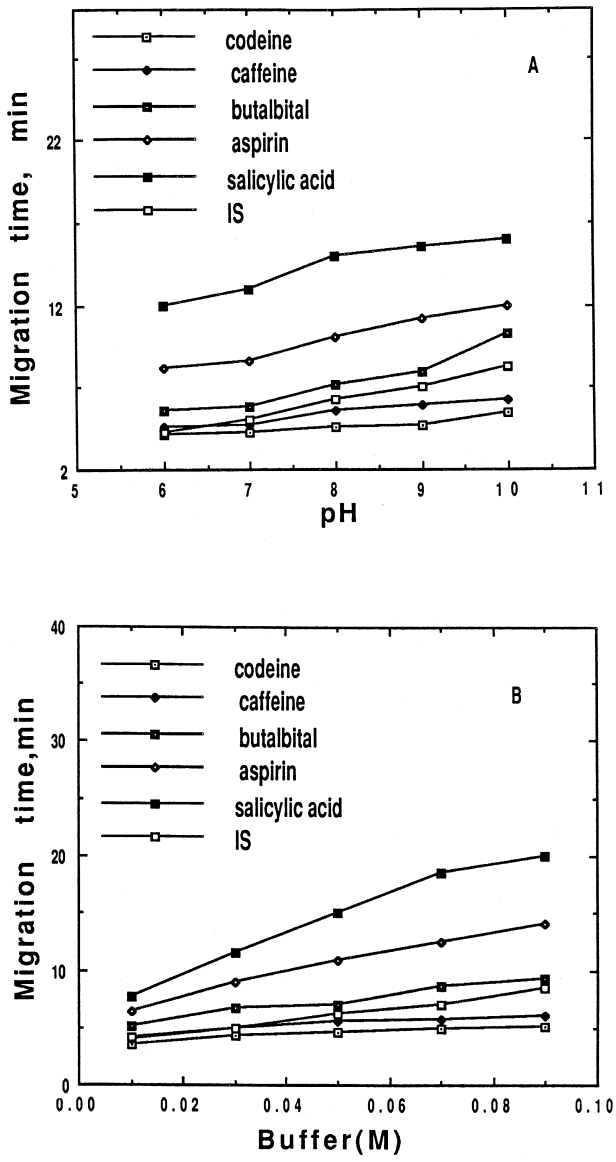

Figure 1. A) Plot showing the effect of pH on the migration times of the analytes; Conditions: Fused silica capillaries (70 cm x 50 ɯm i.d.), potential 20 kV, Detection-220 nm, Electrolyte-Phosphate buffer (0.05 M), Hydrodynamic injection- 5s, Ambient temperature. B) Plot showing the effect of buffer concentration on the migration times of the analytes; Conditions: Fused silica capillaries (70 cm x 50 ɯm i.d.), potential 20 kV, Detection-220 nm, Electrolyte-Phosphate buffer (pH 8), Hydrodynamic injection- 5s, Ambient temperature.

0.01-0.09 M range to study the effect of buffer (Figure 1B). Concentrations greater than 0.05M provided sharper peak shapes and resolution whereas lower buffer concentrations caused some peak tailing. A phosphate buffer solution of pH 8 (0.05M) was selected for the final analysis of the drug mixture. Separation was performed on uncoated fused silica capillaries with an applied potential of 20 kV and detection at 220 nm. Potentials lower than 20 kV increased the resolution between the peaks at the expense of total run time. Hydrodynamic injections were made for 5s. Figure 2A shows an electropherogram of the analytes at ambient temperature.

Mechanism of Separation

When a strong voltage is applied in CE, an electroosmotic flow is generated in the fused silica capillary due to the interaction of buffer electrolyte with the silanol groups on the inner wall of fused silica. All analytes, irrespective of charge, are driven from the anodic to the cathodic end by electroosmotic flow. Anions migrate slower than either cations or neutrals in electrophoresis because anions are pulled back by the anode resulting in reduced velocities of ionic species depending on the charge and mass of the anions. IS (6.3 min), BB (7 min), AP (10 min), and SA (14 min) remained as anions at pH 8 and hence, had longer migration times than either CD (4.5 min) or CF (5.3 min). Since CD remained dissociated as a cation at pH 8, it migrated faster than the rest of the analytes. CF is a very weak base and remained essentially undissociated at pH 8 and migrated with the strong electroosmotic flow. In general, the migration order of analytes in free solution capillary electrophoresis at alkaline pH is cations < neutrals < anions.[18] SA was retained longest because both the hydroxyl and carboxyl functions remained dissociated at the pH of the run buffer.

Quantification

A number of instrumental and operational factors such as buffer composition and concentration, pH, voltage, temperature, sample concentration, and composition and levels of buffer solutions at both ends of capillary may contribute to the accuracy and precision of the quantitative results in CE. The washing cycle of capillary and capillary care also contribute to the reproducibility of CE results. Table 1 lists the capillary wash cycle used in this study. Reproducibility of the method was investigated by repeat (n=10) injections of a sample solution containing equimolar amount of codeine, caffeine, butalbital, aspirin, salicylic acid, and the internal standard. It was observed from Table 2 that the peak area ratio of analytes/internal standard (RSDs% of 0.38-0.94) gave better reproducibility than the peak height ratio mode (RSDs % of 1.2-8.3). Thus, peak area ratios were used in the quantitative

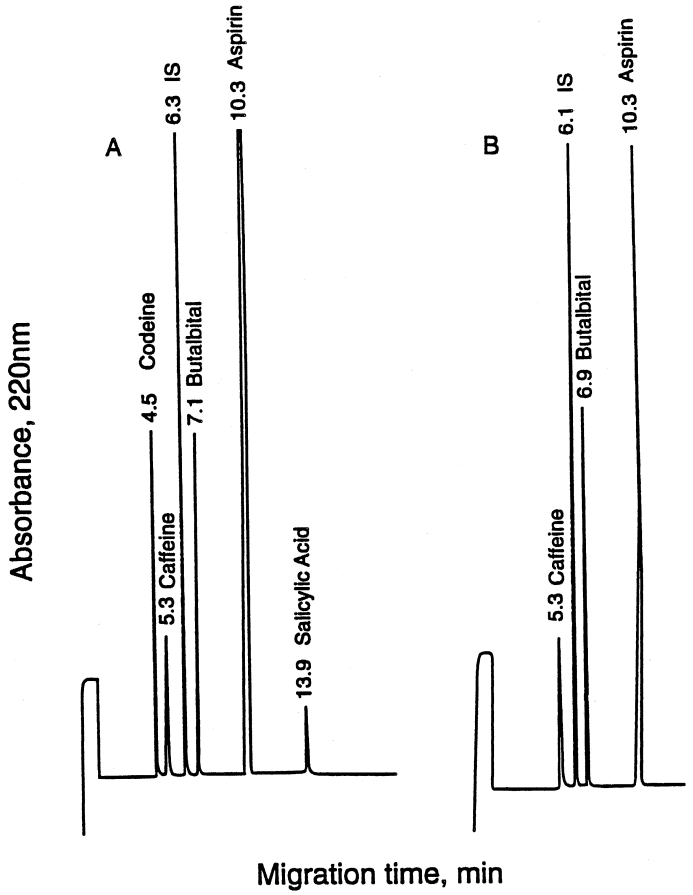

Figure 2. A) Typical electropherogram showing the separation of a mixture containing codeine, caffeine, butalbital, aspirin, salicylic acid and methyl p-hydroxy benzoate as internal standard (IS). B)Typical electropherogram of a tablet extract containing caffeine, butalbital, aspirin with methyl p-hydroxy-benzoate as internal standard. Conditions: Fused silica capillary (70 cm x 50 ɯm i.d.), potential 20 kV, Detection-220 nm, Electrolyte-Phosphate buffer (pH 8, 0.05 M), Hydrodynamic injection- 5s, Ambient temperature.

determination of the analytes. Calibration curves were prepared for CD, CF, AP, and BB with methyl p-hydroxy benzoate as internal standard. The curves for each analyte gave excellent linearity using the internal standard method. Table 3 lists the concentration ranges for calibration curves of each analyte, regression parameters, and limits of detection. Extraction of commercial tablets

Table 2

Reproducibility of Determination of CD, CF, BB and AP by the Internal Standard Method

	Codeine	Caffeine	Butalbital	Aspirin
	Peak Height Ratio of Analytes to Internal Standard (N=10)			
Mean	0.727	0.217	0.520	1.49
Std Dev	0.0049	0.018	0.028	0.018
RSD%	6.74	8.29	5.38	1.20
	Peak Area Ratio of Analytes to Internal Standard (N=10)			
Mean	0.357	0.791	0.425	5.27
Std Dev	0.003	0.003	0.004	0.026
RSD%	0.840	0.379	0.941	0.493

Table 3

Typical Linear Regression Data for the Analysis of CD, CF, AP and BB in a Mixture

Analyte	Code Range µg/mL	r^{2a}	Slope	Intercept	LOD µg/mL[b]
Codeine	20-100	0.999	8.874	0.015	2
Caffeine	20-80	0.999	0.017	0.113	5
Aspirin	162.5-650	0.999	0.015	0.198	2
Butalbital	25-100	0.999	0.007	0.019	10

[a] n = 4. [b] Based on s/n >3.

containing CF, BB, and AP was performed using phosphate buffer (pH 2.5, 0.01M). Recoveries of the drugs from a tablet were calculated by injecting like amounts of reference analytes in phosphate buffer and comparing their peak responses. The recoveries of CF, BB, and AP from the tablet using phosphate buffer were 96-104% (n=3).

Table 4

Accuracy and Precision of the Determination of CF, BB and AP in a Commercial Tablet Dosage Form[a]

Analyte	Amount in Tablet, mg	Amount Found, mg	RSD%	% of Labeled Amount Found
Caffeine	40	40 ± 1.49[b]	3.72	100
Butalbital	50	49 ± 1.12	2.28	98.0
Aspirin	325	324 ± 8.52	2.62	99.7

[a] Fiorinal, Lot no. 779W 6330, Novartis, East Hanover, NJ.
[b] Mean ± std dev based on n = 3.

Assay of a Commercial Antipyretic and Analgesic Tablet

The aforementioned CE method was then applied to the determination of ingredients of a commercial tablet containing caffeine (40 mg), butalbital (50 mg), and aspirin (325 mg). Calibration curves were prepared for each drug and the tablet extract was injected into CE system. A typical electropherogram of the tablet extract is shown in Figure 2B.

The accuracy and precision data obtained from the determination of the tablet components are listed in Table 4. It was found that accuracy and precision data for the analytes were less than 2 and 3.8 %, respectively, for the determination of all three components.

CONCLUSION

A simple and reproducible CE method was developed for the determination of CD, CF, BB, and AP in a mixture. Salicylic acid, which is considered an impurity of AP in USP 23, has a longer migration time than the analytes studied and did not interfere with the assay method. Excellent separations of the four components (and SA) of diverse polarity and ionic character were achieved with neat phosphate buffer (0.05M) at pH 8.

The method was applied to determine the caffeine, butalbital, and aspirin content in a commercial tablet. The recoveries of the tablet components were in the 96-104 % range. The accuracy and precision of determination of the tablet components were less than 3.8%.

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