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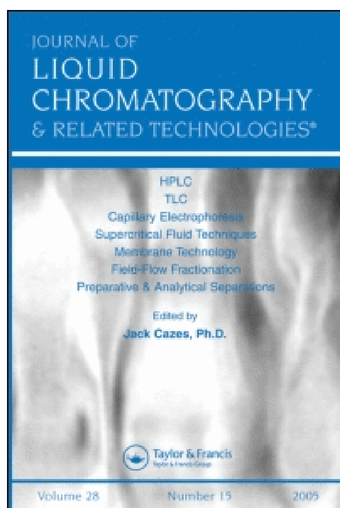

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MEKC DETERMINATION OF GUAIFENESIN, PSEUDOEPHEDRINE, AND DEXTROMETHORPHAN IN A CAPSULE DOSAGE FORM

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MEKC DETERMINATION OF GUAIFENESIN, PSEUDOEPHEDRINE, AND DEXTRO-METHORPHAN IN A CAPSULE DOSAGE FORM

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ABSTRACT

A MEKC method has been developed for the simultaneous determination of guaifenesin (GF), pseudoephedrine (PE), and dextromethorphan (DM) in capsule dosage form using 50 mM sodium dodecyl sulfate (SDS) added to a pH 8.5 100mM Tris run buffer at a voltage of 25 kV. The method utilized UV detection at 220nm and a 72 cm x 50 µm i.d. uncoated fused-silica capillary. The detection limits for GF, PE and DM were 1000, 500, and 500 ng/mL, respectively for a 5 s injection. The calibration curves were linear over ranges of 100-1000 µg/mL, 15-150 µg/mL and 5-50 µg/mL for guaifenesin, pseudoephedrine and dextro-methorphan, respectively, with sodium benzoate (SB) as internal standard. Coefficients of determination were greater than 0.9989 (n=12). Precision and accuracy of the method were 0.60-2.18% and 0.44-4.20%, respectively for GF, 1.62-3.15% and 0.33-2.98%, respectively for PE, and 0.19-4.36% and 1.77-6.92%, respectively for DM. In comparison to high-performance liquid chromatography, the MEKC method is simple, capillary columns are low-cost, and there is high-resolution efficiency with a minimal consumption of environmentally friendly chemicals.

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INTRODUCTION

The number of applications for the separation of pharmaceutical compounds by capillary electrophoresis (CE) has steadily increased since the early reports describing micellar electrokinetic chromatography (MEKC).[1-4] Publications on MEKC have compared the technique to other separation techniques, such as high-performance liquid chromatography (HPLC).[4] It is recognized that the separating power of CE and, especially MEKC, is very often superior to HPLC and small differences in drug molecules can be exploited to effect baseline separation.

Within therapeutic areas, combination drugs are often prescribed which exhibit different chemistries that can make a separation of each by HPLC difficult. Cough-cold preparations are an example of such multiple therapies found in the same dosage form. The active ingredients in one such cough-cold preparation is a combination of guaifenesin (GF) (expectorant), pseudoephedrine HCI (PE) (decongestant), and dextromethorphan HBr (DM) (antitussive). Simultaneous HPLC assays have been described for mixtures of guaifenesin and pseudoephedrine,[5-7] dextromethorphan, and pseudoephedrine,[8-9] and guaifenesin and dextromethorphan.[10-12] Tailing was observed for a dextromethorphan and guaifenesin mixture.[13] Other disadvantages regarding the HPLC methods involved the use of more than one column or mobile phase, and the use of a high flow rate. A MEKC method has been reported, but no quantitation and method optimization were studied..[14] In the current USP23 monograph,[15] GF, PE, and DM in a capsule dosage form are determined by HPLC using two different mobile phase systems at flow rates of 2mL/min.

In this paper, a MEKC method has been developed and validated for the simultaneous determination of GF, PE, and DM in a capsule dosage form. To date, MEKC has not been applied to the quantitation of this particular cough-cold mixture. The method offers the advantage of shorter analysis times and less solvent consumption than those reported by HPLC.

EXPERIMENTAL

Chemicals and Reagents

Guaifenesin, pseudoephedrine hydrochloride, dextromethorphan hydrobromide monohydrate, and benzoic acid-sodium salt were purchased from Sigma Chemical Company (St. Louis, MO, USA). Sodium hydroxide (electrophoresis grade), tetrahydrofuran (THF), glacial acetic acid, methanol, and phosphoric acid were obtained from J. T. Baker (Phillipsburgh, NJ, USA). All solvents used were HPLC grade. Sodium dodecyl sulfate and, tris (hydroxymethyl)aminomethane were also purchased from Sigma Chemical Co. (St. Louis, MO, USA).

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Apparatus

All MEKC experiments were performed using an ABI 270A capillary electrophoresis system (Applied Biosystem, Foster City, CA, USA) equipped with an on-column variable wavelength UV detector. An uncoated fused-silica capillary with total length 72 cm, effective length 50 cm and 50 μm i.d. (Polymicro Technology, Phoenix, AZ, USA) was used for the analysis.

The HPLC separations were performed on a HPLC system consisting of a Waters Model 505 HPLC pump (Waters Corp., Milford, MA), a Rheodyne Model 7225 injection valve (Rheodyne, Cotati, CA, USA), equipped with a 20 μL loop, a Waters 484 variable wavelength Absorbance Detector (Waters Corp., Milford, MA), and a Hewlett Packard Model 3392A Integrator (Avondale, PA USA).

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MEKC and HPLC Conditions

The sampling was by hydrodynamic injection for 5s (calculated volume of 20 nL). The capillary was preconditioned for 40 min with 0.1M NaOH and 20 min with water before the first run, and then for 2 min 0.1M NaOH and 3 min with run buffer prior to each subsequent run. The capillary temperature was 30°C and the applied voltage was 25 kV. The typical run current was 32 μA. A 0.5 cm detection window was created by stripping the polyimide coating of the capillary. The detector was set at 220 nm toward the cathodic end. All MEKC solutions and samples were filtered through a membrane filter of 0.2 μm pore size (Alltech, Deerfield, IL, USA) and degassed in a ultrasonic bath prior to injection.

The run buffer consisted of 100 mM Tris, pH 8.5 containing 50 mM SDS (pH was adjusted with concentrated phosphoric acid).

A comparison of HPLC separation was performed utilizing the official USP23 method for the cough-cold mixture on a C-18 column (Phenomenex 300x3.9mm i.d., 5 micron, Rancho Palos Verdes, CA USA).[15] The mobile phase for the analysis of guaifenesin consisted of 60:40:1.5 v/v/v water-methanol-glacial acetic acid at a flow rate of 2 mL/min. The mobile phase for the analysis of dextromethorphan and pseudoephedrine consisted of 100:70:29:1, v/v/v/v methanol-water-THF-glacial acetic acid containing 0.7g/L docusate sodium salt at a flow rate of 2 mL/min.

Both mobile phases were filtered through a 0.45 μm Nylon-66 filter (Alltech, Deerfield, IL). The UV detector was set at 263 nm to analyze for the three compounds in two separate runs.

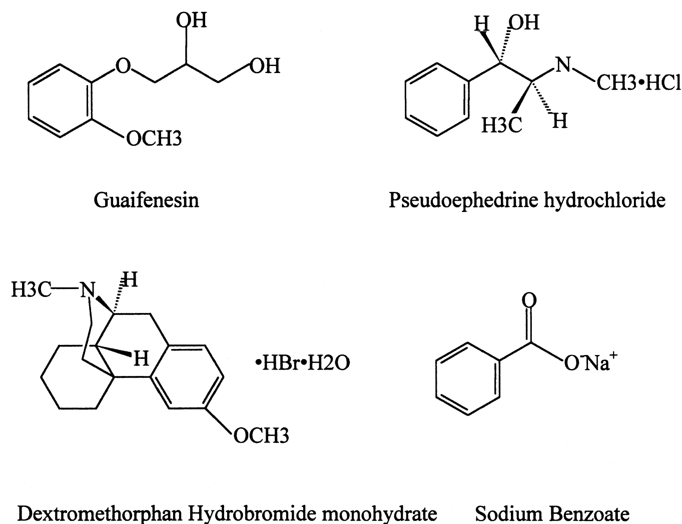

Figure 1. The structures of guaifenesin, pseudoephedrine, dextromethorphan and sodium benzoate (IS).

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Preparation of Stock and Standard Solutions

Stock solutions containing 1 mg/mL of PE hydrochloride and DM hydrobromide monohydrate, 5 mg/mL GF and 2 mg/mL internal standard were prepared separately in HPLC grade water and stored at 4°C.

Dilutions of sample solutions for standard curves and spiked samples were prepared with HPLC grade water and filtered through a 0.22 μm filter prior to use.

Preparation of Commercial Capsule Sample

One gelatin capsule containing 200 mg guaifenesin, 30 mg pseudoephedrine hydrochloride, and 10 mg dextromethorphan hydrobromide was opened using a disposable surgical blade and the entire capsule placed into a 100 mL volumetric flask. 50 mL of HPLC grade water was added and the mixture was heated on a boiling water bath for around 10 min or until the gelatin dissolved.

After cooling, and adding water to volume, the solutions were mixed and sonicated for 10 min. After sonication, an aliquot was removed from the flask, filtered, and injected into either the CE or HPLC systems.

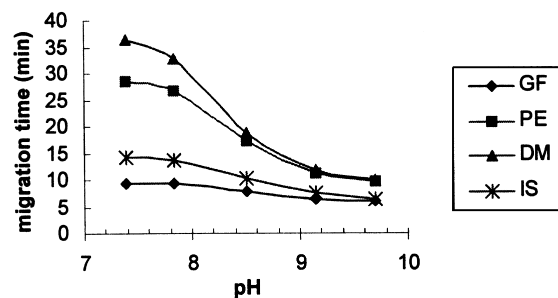

Figure 2. Plot showing the effect of 100 mM tris buffer pH on migration time of the analytes. See Experimental section for run conditions.

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RESULTS AND DISCUSSION

Method Development Using MEKC

Figure 1 shows the chemical structures of the three analytes GF, PE, and DM and internal standard SB. The development of a MEKC method for the simultaneous determination of GF, PE, and DM in a capsule was a challenge due to the differences in polarity between the three analytes. There was also a great disparity in the quantity of each drug present in the commercial capsule and the selectivity needed for separating each compound from the other two and from internal standard. The initial use of CZE was explored, but pseudoephedrine and dextromethorphan were either difficult to elute, or they could not be satisfactorily separated.

Initially, the choice of buffer for the separation of GF, PE, and DM was studied. Borate, phosphate, borate-phosphate, and tris buffers were investigated. The borate buffer, which had been reported previously for separation of the mixture[14] gave a higher system current (100 µA) in this laboratory than the tris buffer (35 µA). The tris buffer was finally selected based on baseline separation of analytes, sharper peaks, lowest system current, and shortest migration times compared to the other buffers.

The effect of pH on the separation of GF,PE, DM and IS was studied in the 7-10 pH range. In Figure 2, migration times of the analytes were plotted against tris buffer pH. The migration times of the PE and DM peaks were greatly affected, but the GF and IS peaks were largely unaffected. It was observed that a run buffer pH of 8.5 would be suitable for baseline resolution of all components.

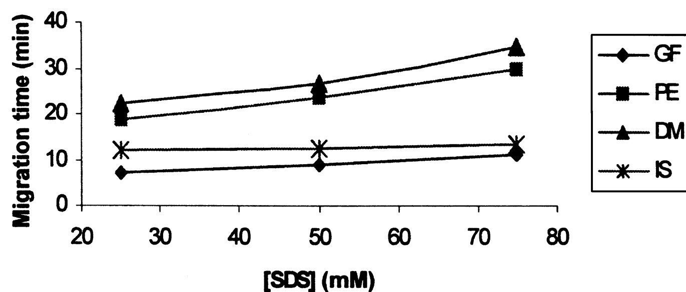

Figure 3. Plot showing the effect of SDS molarity on migration times of analytes. See Experimental section for run conditions.

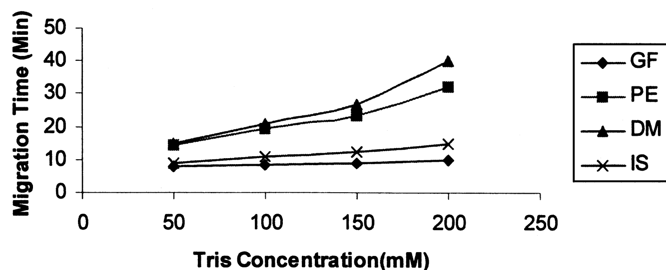

Figure 4. Plot showing the effect of tris buffer concentration on migration time of analytes. See Experimental section for run conditions.

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The effect of SDS molarity in the 20-80 mM range on migration time is shown in Figure 3. Although the shortest migration times were obtained at 25 mM SDS, there was distortion of the PE peaks. Increasing the SDS concentration to 50 mM gave a sharper and more symmetrical PE peak. A run buffer containing 50 mM SDS was found to be optimal for the separation of the analytes.

Finally, the run buffer molarity was optimized as shown in Figure 4. A 100 mM tris buffer concentration was found to be optimal for the separation.

As shown in Figure 5, the final MEKC run buffer of 100 mM tris buffer pH 8.5 containing 50 mM SDS provided an electropherogram with good baseline and excellent specificity.

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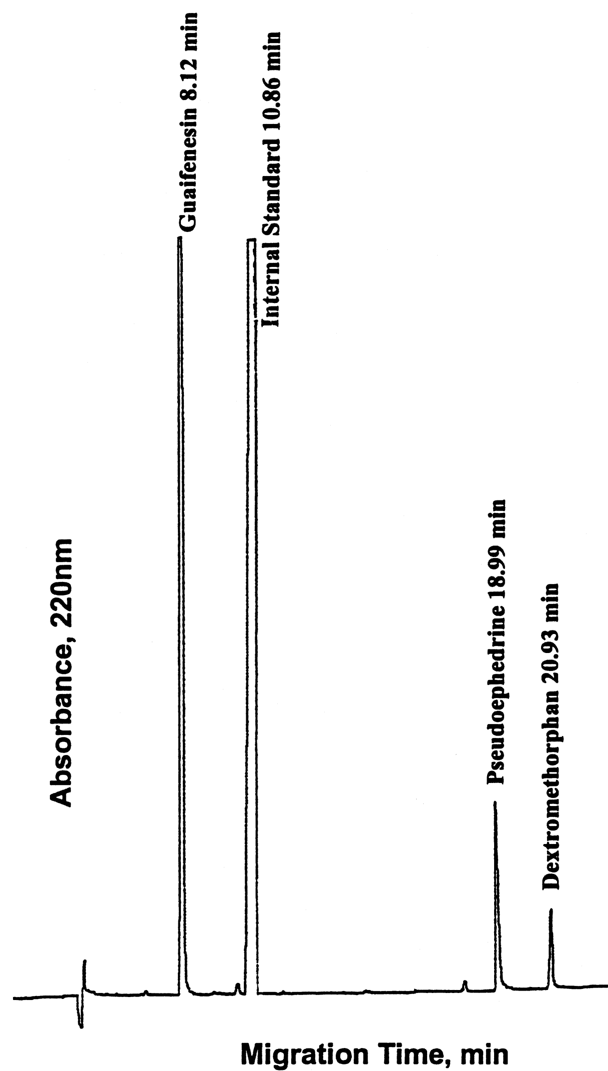

Figure 5. A typical electropherogram of the separation of guaifenesin (200 µg/mL), pseudoephedrine (30 µg/mL), dextromethorphan (10 µg/mL) and sodium benzoate (IS) (200 µg/mL). Conditions: pH 8.5; 100 mM Tris buffer containing 50 mM SDS, 25kV, 30°C and 5 s hydrodynamic injection.

Table 1

Typical Linear Regression Data for the Analysis of GF, PE, and DM in a Mixture

Analyte	Conc. Range μg/mL	r^2 (n=12)	Slope	Intercept	LOD (S/N>3) μg/mL
Guaifenesin	100-1000	0.9996	0.002426	0.002767	1
Pseudoephedrine	15-150	0.9997	0.001824	0.00036	0.5
Dextromethorphan	5-50	0.9989	0.002952	0.00497	0.5

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Although the optimal detection wavelength for each analyte was different, 220 nm was selected as the detection wavelength based on detectability of the lowest concentration DM peak. Detection at 190 nm was slightly more sensitive for some of the analytes, but had the potential limitation of interference by some excipients in the capsule dosage form.

The range of 5-20 s hydrodynamic injections was studied. A 5s injection time was selected for the assays because it provided sharper peaks while retaining suitable limits of detection for each analyte. Ten to 20 s injection times gave some serious precision problems and there was a loss of theoretical plates.

Wall adsorption can be a problem in MEKC and a MEKC method would require either rinsing or replacing the capillary at frequent intervals. Occasionally wall adsorption was a serious problem in our study, even though a series of previous injections exhibited little or no adsorption. Usually, flushing the capillary with 0.1 M NaOH for brief periods eliminated the problem. It is recommended that the capillary should be rinsed for 40 min with 0.1 M NaOH followed by 20 min with distilled water after every 12 injections.

Linearity

To obtain intra- and inter-day linearity data, standard curves were prepared in the 100-1000, 50-150, and 5-50 μg/mL ranges for GF, PE, and DM, respectively, with 200 μg/mL level of sodium benzoate internal standard. Table 1 lists the regression parameters and limits of detection for each analyte. In this assay, analyte/IS peak area ratios were plotted versus analyte concentration in the regression analysis. The use of an internal standard helped to compensate for variance in sample injection.

Table 2

Reproducibility of Analyte/IS Peak Area Ratios of GF, PE, and DM

	Peak Area Ratio of Analyte to Internal Standard[a]		
	Mean	**Std. Dev.**	**RSD%**
Guaifenesin	4.996	0.0818	3.28
Pseudoephedrine	0.279	0.0061	2.20
Dextromethorphan	0.145	0.0007	0.053

[a] n = 9 at 1000, 150 and 50 μg/mL for GF, PE, and DM, respectively, and 200 μg/mL IS.

Table 3

Reproducibility of Migration Times of GF, PE, DM, and Internal Standard[a]

	Mean (min.)	**Std. Dev.**	**RSD%**
Guaifenesin	8.12	0.11	1.35
Pseudoephedrine	18.99	0.43	2.26
Dextromethorphan	20.93	0.39	1.86
Internal Standard	10.86	0.11	1.01

[a] Based on n = 10.

Reproducibility data for peak area ratios of analyte/internal standard is shown in Table 2. Precision of the measurements were in the range of 0.53-3.28% RSD. The precision of migration times was also calculated and % RSD were in the 1-2%range (See Table 3).

Selectivity

A few common cough-cold drugs and preservatives found in OTC cough-cold preparations were injected into the MEKC system to determine further selectivity of the method. Acetaminophen (6 min), chlorpheniramine (25 min), methyl paraben (9.3 min), and ethyl paraben (12 min) were shown not to interfere with the method. There were also some unidentified peaks in the electropherogram of the capsule dosage form, but their migration times were very different from the analytes of interest in this study.

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Table 4

Accuracy and Precision of Spiked Samples

	Concentration Added (μg/mL)	Concentration Found (μg/mL)	RSD %	Error (%)
Intra-Day (n=3)				
Guaifenesin	400	412.33 ± 2.48	0.60	3.10
	160	153.22 ± 2.48	1.62	4.20
Pseudoephedrine	60	60.24 ± 1.90	3.15	0.33
	24	24.24 ± 0.38	1.58	1.00
Dextromethorphan	20	18.62 ± 0.04	0.19	6.92
	8	7.859 ± 0.20	2.50	1.76
Inter-Day (n=9)				
Guaifenesin	400	401.78 ± 8.74	2.18	0.44
	160	155.24 ± 2.52	1.62	2.98
Pesedoephedrine	60	59.33 ± 1.36	2.29	1.11
	24	23.52 ± 0.98	4.15	1.98
Dextromethorphan	20	19.07 ± 0.32	1.66	4.62
	8	7.68 ± 0.40	4.36	3.93

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Selectivity can be easily manipulated through the changing of micellar solutions. In particular, change of surfactant in MEKC corresponds to a column change in HPLC. A variety of surfactants have been used for MEKC, but typical long alkyl-chain surfactants such as SDS are routinely employed.

The limits of detection, defined as the concentration where the signal-to-noise ratio is 3, were found to be 1000 ng/mL for guaifenesin, 500 ng/mL for pseudoephedrine and 500 ng/mL for dextromethorphan with a 5s injection.

Accuracy and Precision

Recoveries of each of the three analytes were calculated using spiked samples. Based on linear regression analysis of calibration curve data in the 100-1000, 15-150, and 5-50 μg/mL ranges for GF, PE, and DM, respectively, percent recoveries of 100.4 ± 2.2% at 400 μg/mL and 97.0 ± 1.6% at 160 μg/mL for GF, 98.9 ± 2.3% at 60 μg/mL and 98.0 ± 4.2% at 24 μg/mL for PE and 95.4 ± 1.7% at 20 μg/mL and 96.0 ± 4.4% at 8 μg/mL for DM were obtained. Accuracy and precision data of spiked analyte samples within the calibration ranges are shown in Table 4.

Table 5

Analysis of GF, PE, and DM in a Commercial Capsule Dosage Form[a]

	Labeled Amount Mg	Amount Found[b] RSD% Mg	% of Label Amount	
Guaifenensin	200	200.15 ± 0.90	1.12	100.12
Pseudoephedrine HCL	30	30.09 ± 0.85	2.83	100.3
Dextromethorphan HBr	10	10.24 ± 0.20	1.94	102.4

[a] Robitussin cold and cough softgel™, Lot 98207, Whitehall-Robins Healthcare, Madison, NJ 07940. [b] Mean ± sd based on n=5.

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The MEKC method was then applied to the determination of ingredients of a commercial capsule dosage form containing 200 mg GF, 30 mg PE hydrochloride, and 10 mg DM hydrobromide. Calibration curves were prepared for each drug and the capsule sample preparation was injected into the MEKC system. The assay data for the individual analytes are shown in Table 5.

Comparson of USP 23 Method to MEKC Method

As a comparison, the USP 23 HPLC method for guaifenesin, pseudoephedrine, and dextromethorphan was used to quantitate each ingredient in the capsule dosage form using a C18 column.[15] Samples of the capsule dosage form were prepared for assay according to the USP 23 monograph.

The capsule solution was injected five times and the percents of label claim were found to be 98.99 ± 0.05%, 99.42 ± 0.07%, and 99.83 ± 1.24% for guaifenesin, pseudoephedrine, and dextromethorphan (n=5), respectively, similar to those determined with the MEKC method.

CONCLUSIONS

This study has demonstrated the feasibility of using MEKC for the analysis of a cough-cold combination. Good linearity and detection limits for the selected GF, PE, and DM mixture can be accomplished within a 23 min migration time. Further work will investigate the analysis of additional types of cough-cold combinations as well as other combination dosage forms using MEKC.

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