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Simultaneous determination of dextromethorphan, diphenhydramine and phenylephrine in expectorant and decongestant syrups by capillary electrophoresis

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Abstract

The separation of basic nitrogenous compounds commonly used as active ingredients in cold medicine formulations by micellar electrokinetic capillary chromatography and capillary zone electrophoresis with direct absorptiometric detection was investigated. The type and composition of the background electrolyte (BGE) were investigated with respect to separation selectivity and BGE stability. BGE of 10 mM sodium dihydrogenphosphate–sodium tetraborate buffer containing 10 mM SDS and 10% acetonitrile, pH 9.0 was found to be optimal. Dextromethorphan hydrobhromide, diphenhydramine hydrochloride and phenylephrine hydrochloride were baseline-separated in less than 11 min, giving separation efficiencies of up to 494,000 theoretical plates, reproducibility of corrected peaks areas below 3% relative standard deviation and concentration detection limits from 2.5 to 5.5 μg ml^{-1}. Detection was performed at 196 and 214 nm. © 2002 Published by Elsevier Science B.V.

Keywords: Capillary electrophoresis; Micellar electrokinetic chromatography; Dextromethorphan; Diphenhydramine; Phenylephrine

1. Introduction

The necessity to ensure the quality of pharmaceutical polydrugs, and consequently the safety and efficacy of the final marketed product, has led to the development and evaluation of new techniques that can reduce the time and cost of analysis. Dextromethorphan (DMF), diphenhydramine (DFH) and phenylephrine (FE) are basic nitrogenous compounds commonly used as active ingredients in cold medicine formulations due to their antitussive, antihistaminic and decongestant activities.

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Several methods describing the simultaneous determination of a wide variety of active compounds in various cough–cold formulations have been reported [1–5]. The separation and determination of these products in dosage forms are complicated due to their similarities in some physical and chemical properties. Ultraviolet (UV–Vis) spectroscopy [6,7], high performance liquid chromatography (HPLC) [8,9], gas chromatography (GC) [10] and multivariate spectrophotometric methods [11–13] have been used to determine these compounds in preparations. Currently, many official methods 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 [14]. In the case of HPLC, these basic drugs strongly interact with the stationary phases causing peak asymmetry and low separation efficiency. The United States Pharmacopoeia Convention in Spring 1995 suggested the reduction of the amount of reagents and materials used in pharmaceutical test and assays that have the potential to cause harm to human health and environment [15].

The use of capillary electrophoresis (CE) in pharmaceutical analysis can have benefits in terms of robustness and ruggedness, cost and time. The versatility of CE in the analysis of a wide array of pharmaceutically relevant analytes varying in polarity, size, and stereochemistry has been represented through the numerous accounts available in the literature [16,17]. The high efficiencies obtained in CE are well suited for complex mixtures in which resolution of a large number of peaks in a short time of analysis is desirable.

The purpose of this study was to develop a CE methodology for the separation and simultaneous determination of DMF (an antitussive agent), DFH (an antihistaminic) and FE (a nasal and bronchial decongestant). Separations of these compounds were not obtained by capillary zone electrophoresis (CZE). However, complete resolution of the sample was reached by micellar electrokinetic chromatography (MEKC). MEKC separation involves differences in hydrophobicity, hydrogen bonding, and charges, and such interactions are manipulated by surfactant concentration and organic modifiers. Therefore, acetonitrile was used to enhance the resolution. The effects of pH, buffer, surfactant and organic modifiers concentration, sampling and separation modes were investigated. The best results were obtained with a background electrolyte (BGE) containing sodium dodecylsulphate (SDS)-borate-phosphate-acetonitrile.

2. Experimental

2.1. Reagents and chemicals

The structure and formulae of the compounds studied are shown in Fig. 1. FE hydrochloride, DMF hydrobhromide, and DFH hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Benadryl DM Compuesto™ was supplied from a local pharmacy and manufactured by Parke–Davis (Buenos Aires, Argentina). HPLC grade acetonitrile and sodium dihydrogenphosphate (NaH_2PO_4) was from Merck (Buenos Aires, Argentina), sodium tetraborate

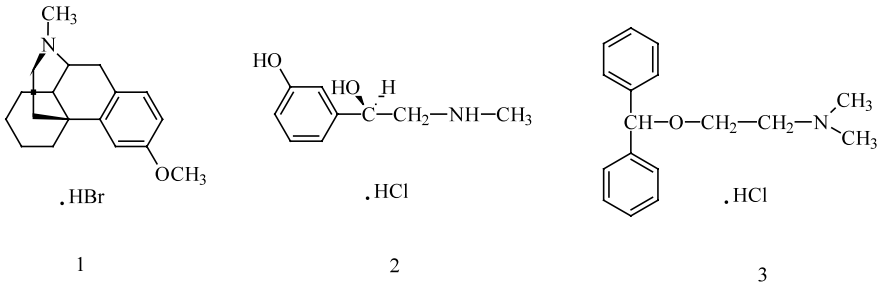

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Fig. 1. Chemical structures of (1) DMF hydrobhromide; (2) FE hydrochloride; and (3) DFH hydrochloride.

($Na_2B_4O_7 \cdot 10H_2O$) from Mallinckrodt (St. Louis, MO), and SDS was supplied by Tokyo Kasei Industries (Chuo-Ku, Tokyo, Japan). The water used in all studies was ultra-high-quality water obtained from a Barnstead Easy pure RF compact ultrapure water system. All solutions were degassed by ultrasonication (Testlab Buenos Aires, Argentina). Running electrolytes and samples were filtered through a 0.45 μm Titan Syringe filters (Sri Inc., Eaton Town, NJ).

2.2. Instrumentation

A Beckman P/ACE MDQ instrument (Beckman Instruments, Inc. Fullerton, CA) equipped with a diode array detector and a data handling system comprising an IBM personal computer and P/ACE SYSTEM MDQ software. Detection was performed at 196 and 214 nm. The fused silica capillaries were obtained from MicroSolv Technology Corporation (New Jersey, USA) and had the following dimensions: 67 cm total length, 50 cm effective length, 75 μm ID, 375 μm OD. The temperature of the capillary and the samples was maintained at 25 °C. Samples were pressure-injected at the anodic side at 0.5 Psi for 5 s.

2.3. Regeneration of capillary and its maintenance

Capillary preparation was carried out by rinsing with 0.1 g mol^{-1} of NaOH for 5 min, then with water for 5 min, and it was finally conditioned with running electrolyte for 10 min before sample injection. To achieve high migration-time reproducibility and to avoid solute adsorption, the capillary was washed between analyses with sodium hydroxide for 2 min, followed by water for 2 min, and then equilibrated with the running buffer for 4 min.

2.4. BGE solutions

For CZE, BGEs were composed of 10 mM sodium dihydrogenphosphate solution (pH 2.5–6.0). Electroosmotic flow (EOF) determination was performed by using acetone as an EOF marker. The EOF marker was prepared by diluting 1 ml of acetone with BGE and sonication for 5 min prior to injection. For MEKC, BGE was composed of 10 mM sodium dihydrogenphosphate–sodium tetraborate buffer (pH 9.0), 10 mM SDS and acetonitrile (10%).

2.5. Preparation of standard solutions

Stock standard solutions for the construction of calibration curves were prepared by dissolution of each compound in BGE (1 mg ml^{-1}) and then suitably diluted to obtain standard solutions within the concentration range 10–100 mg l^{-1}.

A combined standard solution containing FE hydrochloride, DMF hydrobhromide, and DFH hydrochloride was prepared by accurately weighing 50 mg of each powder and made up to 1000 ml with BGE.

A diluted solution of the commercial formulation was prepared as follows: 2 ml of syrup (Benadryl DM Compuesto) was carefully measured into a volumetric flask and diluted to 100 ml with buffer solution.

3. Results and discussion

3.1. CZE

The optimization was performed using a combined mixture containing FE hydrochloride, DMF hydrobhromide, and DFH hydrochloride. The structure of these compounds is given in Fig. 1.

First, the ordinary CZE separation mode was investigated. Therefore, sodium dihydrogenphosphate solutions (10 mM) at pHs 2.5, 4.0, 5.0 and 6.0 were employed to separate these compounds. It was observed that overlapping of the DFH and DMF peaks occurred (Fig. 2). As the pHs increased, the migration times of all compounds decreased, whereas the mobilities increased. Table 1 shows the results obtained for pHs 2.5, 4 and 6. Lowering the buffer pHs often results in the decreasing of EOF, while increasing buffer pHs enhances EOF.

In conclusion, it was found that the peaks of the active compounds were completely overlapped. The results cannot be improved by simply

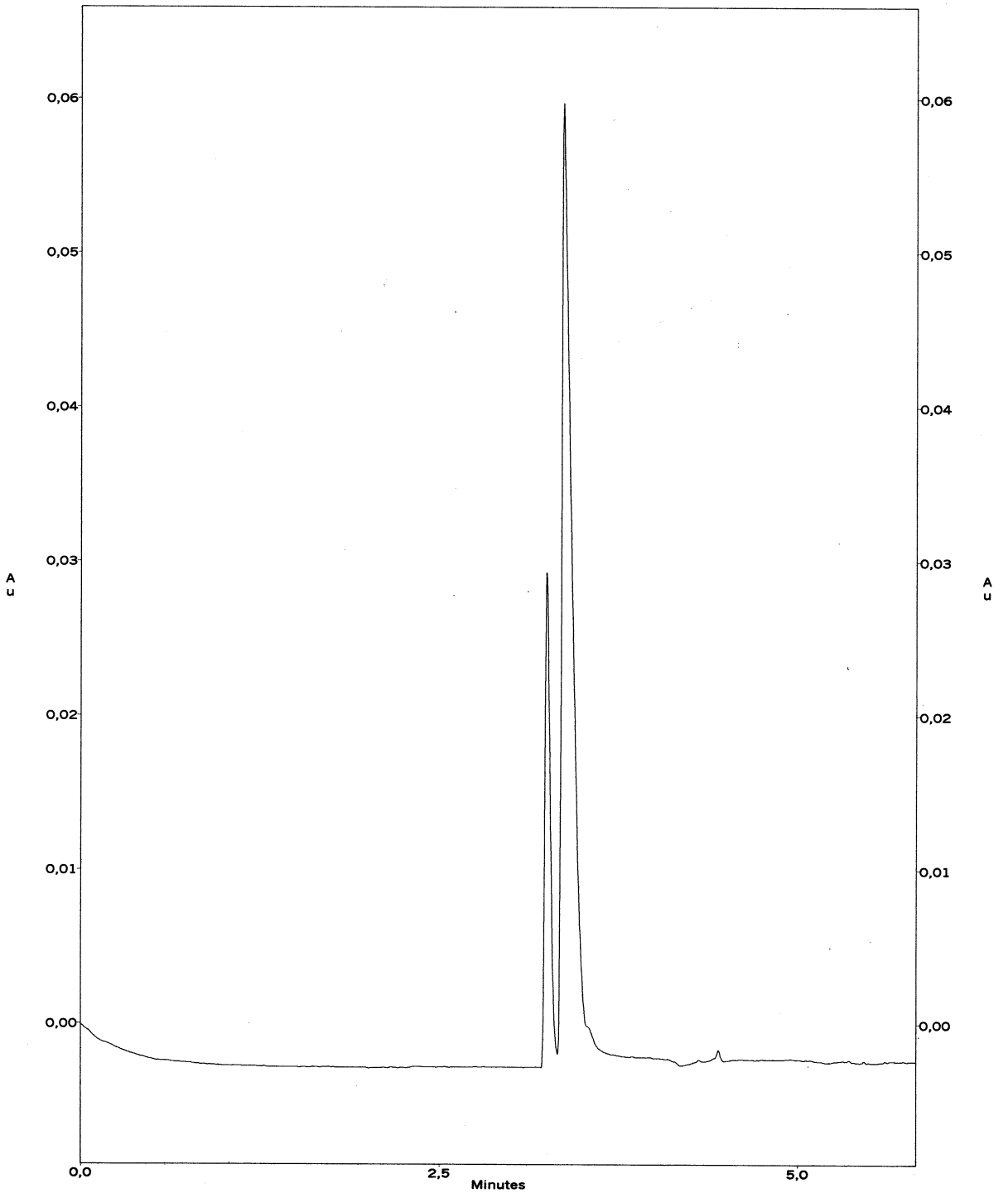

Fig. 2. Electropherogram of a standard mixture of FE, DMF, and DFH. Conditions: 10 mM sodium dihydrogenphosphate buffer, pH 5.0; capillary, 67 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 0.5 Psi, 5 s; 30 kV constant voltage; detection by UV absorbance at 214 nm. Peak identification: (1) FE hydrochloride; (2) DMF hydrobhromide; (3) DFH hydrochloride.

Table 1
Effects of pH on the migration times and electrophoretic mobilities (μ_e) of cold medicine ingredients in the CZE mode

	pH 2.5		pH 4		pH 6	
	Time (min)	μ_e ($cm^2\ V^{-1}\ s^{-1}$)	Time (min)	μ_e ($cm^2\ V^{-1}\ s^{-1}$)	Time (min)	μ_e ($cm^2\ V^{-1}\ s^{-1}$)
DMF	12.01	1.17×10^{-5}	2.97	6.26×10^{-4}	2.30	8.09×10^{-4}
DFH	11.98	1.41×10^{-5}	2.93	6.35×10^{-4}	2.31	8.27×10^{-4}
FE	9.27	6.17×10^{-5}	2.83	6.57×10^{-4}	2.25	8.85×10^{-4}

Conditions: capillary, 67 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 0.5 Psi, 5 s; 30 kV constant voltage; detection by UV absorbance at 214 nm.

changing the pH value, the buffer concentration and the running voltage.

3.2. MEKC

MEKC is a hybrid method between electrophoresis and chromatography, which is mainly used for the separation of neutral compounds that may normally not be resolved. For pharmaceuticals, MEKC has been used for the determination of active drugs in tablets, creams and parenteral formulations [18–21]. Thus, MEKC might be an alternative mode for the separation of active ingredients in this study. These drugs, including cationic, anionic and neutral, could be separated by MEKC with relatively short analysis time [22–25].

The following parameters were consecutively optimized: buffer pH, buffer concentration, surfactant concentration and organic modifier concentration.

3.3. Analytical method parameters

3.3.1. Effect of pH

The buffer pH plays an important role in MEKC because it affects both the overall charges of the solute and EOF. Thus, the effect of the buffer pH was also investigated in the range of 7.6–10.0 at fixed buffer and surfactant concentration (10 mM), adjusted by 0.1 mol l^{-1} of NaOH and 0.1 mol l^{-1} of HCl. It was found that when the pH was lower than 8.5, the resolution was poor.

3.3.2. Effect of buffer concentration

Buffer concentration has also a significant effect on the separation performance through its influence on EOF and the current produced in the capillary. Keeping other parameters constant (C_{SDS} = 10 mM, pH 9.0, 25 kV, 25 °C), the buffer concentration was varied from 5 to 50 mM sodium dihydrogenphosphate—sodium tetraborate. Increases in migration times as well current were observed when the concentration of buffer increased. Resolution also increased for higher buffer concentrations, but no appreciable improvements were observed for buffer concentrations above 10 mM. So, the best results concerning time analysis and current generated were obtained with 10 mM sodium dihydrogenphosphate—sodium tetraborate buffer, pH 9.0. Although a complete resolution was not achieved under these conditions, this BGE was selected for further modifications to enhance the separation of FE, DMF, and DFH.

3.3.3. Effect of surfactant concentration

When 8 mM SDS was added to the buffer, it was found that the peak shapes of the sample were greatly improved, but the separation exhibited no significant improvement even when the SDS concentration was increased to 20 mM. All buffers provided baseline-separation of FE; however, DMF and DFH were not completely separated (Table 2).

Thus, 10 mM sodium dihydrogenphosphate–sodium tetraborate buffer containing 10 mM SDS, pH 9.0, was used as BGE for further study.

3.3.4. Effect of organic modifier concentrations

It has been reported that organic modifiers are very important to improve separation in many systems because they can change the partition coefficient and polarity of the sample [1,26–28]. In this paper, acetonitrile was used as an organic modifier to enhance the resolution of the active ingredients in this study. Various amounts of acetonitrile (5, 10, and 15%) were added into the sodium dihydrogenphosphate–sodium tetraborate buffer containing 10 mM, pH 9.0. Separations of these compounds were not obtained when 5% of acetonitrile was added into BGE. All compounds were baseline-separated when 10% of acetonitrile was added (Table 2).

3.3.5. Quantitation

The calibration plots were measured under the optimal experimental conditions for DMF, DFH and FE concentrations within the range 10–150 μg ml^{-1}.

The calibration plots were obtained, representing the ratio of the corrected areas versus concentration. The calibration equations were calculated by the least-squares linear regression method, and unknown concentrations were calculated by interpolation. The detection and quantitation limits were calculated as the analyte concentrations that give rise to peak heights with a signal-to-noise ratio of 3 and 10, respectively. The limit of detection (LOD) was determined by injecting standard combined solution at three different level concentrations for each analyte (15, 30 and 50 μg ml^{-1}). Table 3 shows the concentration ranges for calibration curves of each analyte, regression parameters and LOD and quantitation.

In order to determine the repeatability (within-day precision) of the method, replicate injections ($n = 6$) of 50.0 μg ml^{-1} combined solution containing DMF, DFH and FE were carried out. In all cases, the precision was better than 1.9% for the migration time and 2.85% for the peak area. Good peak area precision was achieved without adding any internal standard.

The reproducibility (between-day precision) was also evaluated over 3 days by performing six

Table 2
Effects of surfactant concentration and organic modifiers on the migration times and electrophoretic mobilities (μ_e) of cold medicine ingredients in the MEKC mode

BGE	I[a]		II[b]		III[c]	
	Time (min)	μ_e ($cm^2\ V^{-1}\ s^{-1}$)	Time (min)	μ_e ($cm^2\ V^{-1}\ s^{-1}$)	Time (min)	μ_e ($cm^2\ V^{-1}\ s^{-1}$)
DMF	9.18	2.02×10^{-4}	13.46	1.38×10^{-4}	10.42	1.78×10^{-4}
DFH	9.16	2.03×10^{-4}	13.52	1.37×10^{-4}	6.14	3.03×10^{-4}
FE	3.97	4.68×10^{-4}	9.72	1.91×10^{-4}	4.60	4.04×10^{-4}

Conditions: pH 9.0; capillary, 67 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 0.5 Psi, 5 s; 25 kV constant voltage; detection by UV absorbance at 214 nm.

[a] $C_{SDS} = 10$ mM.

[b] $C_{SDS} = 15$ mM.

[c] $C_{SDS} = 10$ mM, $C_{ACN} = 10\%$ (v/v).

Table 3
Typical linear regression data for the analysis of DMF, DFH and FE

Analyte	Concentration range (μg ml^{-1})	r^2 ($n = 6$)	Slope	Intercept	LOQ (μg ml^{-1})	LOD (μg ml^{-1})
DMF	10–150	0.997	226.62 ± 6.55	0.169 ± 0.35	8.33	2.5
DFH	15–150	0.997	139.00 ± 4.11	-0.052 ± 0.21	18.30	5.5
FE	10–150	0.998	371.48 ± 8.40	0.610 ± 0.43	8.33	2.5

Table 4
Commercial formulation[a] recovery test

	Base value (μg ml^{-1})	Quantity added (μg ml^{-1})	Quantity found[b] (μg ml^{-1})	Recovery (%)[c]
Aliquot I				
DMF	–	0.0	59.80	–
DFH	–	0.0	49.94	–
FE	–	0.0	39.60	–
Aliquot II				
DMF	59.80	50.0	110.8	102
DFH	49.94	0.0	–	–
FE	39.60	0.0	–	–
Aliquot III				
DMF	59.80	0.0	–	–
DFH	49.94	50.0	99.74	99.60
FE	39.60	0.0		–
Aliquot IV				
DMF	59.80	0.0	–	–
DFH	49.94	0.0	–	–
FE	39.60	50.0	88.80	98.40

[a] Diluted solution of the commercial formulation, Benadryl DM™.
[b] Mean value ($n = 6$).
[c] $100 \times \frac{\text{Found-base}}{\text{Added}}$.

injections each day. The reproducibility (RSD) on the basis of migration time and peak area was better than 0.62 and 2.98%, respectively.

Robustness of the method was determined by two analysts (six determinations) using the proposed method and the same instrumentation. The results showed no significant differences: 96.1% (found) and 1.0 (RSD%).

3.4. Assay of a commercial product. Recovery test

Once the conditions for separation and quantification were established, the CE method was applied for the determination of FE hydrochloride, DMF hydrobhromide, and DFH hydrochloride in a commercial formulation (Fig. 3). Results were highly satisfactory.

In order to validate this method, 50 ml of the diluted solution of the commercial formulation was collected and divided into ten portions of 5 ml each. The proposed method was applied to six portions, and the average concentrations determined for each compound (FE, DMF and DFH) were taken as a base value. Then, known quantities of the analytes were added to the other aliquots, and the active compounds were determined following the recommended procedure (Table 4).

4. Conclusions

The optimized method shows a good performance with respect to selectivity, linearity and accuracy with regard to the mixture under investigation. The results of this study clearly demonstrate the potentiality and versatility of this method, which could be applied for routine monitoring of active syrup ingredients (FE, DMF, and DFH) in pharmaceutical formulations.

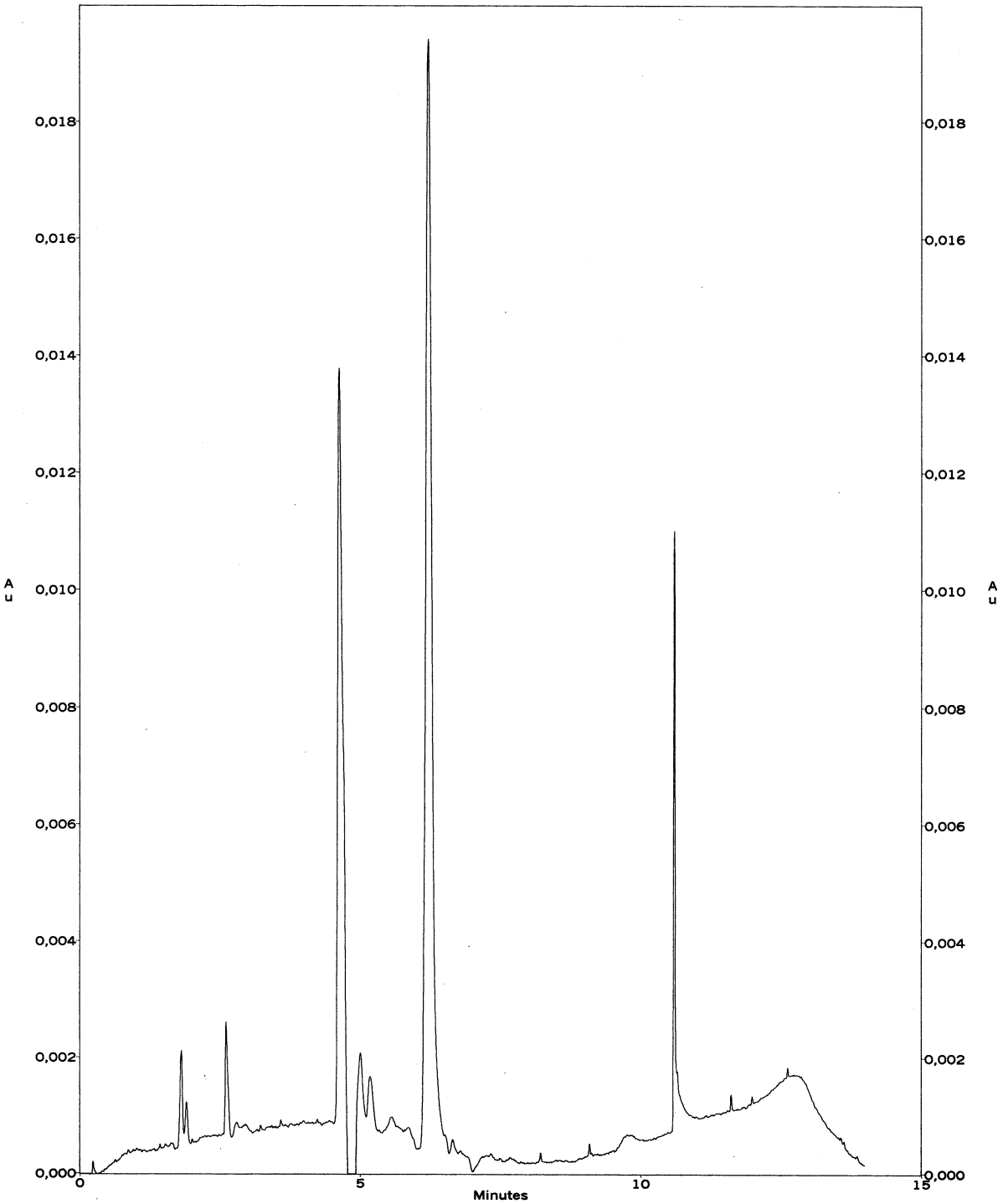

Fig. 3. Electropherogram of Benadryl DM™ syrup. Conditions: 10 mM sodium dihydrogenphosphate–sodium tetraborate buffer, containing 10 mM SDS, pH 9.0; capillary, 67 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 0.5 Psi, 5 s; 25 kV constant voltage; detection by UV absorbance at 214 nm. Peak identification: (1) FE hydrochloride; (2) DFH hydrochloride; (3) DMF hydrobhromide.

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