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Simultaneous determination of paracetamol and chlorpheniramine maleate by micellar electrokinetic chromatography

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Abstract

A micellar electrokinetic chromatography (MEKC) method was established for determination of paracetamol (PARA) and chlorpheniramine maleate (CPM) in cold tablets. Separation of both drugs, as well as other seven cold remedy ingredients, was achieved in 25.5 min using a sodium dihydrogenphosphate–sodium tetraborate buffer (10 mM, pH 9.0) containing sodium dodecyl sulfate (SDS) (50 mM) and acetonitrile (26% v/v). The effective capillary length of 50 cm, the separating voltage of 15 kV and the temperature of 30 °C was optimized. Detection was by a diode array detector at 214 nm. Method linearity was excellent ($r^2 > 0.999$) over the concentration tested (10–250 μg/ml) with good precision and accuracy. Recoveries were good (> 99%) with limits of detection of 0.4 and 0.5 μg/ml and limits of quantitation of 2 (%R.S.D. = 3.1%) and 4 (%R.S.D. = 2.4%) μg/ml, for PARA and CPM, respectively. The developed method was applied to the determination of ingredients in cold tablets and was found to be simple, rapid and efficient.

Keywords: Micellar electrokinetic chromatography (MEKC); Paracetamol (PARA); Chlorpheniramine maleate (CPM); Cold remedy ingredients

1. Introduction

Most cold remedies contain one or more active ingredients, which include paracetamol (PARA), chlorpheniramine maleate (CPM), diphenhydramine hydrochloride (DPH), tripolidine hydrochloride (TPL), phenylpropanolamine hydrochloride (PPA), dextromethorphan hydrobromide (DEX) loratadine (LOR), aspirin (ASA) and caffeine (CAF) (Fig. 1). Simultaneous separation of these compounds can be problematic since they show some similar physical and chemical characteristics. Several methods have been proposed for analysis of these compounds such as spectrophotometry [1–6], HPLC [7–12] and capillary electrophoresis (CE) [13–23]. Spectrophotometric methods

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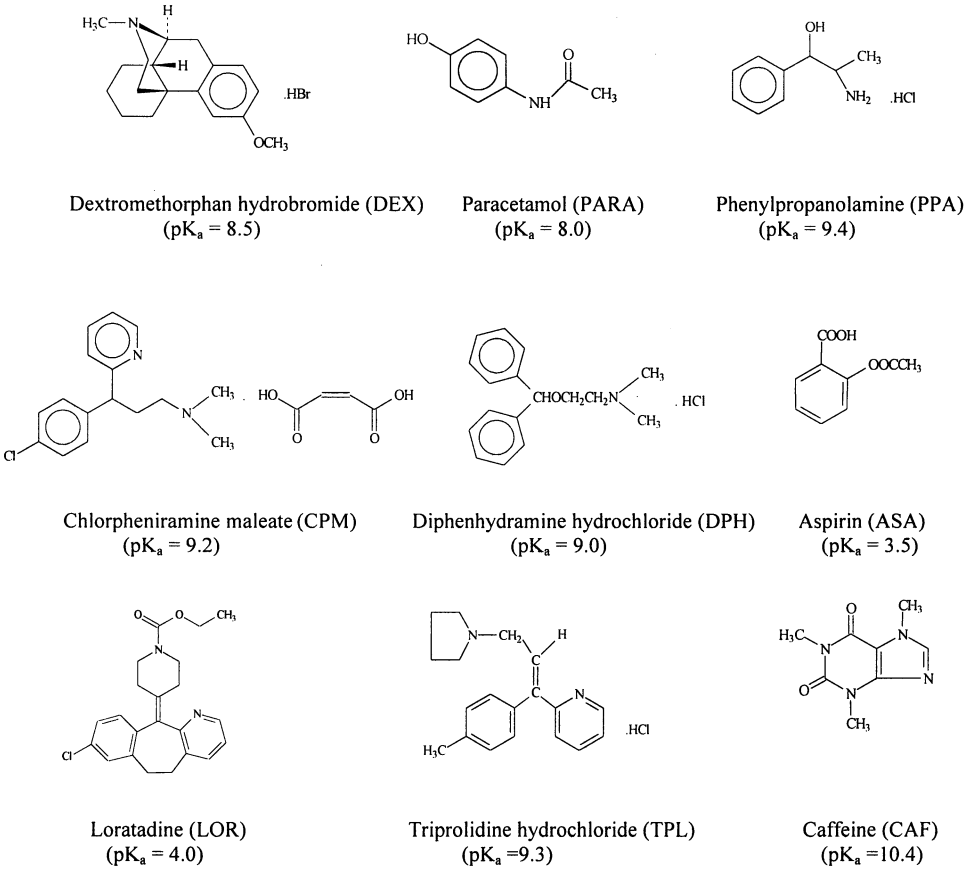

Fig. 1. Structures of the investigated cold remedy ingredients.

usually require sample pre-treatments (e.g. extraction, complex formation, etc.) which can be laborious and time consuming. Additionally, some of the cold remedy ingredients are basic drugs, which strongly react with the stationary phase of the reversed-phase high performance liquid chromatography (RP-HPLC). This causes the peak asymmetry and low separation efficiency. Compared with other techniques CE is a micro-analytical method, which provides advantages in term of simplicity, high efficiency, low cost and short analysis time. Major drawbacks of CE are its sensitivity and reproducibility, which usually are lower than those obtained from HPLC. Micellar electrokinetic chromatography (MEKC) is one mode of CE that is useful for the separation of charged compounds, with similar electrophoretic mobilities, and neutral analytes. Separation in MEKC occurs by the use of either charged or neutral zwitterion surfactants, thus the separation mechanism is based on differences of electrophoretic mobilities, partitioning of analytes and nature of additives.

The previous study of our group [23], revealed that PARA, CPM, PPA and DEX were well separated in a 10 mM sodium dihydrogenphosphate–sodium tetraborate buffer containing 50 mM sodium dodecyl sulfate (SDS) and 5% v/v MeOH, pH 9.0. Since, there is no report on the simultaneous separation of the nine cold remedy ingredients shown in Fig. 1, the current investigation focused on the optimization of the MEKC condition for the separation of these substances. The influence of types and amounts of organic modifiers, buffer concentrations and pH, temperature and voltage were systematically ex-

amined. The optimized condition was validated and applied for quantitation of PARA and CPM in cold tablets.

2. Experimental

2.1. Chemicals

PARA, CPM, DPH, TPL, PPA and sodium tetraborate were from Aldrich (Milwaukee, MI, USA) and DEX, LOR, ASA, CAF, SDS, and sodium dihydrogenphosphate were from Sigma (St. Louis, MO, USA). Methanol (MeOH), ethanol (EtOH), isopropanol (*i*-PrOH) and acetonitrile (ACN) were HPLC grade, all other reagents were of analytical reagent grade and water was deionized distilled water.

2.2. Instrumentation and electrophoretic procedure

CE measurements were performed on a ^{3D}CE system (Hewlett Packard, Waldbronn, Germany) equipped with a diode-array detector, an automatic injector, and an autosampler. Separations were carried out using fused-silica capillary tubes (Polymicro Technologies, USA) with a total length of 58.5 cm, an effective length of 50.0 cm and an inner diameter of 75 μm. The detector wavelength was 214 nm with a bandwidth of 4 nm. All experiments were carried out in a positive mode (anode at the inlet and cathode at the outlet). Sample injections were achieved using the pressure mode for 10 s at 50 mbar. ^{3D}CE Chemstation software (Hewlett Packard) was used for instrumental control, data acquisition and data handling.

For a new capillary tube, the tube was preconditioned by rinsing with 0.1 M sodium hydroxide for 20 min, followed, by deionized water for 10 min. For routine uses, the tube was washed with 0.1 M sodium hydroxide for 2 min, followed, by deionized water for 2 min, and then the buffer for 5 min. As electrolysis can alter the composition of the buffer and subsequently change the electroosmotic flow (EOF), the buffer solution was replaced after five injections to maintain high reproducibility.

2.3. Standard preparations

Stock standard and working standard solutions were prepared in deionized water at a concentration of 1000 and 100 μg/ml, respectively. In case of LOR, MeOH was used the solvent. All solutions were filtered through a 0.2 μm membrane prior injection.

2.4. Linearity, precision, accuracy, recover, limit of detection (LOD) and limit of quantitation (LOQ)

Method linearity was evaluated by injecting five concentrations of the working standard solutions over the range of 10–250 μg/ml ($n = 3$). The calibration curve was plotted between the signals and the standard solution concentrations and regression equation and regression coefficient (r^2) was obtained using Microsoft EXCEL®. Injection precision was determined by repetitive injecting the standard solution at 250 μg/ml ($n = 10$). Intra-day and inter-day precision was performed by analyzing the standard solutions at various concentrations (10, 50, 100 and 250 μg/ml) and on 6 different days ($n = 6$), respectively. The precision was expressed as %R.S.D. Accuracy and recovery were determined by standard addition method. Standard solutions ranging from 50 to 150% of the label amount (500 mg and 2.5 mg per tablet for PARA and CPM, respectively) were added into the samples. The accuracy was calculated in term of percent recovery (%R, the ratio of amount of standard recovered and standard added). The amount of standard, which could be detected with a signal to noise ratio (S/N) of 3 was considered to be the LOD. The lowest amount of standard, which could be quantified with S/N of 10 was defined as the LOQ.

2.5. Determination of paracetamol and chlorpheniramine maleate in tablets

Five different brands of cold tablets were purchased from local drugstores. Twenty tablets of each brand were finely powdered and the equivalent of one tablet was weighed and diluted to give the concentration of 100 μg/ml in deionized

water. All sample solutions were filtered through a 0.2 μm membrane prior injection ($n = 3$).

3. Results and discussion

3.1. Optimization of MEKC condition

Optimization experiments for the separation of the nine cold remedy ingredients was further investigated from the previous study [23]. The initial buffer system was a 10 mM sodium dihydrogenphosphate–sodium tetraborate buffer containing 50 mM SDS and 5% v/v MeOH, pH 9.0. The separation of the investigated compounds in the absence and presence of SDS was not compared since our previous data showed that without SDS peaks of CPM and DEX could not be resolved [23]. In the present study, effects of types (MeOH, EtOH, *i*-PrOH, ACN) and percentages of organic modifiers (0-30% v/v), concentrations (5–30 mM) and pHs (4.0–10.0) of sodium dihydrogenphosphate–sodium tetraborate at a ratio of 1:1 as a buffer, temperature (25–35 °C) and voltage (10–25 kV) were investigated. The buffer pH was controlled by addition of 0.1 M H_3BO_3 or 0.1 M NaOH. Throughout the study, peak resolution ($R_S = 2(t_2 - t_1)/w_1 + w_2$) was determined by the separation of DPH and DEX since they were the two closest peaks.

3.1.1. Effects of types and percentages of organic modifier

Organic modifiers play an important role in improving separation efficiency and selectivity in MEKC. Typically, small amounts of organic modifier increase the migration window and resolution of the separation due to the reduction of the micellar electrophoretic mobility. This effect is caused by the decrease of the buffer conductivity and the EOF through their ability to disrupt the ordered structure of water molecules. Additionally, organic modifiers reduce the thermal diffusion and improve analyte solubility resulting in the enhancement of resolution. Results showed that in the absence and presence of small amount of alcohol (5% v/v), the number of peak separated and the migration order of the interested compounds were similar. The migration order was PARA, CAF, LOR, ASA, and CPM followed by the overlapped peaks of DPH, DEX, PPA and TPL, respectively. Lengthening of the alcohol chain did not greatly improve the separation, but an increase of the total migration time was obtained: 18.0 min in the absence of alcohol, 26.9 min in MeOH, 27.8 min in EtOH and 32.3 min in *i*-PrOH. This was due to the enlargement of the migration window. Further increases in MeOH (10–40% v/v) did not give satisfying results, whereas adding ACN as the organic modifier gave promising results. In the presence of 5% v/v ACN, CAF, LOR, ASA and CPM were eluted as separated peaks, whereas PARA, DPH, DEX, PPA and TPL co-eluted at 25.5 min. At 10% v/v ACN, the resolution was further decreased since CPM was included in the overlapped peak at 43 min. Interestingly, a specific amount of ACN was required for the complete separation of all compounds (Fig. 2). Baseline separation of all analytes with the R_S (calculated from DPH and DEX peaks) of 3.0 was obtained when 26% v/v of ACN was added into the buffer. The total t_m was 25.5 min with the migration order of PPA, CAF, PARA, CPM, DPH, DEX, TPL, LOR and

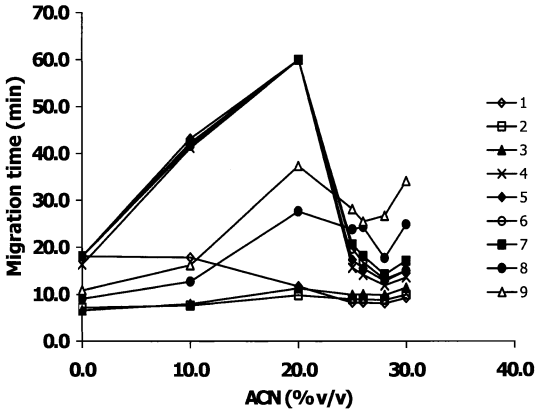

Fig. 2. Plot of migration time vs. percentages of ACN. Conditions: 10 mM sodium dihydrogenphosphate–sodium tetraborate buffer containing 50 mM SDS and various amount of ACN, pH 9.0; capillary, 58.5 cm full length, 50 cm effective length, 75 μm ID, 375 μm OD; hydrodynamic injection at 50 mbar, 10 s; 15 kV constant voltage; 30 °C; detection by UV absorbance at 214 nm. Peak identification: (1) PPA; (2) CAF; (3) PARA; (4) CPM; (5) DPH; (6) DEX; (7) TPL; (8) LOR; (9) ASA.

ASA, respectively. Further increases in the amount of ACN decreased the separation efficiency causing the peaks of DEX and DPH (at 28% v/v) and PPA and CAF (at 30% v/v) to overlap. Addition of extremely high amounts of ACN (> 26% v/v) had marked effects on the separation since it reduced the partitioning of the analytes into micelles (i.e. solute micelle interaction) and increased the migration window. Micelle formation was slowed due to the solvophobic interaction with individual surfactant molecules. Consequently, the separation efficiency was decreased when 28 and 30% v/v ACN was added into the buffer containing SDS as the surfactant.

3.1.2. Effects of buffer concentrations and pH

The buffer concentration or ionic strength greatly affects the EOF and the current generated in the capillary tubes. Fig. 3 shows that increasing of the buffer concentration to 20–30 mM only lengthened the migration time without enhancement of the separation. At these concentrations, the nine compounds were separated into seven peaks with the migration order of PPA, CAF, PARA, CPM, DPH, DEX and TPL, respectively, whereas LOR and ASA could not be detected in 60 min. When the buffer concentration was lowered to 5 and 10 mM, all compounds were eluted in the same order with the total t_m of 22.8 and 25.5 min. At 5 mM, PPA and CAF were not completely separated, whereas at 10 mM the R_S of both drugs was 7.2. Thus, the 10 mM buffer was carried out for further studies.

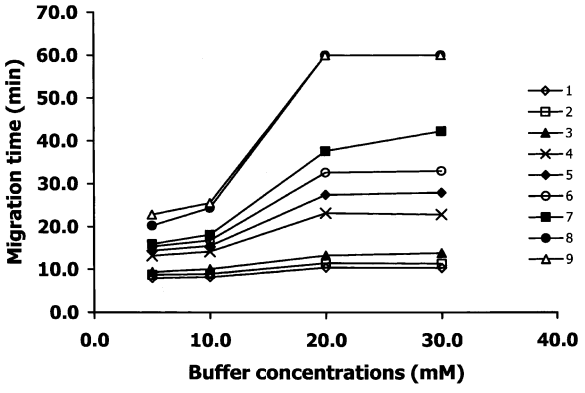

Fig. 3. Plot of migration time vs. buffer concentrations. Conditions: various concentration of sodium dihydrogenphosphate–sodium tetraborate buffer (at a ratio of 1:1) containing 50 mM SDS and 26% v/v ACN, pH 9.0. Other conditions are as Fig. 2.

The buffer pH influences the selectivity, separation efficiency and migration order in CE, especially for closely related compounds. Generally, decreasing the pH reduces the EOF, while increasing of pH enhance the EOF. At pH 7.0, the separation of the cold remedy ingredients could not be achieved and they were detected as one peak which co-eluted with the EOF at 13.9 min. Lowering the pH to 4.0 did not improved the separation but caused overlapping peaks. The total t_m was 19.1 min with the migration order of the overlapped PPA/CAF, PARA, LOR/ASA, DEX, TPL, and DPH. The compounds were eluted into nine and eight peaks with the total t_m of 25.5 and 34.1 min at pH 9.0 and 10.0, respectively. The migration order at both pH's was similar, which was PPA, CAF, PARA, CPM, DPH, DEX, TPL, LOR and ASA, respectively. However, PPA and CAF co-eluted at pH 10.0. ASA was not incorporated into the micelles and was eluted last since it was highly ionized in the basic pH buffer giving it a negative charge resulting in an attraction towards the anode.

3.1.3. Effect of temperature and voltage

High temperature affects the viscosity of the buffer resulting in the increase of the EOF and the observed electrophoretic mobility and the decrease of the migration time. Use of high voltage enhances the EOF and the electrophoretic velocity giving a short analysis time. Thus, high separation efficiency was obtained since diffusion, which causes band broadening, is minimized. However, high voltages can induce a high current and Joule heating, which may cause instability of analytes and lower migration time reproducibility. In this study, both temperature and voltage did not alter the selectivity or migration order of the separation. All analytes were completely separated at 25, 30 and 35 °C with the resolution (calculated from DPH and DEX peaks) of 4.8, 3.0 and 1.9 and the total t_m of 34.7, 25.5, 27.6 min, respectively. The temperature at 30 °C was selected as a compromise for the resolution and analysis time. When applying the voltage of 10, 15, 20 and 25 kV, the

resolution obtained was 3.6, 3.0, 2.6 and 1.9, respectively. At 10 kV, the total migration time was lengthening to more than 60 min and the peaks of CPM, DPH, DEX, TPL and LOR were broadening. At 20 and 25 kV, the total migration time was 20.1 and 15.3 min, respectively, however, DPH and DEX, were not completely separated. Thus, the optimum condition for all compounds was at 15 kV, which provided the reasonable migration time (25.5 min) and an acceptable resolution ($R_S = 3.0$).

3.2. Method validation

Under the optimized MEKC conditions (Fig. 4), the content of PARA and CPM in samples can be quantified. Calibration curves parameters and statistics for PARA and CPM are in Table 1. Results were calculated using peak area since it provided the best correlation with the smallest %R.S.D.s. Calibration curves for both ingredients were linear using linear regression in the range of 10–250 μg/ml, with correlation coefficients > 0.999 for all curves. Injection precision determined by ten replicate injections of the standard solutions at 250 μg/ml, showed the %R.S.D.s calculated from peak area of 1.7 and 2.4% for PARA and CPM, respectively. Intra-day precision from six replicate injections of the standard solutions at 10, 50, 100 and 250 μg/ml was within 3.3% for the migration time and 2.5% for peak area (Table 2). The %R.S.D.s of inter-day variability, determined on 6 different days at one point of the calibration curve (100 μg/ml, $n = 3$) were within 2 and 3% for the migration time and peak area, respectively. The %R.S.D.s are acceptable in CE analysis, although they are slightly higher than those obtained from HPLC technique. Recoveries, determined by standard addition method, showed good accuracy of the method (Table 3). The mean recovery was 100.6% and the mean %R.S.D. was 2.0% assuming the label amount of 500 mg per

Table 1
Calibration curve parameters and statistics of PARA and CPM

Curve	Slope	y-Intercept	Correlation coefficient (r^2)
PARA			
1	4.284	19.590	0.9989
2	4.303	18.292	0.9995
3	4.300	16.483	0.9993
Mean ($n = 3$)	4.296	18.122	0.9993
%R.S.D.	0.2	8.6	
CPM			
1	6.022	20.632	0.9993
2	5.995	17.752	0.9995
3	6.002	16.618	0.9997
Mean ($n = 3$)	6.006	18.334	0.9995
%R.S.D.	0.2	11.3	

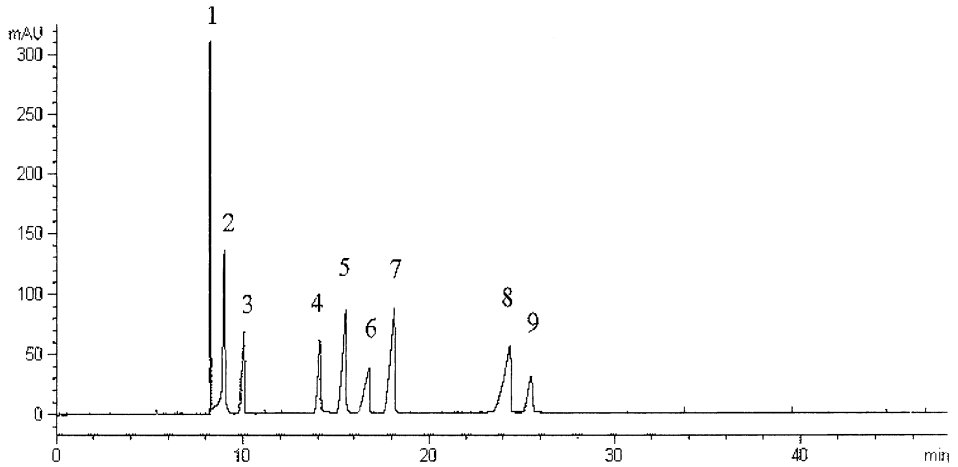

Fig. 4. A typical electropherogram of the standard mixture solution (100 μg/ml). Conditions: 10 mM sodium dihydrogenphosphate–sodium tetraborate buffer containing 50 mM SDS and 26% v/v ACN, pH 9.0. Other conditions are as Fig. 2.

Table 2
Intra-day precision of PARA and CPM ($n = 6$)

Concentration (μg/ml)	%R.S.D. (PARA)		%R.S.D. (CPM)	
	t_m	Peak area	t_m	Peak area
10	1.3	2.1	3.0	1.8
50	0.4	2.1	1.4	2.2
100	0.7	1.9	0.6	2.5
250	3.3	0.8	1.3	1.2

tablet for PARA. The mean recovery was 99.5% and the mean %R.S.D. was 2.1% assuming the label amount of 2.5 mg per tablet for CPM. The LOD based on the S/N = 3 were 0.4 and 0.5 μg/ml, and the LOQ based on the S/N = 10 were 2 and 4 μg/ml, with the %R.S.D. of 3.1 and 2.4%, for PARA and CPM, respectively.

3.3. Determination of paracetamol and chlorpheniramine maleate in cold tablets

Data from assay of PARA and CPM in five different brands of cold tables is shown in Table 4. Brand A, B and C contained 500 mg PARA and 2.5 mg CPM per tablet, brand D contained only 500 mg PARA and brand E contained only 4 mg CPM per tablet. A typical electropherogram of MEKC separation from brand A is shown in Fig. 5. All samples could be directly analyzed without sample pre-treatment. Other ingredients in the tablets did not interfere with the analysis (data not shown). The percent label amount of PARA was within 101.0–105.8% and of CPM was within 65.0–100.2%. The PARA content in brands A–D and the CPM content in brands C and E complied with the United State Pharmacopoeia 25 limit (90.0–110.0%), whereas the CPM content in brand A and B was out of the limit. These two brands were generic drugs from local manufacturers and the stability of CPM in the tablets decreased during improper storage.

Table 4
Assay of PARA and CPM in cold tablets

Brand	Active ingredients	% label amount found (%R.S.D.)	
		PARA	CPM
A	500 mg PARA + 2.5 mg CPM	105.8 (1.2)	65.0 (2.3)
B	500 mg PARA + 2.5 mg CPM	101.0 (0.8)	83.0 (0.5)
C	500 mg PARA + 2.5 mg CPM	104.7 (0.6)	92.3 (0.4)
D	500 mg PARA	102.8 (0.5)	–
E	4 mg CPM	–	100.2 (0.2)

4. Conclusions

The current study demonstrated the systematic procedure of MEKC method development for

Table 3
Accuracy and recoveries of PARA and CPM ($n = 3$)

Amount added (as % of theoretical)	PARA		CPM	
	%Recovery	%R.S.D.	%Recovery	%R.S.D.
50	101.3	2.4	99.9	1.6
80	101.8	1.0	97.7	1.8
100	100.5	1.4	98.4	1.6
120	99.2	2.7	99.8	2.7
150	100.4	2.6	101.5	3.0

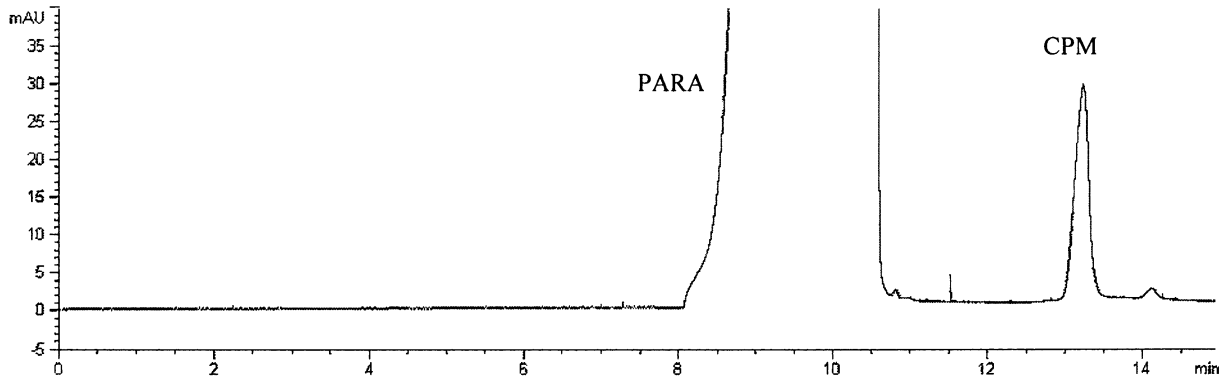

Fig. 5. A representative electropherogram of PARA and CPM in brand A. Other conditions are as Fig. 4.

separation of the commonly used cold remedy ingredients (e.g. PARA, CPM, DPH, DEX, PPA, TPL, CAF, LOR and ASA). The simple buffer system containing 10 mM sodium dihydrogenphosphate–sodium tetraborate 50 mM SDS and 26% v/v ACN, pH 9.0, using temperature at 30 °C and a constant voltage of 15 kV was optimized. Baseline separation of all compounds with the R_S of 3.0 was achieved within 25.5 min. Method assessment for determination of PARA and CPM in tablets shows good linearity, precision and accuracy. The detection and quantitation limits for both drugs were in the range of 0.5–4 μg/ml, respectively. The analysis was achieved in 13 min, which allowed the analysis of five samples per h. Comparing to HPLC, the developed MEKC technique was less expensive, simple, rapid, did not require sample-pretreatment and generated minimal organic waste. The method can be easily transferred to other laboratories and can be used as an alternative for analysis of cold medicine ingredients in pharmaceutical products.

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