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Simultaneous determination of eleven ingredients in ophthalmic solutions by cyclodextrin-modified micellar electrokinetic chromatography with tetrabutylammonium salt

Hitoshi Okamoto*, Atsuhiro Uetake, Ryuji Tamaya, Toshiaki Nakajima, Kazuhiko Sagara, Yuji Ito

Analytical Laboratory, Taisho Pharmaceutical Co., Ltd., 403 Yoshino-cho 1-chome, Omiya-shi, Saitama 330-8530, Japan

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Abstract

Cyclodextrin-modified micellar electrokinetic chromatography was applied to determine simultaneously 11 active ingredients in ophthalmic solutions. All the ingredients were successfully separated by using the mixed carrier system containing sodium dodecyl sulfate with β-cyclodextrin and tetrabutylammonium phosphate. The effects of the cyclodextrin type on selectivity were also examined. Excellent separation of the all ingredients was obtained by the use of dimethyl-β-cyclodextrin. The established method was validated and confirmed to be applicable to the determination of the active ingredients in a commercial ophthalmic solution. These results suggest that capillary electrophoresis can be applied to the quantitative analysis as well as qualitative analysis in pharmaceuticals.

Keywords: Ophthalmic solutions; Pharmaceutical analysis; Cyclodextrins

1. Introduction

The determination of ingredients in pharmaceuticals is significant to keep the quality of the preparations high. High-performance liquid chromatography (HPLC) and gas chromatography (GC) have generally been applied to the analysis of many compounds. However the range of pharmaceutical ingredients is extremely varied and has different chemical properties. Therefore it is difficult to analyze simultaneously many kinds of ingredients using a single method. In an ophthalmic solution, there are also many active ingredients, for example, sympathomimetics, antihistamines, astringents, vitamins, amino acids and sulfonamides, which have different properties. Although several analytical procedures have been developed for the determination of ingredients in ophthalmic solutions [1–6], only a few methods have been reported to carry out the simultaneous determination of these ingredients. Some of the major active ingredients have been analyzed simultaneously by HPLC, but the efficiency of the separation was poor and peak tailing occurred [4]. The relatively recently developed technique of micellar electrokinetic chromatography (MEKC) [7,8] is widely used with pharmaceuticals [9–15]. A simple, economical and efficient alternative approach to chromatographic methods, MEKC can separate not only ionic but also electrically neutral compounds.

*Corresponding author. Fax: +81-48-663-1045.
E-mail address: s17127@ccm.taisho.co.jp (H. Okamoto).

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However in MEKC, hydrophobic or cationic solutes tend to be totally incorporated into the micelle, or to strongly interact with the micelle, and therefore they cannot be separated with a simple micellar solution. Actually, it has been reported that it was difficult to separate several ingredients in ophthalmic solutions simultaneously by single MEKC method [13]. The addition of organic solvents to the micelle solution can improve separations of hydrophobic solutes [16]. However, changes of buffer constitution caused by evaporation can occur with the use of such solvents. The method cyclodextrin-modified MEKC (CD-MEKC), which uses cyclodextrin (CD) together with an ionic micelle solution, has resolved this problem, because it is a non-volatile method [17]. The addition of CD establishes two pseudo stationary phases in the electrolyte, which can improve the separation of highly hydrophobic solutes. To separate ionic compounds successfully, tetraalkylammonium (TAA) salts were added to the micelle solution [18]. TAA salts added to the anionic micellar system may act as a competitor against the cationic solutes and exclude them from the micelle. On the other hand, TAA salts tend to form ion-pairs with anionic solutes and cause easy solubilization to the micelle. Further selectivity was obtained by using a mixed carrier system containing sodium dodecyl sulfate (SDS) with β-CD and TAA salt as modifier to separate antihistamines [19].

In this work, we describe the separation of 11 ingredients used in ophthalmic solutions by the mixed carrier system in CD-MEKC. The effect of five different CD types in the system on the separation is investigated, and a possible separation mechanism is discussed. The application and the validation of this technique to the determination of ingredients in a commercial preparation is also described.

2. Experimental

2.1. Equipment

Capillary electrophoresis (CE) was performed on a $HP^{3D}CE$ instrument (Hewlett-Packard, Waldbronn, Germany) equipped with a diode-array detection system operating at 200 nm. The capillary compartment temperature was maintained at 30°C. Hydrodynamic injection (3.45 kPa, 5 s) at the anodic end of the capillary was used to introduce samples. Fused-silica capillaries [48.5 cm (effective length 40 cm)×50 μm I.D.×375 μm O.D.] were obtained from Hewlett-Packard. For the purpose of determination of a commercial preparation, a bubble cell capillary arrangement was employed in the Hewlett-Packard instrument to increase sensitivity. The capillary was rinsed between injections with 0.1 *M* NaOH (100 kPa, 2 min) followed by deionized water (100 kPa, 1 min) and separation buffer (100 kPa, 3 min). All data were collected and analyzed using HP ChemStation software (Hewlett-Packard).

2.2. Chemicals

The chemical structures and indicative abbreviations of all ingredients are shown in Fig. 1. Tetrahydrozoline hydrochloride (TH) was purchased from Dolder (Basel, Switzerland), pyridoxine hydrochloride (VB6) and chlorpheniramine maleate (CP) from the Society of Japanese Pharmacopeia (Tokyo, Japan), L-aspartic acid (Asp) from Wako (Osaka, Japan), allantoin (AL) from Kawaken Fine Chemicals (Tokyo, Japan), ε-aminocaproic acid (AC) from Daiichi Pure Chemicals (Tokyo, Japan), neostigmine methylsulfate (NM) from Tokyo Chemical Industry (Tokyo, Japan), tocopherol acetate (VE) from the National Institute of Health Sciences (Tokyo, Japan), sodium sulfamethoxazole (SU) from Shionogi (Osaka, Japan), dipotassium glycyrrhizinate (GK2) from Maruzen-Seiyaku (Hiroshima, Japan), naphazoline hydrochloride (NA) from Fukujyu Pharmaceutical (Toyama, Japan) and 2-aminoethanesulfonic acid (TA) from Taisho MTC (Tokyo, Japan). Sudan III was obtained from Wako. Polysorbate 80 was obtained from Nihon Surfactant Kogyo (Tokyo, Japan). To prepare the standard ingredients solution, all compounds except tocopherol acetate were dissolved in water at a concentration of similar level to that present in commercial ophthalmic solutions (0.04–2 mg/ml). Tocopherol acetate was prepared by initially mixing with polysorbate 80 and was subsequently added to water.

SDS, 0.5 *M* tetra-*n*-butylammonium phosphate solution (Low UV Type), α-, β-, γ-, 2,6-di-*O*-methyl-β-, and 2,3,6-tri-*O*-methyl-β-cyclodextrin

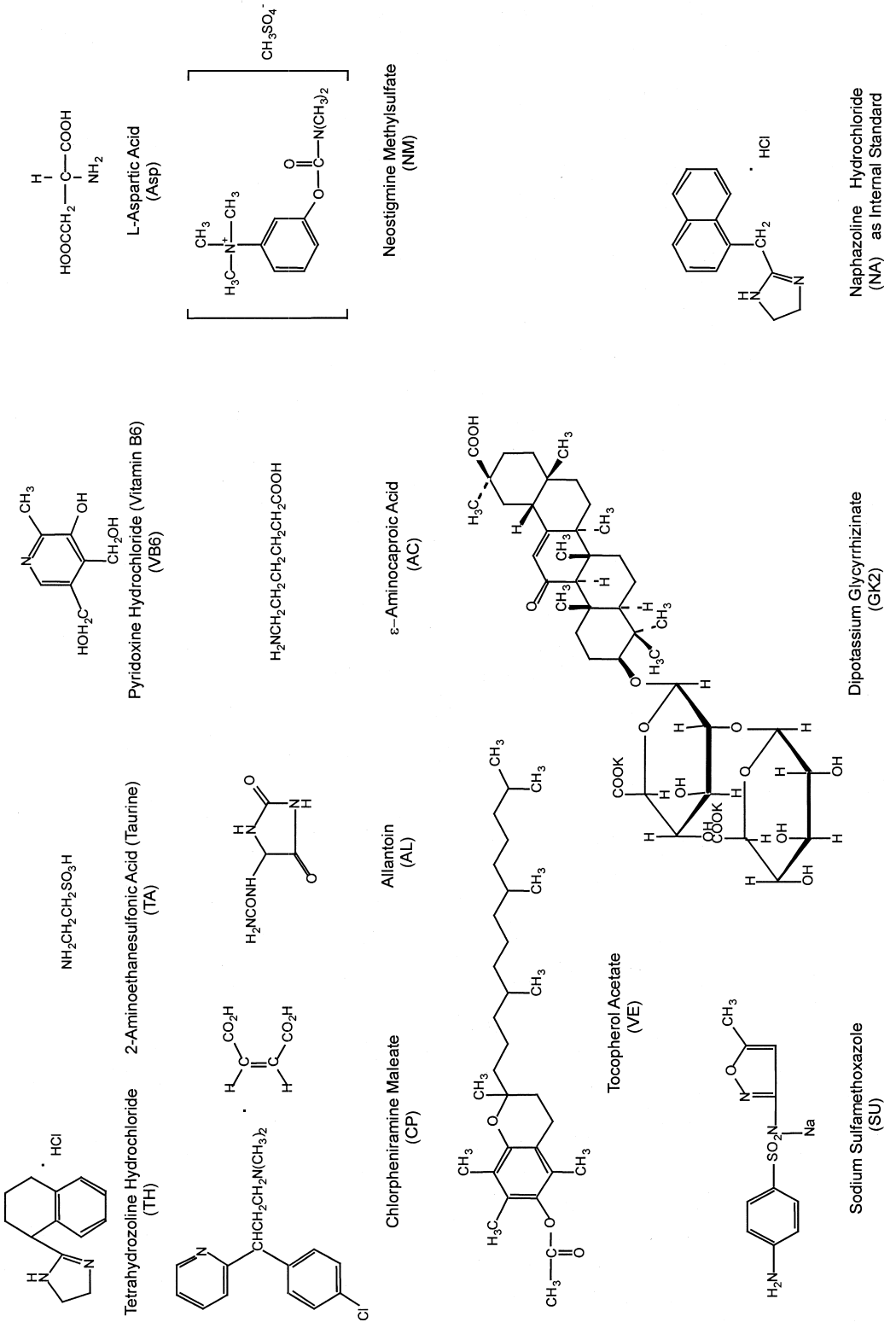

Fig. 1. Active ingredients in ophthalmic solution.

were obtained from Wako. Urea was obtained from Fluka (Buchs, Switzerland). All the chemicals used for buffers, boric acid, sodium tetraborate (Wako) and sodium hydroxide (Kokusan Chemical, Tokyo, Japan) were of analytical grade. All surfactants and CDs were dissolved in a buffer solution prepared by mixing 0.02 *M* sodium tetraborate solution with appropriate volumes of 0.08 *M* boric acid solution to give the required pH values. These solutions were filtered through a 0.45-μm membrane filter prior to use.

2.3. Procedure for the determination of ingredients in a preparation

To determine the ingredients in a commercial ophthalmic solution, 2 ml of the preparation was placed in a 10-ml volumetric flask. An internal standard solution was prepared by dissolving 10 mg of naphazoline hydrochloride in 100 ml of water. A 5-ml volume of the internal standard solution was added to the flask and the mixture was diluted to the volume to the water. Standard compounds (tetrahydrozoline hydrochloride, 2-aminoethanesulfonic acid, pyridoxine hydrochloride, L-aspartic acid, chlorpheniramine maleate and neostigmine methylsulfate) were weighed and diluted in water at a similar concentration to the prepared sample solution. The same volume of internal standard solution was added to the standard solution. All the test solutions were passed through a 0.45-μm membrane filter.

3. Results and discussion

3.1. Separation by MEKC with SDS solution

MEKC with SDS solution was performed to separate ingredients in ophthalmic solutions. SDS was added to the separation buffer (pH 8.0 to 10.0) at different concentrations (0.08, 0.10, 0.15 *M*). A typical chromatogram is shown in Fig. 2. Satisfactory results, however, were not obtained. Notably, later migration solutes, TH, CP, NA, VE migrated with almost the same migration time as Sudan III, which is known as a micelle marker. This result indicated that these solutes were totally solubilized within the micelle, or formed strong ion-pair interaction with the micelle.

3.2. Separation of ingredients with addition of CD and/or TBA to the MEKC buffer

Hydrophobic or cationic compounds can have a high tendency to be fully incorporated into the

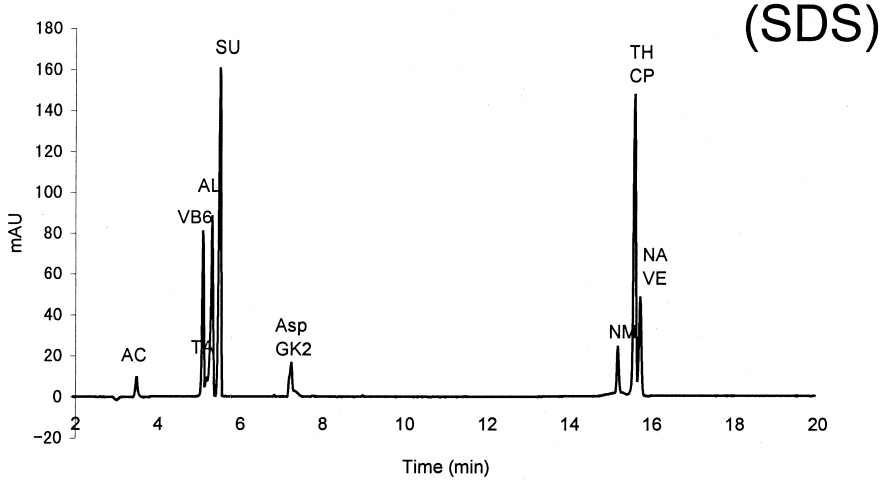

Fig. 2. Separation of ingredients by MEKC with SDS. 0.08 *M* Borate buffer (pH 9.1) containing 0.15 *M* SDS; applied voltage, +18 kV; temperature, 30°C; detection wavelength, 200 nm; capillary, fused-silica capillary (40 cm×50 μm I.D.). Abbreviations of solutes are given in Section 2.2.

micelle or to interact tightly with the micelle, respectively, and therefore can be difficult to separate successfully. One solution to this problem, the addition of CD to the micellar solution has been performed [17]. The addition of β-CD to the SDS micelle solution resulted in a slight improvement in the separation of solute [Fig. 3 (SDS+β-CD)]. Neutral CD migrates at the same velocity as the bulk solution. In CD-MEKC, hydrophobic solutes can be incorporated into either the CD cavity or the micelle. Therefore, the addition of β-CD to the micelle solution affects the resolution. However, the separation of all the ingredients was still insufficient.

It has been reported that the addition of TAA salts to the SDS solution remarkably improved the resolution of the ionic compounds [18]. TAA salts are thought to interact strongly with the SDS micelle, excluding the cationic solute from the micelle. On the other hand, TAA salts and the anionic solute tend to form ion pairs, causing it to solubilize into the micelle [18]. The addition of 0.02 *M* tetrabutylammonium (TBA) salt permitted the separation of all the solutes except CP and VE. [Fig. 3 (SDS+TBA)]. NM, TH and NA may be prevented from interaction with the SDS micelle caused by competition with TBA salt. On the contrary, GK2 was separated from ASP. This result can be explained by the fact that GK2 and TBA salt form ion pairs. The addition of cationic surfactants to the buffer causes electroosmotic flow (EOF) reduction, because cationic surfactant monomers adhere to the capillary wall through ionic interactions. In fact it was seen that all solutes except TA and AL migrated later than buffer containing SDS alone, and as a result of the lower EOF, the separation window was widened. It is supposed that the pH of the buffer affects the phenomenon of the fast migration of these two solutes. The addition of TBA phosphate salt causes a decrease of pH, and so TA and AL may be ionized to a different extent. This estimation is based on their pK_a values [20], and in practice, pH dependent migration shifts of these two solutes were observed.

Ong et al. have demonstrated the usefulness of a mixed carrier system containing SDS with β-CD and TBA salt as modifiers in capillary electrophoresis for the separation of antihistamines [19]. A mixed carrier system was used for the separation of ingredients in ophthalmic solutions. A typical chromatogram is shown in Fig. 3 (SDS+TBA+β-CD). Good separation was achieved for the 11 ingredients by addition of β-CD and TBA salt to the MEKC buffer. High selectivity was obtained by the system, as Ong et al. suggested. CP and VE were fully separated only in the mixed carrier system, which interacted strongly with the SDS micelle. TBA acts as a competitor against CP, and, at the same time, CD is absolutely necessary for enhanced selectivity as another pseudo stationary phase. The enhanced selectivity overcame the strong interaction with the micelle.

3.3. Effect of CD type on the mixed carrier system

Several types of CDs were used together with SDS to manipulate the selectivity in CD-MEKC [21]. Another four CDs: α-CD, γ-CD, 2,6-di-*O*-methyl-β-CD (DM-β-CD), 2,3,6-tri-*O*-methyl-β-CD (TM-β-CD) were tested for their effect on the separation of the ingredients in ophthalmic solution in the mixed carrier system. They are different in the diameter of their cavity and/or the lipophilicity of the external portion of the CD molecule. Typical chromatograms are shown in Fig. 4. Among the four CDs, DM-β-CD (0.03 *M*) was the most effective for sufficient separation and the good peak shapes of ingredients. Therefore we established the suitable separation condition which was a buffer solution of pH 9.1 containing 0.15 *M* SDS, 0.02 *M* TBA and 0.03 *M* DM-β-CD. Inclusion of the solute to the CD is determined primarily by the size of the cavity. The result indicates that the cavity diameter of the β-CD derivative is most suitable for inclusion of the solute. The inclusion also can be affected by other factors such as hydrogen bonding, hydrophobic interactions, dipole–dipole interaction and London dispersion [22]. β-CD has unusually low water solubility because of the hydrogen bonding of the C-2 hydroxyl of 1 glucopyranose unit with the C-3 hydroxyl of an adjacent unit. In the β-CD molecule, a complete set of seven intramolecular hydrogen bonds can form [23]. However methylation disrupts the belt of the hydrogen bond, and can affect intermolecular hydrogen bonding. Derivatization at the 2- and 6-positions also may extend the depth of the CD cavity [23]. Therefore methylation seems to increase the hydrophobicity of the CD cavity. Successful separation

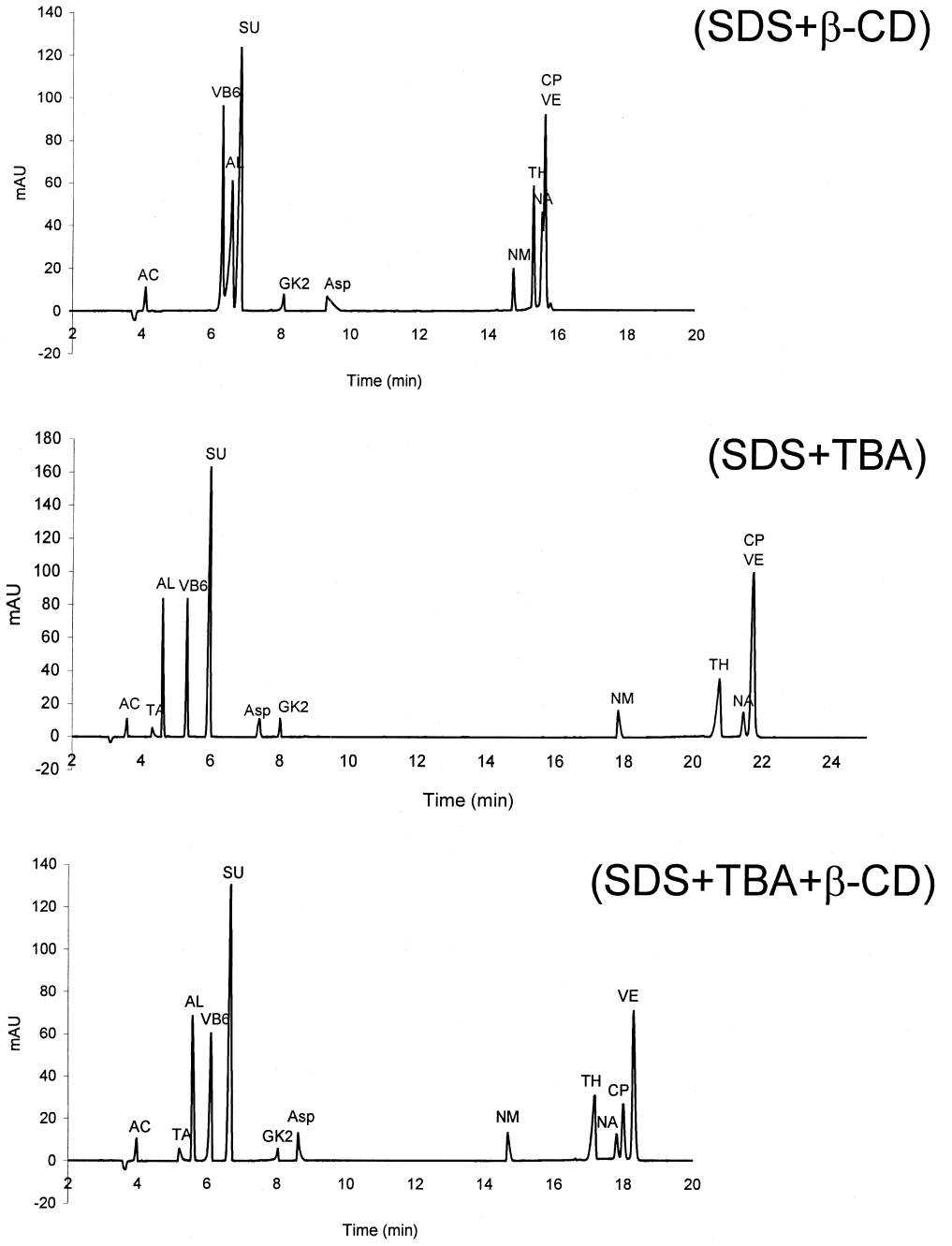

Fig. 3. Separation of ingredients with addition of β-CD and/or TBA to the MEKC buffer. (SDS+β-CD) 0.05 *M* β-CD added to the same SDS solution as in Fig. 2, (SDS+TBA) 0.02 *M* TBA added to the same SDS solution as in Fig. 2, (SDS+TBA+β-CD) 0.04 *M* β-CD and 0.02 *M* TBA added to the same SDS solution as in Fig. 2. Other conditions as in Fig. 2.

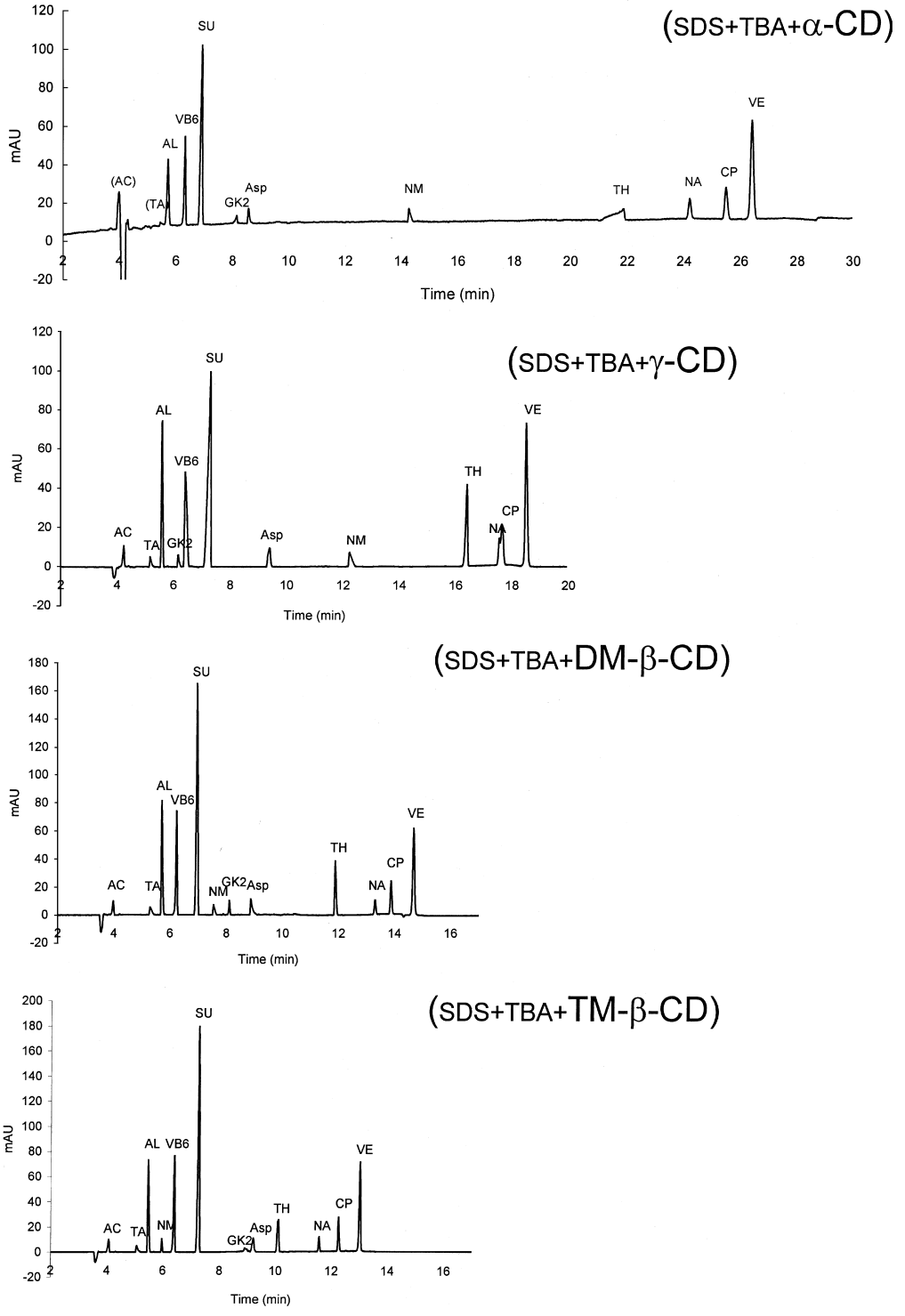

Fig. 4. Effect of CD type on the mixed carrier system. (SDS+TBA+α-CD) 0.06 *M* α-CD with 4 *M* urea or (SDS+TBA+γ-CD) 0.05 *M* γ-CD or (SDS+TMB+DM-β-CD) 0.03 *M* 2,6-di-*O*-methyl-β-CD or (SDS+TBA+TM-β-CD) 0.03 *M* 2,3,6-tri-*O*-methyl-β-CD added to the same SDS+TBA solution as in Fig. 3 (SDS+TBA). Other conditions as in Fig. 2.

with DM-β-CD may be achieved by improvement of the hydrophobic interaction between the cavity interior and the solute, and the hydrogen bond at the cavity edge that determines a solute's access to the cavity entrance [24,25].

3.4. Determination of active ingredients

3.4.1. Validation of the CE method

To assess the specificity, a standard, a sample of a commercial ophthalmic solution and placebo mixtures, which were prepared in the absence of each active ingredient, were analyzed according to the established method. Analysis was performed by an internal standard (I.S.) method using naphazoline hydrochloride as the I.S. A representative chromatogram of sample solution in Fig. 5 shows the separation between the main peaks and the I.S. No interference from the formulation excipients was observed at the migration time of the ingredients.

Detection limit was estimated as a peak with a signal-to-noise ratio of 3. Detection limits for each active ingredient, TA, VB6, NM, Asp, TH and CP were 25 μg/ml, 0.3 μg/ml, 0.3 μg/ml, 7.5 μg/ml, 0.2 μg/ml and 0.3 μg/ml, respectively, which are acceptable results.

Quantitation limit was calculated as a peak with a signal-to-noise ratio of 10. Quantitation limits for each active ingredient, TA, VB6, NM, Asp, TH and CP were 80 μg/ml, 1 μg/ml, 1 μg/ml, 25 μg/ml, 0.5 μg/ml and 1 μg/ml, respectively. Ten replicate injections at the quantitation limit level gave acceptable relative standard deviation (RSD) values ranging from 4.1 to 6.0% for ratio of the corrected peak area (relative to I.S.). The corrected area is defined as the ratio of the measured area to the migration time of the peak.

The linearity of active ingredients in standard solutions was investigated at five concentration levels in the range from 50 to 150% of the normal concentration. For each ingredient, relationship between the relative corrected peak area and the concentration was calculated and is given in Table 1. In all cases, the straight regression lines passing through the origin with the correlation coefficients (r) higher than 0.999 were obtained. The linear relationships confirm that the responses are directly proportional to the concentrations.

Accuracy was assessed over the whole 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 determined were compared to the theoretical amounts. Satisfactory results regarding accuracy were obtained for all ingredients studied (Table 1). The maximum difference between the observed values obtained during investigation and the true value is lower than 1.6%, which is an acceptable value for the determination of active ingredients in pharmaceutical preparations.

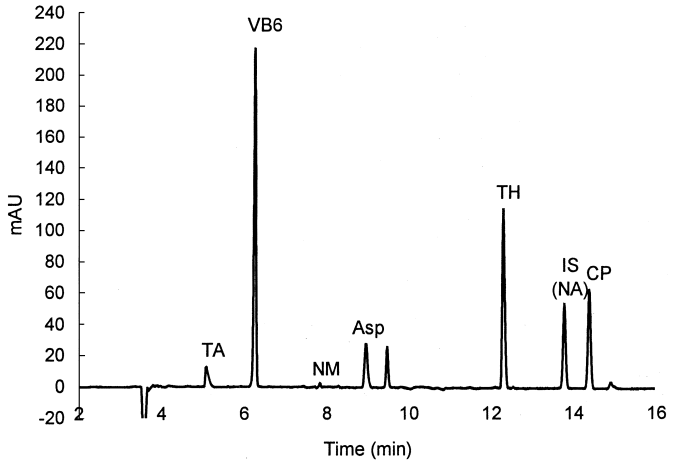

Fig. 5. Typical chromatogram in the assay of a commercial ophthalmic solution. Conditions as in Fig. 4 (SDS+TBA+DM-β-CD). Solutes are given in Section 2.2.

Table 1
Validation data for determination of active ingredients in a commercial ophthalmic solution

	2-Aminoethanesulfonic acid	Pyridoxine hydrochloride	Neostigmine methylsulfate	L-Aspartic acid	Tetrahydrozoline hydrochloride	Chlorpheniramine maleate
Linearity						
Concentration range (μg/ml)	1000–3000	100–300	2–6	420–1300	25–75	20–60
r	0.9992	0.9997	0.9998	0.9997	0.9999	0.9994
Intercept	0.0107	0.0234	0.00244	0.0286	0.0262	−0.0002
Slope	0.000412	0.03397	0.01812	0.001089	0.0445	0.03030
Accuracy (*n*=3, %)						
80%	−0.5	−1.0	−1.6	0.6	0.6	0.6
100%	0	0.1	−0.1	0	−0.6	1.0
120%	0.2	−1.1	0.1	−1.1	1.1	0.9
Precision (*n*=3, RSD, %)						
80%	1.7	0.3	1.0	0.5	2.1	0.9
100%	2.8	0.8	3.6	0.3	0.7	1.0
120%	1.6	1.0	3.3	1.5	1.8	0.2

Precision was determined by measuring (*n*=3) for each active ingredient in spiked placebos at the three concentration levels. RSD was estimated from the established method. Satisfactory results with respect to precision were obtained for all ingredients examined (Table 1).

3.4.2. Quantitative analysis of active ingredients in a commercial ophthalmic solution

System suitability testing over a short time while keeping the operating conditions identical was checked by six continuous injections of standard solution. The ratio of the migration time and the corrected peak area of each active ingredient to that of I.S. was found in RSD to range from 0.1 to 0.3% and from 0.2 to 1.3%, respectively (Table 2). These results are by no means inferior to those of conventional HPLC methods [3–6]. Results of the quantitation of active ingredients in a commercial ophthalmic solution are also given in Table 2. The results suggest that the CE method has the usefulness and advantage of simultaneous determination of the ingredients in pharmaceuticals.

4. Conclusion

It was demonstrated that a mixed carrier system containing SDS with DM-β-CD and TBA salt as modifiers in CE is efficient for the separation of 11 ingredients in ophthalmic solution. The proposed method has great potential for separating many kinds of pharmaceutical ingredients having different chemical properties. It is difficult to separate these

Table 2
Analytical results of a commercial ophthalmic solution

	2-Aminoethanesulfonic acid	Pyridoxine hydrochloride	Neostigmine methylsulfate	L-Aspartic acid	Tetrahydrozoline hydrochloride	Chlorpheniramine maleate
System suitability (*n*=6) RSD (%)						
Relative migration time	0.3	0.3	0.3	0.2	0.1	0.1
Relative corrected peak area	0.5	0.2	1.2	0.3	0.4	1.3
Content (%)[a]						
Average (*n*=6)	100.9	99.7	96.6	101.4	100.6	99.0
RSD (%)	1.3	1.0	1.6	1.2	1.1	1.0

[a] Percentage of the labeled amount.

ingredients simultaneously with high efficiency by only one conventional HPLC method [4]. In order to analyze several ingredients simultaneously by HPLC, gradient elution may be required, however, the method results increased equilibrium time for analysis and waste of solvent causing environmental pollution. The CE method gives similar performance level to HPLC method but can have advantages with respect to simplicity, high throughput and reduced organic solvents consumption. The results confirm that the method is suitable for the simultaneous determination in the pharmaceutical quality control and can be a complement to HPLC methods.

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