Journal of Chromatography, 498 (1990) 313–323
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 044

SEPARATION AND DETERMINATION OF THE INGREDIENTS OF A COLD MEDICINE BY MICELLAR ELECTROKINETIC CHROMATOGRAPHY WITH BILE SALTS

HIROYUKI NISHI*, TSUKASA FUKUYAMA and MASAAKI MATSUO
Analytical Chemistry Research Laboratory, Tanabe Seiyaku Co., Ltd., 16–89, Kashima 3-chome, Yodogawa-ku, Osaka 532 (Japan)
and
SHIGERU TERABE
Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Sakyo-ku, Kyoto 606 (Japan)
(First received June 20th, 1989; revised manuscript received September 15th, 1989)

SUMMARY

The separation of fourteen active ingredients used in a cold medicine was investigated by micellar electrokinetic chromatography (EKC) employing bile salts. Basic drugs were also successfully separated by micellar EKC using bile salts with high theoretical plate numbers ($2.0 \cdot 10^5$–$3.5 \cdot 10^5$) within a relatively short time (*ca.* 20 min). The separation of these solutes by micellar EKC was not successful using sodium dodecyl sulphate. The effects of micellar concentration, pH and organic modifier content on migration times and selectivity were investigated. This technique was also applied to the determination of several active ingredients combined in commercial preparations by an internal standard method.

INTRODUCTION

Micellar electrokinetic chromatography (EKC), first reported by Terabe *et al.* in 1984[1], is a relatively new type of separation method based on micellar solubilization and the instrumental technique of capillary zone electrophoresis (CZE)[2]. The technique has many advantages including the capability of separating neutral substances[3–6]. The selectivity and peak shape are considerably improved in the separation of ionic substances by this technique[7]. The determination of antibiotics in plasma by a direct injection method has also been successfully applied, similar to micellar high-performance liquid chromatography (HPLC)[8,9]. Chiral separations have been achieved by the use of a chiral surfactant, *e.g.*, bile salts[10,11] or a mixed micelle[12]. The separation of closely related isotopic compounds has recently been reported by Bushey and Jorgensen[13].

The determination of the ingredients of a cold medicine preparation[14,15], diltiazem in tablets[16], fluocinonide in cream[16], water-soluble vitamins in vitamin injections[17] and antibiotics in plasma[8,9] have all been performed by micellar EKC em-

0021-9673/90/$03.50

ploying an internal standard method and have given similar reproducibilities to those obtained by HPLC. A few commercial instruments for micellar EKC have recently become available. Detection methods applied have included electrochemical[18,19], mass spectrometric[20,21], and fluorimetric detection[22,23], in addition to photometric detection. The development of a microinjection method is also essential in order to take advantage of a microcolumn method.

In a previous paper, we reported the separation of twelve active ingredients in a cold medicine by micellar EKC with five anionic surfactants including sodium dodecyl sulphate (SDS)[15]. The basic drugs separated, chlorpheniramine maleate, tipepidine hibenzate and noscapine, migrated with similar velocities to that of the micelle or that obtained for Sudan III. Accordingly, it was difficult to separate these substances by micellar EKC with long-chain alkyl anionic surfactants owing to the strong ionic interaction between the basic solutes and the polar groups of the anionic surfactants.

In this paper, we describe the separation of fourteen active ingredients, including several basic compounds, by micellar EKC employing bile salts. The successful separation of corticosteroids and benzothiazepin analogues with bile salt solutions has been reported elsewhere[16]. The effects of the structure of the bile salts, their concentration, the pH of the buffer solution and the organic modifier content are discussed. The application of this technique to the analysis of commercial preparations using an internal standard method is also described.

EXPERIMENTAL

Apparatus and procedure of micellar EKC

Micellar EKC was performed using the apparatus described previously[7]. A fused-silica capillary (650 mm × 50 μm I.D.) (Scientific Glass Engineering, Ringwood, Victoria, Australia) was used as a separation tube. A high voltage (up to +25 kV) was applied by a Model HJLL-25PO d.c. power supply (Matsusada Precision Devices, Otsu, Japan). The electric current passing through the system was monitored using an ammeter throughout the operation. Detection was performed by on-column measurement of UV absorption at either 210 or 220 nm with a Uvidec-100-VI detector (Jasco, Tokyo, Japan). A Chromatopac C-R2AX (Shimadzu, Kyoto, Japan) was used for data processing. Sample solution was introduced by siphoning. Micellar EKC was performed at ambient temperature (*ca.* 20°C).

Reagents

Caffeine, acetaminophen, ethenzamide, phenacetin, chlorpheniramine maleate, noscapine and sulpyrin were purchased from Wako (Tokyo, Japan), guaifenesin and isopropylantipyrine from Tokyo Kasei Kogyo (Tokyo, Japan), dibucaine hydrochloride from Nacalai Tesque (Kyoto, Japan) and trimetoquinol hydrochloride, naproxen, tipepidine hibenzate and triprolidine hydrochloride from our laboratory (Tanabe Seiyaku, Osaka, Japan). The test samples used are summarized in Table I with reference numbers. All samples were used as received and were dissolved in water or methanol at a concentration of *ca.* 1 mg/ml to give adequate peak heights.

Sodium dodecyl sulphate (SDS) was obtained from Nacalai Tesque and sodium cholate (SC), sodium taurocholate (STC), sodium deoxycholate (SDC) and sodium dehydrocholate (SDHC) from Tokyo Kasei Kogyo. Sudan III, obtained from Naca-

TABLE I

TEST SAMPLES WITH REFERENCE NUMBERS

No.	*Sample*	*No.*	*Sample*
1	Caffeine	8	Phenacetin
2	Acetaminophen	9	Isopropylantipyrine
3	Sulpyrin	10	Noscapine
4	Trimetoquinol hydrochloride	11	Chlorpheniramine maleate
5	Guaifenesin	12	Tipepidine hibenzate
6	Naproxen	13	Dibucaine hydrochloride
7	Ethenzamide	14	Triprolidine hydrochloride

lai Tesque, was used as a micelle tracer[3]. The surfactants were dissolved in a buffer solution prepared by mixing 0.02 *M* sodium dihydrogenphosphate solution with appropriate volumes of 0.02 *M* sodium tetraborate solution to give the required pH values. These solutions were filtered through a 0.45-μm membrane filter prior to use. All other chemicals and solvents were of analytical-reagent grade, supplied by Katayama Kagaku Kogyo (Osaka, Japan).

Procedure for the determination of ingredient in preparations

The packages of Novapon granules (cold medicine) (Tanabe Seiyaku) were weighed and ground. One-tenth of the resulting powder was weighed accurately into a 100-ml volumetric flask and 70 ml of methanol were added for extraction. The flask was warmed in a water-bath (*ca.* 40°C) for 10 min with occational shaking, then cooled. An internal standard solution was prepared by dissolving 0.5 g of methyl *p*-hydroxybenzoate in 100 ml of methanol. Internal standard solution (20 ml) was added to the flask and the mixture was diluted to volume with water. This sample solution was passed through a 0.45-μm membrane filter.

Standard compounds (acetaminophen, caffeine, ethenzamide, tipepidine hibenzate) were weighed accurately into a 100-ml volumetric flask at a similar level to that present in a Novapon package and were dissolved in 70 ml of methanol. Internal standard solution (20 ml) was added and the mixture was diluted to volume with water.

The siphoning time (10 s, about 10 cm high) was held constant for both sample and standard solution in the micellar EKC analysis. The ratios of the peak area of each ingredient to that of the internal standard were measured with the data processor and the content of each individual ingredient in a Novapon package was calculated.

RESULTS AND DISCUSSION

Micellar EKC with SDS solutions

An electropherogram of fourteen active ingredients present in the cold medicine using 0.02 *M* phosphate–borate buffer solution of pH 9.0 was very similar to the previously reported result[15], in which twelve active ingredients were investigated. Most solutes migrated with a velocity the same as or similar to that of the electroos-

motic flow, except for the cationic and anionic compounds. The migration times in CZE were reported elsewhere[15].

Micellar EKC was performed with the same buffer solution as that used in CZE but containing in addition 0.1 *M* SDS. A typical chromatogram is shown in Fig. 1. All the solutes except samples 10–14 were succesfully separated by the addition of SDS. Samples 10–14 migrated last with the same migration time as Sudan III, which was added as a micelle tracer. This result indicated that the solutes interacted strongly with the micelle or were totally solubilized within the micelle. It was also difficult to separate these cationic solutes by micellar EKC with other long-chain alkyl surfactants, as reported elsewhere[15].

Micellar EKC with bile salts

Bile salts are biological surfactants synthesized in the liver. The structures of the bile salts used in this work are shown in Table II. All hydroxy groups at the 3α-, 7α- and 11α-positions in the 5β-cholane structure are orientated in the same direction, nearly perpendicular to the steroidal frame. Therefore, the bile salts have both a hydrophilic and a hydrophobic face and tend to combine together at the hydrophobic face in an aqueous phase. Hence bile salts are considered to form a primary micelle with up to ten monomers[24].

Micellar EKC with a bile salt was performed with a buffer solution of pH 9.0. Among the four bile salts tested, the migration pattern of the solutes when using SDHC was similar to that obtained in CZE, which indicates that SDHC had no micellar solubilization effect or did not form micelles as reported elsewhere[16]. Typical chromatograms are shown in Fig. 2, using (A) SC and (B) SDC. The solutes, especially 10–14, were successfully separated within *ca.* 20 min by use of bile salts. The theoretical plates numbers calculated from the equation $N = 2\pi(t_R h/A)^2$, where t_R, h and A are migration time, peak height and peak area, respectively[25], were $2 \cdot 10^5$–$3.5 \cdot 10^5$.

The relative migration order shown in Fig. 2 is interpreted in terms of lipophilicity and solute polarity. The solutes which were anionic under the experimental

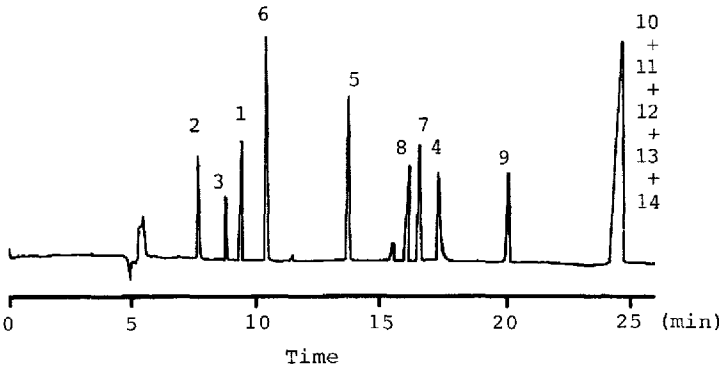
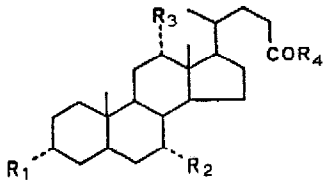


Fig. 1. Separation of fourteen ingredients by micellar EKC employing SDS. Buffer, 0.02 *M* phosphate–borate (pH 9.0) containing 0.1 *M* SDS; applied voltage, +20 kV; temperature, ambient; detection wavelength, 210 nm. Solutes are given in Table I.

TABLE II

STRUCTURE OF BILE SALTS



Bile salt	*Abbreviation*	R_1	R_2	R_3	R_4
Sodium cholate	SC	OH	OH	OH	ONa
Sodium taurocholate	STC	OH	OH	OH	$NHCH_2CH_2SO_3Na$
Sodium deoxycholate	SDC	OH	H	OH	ONa
Sodium dehydrocholate	SDHC	=O	=O	=O	ONa

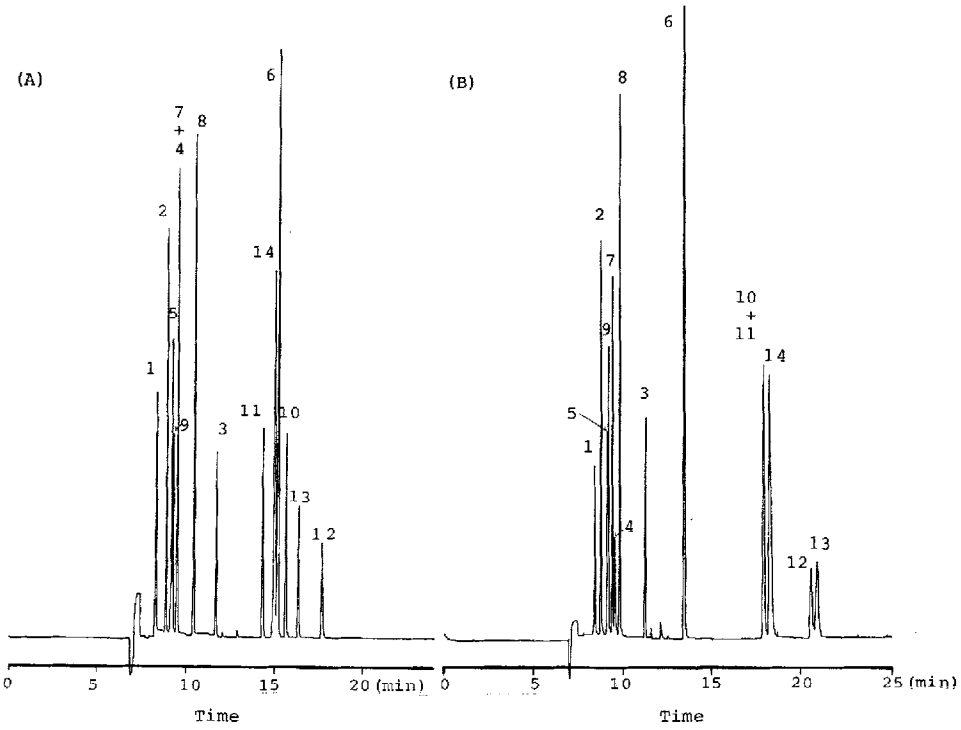


Fig. 2. Separation of fourteen ingredients by micellar EKC employing bile salts. Buffer, 0.02 *M* phosphate–borate (pH 9.0) containing (A) 0.1 *M* sodium cholate and (B) 0.05 *M* sodium deoxycholate. Other conditions as in Fig. 1.

conditions are considered to migrate with a total electrophoretic velocity that is the sum of the solute mobility and electroosmosis, as the anionic solute is unlikely to be incorporated into the anionic micelle. Conversely, the cationic and neutral solutes will be incorporated by the micelle. In particular, cationic solutes are subject to strong ionic interaction with the anionic micelle.

Effect of bile salt concentration and pH

The effect of the bile salt concentration on solute migration times was investigated for SC, STC and SDS solutions (pH 9.0). The results obtained using SC are shown in Fig. 3. The migration times of the basic compounds 10–14 increased substantially with an increase in the SC concentration from 0 to 0.1 *M* but were hardly altered when the SC concentration was increased further to 0.15 *M*. Differences in migration times between anionic solutes 3 and 6, and that among neutral solutes 1–9, except 3 and 6, increased with increase in SC concentration from 0 to 0.1 *M*. However, the migration times did not change significantly above 0.1 *M* SC, as observed for cationic compounds.

As the cationic solutes are considered to be largely incorporated by the anionic SC micelle, the increase in the migration times of these solutes may be attributed to the increase in the migration time of the micelle and partially to a decrease in electroosmotic velocity. A considerable variation of the relative migration order was observed among the cationic solutes 10–14 when the SC concentration was increased from 0.05 to 0.1 *M*. However, this result seems unusual, because the relative migration order is not usually affected by micellar concentration[3].

The more scattered migration times of the neutral solutes at 0.1 than at 0.05 *M* SC shown in Fig. 3 is explained in terms of an increase in micellar concentration and hence capacity factor. However, the slight changes in migration times between 0.1

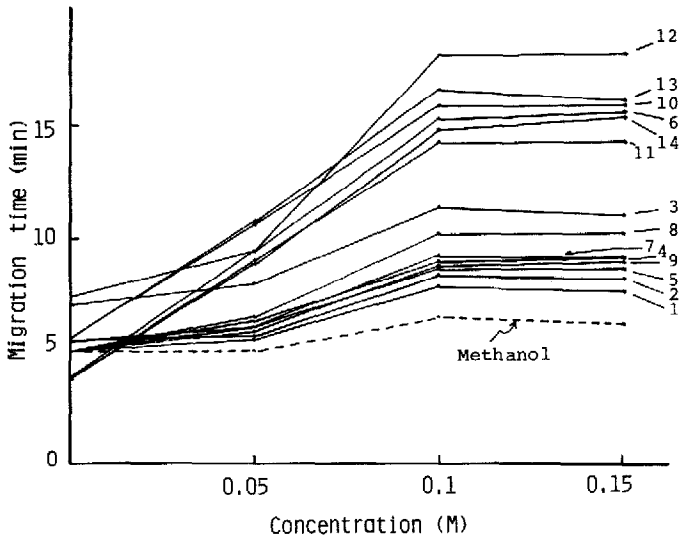


Fig. 3. Effect of sodium cholate concentration on the migration time. Buffer, 0.02 *M* phosphate–borate (pH 9.0). Other conditions as in Fig. 1.

and 0.15 *M* SC as shown in Fig. 3 are difficult to interpret reasonably. A possible explanation is the change in the micellar shape and/or size of SC in this concentration range.

No distinct difference in the migration behaviour of the solutes was observed when STC was used instead of SC. The only structural difference between SC and STC is in the ionic groups (Table II). The use of SDC brought about a significant change in the selectivity, as shown in Fig. 4, especially for samples 10–14, compared with the results obtained with SC. The steroidal part of SDC has only two hydroxyl groups, that is, the hydroxy group at the C-7 position of SC or STC is replaced with a hydrogen atom in SDC. The solubilization capability of SDC seems to be considerably increased by this substitution. The migration times of the solutes at 0.05 *M* SDC were longer than those at 0.1 *M* SC.

The pH dependence of migration times was examined with 0.1 *M* SC solutions in the pH range 7–9 and the results are shown in Fig. 5. The migration times of all the solutes except trimetoquinol hydrochloride increased with increasing pH, although the electroosmotic velocity remained almost constant, as judged from the migration time of methanol, which can be detected from the UV absorption due to the slight change in the refractive index[3]. Large changes in migration times were observed for

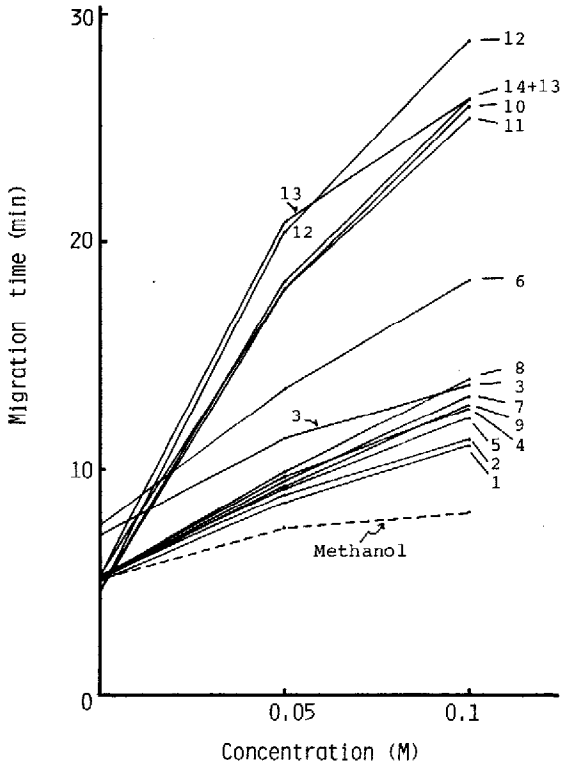


Fig. 4. Effect of sodium deoxycholate concentration on the migration time. Buffer, 0.02 *M* phosphate–borate (pH 9.0). Other conditions as in Fig. 1.

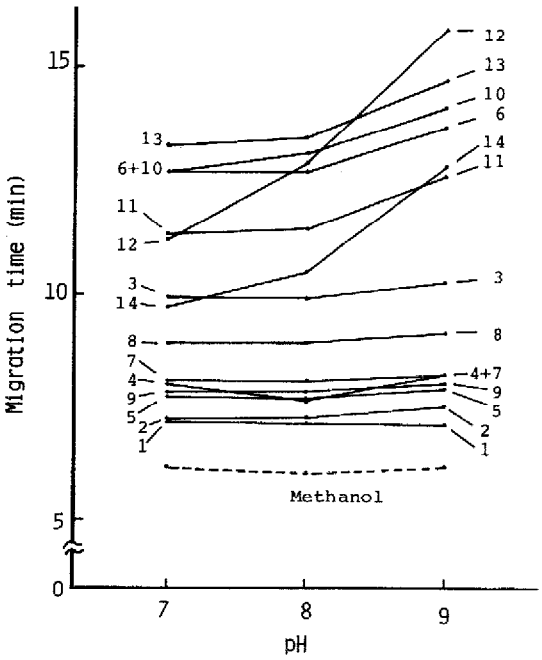


Fig. 5. pH dependence of migration times of fourteen ingredients. Buffer, 0.02 *M* phosphate–borate containing 0.1 *M* sodium cholate; applied voltage, +20 kV.

samples 12 and 14, although it is difficult to interpret the migration behaviour of these solutes.

Effect of organic modifier

Selectivity may also be manipulated by varying the aqueous organic modifier[26,27] in addition to the surfactant species, concentrations and pH. The effect of addition of methanol up to 20% on the separation of the solutes is shown in Fig. 6. The migration times increased with increase in methanol content. This can be ascribed to the decrease in the electroosmotic flow. The migration times of sulpyrin and naproxen, both of which are anionic compounds, were increased significantly by the addition of methanol in comparison with other compounds, that is, the capacity factors of the other solutes became smaller than those of anionic solutes on addition of the organic modifier. The electrophoretic velocities of the anionic solutes did not alter significantly but the migration velocity, which is the sum of the electrophoretic and electroosmotic velocities, was reduced considerably because of the decreased electroosmotic velocity under the experimental conditions. One can generally expect to alter the selectivity in the separation of a mixture of ionic and neutral compounds by adding an organic modifier.

Determination of active ingredients in preparations

On the basis of the above results, a buffer solution of pH 9.0 containing 0.1 *M* SC was selected for the determination of active ingredients combined in preparations.

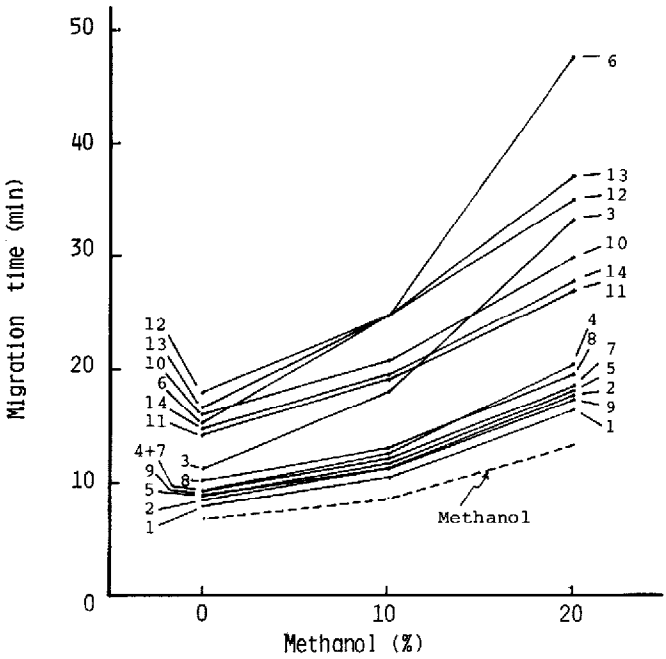


Fig. 6. Effect of organic modifier (methanol) in micellar EKC of fourteen ingredients. Conditions as in Fig. 5 except pH (9.0).

Quantitation was performed by an internal standard (I.S.) method employing methyl *p*-hydroxybenzoate as the I.S. A typical chromatogram of a sample solution is shown in Fig. 7. The reproducibilities of migration times and peak-area ratios obtained from the standard solution with repeated injections ($n = 5$) were 0.8% (coefficient of variation) and 2.2%, respectively, comparable to those reported previously[15,16,28]. Assay results ($n = 3$) are summarized in Table III. The results suggest that micellar EKC can be a useful complement to HPLC in the field of separation science.

TABLE III

ASSAY RESULTS OF NOVAPON GRANULES

Active ingredient	*Content (%)*[a]
Caffeine	97.8, 99.0, 100.1
Acetaminophen	100.3, 102.1, 99.2
Ethenzamide	100.3, 101.3, 98.2
Chlorpheniramine	–
Tipepidine	97.0, 103.4, 96.5

[a] Percentage of the labelled amount.

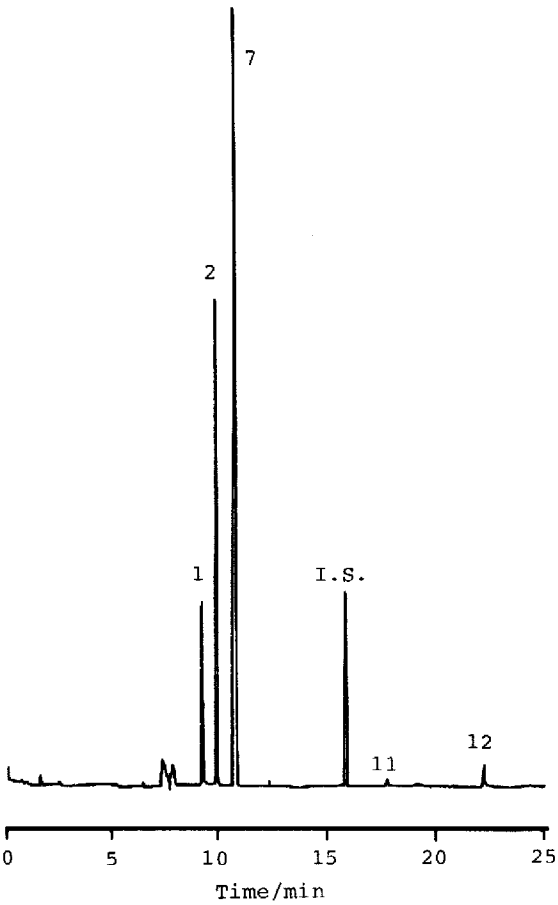


Fig. 7. Typical chromatogram in the assay of Novapon granules. Conditions as in Fig. 2A. Solutes are given in Table I.

ACKNOWLEDGEMENTS

We thank Professor Terumichi Nakagawa (Faculty of Pharmaceutical Sciences, Kyoto University) for his helpful advice. We also thank Dr. Toshio Kakimoto (Analytical Chemistry Research Laboratory, Tanabe Seiyaku) for his encouragement throughout this study.

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