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Method Development for Separation of Active Ingredients in Cold Medicines by Micellar Electrokinetic Chromatography

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Abstract

Separation of nine commonly used active ingredients in cold medicines, were demonstrated by micellar electrokinetic chromatography. The ingredients included paracetamol, chlorpheniramine maleate, diphenhydramine hydrochloride, triprolidine hydrochloride, phenylpropanolamine hydrochloride, dextromethorphan hydrobromide, loratadine, aspirin and caffeine. Effects of buffer concentrations, pH, organic modifiers and capillary length were investigated. The optimum conditions were achieved in 10 mM sodium dihydrogenphosphate-sodium tetraborate buffer, pH 9.0, containing 50 mM sodium dodecyl sulfate and 28% v/v acetonitrile using the effective length of 50 cm, the separating voltage of +15 kV and the capillary temperature of 30°C. Separation of all peaks was obtained within 28.4 min with a resolution of 1.2.

Keywords

cold medicine ingredients, micellar electrokinetic chromatography (MEKC)

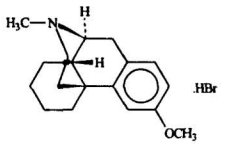
Introduction

Paracetamol, chlorpheniramine maleate, diphenhydramine hydrochloride, triprolidine hydrochloride, phenylpropanolamine hydrochloride, dextromethorphan hydrobromide, loratadine, aspirin and caffeine (Fig. 1.) are commonly used acitve ingredients in cold medicines. Examples of the pharmaceutical formulations of these drugs were shown in Table 1. Phenylpropanolamine was not included in the table since the Thai Food and Drug Administration recently recommended the removal of phenylpropanolamine from all products due to the risk of hemorrhagic stroke associated with its use [1]. The active ingredients in cold medicine show similar physical and chemical properties, which make their separation and determination difficult. Quantitation of these compounds is extensively performed by ultra-violet (UV-Vis) spectroscopy [2-4] and reversed-phase high-performance liquid chromatography (RP-HPLC) [5-12]. UV-Vis spectroscopy usually requires sample pre-treatment or extraction prior analyses, which could be laborious and time consuming. Basic nitrogenous compounds usually strongly interact with the stationary phase of RP-HPLC resulting in peak asymmetry and low separation efficiency. Additionally, RP-HPLC can be expensive due to the high organic solvent consumption. Capillary electrophoresis (CE) is now a well-established method for pharmaceutical analysis [13,14] and has been applied for determination of active ingredients in cold medicines [15-21]. Phenylpropanolamine hydrochloride, dextromethorphan hydrobromide, chlorpheniramine maleate and paracetamol were successfully separated within 11.4 min with a resolution of 1.2 by micellar electrokinetic chromatography (MEKC) using sodium dodecyl sulfate (SDS) as a surfactant [15]. CE with electrospray mass spectrometry (MS) was utilized for determination of paracetamol residue in river water samples [16]. Determination of caffeine and its metabolites, theobromine, parasanthin, theophylline and 1,3,7-trimethyluric acid was achieved by MEKC using SDS as a surfactant [17]. The separation was achieved in 120 s with detection limits of less than 1 μg/mL.

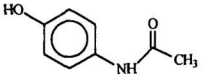
Paracetamol and its metabolites in urine and plasma were directly determined by CE with UV and MS detection [18]. Analysis of caffeine, aspartame and benzoic acid in soft drinks and artificial sweetening powders were compared by capillary zone elctrophoresis (CZE) and HPLC [19]. Results showed that CZE provided 65-110 times higher separation efficiency than that of HPLC, whereas HPLC was 10-20 times higher sensitive than CZE. Paracetamol, pseudoephedrine hydrochloride and dextromethorphan were baseline separated in 20 mM sodium phosphate buffer, pH 7.0, within 5 min using an UV detector at 200 nm [20]. Phenylpropanolamine hydrochloride as well as other anorexics were simultaneously analyzed by CE using acetonitrile containing phosphate buffer [21]. It is evident that CE is a powerful tool in drug analysis since it provides high efficiency, reliability and low cost.

Table 1 Examples of the pharmaceutical formulations of the investigated compounds

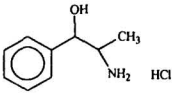
Name	Formulation
Tylenol, Panadol tablets	Paracetamol 500 mg
Coldosain tablets	Paracetamol 500 mg Chlorpheniramine maleate 2 mg Caffeine anhydrous 30 mg
Piriton tablet	Chlorpheniramine maleate 4 mg
Tiffy, Decolgen tablets	Paracetamol 500 mg Chlorpheniramine maleate 2 mg
Benedryl capsule	Diphenhydramine HCl 25 mg
Bayer aspirin tablet	Aspirin 500 mg
Aspent enteric-coated tablet	Aspirin 300 mg
Actifed tablet	Triprolidine HCl 2.5 mg Pseudoephedrine HCl 60 mg
Clarityne tablet	Loratadine 10 mg
Actifed DM cough linctus	Triprolidine HCl 1.25 mg Pseudoephedrine HCl 30 mg Dextromethorphan HBr 10 mg



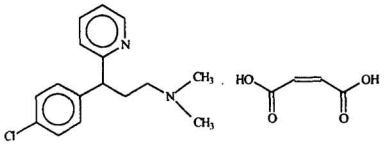
Dextromethorphan hydrobromide
(pK_b = 5.5)



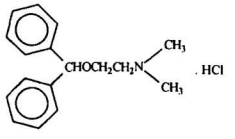
Paracetamol
(pK_b = 4.1)



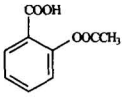
Phenylpropanolamine
(pK_b = 4.6)



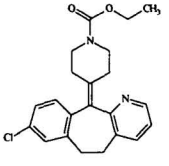
Chlorpheniramine maleat
(pK_b = 4.8)



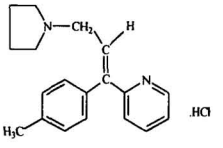
Diphenhydramine hydrochloride
(pK_b = 5.0)



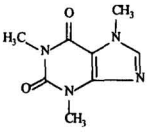
Aspirin
(pK_a = 3.5)



Loratadine
(pK_b = nd.)



Triprolidine hydrochloride
(pK_b = 4.7)



Caffeine
(pK_b =3.6)

Fig. 1. Structures of active ingredients in cold medicines (nd. = not determined)

The aim of this work was to demonstrate the method development procedure for separation of nine commonly used active ingredients in cold medicines. This was the first study that reports the simultaneous separation of these compounds by MEKC. Factors affecting the separation, including buffer concentrations and pH,

types and percentages of organic modifiers and capillary length, were systematically examined.

Experimental

Instruments

The CE system was a Beckman Capillary Electrophoresis System Model P/ACE 2000 instrument equipped with a positive power supply and a fixed wavelength detector controlled by PC through Beckman System Gold software. The separations were performed on an eCAP™ capillary tubing (lot no. S601595, reorder no. 338473, Beckman Instrument, U.S.A.), thermostated at 30 °C. The original dimension of the tube was 5 m in lenght, inner diameter of 75 μm, and outer diameter of 375 μm. The tube was cut to the desired length.

Separations were carried out at a constant voltage of +15 kV or +25 kV. UV detection was performed at 254 nm. Sample injections were achieved using the pressure mode for 10 second. For a new capillary tube, the tube was fast pre-conditioned by rinsing with 0.1 N sodium hydroxide for 20 min, followed, by deionized water for 10 min. For routine use, the tube was washed with 0.1 N sodium hydroxide for 2 min, followed, by deionized water for 2 min, and then equilibrated with the buffer for 5 min. As electrolysis can alter the composition of the buffer and subsequently change the electro-osmotic flow (EOF), the buffer solution was replaced at regular intervals to maintain high reproducibility. Prior to each sequence, voltage had to be controlled by increasing from +10 kV to +15 kV in 8 minutes.

Materials

Paracetamol, chlorpheniramine maleate, diphenhydramine hydrochloride, triprolidine hydrochloride, phenylpropanolamine hydrochloride and sodium tetraborate were from Aldrich (Milwaukee, MI, USA) and dextromethorphan hydrobromide, loratadine, aspirin, caffeine sodium dodecyl sulfate, and sodium

dihydrogenphosphate were from Sigma (St. Louis, MO, USA). Methanol, ethanol acetonitrile were HPLC grade, all other reagents were of analytical reagent grade and water was deionized distilled water.

Standard preparation

Stock standard and working standard solutions were prepared in deionized water at a concentration of 1 and 0.1 mg/mL, respectively. Except for loratadine, methanol was used as a solvent. All soultions were filtered through a 0.45 μ membrane prior injection.

Optimization procedure

Separation of the working standard solutions was performed on different capillary length (43-65 cm, effective length) using a constant voltage of +15 or +25 kV and an capillary temperature at 30°C). Different concentrations (10-40 mM) and pH (7.0-10.0) of sodium dihydrogenphosphate-sodium tetraborate at a ratio of 1:1 were optimized. The pH buffere was adjusted by adding 1 N HCl or 1 N NaOH. Effects of types (e.g. MeOH, EtOH, *i*-PrOH, ACN) and percentages of organic modifier (5-30% v/v) were also investigated. The optimum condition was determined by the resolution value (R_S) of the two closest peaks calculated from: $R_S = 2\ (t_2-t_1)/\ (w_1+ w_2)$, where t and w were migration time and baseline peak width, respectively.

Results and Discussion

Effects of buffer concentrations and pH

The preliminary condition for the separation of the investigated compounds was modified from our previous study [14]. The condition was 10 mM phosphate-borate buffer, pH 9.0, containing 50 mM SDS and 5% v/v MeOH using an effective capillary length of 45 cm with an inner diameter of 75 μ, and a constant voltage of +25 kV. Under this condition, the nine compounds were separated into three groups of overlapped peaks with a total migration time of 11.0 min. Changing of the

buffer concentration from 10 mM to 20, 30 and 40 mM resulted in decreasing of the separation efficiency and increasing of the total migration time (Fig. not shown). The increase of the migration time at higher buffer concentrations was due to the reduced EOF and the decrease of the observed electrophoretic mobility. Additionally, when the buffer concentration of 20-40 mM was employed, the high current (>100 μA) was obtained causing a lot of spiking peaks . We reasoned that this was due to an increase of the Joule's heating at high concentration buffers. Thus, the phosphate-borate buffer concentration was maintained at 10 mM and further modification was investigated by varying the buffer pH. Fig. 2 illustrated the influence of pH on separation of the investigated compounds. The nine compounds were separated into four groups of peaks at pH 7.0, 8.0, 9.0 with the total migration time of 52.8, 32.9 and 11.6 min, respectively. The inonization of the investigated compounds was minimized at or around neutral pH (7.0 and 8.0), therefore these pH was excluded from the study. The separation at pH 10.0 was slightly improved with the total migration time of 29.6 min. However, at this high pH (10.0), the analytes quiclkly degraded causing the instability of the standard solution. Futher experiments were performed at pH 9.0, but different organic modifiers were included in order to improve the separation efficiency of the analytes.

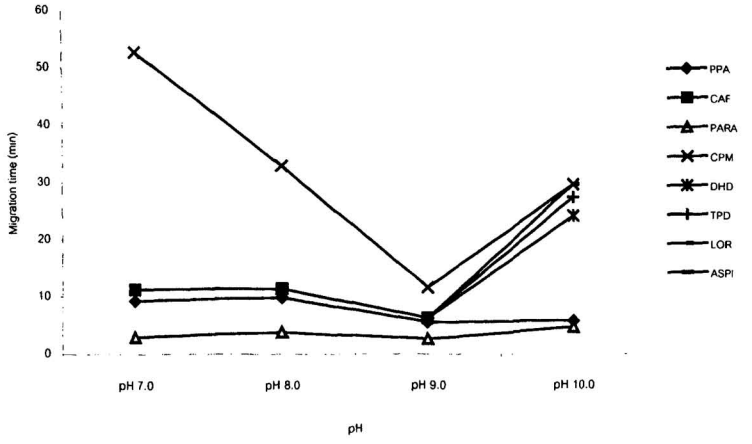


Fig. 2. Influence of pH on the separation of the investigated compounds. Condition: 10 mM sodium dihydrogenphosphate-sodium tetraborate containing 50 mM SDS, applied voltage: 25 kV, temperature: 30 °C, capillary: 45 cm (effective length) x 75 μ (I.D). PPA = Phenylpropanolamine, CAF = Caffeine, PARA = Paracetamol, CPM = Chlorpheniramine maleate, DHD = Diphenhydramine, DEX = Dextromethorphan, TPD= Tripolidine, LOR = Loratadine, ASPI = Aspirin.

Effects of types and percentages of organic modifiers

In MEKC, selectivity is greatly affected by types and concentrations of organic modifiers. Separation obtained from buffer in the presence (5% v/v MeOH, EtOH, *i*-PrOH and ACN) and absence of organic modifiers were compared. In all cases, buffer containing organic modifiers provided the better results with the total migration time of 10.3 (without organic modifier), 11.0 (MeOH), 12.3 (EtOH), 12.8 (*i*-PrOH) and 28.5 (ACN), min. This is not unexpected since organic modifiers can manipulate the hydrophobicity, hydrogen bonding and charge interaction of solutes [14,16]. Small amount of organic modifiers increases the migration window and resolutions of the separation due to the increase of the micellar electrophoretic mobility. Lengthening of the alcohol chain from MeOH to *i*-PrOH did not greatly improve the separation, but increased the migration time. The employment of

MeOH at higher levels (10-40% v/v) still did not give satisfying results, but the lengthening of the migration time from 11.0 to 14.7 min was obtained. Results indicated that a different organic modifier was necessary and acetonitrile (ACN) was chosen as the alternative. Interestingly, varying amount of ACN significantly improved the separation of the analytes (Fig. 3). The separation was greatly enhanced when 25-30% v/v of ACN was added into the buffer. Keeping other factors constant (10 mM phosphat-borate buffer, pH 9.0, 30 °C), the combination of 28% v/v ACN as the organic modifier and the reduced separating voltage (+15 kV) provided separation of the analytes within 28.4 min. We reasoned that ACN has lower polarity than MeOH and has a stronger effect in the enlargement of the migration window resulting in the improvement of the separation. Additionally, ACN greatly reduced the EOF and the electrophoretic mobility of the micelles and analytes providing more partitioning between the analytes and the micelles. However, when excess organic modifier (30% v/v of ACN) was added into the buffer, the micelle formation decreased causing the overlapped peaks and lower separation efficiency.

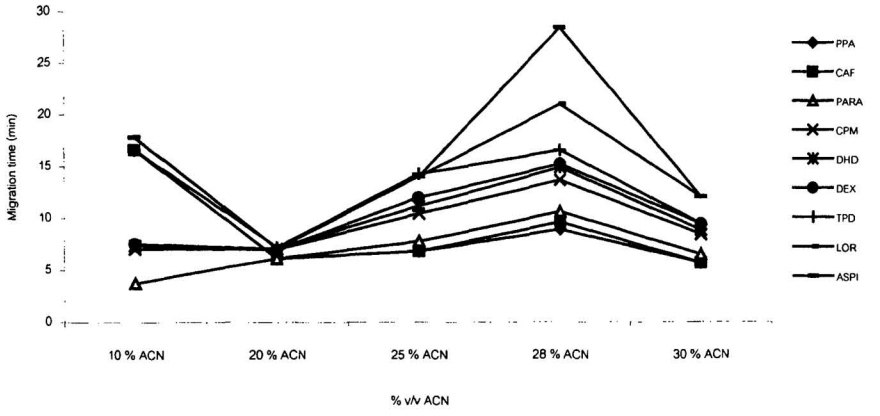


Fig. 3. Influence of percentages of acetonitrile on the separation of the investigated compounds. Condition: 10 mM sodium dihydrogenphosphate-sodium tetraborate (pH 9.0) containing 50 mM SDS and various amount of ACN, applied voltage: 15 kV, temperature: 30 °C, capillary: 50 cm (effective length) x 75 μ (I.D). Numbers are the same as Fig. 2.

Effects of capillary length

Capillary dimension, particularly the effective length, can improve or worsen the separation efficiency. In this study, various effective length ranking from 43-65 cm was investigated. As the length increased, the migration time and separation efficiency increased (Fig. 4). However, diphenhydramine and dextromethorphan overlapped at the length of 43 and 65 cm. Additionally, phenylpropanolamine hydrochloride and caffeine were not completely separated at 43 cm. The optimum separation of all compounds was obtained in a reasonable migration time (28.4 min) at the length of 50 cm with the resolution of 1.2 calculated from the two closest peaks, diphenhydramine and dextromethorphan.

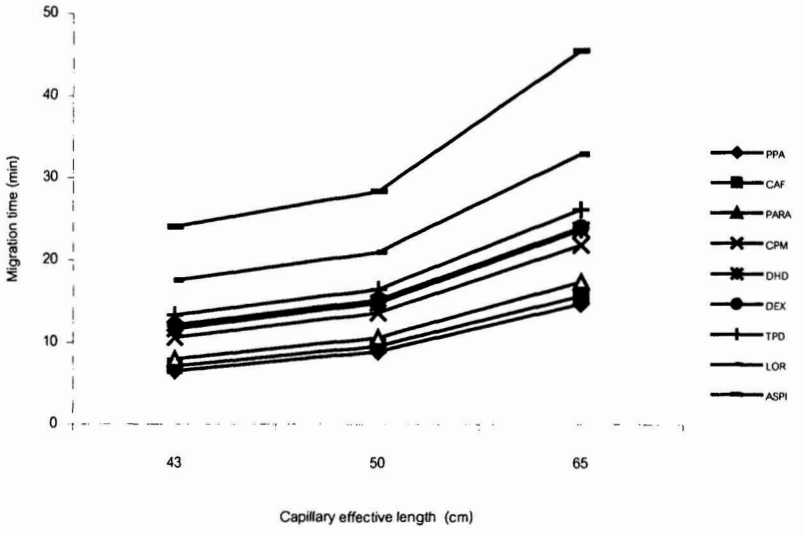


Fig. 4. Influence of capillary length on the separation of the investigated compounds. Condition: 10 mM sodium dihydrogenphosphate-sodium tetraborate (pH 9.0) containing 50 mM SDS and 28% v/v ACN, applied voltage: 15 kV, temperature: 30 °C, capillary: various effective length x 75 μ (I.D). Numbers are the same as Fig. 2.

Conclusion

Our results suggest that the commonly used active ingredients in cold medicines including paracetamol, chlorpheniramine maleate, diphenhydramine hydrochloride, triprolidine hydrochloride, phenylpropanolamine hydrochloride, dextromethorphan hydrobromide, loratadine, aspirin and caffeine, could be successfully separated by MEKC. Main factors affecting the separation of these compounds were buffer concentrations and pH and organic modifiers, whereas, capillary lengths help enhancing the separation efficiency. The optimum conditions were a 10 mM sodium dihydrogenphosphate-sodium tetraborate buffer, pH 9.0 containing 50 mM SDS and 28% v/v ACN using a constant voltage of +15 kV,

capillary temperature of 30 °C and a capillary length of 50 cm (effective length). The order of elution was phenylpropanolamine, caffeine, paracetamol, chlorpheniramine maleate, diphenhydramine, dextromethorphan, triprolidine, loratadine and aspirin, respectively. The conditions provided separation of all compounds (Fig. 5) with a reasonable migration time (t_m = 28.4 min) and an acceptable resolution (R_S = 1.2) calculated from the two closest peaks (diphenhydramine and dextromethorphan). The developed method is currently transferred to our new CE instrument, method validation and applications for determination of active ingredients in cold medicine preparations is under investigation.

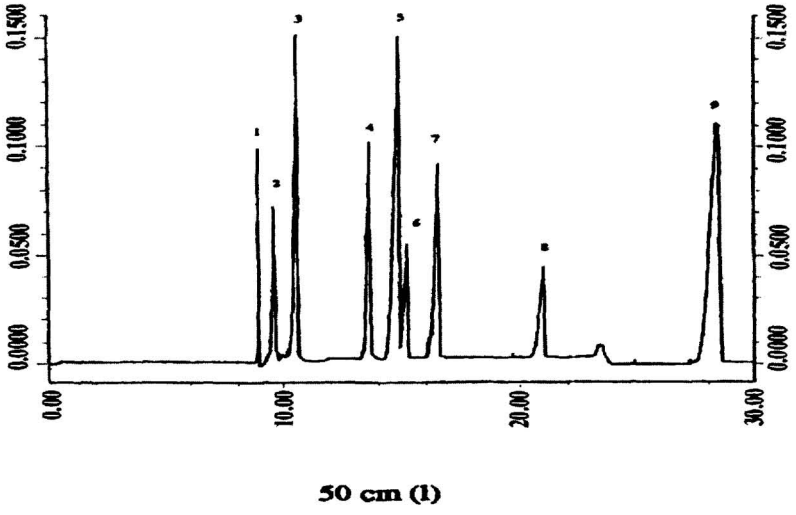


Fig. 5. A typical electropherogram of the investigated compounds under the optimized conditions. 1 = Phenylpropanolamine, 2 = Caffeine, 3 = Paracetamol, 4 = Chlorpheniramine maleate, 5 = Diphenhydramine, 6 = Dextromethorphan, 7= Tripolidine, 8 = Loratadine, 9 = Aspirin.

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