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REGULAR ARTICLE

Direct enantioseparation of mandelic acid by high-performance liquid chromatography using a phenyl column precoated with a small amount of cyclodextrin additive in a mobile phase

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Abstract

Direct enantioseparation of mandelic acid by high-performance liquid chromatography (HPLC) with a reversed phase column and a mobile phase containing a small amount of hydroxylpropyl-β-cyclodextrin (HP-β-CD) was studied as an efficient method for saving consumption of the CD additive. As a result, it was proposed that racemic mandelic acid can be analyzed with a phenyl column by using a mobile phase composed of 10 mM ammonium acetate buffer (pH 4.2) and 0.02% (w/v) HP-β-CD at a flow rate of 1.0 mL/min at 40°C after the passage of 10 mM ammonium acetate buffer (pH 4.2) containing 0.1% (w/v) HP-β-CD as a precoating mobile phase for 60 min. It is suggested that HP-β-CD is bound with a phenyl group on the surface of the stationary phase to allow a phenyl column to act as a transient chiral column, and injected mandelic acid can form the ternary complex with the adsorbed HP-β-CD. The longer retention time of D-mandelic acid than the L-isomer for HPLC can be explained from the higher stability of the HP-β-CD complex with D-mandelic acid, which was confirmed by CE experiment with HP-β-CD as a selector. The efficiency of a phenyl column compared with other stationary phases was also discussed.

KEYWORDS

enantioseparation, mandelic acid, phenyl column, hydroxypropyl-β-cyclodextrin, HPLC

1 | INTRODUCTION

Since Davankov and Rogozhin introduced chiral ligand exchange chromatography for direct enantioseparation of amino acids,[1,2] a large number of chiral high-performance liquid chromatography (HPLC) methods have been reported as reviewed.[3–10] The main strategies of chiral HPLC can be divided into two categories, direct and indirect methods. The direct method, which does not require chemical derivatization, is based on a chiral stationary phase or a chiral selector in a mobile phase with an achiral stationary phase.[3–10] Separation is possible through reversible diastereomeric association between the chromatographic chiral environment and analyte enantiomers. The direct methods using chiral stationary phases are the most applied in separation

science.[6] The use of chiral stationary phases may not be sometimes effective because of interferences from matrix and/or endogeneous compounds. Therefore, in some situations, it may be necessary to use two or more columns to remedy the problem of lack of selectivity.[3,9] Although many kinds of chiral stationary phases have been commercially available, chiral columns are expensive, and it is not possible to find universal chiral columns for separation of various compounds. The indirect method is based on diastereomer formation of analyte enantiomers with a chiral derivatization reagent introduced as a second asymmetric source, which enable chromatographic enantioseparation with achiral stationary phases. The advantage of the indirect method includes commercially availability of chiral derivatizing reagents and a greater choice of chromatographic conditions. However, derivatizing reagents having high enantiomeric purity are required, and it must be confirmed that racemization of neither chiral analytes nor derivatizing reagents occurs during their derivatization processes.

As mentioned above, HPLC with a chiral mobile phase additive as a chiral selector and an achiral stationary phase is also possible for direct chiral separation. However, this method requires high chiral purity of the chiral selectors, and expensive selectors cannot be used because the mobile phase with a selector is use-and-discard. Among these methods, cyclodextrins (CDs) and their derivatives have been used as a chiral selector.[6,8,10] In the inclusion complexation, the cavity of native or derivatized CDs hydrophobically interacts with a hydrophobic part of chiral analytes, such as an aromatic ring.[11,12] This interaction plays an important role for enantioselectivity. Until now, several reversed phase HPLC methods with an octadecylsilyl (ODS) column and mobile phases containing neutral[13–15] or ionic CDs[16–19] have been reported. Shi et al. studied enantioseparation of substituted mandelic acids by HPLC with an ODS column and a mobile phase containing hydroxypropyl-β-CD (HP-β-CD).[13] The results of thermodynamic analysis and molecular modeling showed that the enantioseparation was attributed to the enthalpic factor in the hydrophobic interaction. Ma et al. reported a fast enantioseparation of a chiral aromatic amine using HPLC with C8 or C18 columns and highly sulfated β-CD as a chiral additive in the mobile phase.[17] They explored the molecular interaction between the amine enantiomers and the sulfated β-CD using vibrational circular dichroism spectroscopy as well as molecular modeling. It was indicated that the two amine enantiomers interact with the sulfated-β-CD by an inclusion of the aromatic part of the analyte, as well as through electrostatic interaction between the protonated amine and the sulfate groups located at the narrow side of the sulfated β-CD cavity. Recently, dynamic coating methods for enantioseparation have been reported.[20,21] Kucerova et al. studied enantioseparation of amino acids and dipeptides using sulfobutylether-β-CD onto a strong anion exchange stationary phase.[20] Folprechtova et al. reported the effect of substitution degrees of sulfobutylether-β-CD onto strong anion exchange stationary phases for various types of analytes.[21] It was found that lower sulfobutylether-β-CD substitution yielded higher enantioseparation values for basic and neutral compounds and that higher sulfobutylether-β-CD substitution showed higher enantioseparation values for acidic compounds.

Most of these enantioseparation methods required relatively high concentration of CD derivatives (more than 15-g CD derivatives per 1 L of mobile phases).[13–19] Thus, these methods were difficult to use daily analysis. In this study, we tried to investigate enantioseparation of mandelic acid as the model compound by reversed phase HPLC with a mobile phase containing a small amount of CD. Mandelic acid serves as building blocks for semisynthetic antibiotics, anticancer, antiobesty, and antithrombotic agents[22] and are used as one of chemical peeling agents in cosmetic dermatological products.[23] Various enantioseparation methods of mandelic acid have been developed by using capillary electrophoresis (CE) with neutral or ionic cyclodextrins as chiral selectors[24–26] and HPLC with chiral stationary phases[27–29] or chiral mobile phase.[30] However, these methods have not been applied to enantioseparation of mandelic acid in any real samples. In the present study, mandelic acid was found to be reproducibly enantioseparated by HPLC with a mobile phase containing 0.02% (w/v) HP-β-CD after treatment of a phenyl column with 0.1% (w/v) HP-β-CD. The proposed method was successfully applied to analysis of mandelic acid enantiomers in cosmetics.

2 | MATERIALS AND METHODS

2.1 | Reagents and chemicals

D-Mandelic acid was obtained from Tokyo Kasei (Tokyo, Japan). HP-β-CDs (average molecular weight: 1,540 and 1,380; average degree of substitution: 7 and 4, respectively), HP-γ-CD, heptakis (2,6-di-*O*-methyl)-β-CD, heptakis (2,3,6-tri-*O*-methyl)-β-CD, and acetonitrile were obtained from Sigma-Aldrich (St. Louis, MO, USA). Racemic mandelic acid, α-, β-, and γ-CDs and other chemicals

(analytical grade) were obtained from Fujifilm Wako (Osaka, Japan).

2.2 | HPLC apparatus and chromatographic conditions for mandelic acid analysis

The HPLC system consisted of a Jasco (Tokyo, Japan) model PU-4580 pump, a Jasco Model UV-2075 detector, a Rheodyne (Cotati, CA, USA) manual injector, a Shimadzu (Tokyo, Japan) column oven Model CTO-10A vp, and a Flom (Tokyo, Japan) degasser Model Gastorr. InertSustain Phenyl (4.6 mm i.d. × 150 mm, GL Sciences, Tokyo, Japan), InertSustain C18 (4.6 mm i.d. × 150 mm, GL Sciences), InertSustain Phenylhexyl (4.6 mm i.d. × 150 mm, GL Sciences), HC-C18 (4.6 mm i.d. × 150 mm, Agilent Technologies, Waldbronn, Germany), and Kaseisorb LC ODS Super (4.6 mm i.d. × 150 mm, Tokyo Kasei) columns were used as reversed phase columns.

The potency of the CD additives and the stationary phases for enatioseparation was examined by using various kinds of CDs and some kinds of reversed-phase columns. Ammonium acetate buffer (10 mM, pH 4.2) containing 0.1% (w/v) each CD was used as a mobile phase. After the passage of the mobile phase through each column at a flow rate of 1.0 mL/min at 40°C for 20 min, 10 μL of 1 mM racemic mandelic acid solution was analyzed five times at consecutive 13 min intervals under the same conditions, and the analytes were detected at 220 nm.

The effects of concentration of the CD additive, pH of a mobile phase, and the column temperature on the enantioseparation were examined. After the passage of a mobile phase composed of 10 mM ammonium acetate buffer (pH 4.0–5.0) and 0.02–0.15% (w/v) HP-β-CD (average molecular weight: 1540) through a phenyl column at a flow rate of 1.0 mL/min at 35–50°C for 60 min, 10 μL of 1 mM racemic mandelic acid solution was analyzed under the respective conditions, and the analytes were detected at 220 nm.

For determination of racemic mandelic acid in a cosmetic sample, after the passage of mobile phase composed of 10 mM ammonium acetate buffer (pH 4.2) and 0.1% (w/v) HP-β-CD (average molecular weight: 1,540) through a phenyl column at a flow rate of 1.0 mL/min at 40°C for 60 min, the sample solution was analyzed by HPLC with another mobile phase composed of 10 mM ammonium acetate buffer (pH 4.2) containing 0.02% (w/v) HP-β-CD under the same flow conditions. At the end of analysis, column was washed with 80% (v/v) acetonitrile.

2.3 | Capillary electrophoretic apparatus and its running conditions

Electrophoretic experiments were carried out using a Capillary Electrophoresis System (Agilent Technologies). Separation was performed in fused silica capillary of 64.5 cm (effective length 56 cm) × 50 μm i.d. (Agilent Technologies). The background electrolyte (BGE) was 10 mM phosphate buffer (pH 7.0) containing 0 to 60 mM HP-β-CD. The standard solution of mandelic acid (1 mM) and the BGE was injected by pressure of 50 mbar for 4 s, respectively. The capillary was kept at 20°C. The analytes were detected at 200 nm. The power supply was operated in the constant-voltage mode, at +30 kV.

2.4 | Sample preparation

Stock solutions of racemic mandelic acid (10 mM) and D-mandelic acid (10 mM) were separately prepared with purified water, stored at −15 °C, and diluted 10 times before use. A cosmetic sample (0.1 g), which were purchased from an internet shop, was prepared by adjusting the volume to 100 mL with purified water. Recovery was examined by addition of 0.05 or 0.1 mM solutions of each enantiomer of manderic acid to the diluted sample solution ($n = 3$).

2.5 | Determination of HP-β-CD adsorbed on stationary phase of reversed phase columns

A mobile phase composed of 10 mM ammonium acetate buffer (pH 4.2) and 0.1% (w/v) HP-β-CD was passed through the reversed phase HPLC column at a flow rate of 1.0 mL/min at 40°C for 60 min, and the column was washed with purified water for 4 min. Then, the HP-β-CD adsorbed on the stationary phase was collected with passage of 80% (v/v) acetonitrile at 1 mL/min for 10 min (the first 10 mL eluate) and was collected for 10–20 min (second 10 mL eluate). HP-β-CD in the eluates was determined by hydrophilic interaction chromatography (HILIC) with InertSustain NH2 (4.6 mm i.d. × 150 mm, GL Sciences) column and a mobile phase composed of 70% acetonitrile. Elution was carried out at a flow rate of 1.0 mL/min at 40°C. HP-β-CD was detected with a Shimadzu RID-6A refractive index (RI) detector. Data acquisition from UV and RI detectors and the data processing were conducted with a Chromato-PRO (Runtime Instruments Co. Kanagawa, Japan).

3 | RESULTS AND DISCUSSION

3.1 | Factors affecting enantioseparation

The potency of a variety of CDs for enantioseparation of mandelic acid was investigated by HPLC using a phenyl column and a mobile phase composed of 10-mM ammonium acetate buffer (pH 4.2) and 0.1% (w/v) of HP-β-CDs (average molecular weight: 1,540 and 1,380), 2,6-di-*O*-methyl-β-CD, 2,3,6-tri-*O*-methyl-β-CD, HP-γ-CD, α-CD, β-CD, and γ-CD, separately. After the passage of the mobile phase containing each CD for 20 min to obtain the stable baseline, 1 mM racemic mandelic acid was analyzed five times consecutively at 13 min intervals (at 0, 13, 26, 39, and 52 min after the baseline stabilization). An increase in number of the analysis brought about increases in the retention time and the resolution of racemic mandelic acid for HP-β-CD (average molecular weight: 1,540) (Figure 1A) and 2,6-di-*O*-methyl-β-CD, with which the resolution was not sufficient (Figure 1B), suggesting that the adsorption of the β-CDs on the stationary phase of the phenyl column proceeded during the above five analyses. On the other hand, mandelic acid was not enantioseparated by the other CDs (e.g., Figure 1C). It was found that HP-β-CD with average molecular weight of 1,540 not 1,380 is the most effective for the enantioseparation of mandelic acid. This result was consistent with the previous report on chiral capillary electrophoresis (CE).[31]

The effect of stationary phases on the retention time and the resolution were compared by using InertSustain phenyl, Agilent HC-C18, and InertSustain phenylhexyl columns and a mobile phase containing HP-β-CD (Figure 2). Chromatogram obtained with an HC-C18 column was the same as chromatograms obtained with InertSustain C18 and Kaseisorb LC ODS Super columns. Only a phenyl column was found to be effective for the enantioseparation of racemic mandelic acid. This result was discussed later Section 3.3.

The factors affecting the retention time and the resolution such as concentration of the CD additive, pH of the mobile phase, and the column temperature on the enantioseparation were examined (Figure 3). Mobile phases composed of 10 mM ammonium acetate buffer (pH 4.2) and various concentrations (0.02–0.15% [w/v]) of HP-β-CD were passed through a phenyl column at a flow rate of 1.0 mL/min at 40°C for 60 min and then racemic mandelic acid was analyzed with the respective mobile phases. Mandelic acid was not enantioseparated with a mobile phase containing 0.02% (w/v) HP-β-CD, and the higher HP-β-CD concentration caused increases in both the retention time and the resolution of mandelic acid (Figure 3A). The effect of pH (4.0–5.0) of the mobile phase on the retention time and the resolution of mandelic acid was also investigated (Figure 3B). Because pK_a of mandelic acid is 3.41,[32] an increase in pH value of the mobile phase decreased the retention time of mandelic acid as well as the resolution because of a decrease in hydrophobic interaction. The effect of column temperature (35–50°C) on the retention time and the resolution of mandelic acid was examined (Figure 3C). A lower column temperature caused increases in both the retention time and the resolution of mandelic acid. Therefore, the optimum analytical conditions giving both the moderately short retention time and the acceptable resolution were considered a mobile phase containing 10 mM ammonium acetate buffer (pH 4.2) and 0.1% (w/v) HP-β-CD (average molecular weight: 1,540) at 40°C.

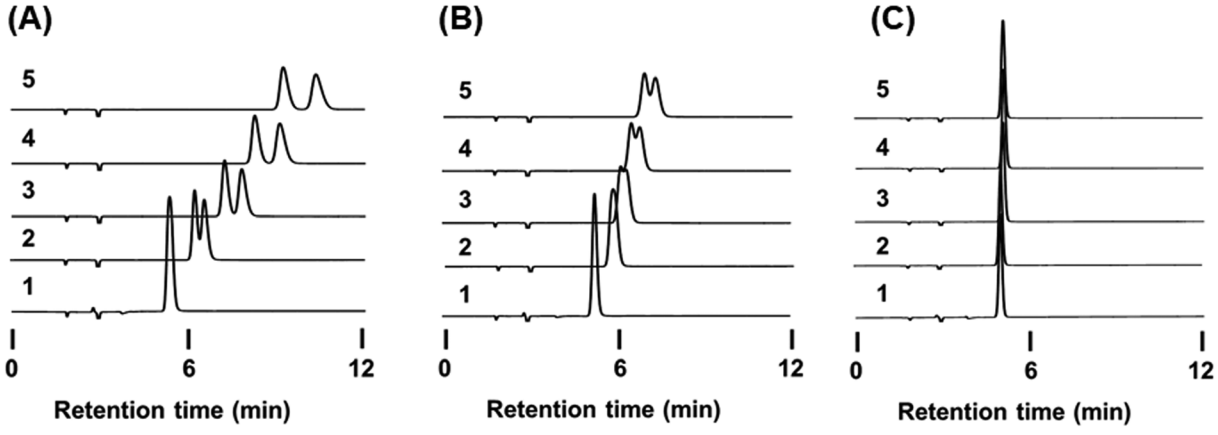

FIGURE 1 Effect of the type of CD on the retention time and the resolution of HPLC analysis of racemic mandelic acid. Mandelic acid (1 mM) was analyzed five times consecutively at 0 (1), 13 (2), 26 (3), 39 (4), and 52 (5) min by using a phenyl column and mobile phases composed of 10 mM ammonium acetate buffer (pH 4.2) and 0.1% (w/v) HP-β-CD (A), 2,6-di-O-methyl-β-CD (B), or β-CD (C) at a flow rate of 1 mL/min at 40°C after passage of each mobile phase through phenyl column at a flow rate of 1 mL/min for 20 min

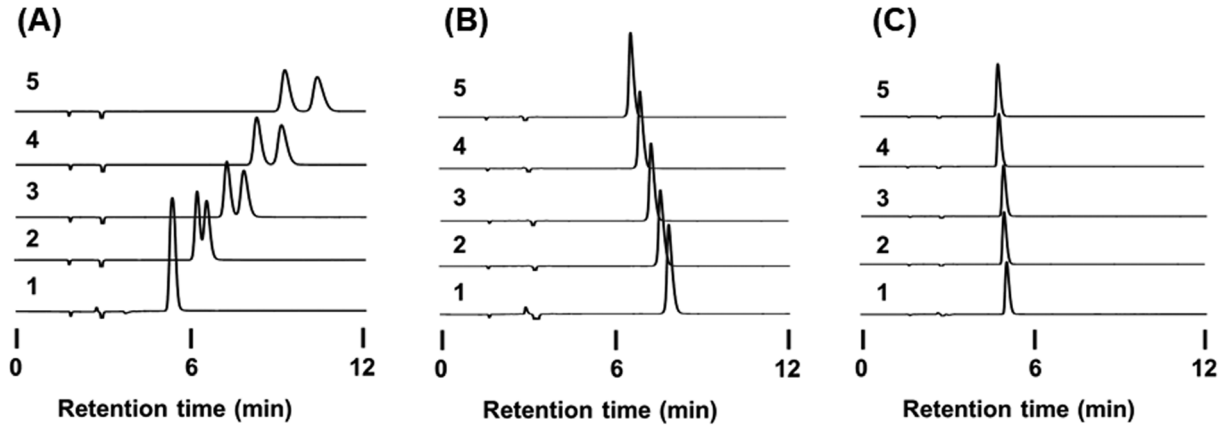

FIGURE 2 Effect of the type of reversed phase columns on the retention time and the resolution of HPLC analysis of racemic mandelic acid. Mandelic acid (1 mM) was analyzed five times consecutively at 0 (1), 13 (2), 26 (3), 39 (4), and 52 (5) min by using InertSustain phenyl (A), HC-C18 (B), or InertSustain phenylhexyl (C) columns and a mobile phase composed of 10 mM ammonium acetate buffer (pH 4.2) and 0.1% (w/v) HP-β-CD at a flow rate of 1 mL/min at 40°C after passage of the same mobile phase through each column at a flow rate of 1 mL/min for 20 min

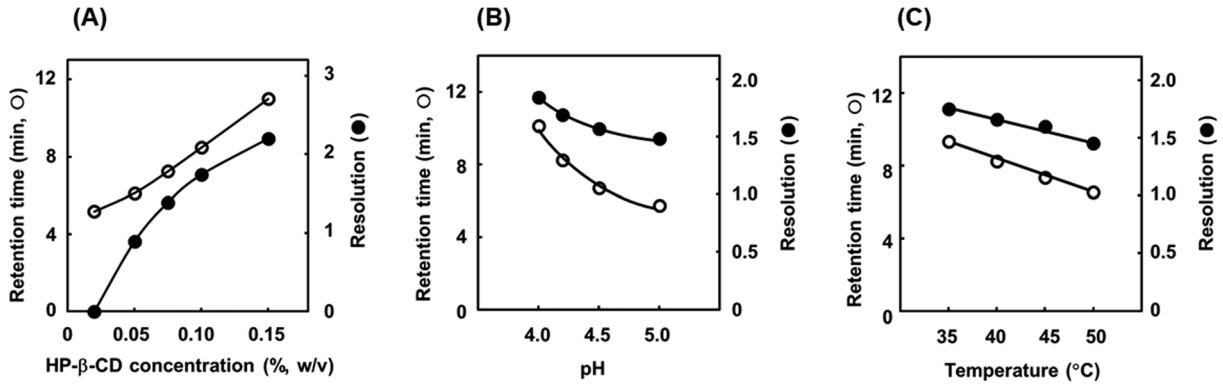

FIGURE 3 Effects of concentration of HP-β-CD (A), pH of mobile phase (B), and column temperature (C) on the retention time and the resolution of HPLC analysis of racemic mandelic acid. Mandelic acid was analyzed by using a phenyl column and a mobile phase composed of 10 mM ammonium acetate buffer (pH 4.2 for a and C and pH 4.0–5.0 for B) and 0.02–0.15% (*w*/*v*) (a) and 0.1% (w/v) (B and C) HP-β-CD at a flow rate of 1 mL/min at 40°C for (A) and (B) and 35–50°C for (C) after passage of each mobile phase through a phenyl column at a flow rate of 1 mL/min for 60 min. Open and closed circles denote the retention time of the former peak and the resolution, respectively

The retention time and the resolution of mandelic acid were affected by the number of analysis, that is, the total passage time of the mobile phase containing 0.1% (w/v) HP-β-CD through the phenyl column as described above. After the passage of 10 mM ammonium acetate buffer (pH 4.2) containing 0.1% (w/v) HP-β-CD for 60 min, racemic mandelic acid was enantioseparated even by using the mobile phase without HP-β-CD (Figure 4A). The retention time and the resolution were gradually decreased with increasing the total passage time of HP-β-CD-free mobile phase. This indicates that HP-β-CD adsorbed phenyl groups on the stationary phase during the initial 60 min flow with 0.1% (w/v) HP-β-CD desorbs gradually, and HP-β-CD need to be supplied to keep the constant retention time and the resolution. Thus, the effect of the concentration of HP-β-CD (0.01–0.03% [w/v]) in the mobile phase after the flow with 0.1% (w/v) HP-β-CD on the retention time, and the resolution was examined. As a result, using a mobile phase containing 0.02% (w/v) HP-β-CD, the retention time and the resolution were found to be kept constant regardless of the number of the analysis (Figure 4B). Mobile phases containing 0.01% and 0.03% (w/v) HP-β-CD brought about slightly decrease and increase, respectively, in the retention time. Therefore, it can be proposed that racemic mandelic acid was analyzed by using the mobile phase containing 0.02% (w/v) HP-β-CD at a flow rate of 1.0 mL/min at 40°C after the passage of 10 mM

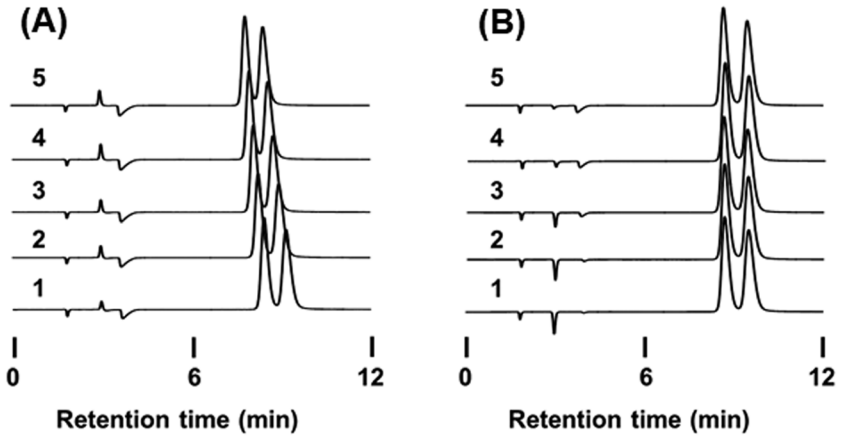

FIGURE 4 Repeatability of the retention time and the resolution of HPLC analysis of mandelic acid by using a mobile phase containing a small amount of HP-β-CD. Mandelic acid was analyzed five times consecutively at 0 (1), 13 (2), 26 (3), 39 (4), and 52 (5) min by using a phenyl column and mobile phase composed of 10 mM ammonium acetate buffer (pH 4.2) containing 0% (A) and 0.02% (w/v) (B) HP-β-CD at a flow rate of 1 mL/min at 40°C after passage of 10 mM ammonium acetate buffer (pH 4.2) containing 0.1% (w/v) HP-β-CD through a phenyl column at a flow rate of 1 mL/min for 60 min

ammonium acetate buffer (pH 4.2) containing 0.1% (w/v) HP-β-CD as a precoating mobile phase for 60 min. This method is efficient to reduce consumption of the CD derivatives.

3.2 | Analysis of a cosmetic sample

Mandelic acid was subjected to the proposed HPLC method above with a small amount of HP-β-CD. The limit of detection (LODs) of L- and D-mandelic acid enantiomers defined as a signal-to-noise ratio of 3 were 0.0005 mM (0.0761 mg/L) and 0.00055 mM (0.0837 mg/L), respectively, and LOQs (the limit of quantification) of L- and D-enantiomers defined as a signal-to-noise ratio of 10 were 0.0015 mM (0.228 mg/L) and 0.00165 (0.251 mg/L), respectively. Linearity ($r^2 > 0.999$) was demonstrated in the concentration range of 0.0015–2 mM for L-mandelic acid and 0.00165–2 mM for D-mandelic acid by each standard curve (12 points). Intraday repeatabilities of the peak area and the retention time ($n = 5$) were less than 2.7 and 0.4% RSD, respectively, at 0.02, 0.1, and 0.5 mM for D- and L-mandelic acids. Interday repeatabilities of the peak area and the retention time in 3 days ($n = 5$, each day) were less than 1.5% and 0.5% RSD, respectively, at 0.2 mM for the enantiomers. Recoveries were between 98% and 103%.

Using the proposed method, D- and L-mandelic acids in a cosmetic sample were analyzed (Figure 5). D-Mandelic acid was found to correspond to the latter separated peak. The contents of D- and L-mandelic acids were 24.6 ± 0.4 mg/g and 24.7 ± 0.3 mg/g, respectively, suggesting that mandelic acid in the sample was racemic mixture. The total content of D- and L-mandelic acids (49.3 mg/g) was almost the same as the content displayed on the label (5%). Only 0.3 g of HP-β-CD was required for the proposed method to prepare the precoating mobile phase (100 mL) containing 0.1% (w/v) (0.65 mM) HP-β-CD and the mobile phase (1 L) containing 0.02% (w/v) (0.13 mM) HP-β-CD, which was significantly lesser than the amount of HP-β-CD (more than 15 g/L mobile phase) used in the previously reported methods with reversed phase HPLC.[13–15,30] Chiral CE method by Valko et al. required the HP-β-CD concentration higher than 60 mM as a chiral selector for the baseline separation of D- and L-mandelic acids in which preparation of 10 mL of the BGE requires 0.924 g of HP-β-CD.[24] Mandelic acid has been also enantioseparated by CE with cationic CDs[25,26]

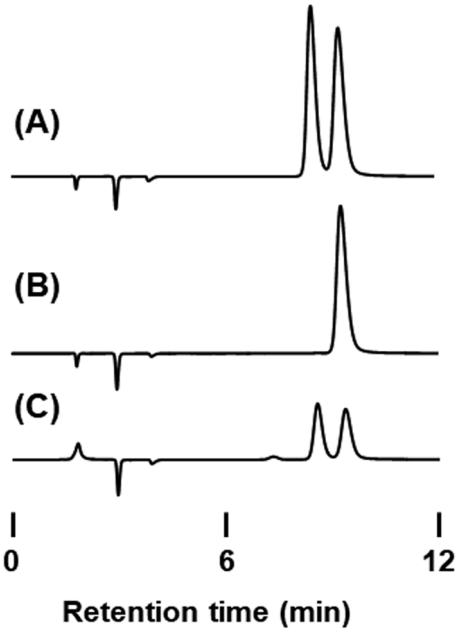

FIGURE 5 Chromatograms of racemic mandelic acid (A), D-mandelic acid (B), and a cosmetic sample (C) for HPLC analysis by using a phenyl column and a mobile phase composed of 10 mM ammonium acetate buffer (pH 4.2) and 0.02% (w/v) HP-β-CD at a flow rate of 1 mL/min at 40 °C after passage of 10 mM ammonium acetate buffer (pH 4.2) containing 0.1% (w/v) HP-β-CD through a phenyl column at a flow rate of 1 mL/min for 60 min

and by HPLC with novel stationary phases.[27–29] However, the preparation of these CDs and chiral stationary phases is not so simple. In the present work, the proposed HPLC method using a phenyl column and an HP-β-CD as a chiral selector was successfully applied to the enantioseparation of mandelic acid, while selection and combination of CDs may make this method adaptable to various types of chiral compounds.

3.3 | The possible mechanism for enantioseparation of mandelic acid by using phenyl column and HP-β-CD as a selector

The ability of β-CD to form inclusion complexes is highly affected by sizes, shapes, and hydrophobic nature of guest molecules.[33] It is usually regarded that a single guest molecule is accommodated into the β-CD cavity to form the 1:1 host-guest complex. Prabhu et al. studied the inclusion complexation behavior of salbutamol, sotalol, and atenolol drugs with β-CD by UV-visible absorption, fluorometry, time resolved fluorescence, FT-IR, ^{1}H-NMR, SEM, and PM3 (Parametric method 3).[33] Addition of β-CD to aqueous solutions containing the above drugs resulted in observation of the excimer fluorescence of each compound. They concluded that the excimer fluorescence was attributed to formation of the 1:2 β-CD-drug inclusion complex. Alremeithi et al. determined *p*-aminohippuric acid with β-CD by sensitized fluorescence spectrometry.[34] The 1:2 host-guest inclusion between β-CD and *p*-aminohippuric acid became evident by mass spectrometry and density functional theory calculations that supported the formation of an excimer state at 355 nm. Recently, Yildiz et al. has studied complexation of cinnamaldehyde with HP-β-CD and HP-γ-CD and suggested formation of the 1:1 and 1:2 CD-cinnamaldehyde inclusion complexes by computational molecular modeling.[35] These reports show the possibility that two phenyl groups can be simultaneously included in the cavity of β-CD.

In order to clarify the reason why mandelic acid could enantioseparated by HPLC with only a phenyl column, the content of HP-β-CD adsorbed in a phenyl column was determined as described in *Experimental* section. Figure 6A shows a chromatogram of standard solution of HP-β-CD. Because the HP-β-CD is a mixture of several substitution degrees, the peak of HP-β-CD was relatively broad. Linearity ($r^2 > 0.999$) was demonstrated in the concentration range of 0.01–2% (w/v) by the standard curve (9 points, Figure 6C). The first and second 10 mL eluates from phenyl column after absorbing HP-β-CD were collected ($n = 3$). As a result, HP-β-CD was detected only in the first eluate (Figure 6B). The content was 45.7 ± 0.9 mg, and the adsorption ratio to the total amount of HP-β-CD in mobile phase that passed through the columns was 76.3%. Thus, HP-β-CD may form a complex with phenyl groups on the surface of the stationary phase in the column to allow a phenyl column to act as a transient chiral column. When mandelic acid was injected in the phenyl column, the ternary complex could be formed by aggregation of a phenyl group on the stationary phase, HP-β-CD, and a phenyl group of mandelic acid as the β-CD inclusion complexes with two guest molecules having a phenyl group were reported.[34,35] The longer retention time for D-mandelic acid in the chromatographic process suggests that the ternary inclusion complex with D-mandelic acid was more stable than that with L-mandelic acid.

To verify the above proposition, racemic mandelic acid was analyzed by CE with HP-β-CD as a selector. In the BGE at pH 7, where mandelic acid is completely deprotonated, the analyte electrophoretically migrates as an anion. However, the electroosmotic flow was sufficient to reverse the direction of the apparent mobility, and mandelate complexes with neutral HP-β-CD migrates faster than mandelate anion. As shown in Figure 7, D-mandelic acid moved faster than the L-isomer to enable the enantioseparation. Thus, it was indicated that D-mandelic acid formed more stable diastereomer complexes with HP-β-CD than the L-isomer. This fact was consistent with the enantioselectivity proposed by the above HPLC result.

Valko et al. reported chiral CE of organic acids containing mandelic acid by using HP-β-CD as a chiral selector.[24] Resolution of D- and L-mandelic acid was affected by the degree of substitution (DS), that is, the substituted number of hydroxypropyl groups per β-CD molecule. Regardless of DS (3–7.3), more than 40 mM HP-β-CD in the BGE was required for the enantioseparation of mandelic acid. This result agrees well with our result in Figure 7. The concentration of HP-β-CD of the precoating mobile phase (0.1% [w/v]) and the mobile phase (0.02% (w/v)) in this HPLC method correspond to 0.65 and 0.13 mM, respectively. The concentration of adsorbed HP-β-CD per inner column volume after the precoating was 11.9 mM. Therefore, the proposed HPLC was found to be more efficient than the chiral CE method with respect to the concentration of HP-β-CD.

The contents of HP-β-CD adsorbed in other reversed phase columns were also determined. The contents in phenylhexyl and ODS columns were 56.8 ± 1.1 and 56.8 ± 0.7 mg, respectively, which were higher than the content in a phenyl column. Both adsorption ratio to the amount of HP-β-CD in mobile phase that passed through the columns were 94.9% (cf. 76.3% for a phenyl column).

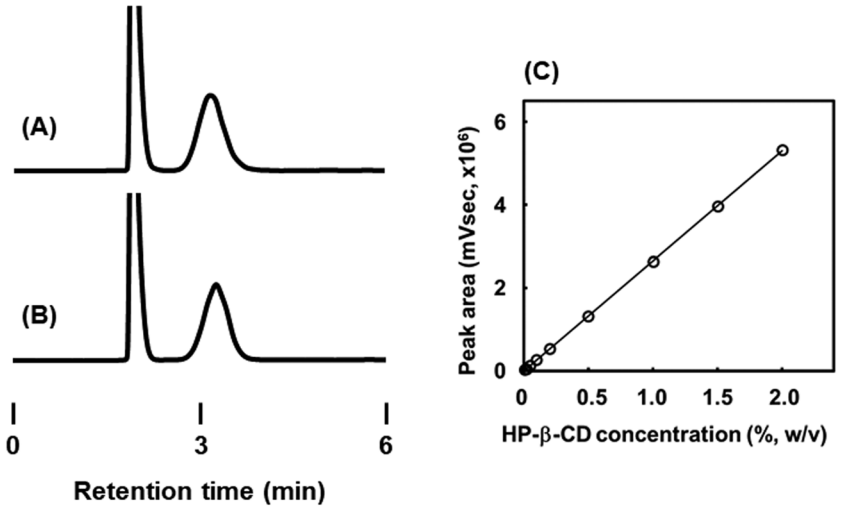

FIGURE 6 Chromatograms of HP-β-CD standard solution (A) and the first 10-mL eluate from phenyl column after adsorbing HP-β-CD (B) and standard curve of HP-β-CD (C) for HILIC analysis by using an InertSustain NH2 column and a mobile phase composed of 70% (v/v) acetonitrile at a flow rate of 1 mL/min at 40°C. HP-β-CD was detected with RI detector

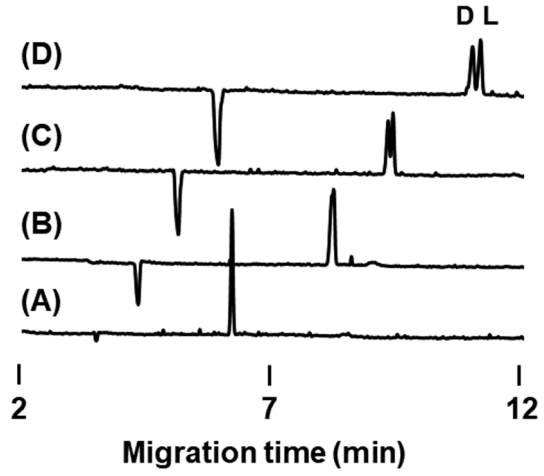

FIGURE 7 Electropherograms of racemic mandelic acid analyzed by CE with 10-mM phosphate buffer (pH 7) containing 0 mM (A), 20 mM (B), 40 mM (C), and 60 mM (D) HP-β-CD

In the case of phenyl column, phenyl groups are directly bound to the surface of the stationary phase. Therefore, the complexation of HP-β-CD with a phenyl group on the phenyl column surface is not so stabilized thermodynamically (Figure 8A) as observed in Figure 4. On the other hand, HP-β-CD may be deeply penetrated by phenylhexyl and alkyl groups on the stationary phase for phenylhexyl and ODS columns, respectively (Figures 8B,C), where HP-β-CD may not stay on the surface of the stationary phase, and the complexation equilibrium of HP-β-CD is stabilized. Considering such a structural difference, it is plausible that mandelic acid can easily interact with HP-β-CD on the surface only for phenyl column to enable the enantioseparation (Figure 8).

In this study, mandelic acid was used as a model compound. We studied the enantioseparation of other compounds by HPLC with a phenyl column in preliminary experiments. As a result, hydroxymandelic acid was partially enantioseparated by a mobile phase composed of 0.1% (w/v) HP-β-CD and 10 mM ammonium acetate buffer (pH 4.2). Phenylalaninol, abscisic acid, and pantothenic acid were found to be fully or partially enantioseparated by a mobile phase composed of 0.1% (w/v) HP-β-CD and 0.1% (v/v) phosphoric acid. Moreover, catechin was enantioseparated by a mobile phase

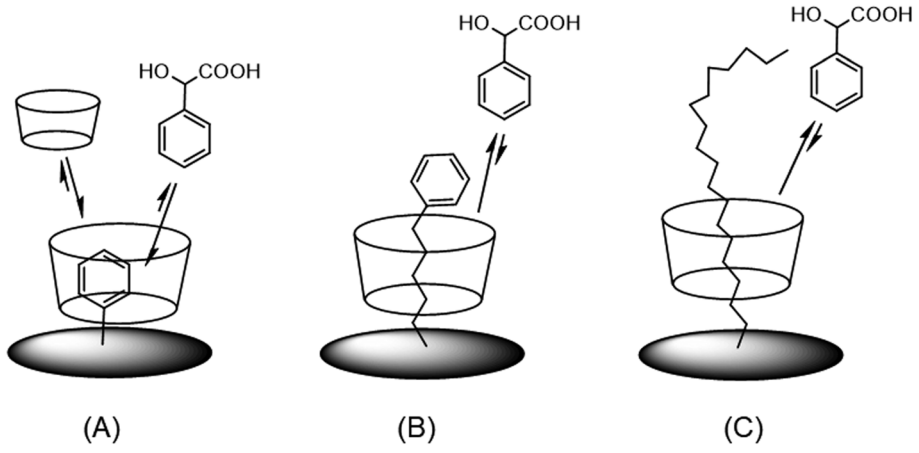

FIGURE 8 Plausible structure of HP-β-CD complexes on the surface of the stationary phase of phenyl (A), phenylhexyl (B), and ODS (**C**) columns

containing 0.05% (w/v) β-CD. Thus, the proposed enantioseparation method with phenyl column and a small amount of CD additives may be promising for application to the enantioseparation of various types of compounds.

4 | CONCLUSION

It was proposed that racemic mandelic acid can be analyzed by HPLC with a phenyl column by using a mobile phase containing 10 mM ammonium acetate buffer (pH 4.2) and 0.02% (w/v) HP-β-CD (average molecular weight: 1,540) at a flow rate of 1.0 mL/min at 40°C after the passage of 10 mM ammonium acetate buffer (pH 4.2) containing 0.1% (w/v) HP-β-CD as a precoating mobile phase for 60 min. This method gave the constant retention time and resolution for the repeated analyses and is quite efficient to reduce consumption of HP-β-CD. The proposed method was successfully applied to chiral analysis of mandelic acid enantiomers in a cosmetic sample. The enantioseparation mechanism is also proposed as follows. HP-β-CD is bound with a phenyl group on the surface of the stationary phase to allow a phenyl column to act as a transient chiral column, and injected mandelic acid can form the ternary complex with the adsorbed HP-β-CD. The longer retention time of D-mandelic acid for HPLC can be explained from the higher stability of HP-β-CD complex with D-mandelic acid, which was confirmed by the CE experiment with HP-β-CD as a selector. Phenylhexyl and ODS columns are not suitable for using as a transient chiral column because HP-β-CD may be penetrated by phenylhexyl and alkyl groups too deeply to stay on the surface of the stationary phase.

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