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Separation of mandelic acid and its derivatives with new immobilized cellulose chiral stationary phase

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Abstract: A new liquid chromatographic method has been developed for the chiral separation of the enantiomers of mandelic acid and their derivatives 2-chloromandelic acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid. The enantiomers were separated by a CHIRALPAK® IC (250 mm×4.6 mm, 5 μm). Mandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid were baseline resolved (resolution factor (R_S)=2.21, R_S=2.14, and R_S=3.70, respectively). In contrast, the enantioselectivities between CHIRALPAK® IC and 2-chloromandelic acid and 4-hydroxymandelic acid investigated were low. By comparing the chromatographs of mandelic acid enantiomers and mandelic acid spiked with (*R*)-mandelic acid, it was determined that the first effluent was (*R*)-mandelic acid.

Key words: CHIRALPAK® IC, Enantiomeric separation, High-performance liquid chromatography (HPLC), Mandelic acid, Derivatives

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1 Introduction

Enantiomerically pure mandelic acid and its derivatives are important chiral analogs that are widely employed in the pharmaceutical synthetic industry, such as the manufacture of semisynthetic penicillins, cephalosporins or (*S*)-oxybutynin, and for the synthesis of various other chiral pharmaceuticals and drug intermediates (Grover *et al*., 2000; Huang and Xu, 2006; Davulcu *et al*., 2009; Mishra *et al.*, 2009). They are also valuable chiral synthons that have been widely employed for the resolution of racemic compounds, like alcohols and amines (Whitesell and Reynolds, 1983; Hansen *et al.*, 2009; Schramm and Christoffers, 2009). Thus, many methods have been reported for the separation of optically pure (*S*)- or (*R*)-mandelic acid (Takahashi *et al*., 1995; Guo *et al*., 2009). None of these methods is more rapid or simple than the method of chiral stationary phase high-performance liquid chromatography (HPLC).

Debowski *et al.* (1983) resolved the enantiomers of mandelic acid derivatives in a reversed-phase HPLC system via α- and β-cyclodextrin, but the enantiomers were not baseline resolved. Thunberg *et al.* (2008) separated the enantiomers of mandelic acid using CHIRALPAK® AD and CHIRALPAK® IA, but optimization of the chiral separation of the enantiomers of mandelic acid was not investigated.

In this study, we used a CHIRALPAK® IC, a new chiral stationary phase made by immobilizing cellulosic tris(3,5-dichlorophenylcarbamate) on silica gel. Relative to similar columns in which the chiral selector is coated onto silica, it is thought that this new column might possess advantages in terms of robustness and the range of mobile phase solvents that can be utilized (Zhang *et al*., 2008; 2010; Ferretti *et al.*, 2009). The chiral selector used to prepare CHIRALPAK® IC was found too soluble in classical solvents (such as dichloromethane and chloroform) to

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afford a coated version.

Herein we report the results of our studies concerning the effect of variation of experimental conditions and optimization of the chiral separation of the enantiomers of mandelic acid, 2-chloromandelic acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid (the structures are shown in Fig. 1). The structures of the enantiomers of mandelic acid were also determined. Mandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid were all baseline resolved.

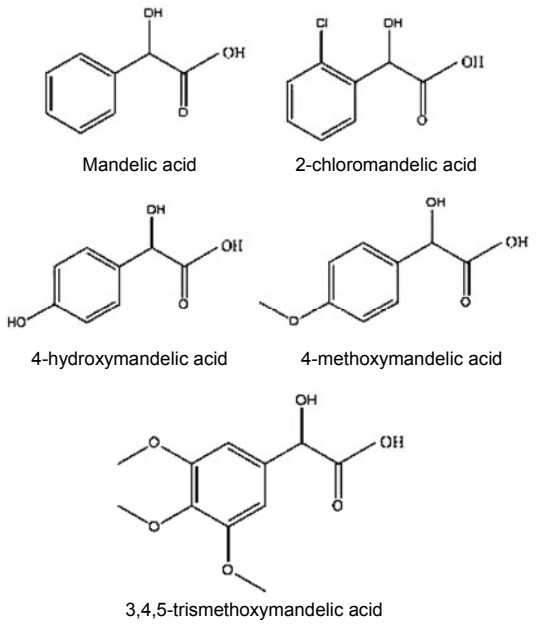

Fig. 1 Structures of mandelic acid and its derivatives

2 Materials and methods

2.1 Apparatus

The HPLC instrument used in this study was an Agilent 1100 series apparatus (Palo Alto, CA, USA). It was equipped with a quaternary pump, a vacuum degasser, a column oven, a multiple wavelength UV detector, an auto-sampler, and HP Chemstation software. The analysis was carried out with a CHIRALPAK® IC (250 mm×4.6 mm, 5 μm; Daicel, Japan).

2.2 Reagents

HPLC-grade *n*-hexane, ethanol, and isopropanol were obtained from T&J Kermel Reagent Company. Trifluoroacetic acid (TFA) was analytically pure, and obtained from T&J Kermel Reagent Company. Mandelic acid, 2-chloromandelic acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid were obtained from New Drugs Research and Development Center of Zhengzhou University, China. (*R*)-mandelic acid was synthesized by School of Pharmacy, Zhengzhou University.

2.3 Sample preparation

Mandelic acid, 2-chloromandelic acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid were dissolved in appropriate amounts of ethanol. The solutions were all filtered (0.45 μm) to prepare sample solution.

2.4 Chromatographic condition

The basic solvent of the mobile phase was *n*-hexane, with the polarity modifier isopropanol or ethanol. The five compounds were all acidic compounds, so 0.1% TFA was used as the mobile phase additive. Mobile phases were filtered with 0.45 μm solvent filter and ultrasonically degassed. Separations were performed on a CHIRALPAK® IC (250 mm× 4.6 mm, 5 μm) at 15–35 °C with a flow rate of 0.4–1.2 ml/min. The detection wavelengths of mandelic acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid were set at 230 nm, and 2-chloromandelic acid was set at 210 nm. The volume of sample injected was 10 μl.

The dead time was determined by injecting 1,3,5-tert-butylbenzene as a non-retained marker.

3 Results and discussion

3.1 Effect of alcohol modifier

The effect of the content of mobile phase modifiers (ethanol or isopropanol) on mandelic acid, 2-chloromandelic acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid was studied. The results are shown in Table 1.

By modifying the mobile phase with ethanol, the column could afford shorter retention time than that with isopropanol, but the resolution was decreased.

With a decrease in the alcohol content in the mobile phase, there was an observed increased retention

of both enantiomers, and an increased resolution due to the decreased interaction between the respective enantiomers and the stationary phase.

The CHIRALPAK® IC column possesses advantages in the range of mobile phase solvents that can be utilized. Dichloromethane was used as the mobile phase modifier to optimize the chiral separation of the enantiomers of 2-chloromandelic acid (Zhang *et al.*, 2008).

The results also showed that the enantioselectivity between 4-hydroxymandelic acid and chiral stationary phase investigated was low.

3.2 Effect of additive TFA

Experiments were carried out to investigate the effect of additive TFA on the enantioselectivities of mandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid (Table 2).

Table 1 Effect of contents of alcohol modifiers on the enantioselectivities of mandelic acid and its derivatives

Compound	c (%)	t_{R1} (min)		t_{R2} (min)		α		R_S	
		I	E	I	E	I	E	I	E
Mandelic acid	5	17.7	11.0	21.9	12.5	1.23	1.13	2.10	1.46
	10	7.3	4.4	8.9	5.0	1.22	1.11	1.15	1.06
	20	2.9	1.9	3.3	2.0	1.22	1.11	1.05	0.58
	30	1.8	1.2	2.1		1.11	1.00	0.40	nr
	40	1.4	0.8			1.00	1.00	nr	nr
2-chloromandelic acid	5	18.8	10.5	20.2	10.8	1.08	1.03	0.71	0.30
	10	7.3	4.3	8.0		1.10	1.00	0.57	nr
	20	2.8	1.8	3.0		1.09	1.00	0.47	nr
	30	1.6	0.9			1.00	1.00	nr	nr
	40	1.4	0.8			1.00	1.00	nr	nr
4-hydroxymandelic acid	5	57.5	29.5	66.1	32.2	1.15	1.09	1.18	0.76
	10	14.0	7.1	15.4	7.3	1.10	1.02	0.60	0.22
	20	3.4	2.0			1.00	1.00	nr	nr
	30	1.5	0.9			1.00	1.00	nr	nr
	40	1.4	0.8			1.00	1.00	nr	nr
4-methoxymandelic acid	5	38.2	22.3	43.5	24.7	1.14	1.11	2.07	1.65
	10	15.1	8.5	17.1	9.4	1.13	1.10	1.61	1.18
	20	5.6	3.1	6.2	3.4	1.12	1.09	1.11	0.64
	30	3.0	1.7	3.4	1.8	1.11	1.05	0.85	0.45
	40	2.2	1.2	2.4		1.11	1.00	0.59	nr
3,4,5-trismethoxymandelic acid	5	−	64.6		89.2	−	1.38	−	6.82
	10	58.0	18.7	74.1	25.5	1.28	1.36	4.31	5.11
	20	16.4	5.8	21.1	7.8	1.29	1.34	3.66	3.70
	30	8.1	3.0	10.5	3.9	1.30	1.32	3.09	2.73
	40	5.1	1.9	6.6	2.5	1.30	1.33	2.42	1.97

c: content of alcohols (isopropanol/ethanol); I: isopropanol; E: ethanol; t_{R1}, t_{R2}: retention times of the first peak and the following peak, respectively; α: separation factor; R_S: resolution factor; nr: not resolved; −: retention time is too long (>100 min). Chromatographic condition: the basic solvent of the mobile phase was *n*-hexane with 0.1% TFA, the column temperature was 25 °C, and the flow rate was 0.8 ml/min

Table 2 Effect of TFA on the enantioselectivities of mandelic acid and its derivatives

TFA content (%)	Mandelic acid				4-methoxymandelic acid				3,4,5-trismethoxymandelic acid			
	t_{R1} (min)	t_{R2} (min)	α	R_S	t_{R1} (min)	t_{R2} (min)	α	R_S	t_{R1} (min)	t_{R2} (min)	α	R_S
0.1	17.7	21.9	1.23	2.21	38.2	43.5	1.14	2.07	5.8	7.8	1.34	3.65
0.2	17.9	21.7	1.20	2.44	38.7	44.1	1.14	2.02	5.5	7.3	1.32	3.44
0.3	17.7	22.1	1.23	2.50	40.3	45.8	1.14	1.98	5.5	7.3	1.33	3.66
0.4	18.7	23.4	1.23	2.32	41.4	47.2	1.14	2.04	5.5	7.3	1.33	3.83

t_{R1}, t_{R2}: retention times of the first peak and the following peak, respectively; α: separation factor; R_S: resolution factor. Mandelic acid and 4-methoxymandelic acid: mobile phase was *n*-hexane-isopropanol (95/5, v/v); 3,4,5-trismethoxymandelic acid: mobile phase was *n*-hexane-ethanol (80/20, v/v). The column temperature was 25 °C, and the flow rate was 0.8 ml/min

The results show that the retention and resolution differed little over the TFA concentration range of 0.1%–0.4%. Higher TFA content may be harmful to the chiral stationary phase, so 0.1% TFA was used.

3.3 Effect of column temperature

The effect of column temperature on mandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid was studied. The separation data were shown in Table 3.

Calculate parameters by the van′t Hoff equation are shown as below (Zhou *et al*., 2012):

$$\ln\alpha=-\Delta_{R,S}\Delta H°/(RT)+\Delta_{R,S}\Delta S°/R.$$

Here, α was chiral selector, and $\alpha=k'_2/k'_1$ (k'_1 and k'_2 are capacity factors of the two enantiomers, respectively). $\Delta_{R,S}\Delta H°$ and $\Delta_{R,S}\Delta S°$ are enthalpy change and entropy change, respectively. van′t Hoff plots were drawn for logarithm of α vs. inverted temperature ($1/T$) for two isomers. The regression equation of mandelic acid is as follows: $\ln\alpha=188.47/T-0.4308$ (R=0.9926). The results show that the linearity of the regression equation was good. From the equation, the values of $\Delta_{R,S}\Delta H°$ and $\Delta_{R,S}\Delta S°$ were calculated, and $\Delta_{R,S}\Delta H°=-0.187$ kJ/mol, $\Delta_{R,S}\Delta S°=-0.428$ J/mol, respectively, over the temperature range of 15–35 °C, with $|\Delta_{R,S}\Delta H°|>|T\Delta_{R,S}\Delta S°|$.

The regression equation of 4-methoxymandelic acid is as follows: $\ln\alpha=201.64/T-0.5426$ (R=0.9926). The results show that the linearity of the regression equation was good. $\Delta_{R,S}\Delta H°=-0.200$ kJ/mol, $\Delta_{R,S}\Delta S°=-0.539$ J/mol, respectively, over the temperature range of 15–35 °C, with $|\Delta_{R,S}\Delta H°|>|T\Delta_{R,S}\Delta S°|$.

The regression equation of 3,4,5-trismethoxymandelic acid is as follows: $\ln\alpha=357.30/T-0.9074$ (R=0.9951). The results show that the linearity of the regression equation was good. $\Delta_{R,S}\Delta H°=-0.356$ kJ/mol and $\Delta_{R,S}\Delta S°=-0.903$ J/mol over the temperature range of 15–35 °C, with $|\Delta_{R,S}\Delta H°|>|T\Delta_{R,S}\Delta S°|$.

The chiral separation process of the three compounds was all controlled by enthalpy. As the column temperature was increased, a corresponding decrease in retention was observed. The resolution was also found to decrease. This would be expected since the mass transfer kinetics is faster at higher temperatures, which resulted in lower enantiomeric retention (Munro and Walker, 2001).

These data show that the column temperature should be carefully controlled for optimum chiral separation of enantiomer retention, and 25 °C was closest to room temperature, so the other parameters were optimized at 25 °C.

3.4 Effect of flow rate

The effect of flow rate on the enantioselectivity was investigated (Table 4).

Table 3 Effect of temperature on the enantioselectivities of mandelic acid and its derivatives

T (°C)	Mandelic acid				4-methoxymandelic acid				3,4,5-trismethoxymandelic acid			
	t_{R1} (min)	t_{R2} (min)	α	R_S	t_{R1} (min)	t_{R2} (min)	α	R_S	t_{R1} (min)	t_{R2} (min)	α	R_S
15	28.2	35.5	1.25	2.59	64.7	75.6	1.17	2.56	6.5	9.0	1.40	4.20
20	23.2	28.7	1.24	2.28	54.7	63.5	1.16	2.40	6.1	8.3	1.36	3.91
25	17.7	21.9	1.22	2.21	38.2	43.5	1.14	2.07	5.8	7.8	1.34	3.65
30	16.9	20.3	1.21	1.82	35.0	39.5	1.13	1.85	5.6	7.3	1.31	3.22
35	16.2	19.6	1.20	1.78	33.9	37.9	1.12	1.66	5.4	6.9	1.29	3.04

t_{R1}, t_{R2}: retention times of the first peak and the following peak, respectively; α: separation factor; R_S: resolution factor. Mandelic acid and 4-methoxymandelic acid: mobile phase was *n*-hexane-isopropanol (95/5, v/v; 0.1% TFA); 3,4,5-trismethoxymandelic acid: mobile phase was *n*-hexane-ethanol (80/20, v/v; 0.1% TFA). The flow rate was 0.8 ml/min

Table 4 Effect of flow rate on the enantioselectivities of mandelic acid and its derivatives

Flow rate	Mandelic acid				4-methoxymandelic acid				3,4,5-trismethoxymandelic acid			
(ml/min)	t_{R1} (min)	t_{R2} (min)	α	R_S	t_{R1} (min)	t_{R2} (min)	α	R_S	t_{R1} (min)	t_{R2} (min)	α	R_S
0.4	32.5	40.0	1.23	2.11	75.5	86.2	1.14	2.07	11.5	15.2	1.33	3.79
0.6	22.5	27.7	1.23	2.07	49.0	55.8	1.14	2.08	7.7	10.3	1.33	3.75
0.8	17.5	21.6	1.23	2.18	38.2	43.5	1.14	2.07	5.8	7.8	1.34	3.65
1.0	14.0	17.3	1.22	2.04	30.0	34.1	1.14	1.93	4.7	6.3	1.34	3.50
1.2	11.9	14.7	1.24	1.99	26.1	29.9	1.14	1.93	3.9	5.2	1.34	3.32

t_{R1}, t_{R2}: retention times of the first peak and the following peak, respectively; α: separation factor; R_S: resolution factor. Mandelic acid and 4-methoxymandelic acid: mobile phase was *n*-hexane-isopropanol (95/5, v/v; 0.1% TFA); 3,4,5-trismethoxymandelic acid: mobile phase was *n*-hexane-ethanol (80/20, v/v; 0.1% TFA). The column temperature was 25 °C

The results show that the change in separation factor was not significant over the flow rate range of 0.4–1.2 ml/min. By the van Deemter chromatography theory, the vertical proliferation plays a major role at lower flow rates, and the mass transfer resistance plays a major role at higher rates.

The maximum resolution of mandelic acid was at the flow rate of 0.8 ml/min and the resolution was reduced when the flow rate was reduced to 0.6 ml/min, because of the extension of the peak due to the increased peak time.

The resolutions of 4-methoxymandelic acid and 3,4,5-trismethoxymandelic acid were decreased as the flow rate increased over the range of 0.4–1.2 ml/min. With the increase in flow rate, the interaction between enantiomers and the mobile phase was increased, leading to decreased resolution. In order to reduce the analytical time and to get better separation, the flow rate of 0.8 ml/min was used.

The HPLC chromatograms of mandelic acid, 2-chloromandelic acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid are shown in Fig. 2.

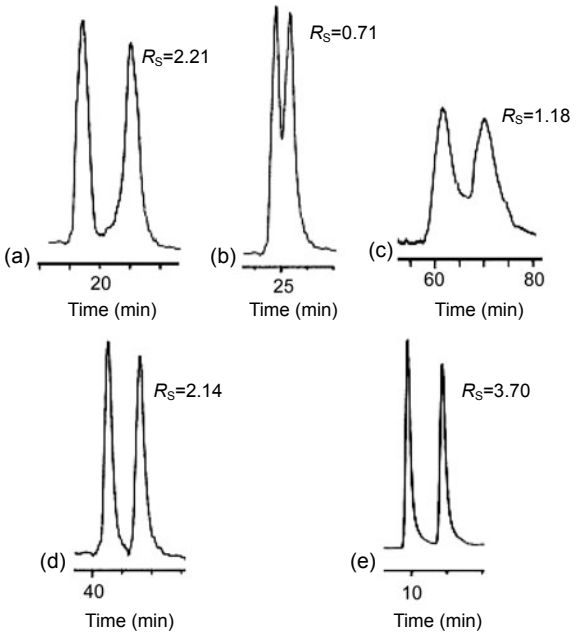

Fig. 2 HPLC chromatograms of mandelic acid and its derivatives

(a) Mandelic acid; (b) 2-chloromandelic acid; (c) 4-hydroxymandelic acid; (d) 4-methoxymandelic acid; (e) 3,4,5-trismethoxymandelic acid. Chromatographic conditions: (a–d) mobile phase was *n*-hexane-isopropanol (95/5, v/v; 0.1% TFA) at 25 °C, the flow rate was 0.8 ml/min; (e) mobile phase was *n*-hexane-ethanol (80/20, v/v; 0.1% TFA) at 25 °C, the flow rate was 0.8 ml/min

3.5 Structure determination of mandelic acid

An appropriate amount of (*R*)-mandelic acid was added to the solution of mandelic acid. The chromatograph is shown in Fig. 3. By comparing Fig. 2a with Fig. 3, the first effluent was determined as (*R*)-mandelic acid, and the following effluent as (*S*)-mandelic acid.

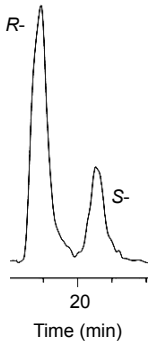

Fig. 3 HPLC chromatogram of mandelic acid containing (*R*)-mandelic acid

4 Conclusions

The enantiomers of mandelic acid and their derivatives (2-chloromandelic acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid) were first separated with CHIRALPAK® IC, and the optimum chromatographic conditions of mandelic acid, 2-chloromandelic acid, 4-hydroxymandelic acid, and 4-methoxymandelic acid were *n*-hexane-isopropanol (95/5, v/v; 0.1% TFA) at 25 °C with a flow rate of 0.8 ml/min (R_S=2.21, R_S=0.71, R_S=1.18, and R_S=2.14, respectively). The optimum chromatographic conditions of 3,4,5-trismethoxymandelic acid were *n*-hexane-ethanol (80/20, v/v; 0.1% TFA) at 25 °C with a flow rate of 0.8 ml/min (R_S=3.70).

The results show that in the separation of the enantiomers of mandelic acid and their derivatives, isopropanol used as the mobile phase modifier because it showed higher resolution than ethanol. In addition, in the chromatograms of mandelic acid, the first effluent was determined as (*R*)-mandelic acid, and the following effluent as (*S*)-mandelic acid.

A specific and sensitive HPLC method was developed for the separation of the enantiomers of mandelic acid and their derivatives 2-chloromandelic

acid, 4-hydroxymandelic acid, 4-methoxymandelic acid, and 3,4,5-trismethoxymandelic acid.

Compliance with ethics guidelines

Jie ZHOU, Qian LIU, Guang-jun FU, and Zhen-zhong ZHANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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