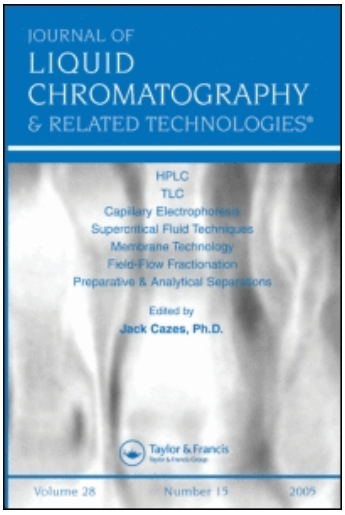

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Ahsanul Haque[a]; James T. Stewart[a]
[a] Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, University of Georgia, Athens, GA, U.S.A.

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DETERMINATION OF ACETAMINOPHEN, CAFFEINE, AND BUTALBITAL IN A COMMERCIAL TABLET DOSAGE FORM BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY

Ahsanul Haque, James T. Stewart

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

ABSTRACT

A micellar electrokinetic chromatography (MEKC) method was developed to analyze acetaminophen (AP), caffeine (CF), and butalbital (BB) simultaneously in a commercial tablet dosage form. Uncoated fused silica capillary (70 cm x 50 μm i.d, 50 cm to detector) was used for the separation at an applied voltage of 20 kV. A phosphate run buffer (pH 9, 0.05 M) containing 0.05 M sodium dodecyl sulphate was used for analysis. Separations of the analytes were achieved within 12 min at ambient temperature with detection set at 220 nm. Calibration curves were prepared for AP (260-520 μg/mL), CF (30-64 μg/mL) and BB (40-80 μg/mL) by the internal standard method with methyl-p-hydroxybenzoate as internal standard. Correlation coefficients of calibration curves were greater than 0.990. A five second hydrodynamic injection was used for analysis. Tablet powder equivalent to one tablet weight was extracted with absolute methanol in an ultrasonic bath for 30 min. The methanol extract was diluted with water and injected into the CE system for quantitation. Recoveries of the analytes were in the 95-103% range.

INTRODUCTION

Micellar electrokinetic chromatography (MEKC) is a type of capillary electrophoresis (CE) that has been used in the analysis of pharmaceuticals.[1-3] Capillary electrophoresis (CE) employs a narrow bore capillary (20-200 μm, i.d), buffer electrolyte and high voltage to achieve high efficiency separations of both small and large molecules. The separation principle of CE is based on differential electrophoretic mobility, therefore, neutral species can not be separated by this method. Terabe et al[4] introduced MEKC to separate neutral species by CE. MEKC uses the same instrument as CE but employs a run buffer which contains ionic surfactants above critical micellar concentrations (CMC). The technique is regarded as a chromatographic technique because the separation mechanism is based on the differential partitioning of ionic and neutral solutes between the micellar pseudophase and the aqueous phase. MEKC is considered as the method of choice to analyze neutral analytes in CE.

It was of interest in this laboratory to apply MEKC methods to the analysis of a USP drug mixture to show advantages and disadvantages of the method to a compendial monograph. Thus, a mixture of acetaminophen (AP), caffeine (CF), and butalbital (BB) was selected as a model mixture to investigate since it was also available as a commercial tablet dosage form. The existing USP assay is an HPLC method based on an octadecylsilane stationary phase using a phosphate buffered methanolic mobile phase at a 2 mL/min flow rate.[5] Two other reported HPLC procedures used either a silica or a cyanopropyl stationary phase to separate the drug mixture at a 1.5 mL/min flow rate.[6, 7] There have been no MEKC methods reported for this particular mixture thus far. Some CE or MEKC methods have been reported for either AP or CF or both in dosage forms.[8-11]

In this paper, a MEKC method was developed to determine AP, CF, and BB in a commercial tablet dosage form. Compared to the reported HPLC procedures, the MEKC method is more environmentally friendly as it requires no organic modifier in the run buffer. In addition, accuracy and precision and recoveries of the three analytes are comparable to that obtained by the HPLC methods. Thus, the MEKC procedure can be used for this mixture, even if the benefits are small compared to the existing HPLC methods.

EXPERIMENTAL

Instrumentation

MEKC was performed with an Applied Biosystems Model 270A capillary electrophoresis unit equipped with a Hewlett Packard integrator (Model HP 3395). 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. The new fused silica capillary was regenerated with a wash of 1 M NaOH solution for 20 min followed by a wash with water for 20 min prior to analysis. Before each injection, the capillary was washed with 0.1M NaOH (3 min) followed by a run buffer (2 min). At the end of the day, the capillary tube was washed with distilled water for 10 min. In these experiments, an uncoated fused silica capillary (72 cm x 50 μm i.d, 50 cm to detector) was used for separation. Phosphate buffer (0.05 M, pH 9) containing 0.05M sodium dodecyl sulphate was employed as a run buffer with an applied voltage of 20 kV and detection at 220 nm. All analyses were performed with a 5s hydrodynamic injection at the anodic end.

Reagents and Chemicals

Acetaminophen (AP), caffeine (CF), butalbital (BB), sodium dodecyl sulphate (SDS), and methyl p-hydroxy benzoate were purchased from Sigma 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 the silica capillary. Fioriset® tablets manufactured by Novartis (East Hanover, NJ) were used in the experiment. Fused silica capillaries were 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

Standard stock solutions containing AP (6.5 mg/mL), CF (1 mg/mL) and BB (800 μg/mL) were prepared in absolute methanol. A stock solution of methyl p-hydroxybenzoate (1 mg/mL) internal standard was prepared in distilled water and stored at 4°C.

For calibration curves, aliquots of the standard stock and internal standard solutions were diluted with distilled water to provide calibration ranges of AP (260-520 μg/mL), CF (30-64 μg/mL), and BB (40-80 μg/mL). Linear regression analysis of peak area vs analyte concentration was performed to obtain slope, intercept, and correlation coefficient for each analyte. Commercial tablets containing AP, CF, and BB were ground in a mortar with a pestle and the powder equivalent to one average tablet weight was taken and dissolved in 50 mL of methanol in an ultrasonic bath for 30 min with stirring. The final concentrations of analytes were AP (6.5 mg / mL), CF (1 mg /mL), and BB (800 μg/mL). An aliquot (60 μL) of the tablet extract was added to 100 μL of internal standard in a 1 mL volumetric tube and diluted to 1 mL with distilled

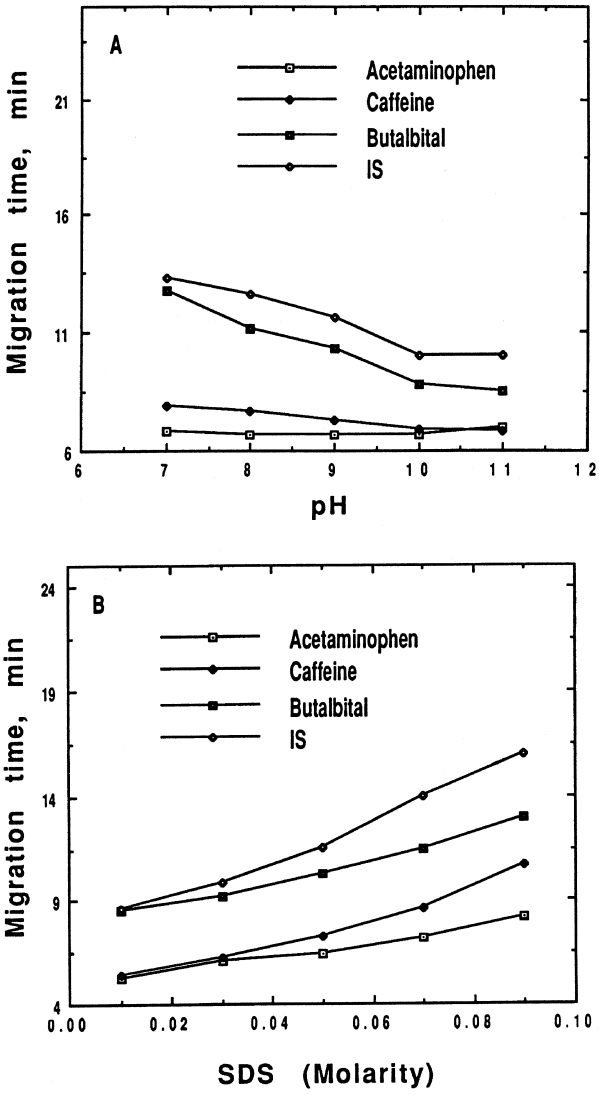

Figure 1. A) Plots showing the effect of pH of the run buffer on the migration times of the analytes; B) Plots showing the effect of sodium dodecyl sulphate molarity in the run buffer on the migration times of the analytes. Conditions: see Experimental Section.

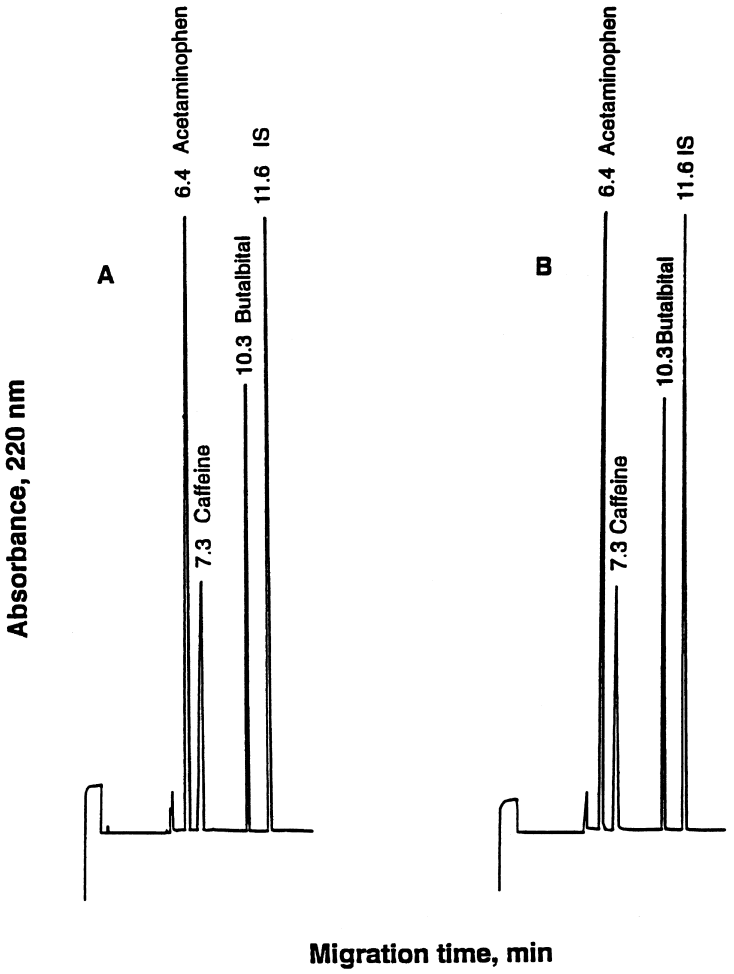

Figure 2. A) Typical electropherogram showing the separation of a mixture containing AP, CF, BB and methyl p-hydroxy benzoate as internal standard (IS). B)Typical electropherogram of a tablet extract containing AP, CF and BB with methyl p-hydroxybenzoate as internal standard. Conditions: see Experimental Section.

water before injecting 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.

Table 1

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

Peak Height Ratio of Analytes to Internal Standard*

	Mean	RSD%
Acetaminophen	1.81	1.82
Caffeine	0.354	3.67
Butalital	0.592	4.00

Peak Area Ratio of Analytes to Internal Standard*

Acetaminophen	0.850	0.47
Caffeine	0.219	0.46
Butalbital	0.221	0.90

* (n = 10).

RESULTS AND DISCUSSION

In the method development, both CE and MEKC were investigated for the separation. With CE, buffer pHs were varied in the 7-11 range and it was observed that the analytes were only separated at pH 10. With MEKC, 0.05 M sodium dodecyl sulphate (SDS) added to the run buffer gave separation of the analytes in the micellar buffer in the pH range from 7 to 9.5 (Figure 1A). Furthermore, peak shapes of the analytes, especially, AP and CF, were found to be much sharper with MEKC compared to CE. Therefore, MEKC was chosen for the separation of analytes. A pH 9 0.054M phosphate buffer containing 0.05 M SDS was found to be the best run buffer for the separation of all analytes (Figure 1B). A typical electropherogram is shown in Figure 2A.

The capillary wash cycles used in this study are described in the experimental section. A buffer solution was prepared every day before analysis to monitor reproducible migration times. Repeatability of the method was investigated by replicate (n=10) injections of a sample solution containing AP, CF, BB, and internal standard. The peak area ratio of analytes/internal standard (RSDs% of 0.47-0.90) gave better repeatability (Table 1) than the peak height ratio mode (RSDs% of 1.8-4). Thus, peak area ratios were used in the quantitative determination of the analytes.

Calibration curves for each analyte gave good linearity using the internal standard method. The concentration ranges for calibration curves of each analyte, regression parameters, and limits of detection are listed in Table 2. The

Table 2

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

Analyte	Conc. Range μg/mL	r^2*	Slope	Intercept	LOD** μg/mL
Acetaminophen	260-520	0.988	0.003	0.125	0.50
Caffeine	30-64	0.991	0.008	0.078	5
Butalbital	40-80	0.993	-0.005	-0.008	0.50

* (n = 4).** (s/n>3).

Table 3

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

Analyte	Amount in Tablet, mg	% Recovery	RSD%
Acetaminophen	325	95.0	1.30
Caffeine	40	98.0	0.17
Butalbital	50	103.0	0.31

[a] Fioriset®, Lot No. 134W6142, Novartis, East Hanover, NJ.

MEKC method was applied to a commercial tablet containing AP (325 mg), CF (40 mg), and BB (50 mg). Recoveries of the drugs from the tablet were calculated by injecting like amounts of reference analytes and comparing their peak responses. The recoveries of AP, CF, and BB were in the 95-103% (n=3) range.

A typical electropherogram of the tablet extract is shown in Figure 2B. Table 3 lists the accuracy and precision data obtained from the determination of the tablet components. The accuracy and precision were better than 4.6 and 1.3%, respectively, for all three components.

In summary, the MEKC method developed herein for AP, CF, and BB showed good accuracy and precision when applied to the quantitation of the three drugs in a commercial tablet dosage form. The literature reported HPLC recoveries of 97-102.5% were comparable to those obtained for the MEKC method.

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