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Simultaneous determination of ingredients in a cold medicine by cyclodextrin-modified microemulsion electrokinetic chromatography

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Abstract

Cyclodextrin-modified microemulsion electrokinetic chromatography (CD-MEEKC) was used to simultaneously determine 14 active ingredients (thiamine nitrate, anhydrous caffeine, acetaminophen, riboflavin, guaifenesin, pseudoephedrine hydrochloride, ascorbic acid, ethenzamide, DL-methylephedrine hydrochloride, dihydrocodeine phosphate, ibuprofen, noscapine, carbinoxamine maleate, and bromhexine hydrochloride) in a cold medicine. Separation of the ingredients was optimized by changing the SDS concentration and oil type and the addition of 2-propanol and cyclodextrin (CD) to the separation solution. The separation selectivity was improved dramatically by changing CD type. All of the active ingredients and formulation excipients were successfully separated with the use of a separation solution consisting of 0.81% (w/w) pentane, 6.61% (w/w) 1-butanol, 2% (w/w) 2-propanol, 4.47% (w/w) SDS, and 86.11% (w/w) 10 mM sodium tetraborate solution with 3 mM 2,6-di-*O*-methyl-β-CD. The established method was then validated and demonstrated to be applicable to the determination of the active ingredients in a model cold medicine. No interference from the formulation excipients was observed. Good linearities were obtained with correlation coefficients above 0.999. Recovery and precision ranged from 99.1 to 100.7% and from 0.5 to 2.8% R.S.D., respectively. The detection limit for ingredients ranged from 0.6 to 4.2 $\mu g\,ml^{-1}$. Good agreement was obtained between the established method and the traditional HPLC method. These results suggest that CD-MEEKC can be used for the determination of multiple ingredients in cold medicine.

Keywords: Cyclodextrin-modified microemulsion electrokinetic chromatography; Pharmaceutical analysis; Capillary electrophoresis (CE); Cold medicine

1. Introduction

In recent years, CE has become a mature and well-established analytical tool in the pharmaceutical field [1,2]. Details of registered CE methods are now included in most pharmacopoeias [3]. The high separation efficiencies obtained in CE are well suited for complex mixtures in which separation of a large number of peaks in a separation window is desirable. The versatility of CE in the simultaneous analysis of a wide range of pharmaceutical ingredients with varied chemical and physical properties is well represented in the numerous accounts available in the literature. The separation capability of CE was dramatically enhanced with the development of MEKC [4] and microemulsion electrokinetic chromatography (MEEKC) [5,6]. These systems have been widely used for the determination of ingredients in pharmaceuticals because of their usefulness for separation not only of ionic but also nonionic and hydrophobic compounds.

Most cold medicines contain multiple active ingredients that include antipyretics, analgesics, antitussive agents, mucolytic agents, bronchodilators, antihistamines, and several vitamins. In the past, RP-HPLC procedures were usually used to analyze combinations of these compounds [7]. However, as is difficult to analyze simultaneously many different kinds of ingredients using a single method, ingredients were often

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divided into several groups based on their chemical properties, i.e. cationic compounds in one run and anionic or neutral compounds in another run [8]. Gradient elution was also required for simultaneous analyzes [9]. Use of CE for the purpose of simultaneous determination of ingredients in cold medicine has become a more attractive separation technique and has been undertaken with capillary zone electrophoresis [10], MEKC [11–14] and MEEKC [6,15]. Methods based on CE offer a broad range of selectivity in combination with high separation efficiency, can be used with low sample volumes, and are suitable for simultaneous determination.

Recently, there has been increased investigation into the use of MEEKC separation due to the high solubilizing power of microemulsions. An immiscible oil is capable of forming stable and dispersed nanometer-sized droplets in an aqueous buffer via surfactants and co-surfactants to reduce surface tension between the oil droplets and the water, resulting in a stable microemulsion solution. These surfactant-coated oil droplets are used as a pseudostationary phase in MEEKC. Separation in MEEKC is achieved due to the differential partitioning of analytes between the oil droplets and the surrounding aqueous buffer zone as well as their individual mobilities, which are determined by their mass-to-ratios. One of the advantages of MEEKC over conventional MEKC includes the fact that analytes are more easily able to penetrate the surface of the droplets than the surface of micelles, which are much more rigid [6]; therefore, MEEKC is applicable over a wider range of analytes. Cyclodextrin (CD) offers a highly hydrophobic cavity and helps to solubilize hydrophobic compounds. The addition of CD to microemulsion solution establishes two pseudostationary phases in the separation solution that can improve the separation of hydrophobic compounds [16,17].

In this study, simultaneous determination of 14 active ingredients (see Fig. 1) in a cold medicine was successfully performed using a single CD-modified MEEKC (CD-MEEKC) method. The effects of SDS concentration, oil type, and addition of 2-propanol to the microemulsion solution upon separation were investigated as well. Attention was also focused on the effect on the separation of five different CD types in the system. This method was validated for the determination of active ingredients in a model preparation.

2. Experimental

2.1. Equipment

CE was performed using an Agilent CE instrument (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detection system operating at 200 or 280 nm. Fused-silica capillaries (50 μm i.d., 375 μm o.d., 40 cm in length to the detector and total length of 48.5 cm) were obtained from Agilent Technologies. A bubble cell capillary arrangement was used to increase sensitivity. The capillary compartment temperature was maintained at 32 °C. Hydrodynamic injection (3 kPa × 2 s) at the anodic end of the capillary was used to introduce samples. The applied voltage was set at 18 kV. Prior to each day of use, the capillary was conditioned by rinsing with deionized water, methanol, 1 M NaOH, 0.1 M NaOH, deionized water, and finally the separation solution for 15 min each. The capillary was rinsed between runs with deionized water (3 min), methanol (3 min), 0.1 M NaOH (2 min), deionized water (1 min), and finally the separation solution (3 min). All data were collected and analyzed using ChemStation software (Agilent Technologies).

2.2. Chemicals

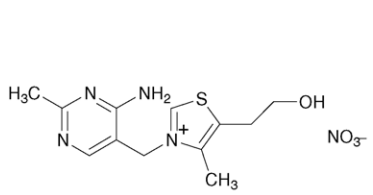
The chemical structures of the active ingredients are shown in Fig. 1. Thiamine nitrate, caffeine, acetaminophen, riboflavin, and ascorbic acid were purchased from the Society of Japanese Pharmacopoeia (Tokyo, Japan), guaifenesin and bromhexine hydrochloride from Yamamoto Chemical Industries (Wakayama, Japan), pseudoephedrine hydrochloride and DL-methylephedrine hydrochloride from Alps Pharmaceutical Industries (Gifu, Japan), ethenzamide from Shizuoka Caffeine (Shizuoka, Japan), dihydrocodeine phosphate and noscapine from Takeda Chemical Industries (Osaka, Japan), ibuprofen from BASF Japan (Tokyo, Japan), and carbinoxamine maleate from Kongo Chemical (Toyama, Japan). Ethyl parahydroxybenzoate, used as an internal standard (IS), was purchased from API Corporation (Osaka, Japan).

Pentane; 1-butanol; SDS; sodium tetraborate; α-, β-, γ-, 2,6-di-*O*-methyl-β- (DM-β-); 2,3,6-tri-*O*-methyl-β- (TM-β-) CD; and metaphosphoric acid were obtained from Wako Pure Chemical Industries (Osaka, Japan). 2-propanol was purchased from Kokusan Chemical (Tokyo, Japan). All chemicals were of analytical grade.

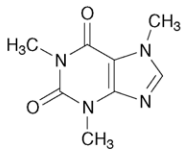
The microemulsion used as the MEEKC separation solution was prepared by weighing 4.47 g SDS, 86.11 g 10 mM sodium tetraborate solution, 0.81 g pentane, 6.61 g 1-butanol, and 2.00 g 2-propanol into a 100 ml flask. This mixture was sonicated for 30 min to produce a clear and highly stable microemulsion. Finally, DM-β-CD was added to the solution at the concentration of 3 mM. The microemulsion used as the extraction solution was prepared in a similar manner; its composition was: 0.81% (w/w) pentane, 6.61% (w/w) 1-butanol, 2% (w/w) 2-propanol, 4.47% (w/w) SDS, and 86.11% (w/w) of 2% (w/v) of metaphosphoric acid solution with 3 mM DM-β-CD. The other microemulsions used during the optimization studies were prepared in a similar manner.

2.3. Procedure for determination of ingredients in a model preparation by CD-MEEKC

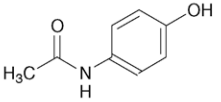
To determine the ingredients in a model preparation (see Table 1, including hydroxypropylcellulose as an excipient), about 0.3 g of pulverized granules were weighed accurately into a centrifuge tube. The tube was sonicated for 10 min and subsequently shaken vigorously for 10 min after about 40 mL of the extraction solution was added. Exactly 2 mL of



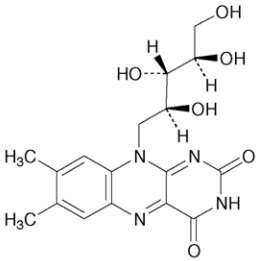
1. Thiamine Nitrate



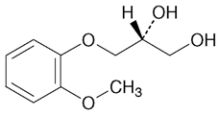
2. Anhydrous Caffeine



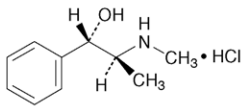
3. Acetaminophen



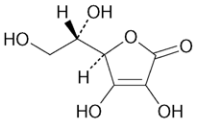
4. Riboflavin



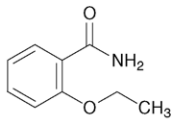
5. Guaifenesin



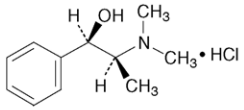
6. Pseudoephedrine Hydrochloride



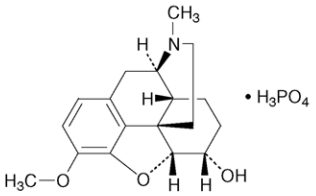
7. Ascorbic Acid



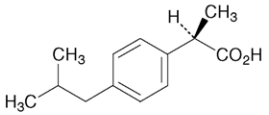
8. Ethenzamide



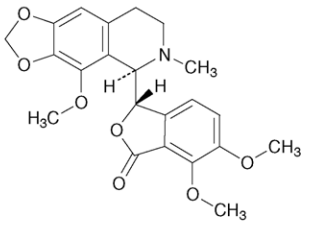
9. *dl*-Methylephedrine Hydrochloride



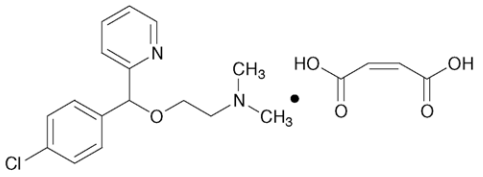
10. Dihydrocodeine Phosphate



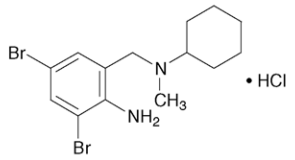
11. Ibuprofen



12. Noscapine



13. Carbinoxamine Maleate



14. Bromhexine Hydrochloride

Fig. 1. Active ingredients in the cold medicine.

Table 1
Content of each active ingredient in a model cold medicine (mg per daily dose)

Active ingredient	Content
Thiamine nitrate	24
Anhydrous caffeine	75
Acetaminophen	900
Riboflavin	12
Ascorbic acid calcium salt	515
DL-Methylephedrine hydrochloride	60
Dihydrocodeine phosphate	24
Noscapine	48
Carbinoxamine maleate	7.5
Bromhexine hydrochloride	12

the internal standard solution, which was prepared by dissolving 0.12 g of ethyl parahydroxybenzoate in 100 mL of water–methanol (4:1, v/v), was added to the solution in the centrifuge tube and the mixture was diluted to 50 mL with extraction solution and centrifuged for 5 min at 200 × *g*.

Standard compounds were weighed and diluted in extraction solution to concentrations similar to those in the prepared sample solution. The same volume of internal standard solution was also added to the standard solution. All test solutions were passed through a polysulfone 0.45 μm membrane filter (Nihon Pall).

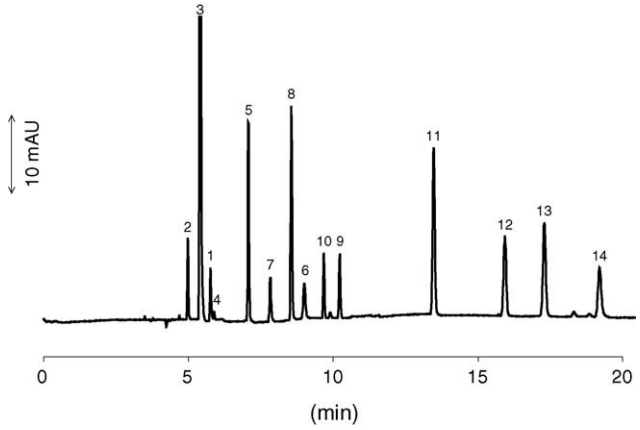


Fig. 2. Standard MEEKC separation of ingredients in a cold medicine. Separation solution 0.81% (w/w) *n*-octane, 6.61% 1-butanol, 3.31% (w/w) SDS, and 89.27% (w/w) 10 mM sodium tetraborate solution; applied voltage, +18 kV; temperature, 32 °C; detection wavelength, 200 nm; capillary, fused-silica (50 μm i.d. × 40 cm). Solutes were as noted in Fig. 1.

CD-MEEKC separation was performed using the separation solution described in Section 2.2 under the separation conditions described in Section 2.1. Riboflavin and ascorbic acid were detected and analyzed at 280 nm and the other ingredients at 200 nm.

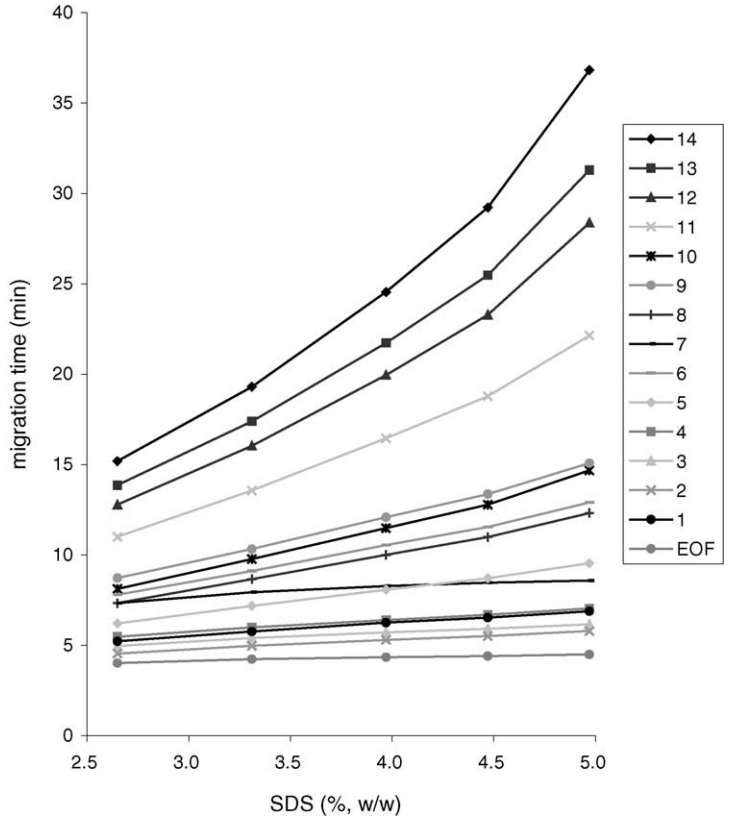


Fig. 3. Effect of SDS concentration on MEEKC separation of ingredients. The separation solution consisted of 0.81% (w/w) *n*-octane, 6.61% (w/w) 1-butanol, *x*% (w/w) SDS, and (92.58 − *x*)% (w/w) 10 mM sodium tetraborate solution. Other conditions were as provided in Fig. 2. Solutes were as noted in Fig. 1.

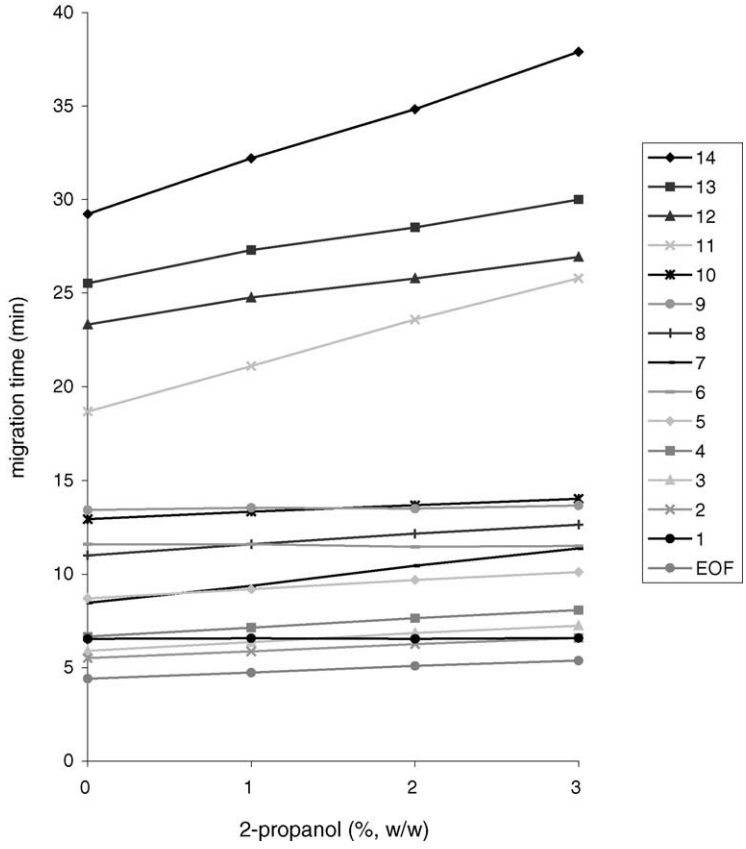


Fig. 4. Effect of 2-propanol addition on MEEKC separation of ingredients. The separation solution consisted of 0.81% (w/w) *n*-octane, 6.61% (w/w) 1-butanol, *x*% (w/w) 2-propanol, 4.47% (w/w) SDS, and (88.11 − *x*)% (w/w) 10 mM sodium tetraborate solution. Other conditions were as provided in Fig. 2. Solutes were as noted in Fig. 1.

2.4. Procedure for HPLC analysis

HPLC was performed using an Alliance HPLC system (Waters, MA, USA).

HPLC analysis was performed on the active ingredients in the model cold medicine, with the exception of ascorbic acid, as described by Ito et al. [8] but with a slight modification in the composition of the mobile phase to optimize column separation. The YMC application data method was used for the ascorbic acid (YMC Co. Ltd., Application data T910425A).

3. Results and discussion

3.1. Optimization of separation

3.1.1. Standard MEEKC separation

Separation conditions were optimized using the ingredient standard solution. A common microemulsion solution [16] consisting of 0.81% (w/w) *n*-octane, 6.61% (w/w) 1-butanol, 3.31% (w/w) SDS, and 89.27% (w/w) running buffer was initially used to separate ingredients. Borax solution was used as a high pH buffer due to the greater magnitude of electroosmotic flow (EOF) and provided an acceptable analysis time. A typical electropherogram is shown in Fig. 2. The separation of thiamine nitrate (1) and riboflavin (4) was found to be insufficient.

3.1.2. Effect of SDS concentration

As shown in Fig. 3, the effect of SDS concentration on the separation of ingredients was examined. When the SDS concentration was increased, all ingredients except ascorbic acid (7) migrated slowly. Higher concentrations of SDS increase the retention factor of neutral or cationic compounds due to increased charge density on the oil droplets. Increasing the SDS concentration also increases the ionic strength of buffers, which reduces the EOF level and increases analysis time. In the case of ascorbic acid, an electric repulsion may occur between the microemulsion and the solute. The concentration of 4.47% (w/w) SDS was selected for further optimization and a better separation trend was obtained as compared with these conditions.

3.1.3. Effect of 2-propanol addition

In MEEKC or MEKC, highly water-insoluble solutes partition strongly into either microemulsions or micelles and

are therefore highly retained with poor separation. Often, attempts have been made to improve this situation by adding organic solvents to separation solutions so as to reduce retention and improve separation. Addition of 2-propanol also affects the charge density and size of the microemulsion [16]. Changing the viscosity and dielectric constant of the separation solution reduces the EOF level. As shown in Fig. 4, increasing the concentration of 2-propanol led to increases in the migration times of the analytes. Selectivity was also affected by the addition of 2-propanol, particularly the migration behaviors of thiamine nitrate (1), guaifenesin (5), and pseudoephedrine hydrochloride (6). The addition of 2% (w/w) 2-propanol was chosen for further optimization because better overall separation was seen at this level.

3.1.4. Effect of oil type

The effects of the choice of oil on the separation of ingredients were examined as shown in Fig. 5. Although similar migration times were observed for all of the oils examined, thiamine nitrate (1) migrated faster than caffeine (2) and pseudoephedrine hydrochloride (6) migrated faster than ascorbic acid (7) as the carbon numbers of the oils used decreased. Separation of thiamine nitrate (1) and caffeine (2) remained insufficient.

3.1.5. Effect of CD addition

During the separation process with CD-MEEKC [16,17] or CD-MEKC [18], analytes will be found distributed between the microemulsion (micelle) and the CD, which can improve the selectivity. A previous study performed by our group demonstrated sufficient separation of 11 ingredients in an ophthalmic solution with the use of a CD-MEKC system [19]. In the present study, the effects of CD addition to the MEEKC system were examined using DM-β-CD as the secondary pseudostationary phase (Fig. 6). An increased concentration of DM-β-CD in the separation solution reduced the migration times of the analytes, which showed a strong interaction with the microemulsion, as their solubility in the buffer increased. With the addition of 15 mM DM-β-CD, thiamine nitrate (1) migrated faster than caffeine (2) to become the fastest detected analyte. Nonetheless, simultaneous separation of the ingredients could not achieved because ascorbic acid (7) migrated to the crowded area occupied by many peaks under the same separation conditions. As noted in the previ-

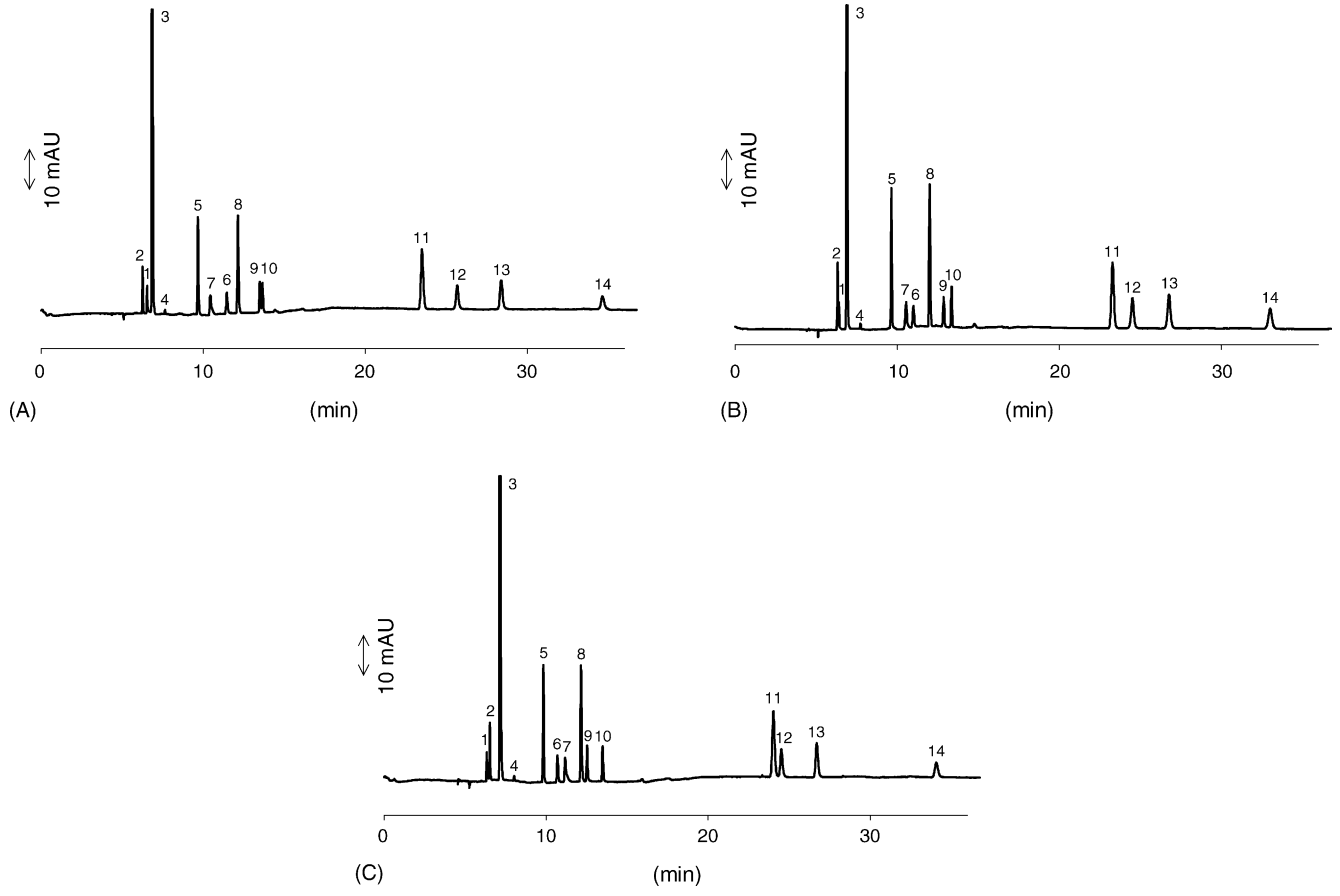


Fig. 5. Effect of oil type on MEEKC separation of ingredients: (A) *n*-octane, (B) hexane and (C) pentane. The separation solution was comprised of 0.81% (w/w) oil, 6.61% (w/w) 1-butanol, 2% (w/w) 2-propanol, 4.47% (w/w) SDS, and 86.11% (w/w) 10 mM sodium tetraborate solution. Other conditions were as provided in Fig. 2. Solutes were as noted in Fig. 1.

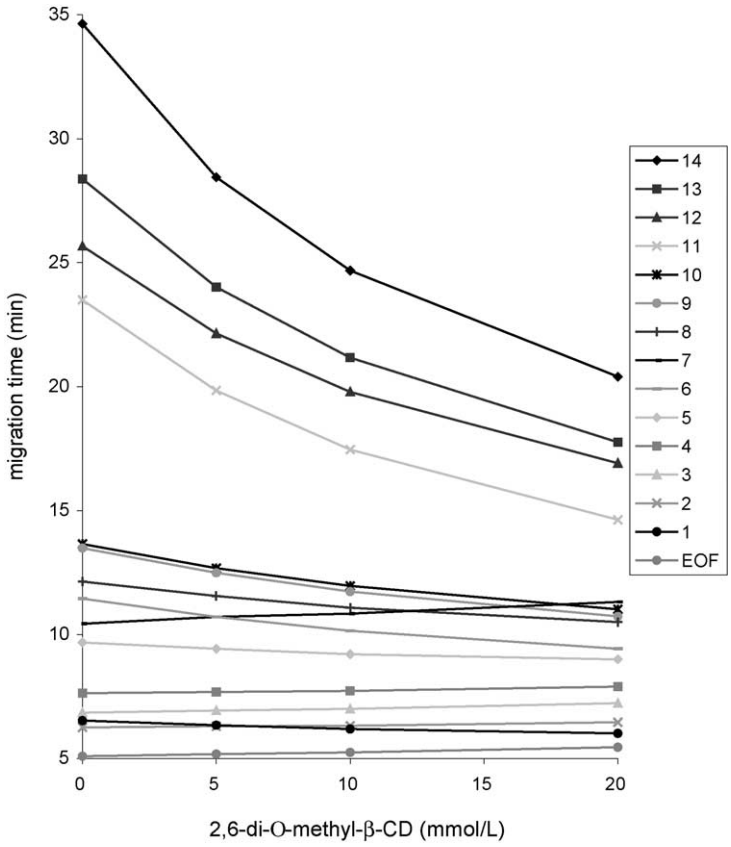


Fig. 6. Effect of 2,6-di-*O*-methyl-β-cyclodextrin addition on MEEKC separation of ingredients. The separation solution consisted of 0.81% (w/w) *n*-octane, 6.61% (w/w) 1-butanol, 2% (w/w) 2-propanol, 4.47% (w/w) SDS, and 86.11% (w/w) 10 mM sodium tetraborate solution with *x* mM 2,6-di-*O*-methyl-β-cyclodextrin. Other conditions were as provided in Fig. 2. Solutes were as noted in Fig. 1.

ous section, oil type had a little influence on the selectivity of thiamine nitrate (1) and caffeine (2). When pentane was used as the oil core of the microemulsion, only a small amount of DM-β-CD addition was predicted to be adequate for the optimum separation of all the peaks, including groups of both thiamine nitrate (1) and ascorbic acid (7). As expected, all peaks were entirely separated using a microemulsion consisting of pentane with 2 mM of DM-β-CD (Fig. 7A).

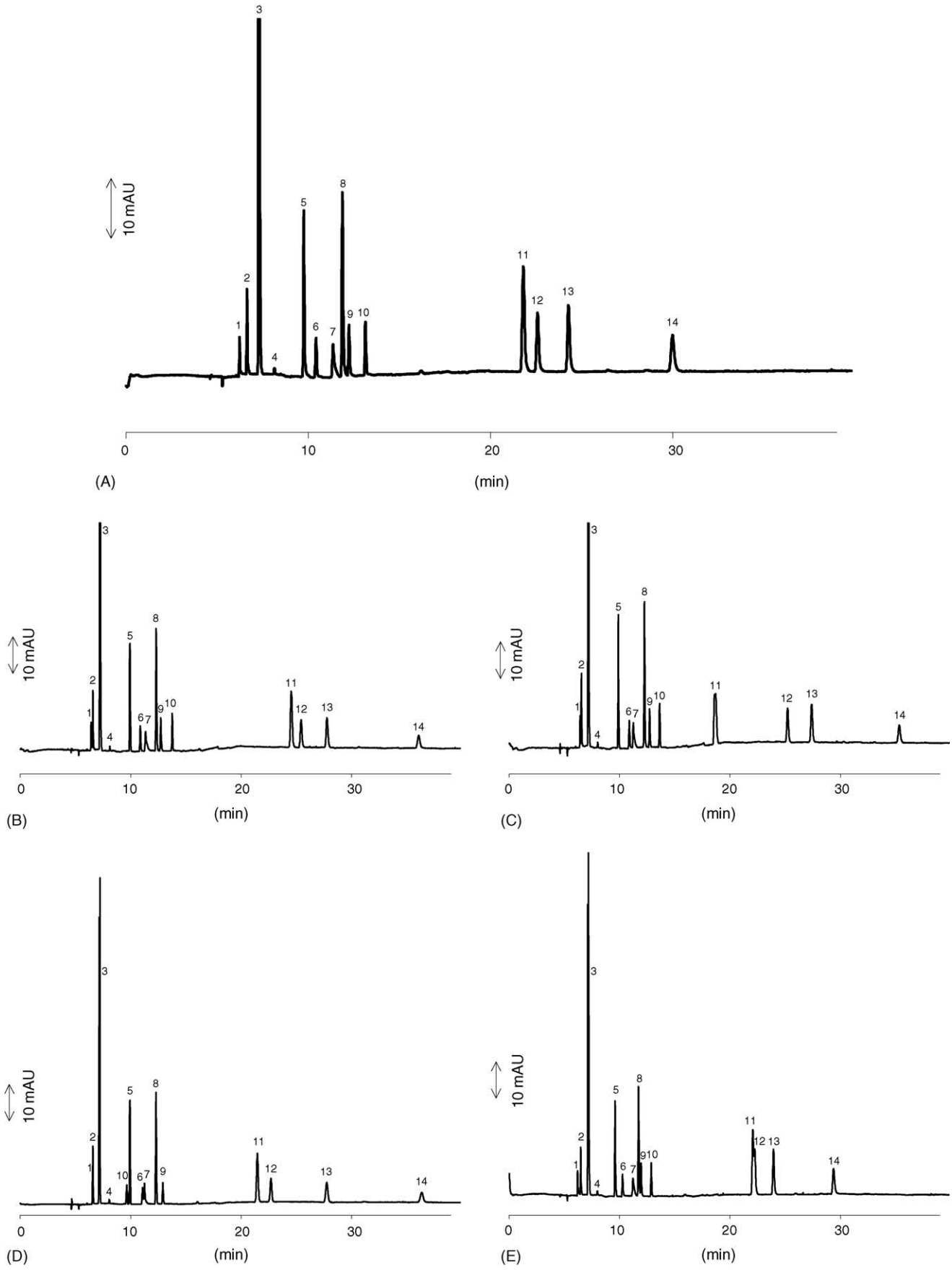
Several types of CDs, i.e. α-CD, β-CD, γ-CD, and TM-β-CD, were examined for their effects on the separation. Each CD is different in terms of the diameter of its cavity and/or the lipophilicity of the external portion of the molecule. Some typical electropherograms are shown in Fig. 7. Among the five CDs, DM-β-CD was found to be the most effective for sufficient separation and good peak shapes. As compared with non-CD addition (see Fig. 5C), the migration time of ibuprofen (11) was significantly reduced through interaction with β-CD (Fig. 7C). Similarly, the migration time of dihydrocodeine phosphate (10) was reduced through interaction with γ-CD (Fig. 7D). Analytical time was notably reduced with the use of DM-β-CD and TM-β-CD (Fig. 7A and E). These results indicate that the cavity atmosphere of the β-CD derivative is most suitable for inclusion of solutes that strongly interact with microemulsions. Inclusion of solute is determined primarily by cavity size; additionally, hydrogen bonding at the cavity edge presumably determined a compound's access at the cavity entrance [20]. Methylation disrupts the hydrogen bond belt of the C-2 hydroxyl of the 1-glucopyranose unit with the C-3 hydroxyl of an adjacent unit formed in CD molecules and can also affect intermolecular hydrogen bonding [21]. However, the overall mechanism underlying CD-MEEKC separation of each solute in this system requires further clarification. Successful separation was achieved using a separation solution consisting of 0.81% (w/w) pentane, 6.61% (w/w) 1-butanol, 2% (w/w) 2-propanol, 4.47% (w/w) SDS, and 86.11% (w/w) 10 mM sodium tetraborate solution with 3 mM DM-β-CD.

3.2. Determination of ingredients in a model medicine

3.2.1. Validation of the method

A CD-MEEKC method was established for the purpose of obtaining complete separation of 14 ingredients in a cold medicine. The application and validation of the technique were demonstrated for the determination of the active ingredients in a model cold medicine.

Microemulsions have also been used as extraction solutions because of their wide range of solubilities [22,23]. In this study, all the ingredients were dissolved in a microemulsion similar to the separation solution, with the exception of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(D)
(E)

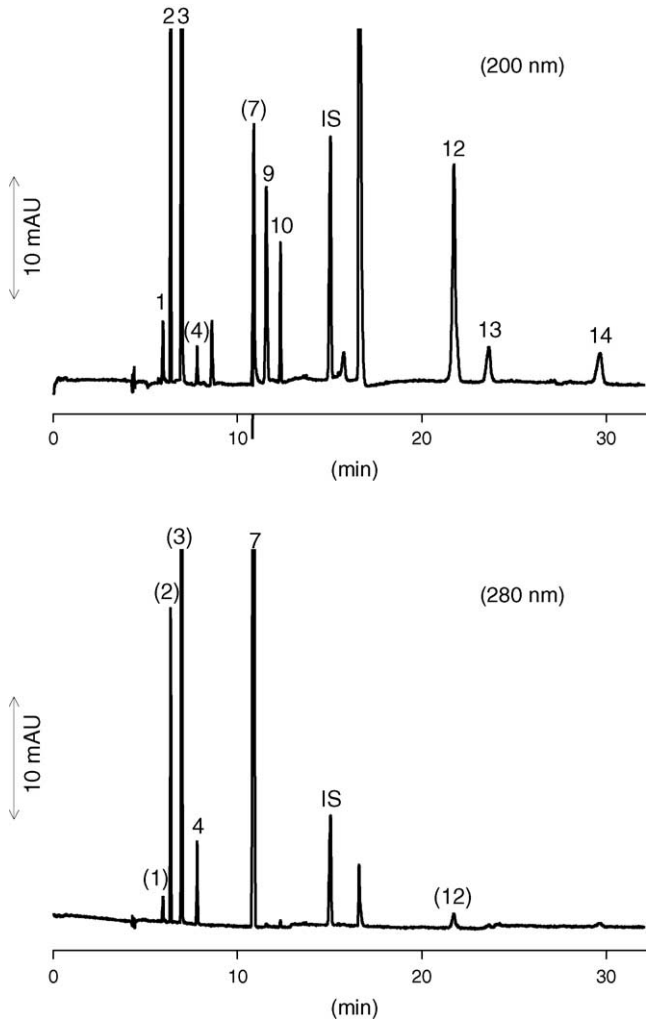

Fig. 8. Typical electropherogram obtained in an assay of a model cold medicine. The separation solution used was as provided in Fig. 7A. Conditions were as provided in Fig. 2. Solutes were as noted in Fig. 1.

the replacement of borax solution with metaphosphoric acid solution in order to maintain the stability of ascorbic acid. Microemulsion extraction resulted in high solubility of the each active ingredient via relatively simple extraction steps, i.e. sonication and shaking without a heating process. The representative electropherograms of sample solutions detected at 200 or 280 nm in Fig. 8 show the specificity of the method, by which all of the active ingredients and the IS were separated well. Several peaks observed in addition to the active ingredients and the IS were of formulation excipients. Each peak purity index for the active ingredients, as determined with a photo diode array detector, was unified for the sample solution, which was indicative of a single, pure peak.

The method was found to be robust, as small but deliberate changes in the method parameters had no detrimental effects on performance (Table 2). The separation between thiamine nitrate (1) and caffeine (2) decreased with decreasing capillary temperature and 1-butanol content in the separation solution. The separation between ascorbic acid (7) and DL-methylephedrine hydrochloride (9) decreased with decreasing SDS concentration and increasing 1-butanol content in the separation solution. Changes in the content of 2-propanol and DM-β-CD in the separation solution did not alter the chromatographic profile.

Table 2
Robustness

Parameter	Variation	Resolution[a]	Peak
Temperature (°C)	29	1.5	1/2
	32	2.2	
	35	3.7	
SDS concentration (%) (w/w)	4.18	1.5	7/9
	4.47	5.0	
	4.90	8.1	
1-Butanol content (%) (w/w)	6.10	1.5	1/2
	6.61	2.2	
	6.90	4.8	
	6.10	7.6	7/9
	6.61	5.0	
	6.90	1.5	

[a] 1/2: Resolution between thiamine nitrate and caffeine; 7/9: resolution between ascorbic acid and DL-methylephedrine hydrochloride.

In order to estimate the stability of the analytical solutions, sample and standard solutions were kept in a light-resistant container at room temperature and evaluated based on changes in relative corrected peak area. All active ingredients were stable for at least 24 h under these conditions.

The detection limit, which was observed as a peak with a signal-to-noise ratio of three, is shown in Table 3. The limit was determined by injecting sample solutions with known low concentrations of analytes.

The quantitation linearity of the active ingredients in standard solution was examined at five concentration levels ranging from 50 to 150% of the normal concentration. For each ingredient, the relationship between relative corrected peak area and concentration was calculated and is given in Table 3. In all cases, straight regression lines with correlation coefficients (r) above 0.999 were obtained. The intercept values were not significantly different from zero (95% confidence).

Accuracy was assessed over the entire concentration range (80, 100, and 120%) by analyzing placebos spiked with active ingredients at three concentration levels. The solutions were replicated three times each, and the amounts of ingredients determined were compared to the theoretical amounts. Adequate results for recovery were obtained for all ingredients studied (Table 1).

Fig. 7. Effect of cyclodextrin type on CD-MEEKC separation of ingredients: (A) 2,6-di-*O*-methyl-β- , (B) α- , (C) β- , (D) γ- and (E) 2,3,6-tri-*O*-methyl-β-cyclodextrin. The separation solution consisted of 0.81% (w/w) pentane, 6.61% (w/w) 1-butanol, 2% (w/w) 2-propanol, 4.47% (w/w) SDS, and 86.11% (w/w) 10 mM sodium tetraborate solution with 3 mM cyclodextrin. Other conditions were as provided in Fig. 2. Solutes were as noted in Fig. 1.

Table 3
Validation data for determination of active ingredients in a model cold medicine

	Thiamine nitrate (thiamine hydrochloride)	Anhydrous caffeine (caffeine)	Acetaminophen	Riboflavin	Ascorbic acid calcium salt (ascorbic acid)	DL-Methylephedrine hydrochloride	Dihydrocodeine phosphate	Noscapine	Carbinoxamine maleate	Bromhexine hydrochloride
Linearity										
Concentration range ($\mu g\,ml^{-1}$)	14–64	50–200	200–2000	10–30	300–1200	40–160	20–100	30–130	5–20	8–32
r	0.9992	0.9997	0.9999	0.9992	0.9996	0.9995	0.9997	0.9996	0.9993	0.9998
Intercept	0.0025	−0.0515	0.8551	0.0149	−0.7545	0.0234	0.0048	0.0164	0.0049	−0.0031
Slope	0.00766	0.02113	0.02985	0.03433	0.02405	0.00912	0.00925	0.01270	0.01540	0.00887
Recovery ($n = 3$, %)										
Concentration levels (%)										
80	100.0	100.5	100.4	100.2	100.3	99.8	100.3	100.4	100.1	100.7
100	100.7	99.7	100.5	99.1	99.6	100.2	99.7	99.9	99.6	99.1
120	100.5	99.9	100.4	99.6	100.0	99.5	100.6	100.4	99.9	100.5
Precision ($n = 3$, R.S.D.%)										
Concentration levels (%)										
80	1.9	1.3	0.7	1.1	1.6	1.6	1.4	1.1	2.5	0.6
100	1.9	0.8	1.8	0.5	0.7	1.2	1.8	1.7	2.8	0.7
120	0.8	1.9	1.2	0.9	1.0	1.6	0.8	1.8	2.8	1.2
Detection limit ($S/N = 3$) ($\mu g\,ml^{-1}$)										
	4.2	1.2	0.6	0.8	1.4	2.8	1.6	2.6	1.4	2.1

Table 4
Comparison of the CD-MEEKC method with HPLC determination of active ingredients in a model cold medicine ($n = 6$)

	Thiamine nitrate	Anhydrous caffeine	Acetaminophen	Riboflavin	Ascorbic acid calcium salt	DL-Methylephedrine hydrochloride	Dihydrocodeine phosphate	Noscapine	Carbinoxamine maleate	Bromhexine hydrochloride
CD-MEEKC										
Content (mg per daily dose)	25.05	75.3	891	12.16	495	59.2	23.86	48.5	7.63	12.39
R.S.D. (%)	2.5	2.3	1.1	2.1	2.4	2.0	1.7	1.8	2.4	0.9
HPLC										
Content (mg per daily dose)	25.64	75.8	893	12.33	502	59.9	23.97	47.8	7.58	12.49
R.S.D. (%)	0.7	0.8	0.2	0.6	1.2	0.5	0.6	0.8	0.7	1.0

Precision was determined by measuring ($n = 3$) each active ingredient in spiked placebos at the three concentration levels. R.S.D. was estimated using the established method. Good results for precision were obtained for all ingredients examined (Table 1).

3.2.2. Quantitative analysis of active ingredients in a model cold medicine

The established CD-MEEKC method was applied to determine the ingredients in a model cold medicine. For the purpose of comparison, the same sample was analyzed using the conventional HPLC method. For the HPLC determination, the ingredients were divided into three groups based on their chemical properties, i.e. anhydrous caffeine, acetaminophen, and riboflavin in the first group, thiamine nitrate, DL-methylephedrine hydrochloride, dihydrocodeine phosphate, noscapine, carbinoxamine maleate, and bromhexine hydrochloride in the second group, and ascorbic acid in third group, and analyzed individually by group. The results of the quantitation of the active ingredients in a model cold medicine as shown in Fig. 8 are given in Table 4. On the whole, good agreement was obtained between the CD-MEEKC method and traditional HPLC methods. In terms of precision, however, the R.S.D. of the established method was not as good as the precision seen with the HPLC methods.

4. Conclusion

The application of the CD-MEEKC method to the simultaneous determination of active ingredients in a pharmaceutical preparation was demonstrated for the first time. The use of a microemulsion as an extraction solution resulted in high recovery of all ingredients simultaneously via a relatively easy extraction process. Slightly less precision was obtained as compared with the HPLC method, but the major advantage of CE methods, including CD-MEEKC, over traditional methods is the ability to simultaneously analyze large numbers of peaks using a single method. Injection repeatability with CE is generally inferior to HPLC [24], which presents a major obstacle to the widespread use of CE in routine analysis. Improvement of instrumentation as well as the further development of methods should yield more precise quantitative results. Today, once the CE method is published in the pharmacopoeias and equipment performance is developed further, this method will be counted upon more heavily for routine analysis in quality control applications. At present, this method can be expected to find applications in routine analysis once its advantages and disadvantages are afforded due consideration.

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