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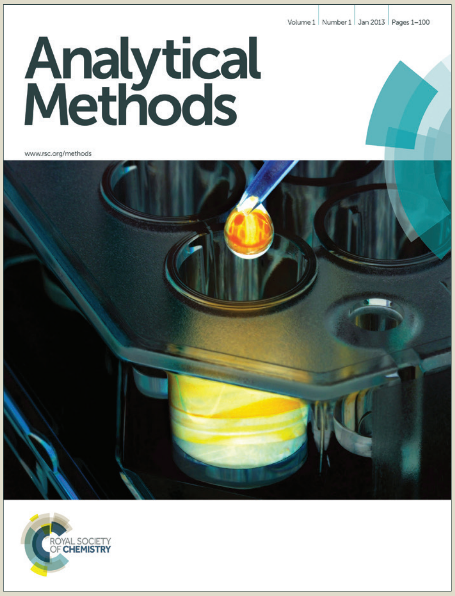

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Isoleucine Ionic Liquids as Additives to Separate Mandelic Acid and Their Derivatives Enantiomers by HPLC

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Abstract

Amino acid ionic liquids (AAILs) have been drawn great interest recent years in analytical chemistry due to their potentials in separation. In this study, functional isoleucine ionic liquid was synthesized and used as the novel chiral ligands coordinated with Cu (II) in chiral ligand exchange chromatogram. Enantiomers of mandelic acid and their derivatives were baseline separation. The influential factors, such as the content of methanol modifier, the concentration of isoleucine ionic liquid, the ratio of isoleucine ionic liquid and copper ion, flow rate and temperature on separation were investigated.

Keywords: Isoleucine ionic liquids; chiral separation; mandelic acid and their derivatives; HPLC

1. Introduction

Chiral ionic liquids (CILs) is a new type of chiral recognition agent. They have extraordinary potential in separation science [1] and asymmetric synthesis [2] owing to their unique properties and high-degree organization. Recently, CILs have been synthesized [3] and employed as chiral selectors in CE [4, 5], HPLC [6]. Since most of amino acids have chiral carbon atoms, they are highly promising candidates for CILs, and thus called amino acid ionic liquids (AAILs). AAILs have attracted more interest in chemosynthesis [7, 8] and separation [9] because of their many obvious advantages, including convenient synthesis, low cost, good biodegradability [10], reduced toxicity and high biocompatibility. Moreover, they have been applied as chiral selector in HPLC [11], CE [12, 13].

Mandlic acid and their derivatives are important chiral analogues which are widely used in the synthesis of varies drugs, such as penicillin, cephalosporin, antiobesity medicine [14]. Besides, they are also chiral selector that have been used for the separation of racemic compounds, like alcohols and amines [15, 16]. Different methods have been proposed for the separation of mandelic acid [17-20]. And among the different analytical methods, AAILs has become an environmentally friendly separation method. Yang [21] et al. separated the enantiomers of mandelic acid by using 1-butyl-3-methyl-imidazole-L-proline as chiral ligand.

Herein isoleucine ionic liquid was synthesized and applied as chiral ligand coordinated with Cu(II) ($CuSO_4 \cdot 5H_2O$) in a chiral ligand exchange high performance liquid chromatography system to separate mandelic acid, 4-methoxymandelic acid, 2-chloromandelic acid and 3, 4, 5-trimethoxymandelic acid (as shown in Fig.1).

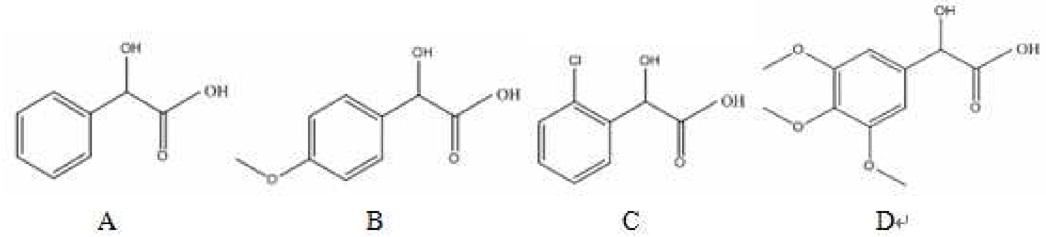


Fig. 1 The structure of mandelic acid and their derivatives

A: Mandelic acid, B: 4-methoxymandelic acid, C: 2-chloromandelic acid, D: 3,4,5-trimethoxy-mandelic acid.

2. Experimental

2.1 Chemicals

Isoleucine was purchased from Zhengzhou Chuangsheng Biological Engineering Company (Zhengzhou, China). Methanol, absolute ethyl alcohol, isopropanol, acetic ether and acetonitrile were obtained from T&J Kermel Reagent Company (Tianjin, China). N-methyl-imidazole and n-butyl-bromide were got from Sinopharm Chemistry Company (Beijing, China). $CuSO_4 \cdot 5H_2O$ was obtained from Kaitong Chemistry Reagent Company (Tianjin, China). mandelic acid, 2-chloromandelic acid, 4-methoxymandelic acid and 3, 4, 5-trismethoxymandelic acid were obtained from

New Drugs Research and Development Center of Zhengzhou University. N-methyl -imidazole and n-butyl-bromide were chemical grade. Isoleucine, acetic ether, isopropanol and absolute ethyl alcohol used in this work were analytical grade. All methanol used in HPLC was HPLC grade.

2.2 Apparatus

The HPLC instrument used in this study was an Agilent 1200 series apparatus (Palo Alto, CA, USA) 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 with a Kromasil C_{18} (250×4.6 mm, 5 μm) column.

2.3 Sample Preparation

Mandelic acid, 2-chloromandelic acid, 4-methoxymandelic acid and 3, 4, 5-trismethoxymandelic acid was dissolved in appropriate amount of methanol respectively. The solutions were all filtered (0.22 μm) to prepare sample solution.

2.4 Chromatographic Condition

The basic solvent of mobile phase was methanol. Mobile phase was filtered with 0.45 μm solvent filter and ultrasonically degassed. The detection wavelengths of mandelic acid and their derivatives were all set at 273 nm. The volume of sample injected was 10 μL.

2.5 Preparation and Characterization of Isoleucine Ionic Liquid

2.5.1 Prepration of 1-butyl-3-methyl-imidazole bromine ([Bmim]Br)

The mixture of N-Methylimidazole (9.9 mL), 1-bromobutane (16.8 mL) and isopropanol (10 mL) was refluxed for 72h at 70˚C. Then the solvent was removed by vacuumed distillation at 55˚C. After water (30 mL) was added, the organic layer was extracted by acetic ether (20 mL) for five times. Finally, The resultant solution was evaporated at 70˚C under vacuum to remove water and dried in vacuum for 72h at 60˚C.

2.5.2 Prepration of [Bmim]OH

[Bmim]Br and potassium hydroxide were all dissolved in absolute ethanol, the ratio of them was 1.0/1.0, the reaction last for 6h at 0˚C. The white precipitate KBr was removed by filtration. Then, the solvent was removed by vacuumed distillation and dried in vacuum to obtain [Bmim]OH.

2.5.3 Prepration and Purification of Isoleucine Ionic Liquid

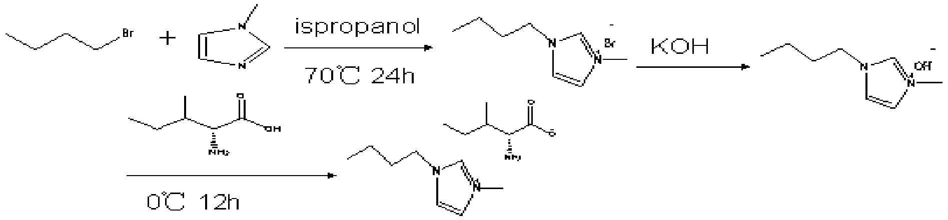
The neutralization reaction of [Bmim]OH (absolute ethanol used as the solvent) and L-leucine was used to prepare isoleucine ionic liquid. The reaction last for 12h at the low temperature.

The solvent of the resultant solution was removed by vacuumed distillation. Then an equal volume of methanol/acetonitrile (9/1, v/v) was added with stirring, the excess isoleucine was removed by filtration. Thereafter, the solvent was removed by vacuumed distillation and dried in vacuum at 60˚C to obtain viscous and purified isoleucine ionic liquid.

2.5.4 Characterization of Isoleucine Ionic Liquid

The chemical structures of isoleucine ionic liquid were characterized by ^{1}HNMR. ^{1}H NMR spectra of L-leucine ionic liquid was recorded on an AVANCE III 400-MHz spectrometer using $CDCl_3$ as the solvent. ^{1}HNMR ($CDCl_3$, 400HZ) δ: 0.95 (m, 6H, $-CH_3$); 1.35 (m, 2H, $-CH_2$); 1.85 (m, 2H, $-CH_2$); 4.07 (t, 2H, $N-CH_2$); 4.30 (s, 3H, $N-CH_3$);7.35 (d, 1H, C=CH); 7.43 (d, 1H, C=CH); 3.07 (d, 1H, N-CH); 2.10 (m, 1H, -CH);1.10 (m, 3H, $-CH_3$); 1.70 (m, 2H, $-CH_2$).

Scheme 1. Preparation of amino acid ionic liquids



3. Results and Discussions

3.1 Effect of Methanol Modifier

In this experiment, the ratio of isoleucine ionic liquid and copper ion was 2.5/1.0 (c/c), the flow rate was 0.7 mL /min at 25˚C, and the ratio of methanol and water on the enantioselectivity of mandelic acid and their derivatives was investigated. The results were shown in Table 1.

Table 1. Effect of methanol modifier on the enantioselectivity of the four compounds

Compound	t_{R1}(min)	t_{R2}(min)	R_S	CH_3OH/H_2O(v/v)
mandelic acid	4.5	4.6	0.21	60/40
	5.2	5.5	0.47	50/50
	6.1	6.7	0.98	40/60
	9.1	10.7	1.08	30/70
	10.0	12.2	1.26	20/80
4-methoxymandelic acid	4.4	-	-	60/40
	5.1	5.4	0.63	50/50
	6.0	6.6	0.87	40/60
	9.4	10.9	1.02	30/70
	11.3	13.3	1.15	20/80
2-chloromandelic acid	5.2	-	-	60/40
	6.8	7.0	0.19	50/50
	8.2	8.9	0.91	40/60
	13.9	16.9	0.87	30/70
	16.9	20.3	1.19	20/80
3,4,5-trimethoxymandelic acid	4.4	-	-	60/40
	4.5	-	-	50/50
	5.4	-	-	40/60
	8.7	-	-	30/70
	12.5	-	-	20/80

Chromatographic condition: the ratio of isoeucine ionic liquid and Cu (II): 2.5/1.0 (c/c); the content of isoleucine ionic liquid: 0.75 mmol/L; flow rate: 0.7 mL/min; temperature: 25˚C.

The results showed that with the decrease in the proportion of methanol, the retention time of the four compounds were all increased, and the resolution of mandelic, 4-methoxymandelic and 2-chloromandelic acid was increased whereas 3, 4, 5-trimethoxymandelic acid had no separation sign.

3.2 Effect of the Concentration of Isoleucine Ionic Liquid

The effect that the concentration of isoleucine ionic liquid on mandelic acid and their derivatives was studied. The concentration of isoleucine ionic liquid was in the range of 0.5~1.5 mmol/L, in which concentration, the complexes of isoleucine ionic liquid and Cu(II) could dissolve in the mobile phase completely. The results were shown in Table.2.

Table 2. Effect of the concentration of isoleucine ionic liquid on the enantioselectivity of mandelic acid and their derivatives

Compound	t_{R1}(min)	t_{R2}(min)	R_S	the content of L-IsoIL(mmol/L)
mandelic acid	10.0	12.0	1.26	0.5
	12.1	16.1	1.34	0.75
	14.1	18.2	1.51	1.0
	15.7	18.9	1.62	1.25
	17.1	22.1	1.92	1.5
4-methoxymandelic acid	11.3	13.3	1.15	0.5
	14.8	18.4	1.65	0.75
	14.9	18.7	1.83	1.0
	15.2	19.3	2.02	1.25
	16.4	20.6	2.16	1.5
2-chloromandelic acid	16.9	20.3	1.19	0.5
	21.2	25.4	1.23	0.75
	21.5	26.5	1.43	1.0
	22.1	27.1	1.59	1.25
	25.1	30.8	1.76	1.5
3,4,5-trimethoxymandelic acid	12.5	-	-	0.5
	13.8	14.6	0.39	0.75
	15.2	16.1	0.56	1.0
	17.5	18.4	0.83	1.25
	18.2	18.9	1.15	1.5

Chromatographic condition: CH_3OH-H_2O (20/80, v/v); the ratio of isoleucine ionic liquid and Cu(II): 2.5/1.0 (c/c); flow rate: 0.7 mL/min; temperature: 25˚C.

The results indicated that with the increase of the content of isoleucine ionic liquid, the retention time and the resolution were all increased. The phenomenon demonstrated that isoleucine ionic liquid had a certain capacity of separation.

3.3 Effect of the Ratio of Isoleucine Ionic Liquid and Copper Ion

The content of copper ion was changed to investigate the influence of the ratio of isoleucine ionic liquid and copper ion on enantioselectivity of mandelic acid and their derivatives. The separation data has been shown in Table 3.

Table 3 Effect of the ratio of isoleucine ionic liquid and copper ion on the enantioselectivity of mandelic acid and their derivatives

Compound	t_{R1}(min)	t_{R2}(min)	R_S	ratio of L-Isoil/Cu(II)(c/c)
mandelic acid	13.9	18.4	1.23	1.0/2.0
	13.6	18.8	1.25	1.0/1.0
	10.7	14.2	1.15	1.0/0.5
	6.6	7.13	0.86	1.0/0.25

4-methoxymandelic acid	14.2	18.1	1.12	1.0/2.0
	13.9	17.2	1.14	1.0/1.0
	9.0	11.9	1.05	1.0/0.5
	7.9	8.9	0.53	1.0/0.25
2-chloromandelic acid	19.5	22.1	1.14	1.0/2.0
	19.3	21.9	1.12	1.0/1.0
	14.5	16.2	1.10	1.0/0.5
	10.4	12.2	0.43	1.0/0.25
3,4,5-trimethoxymandelic acid	13.3	14.4	0.30	1.0/2.0
	13.5	14.5	0.32	1.0/1.0
	11.9	13.1	0.17	1.0/0.5
	9.3	-	-	1.0/0.25

Chromatographic condition: CH_3OH-H_2O (20/80,v/v); the content of isoleucine ionic liquid: 0.75 mmol/L; flow rate: 0.7 mL/min; temperature: 25°C.

The results showed that the optimum ratio of isoleucine ionic liquid and copper ion was in the range of 1.0/1.0~1.0/0.5 (c/c), which was suitable for the separation of mandelic acid and their derivatives**.**

3.4 Effect of Flow Rate

The effect of flow rate on the enantioselectivity of mandelic acid and their derivatives was investigated (as shown in Table 4).

Table 4 Effect of flow rate on the enantioselectivity of mandelic acid and their derivatives

Compound	t_{R1}(min)	t_{R2}(min)	R_S	Flow rate (mL/min)
mandelic acid	14.3	20.3	2.11	0.6
	10.9	13.8	1.27	0.8
	9.1	12.7	1.07	1.0
	7.7	9.5	0.55	1.2
4-methoxymandelic acid	15.3	18.5	2.10	0.6
	13.1	17.4	1.44	0.8
	10.1	14.3	1.44	1.0
	9.4	13.1	0.76	1.2
2-chloromandelic acid	23.9	32.3	1.43	0.6
	19.9	28.6	1.23	0.8
	14.4	19.5	1.43	1.0
	11.7	15.8	1.20	1.2
3,4,5-trimethoxymandelic acid	15.9	17.5	1.24	0.6
	12.9	15.6	1.20	0.8
	11.7	13.5	1.02	1.0
	8.7	9.7	1.04	1.2

Chromatographic condition: CH_3OH-H_2O (20/80, v/v); the content of isoleucine ionic liquid: 0.75 mmol/L; the ratio of isoleucine ionic liquid and Cu (II): 2.5/1.0 (c/c); temperature: 25˚C.

3.5 Effect of Temperature

The effect of column temperature on mandelic acid, 4-methoxymandelic acid, 2-chloramandelic acid and 3, 4, 5-trismethoxymandelic acid was studied in this part. The separation data was shown in Table 5.

Table 5 Effect of temperature on the enantioselectivity of mandelic acid and their derivatives

Compound	t_{R1}(min)	t_{R2}(min)	R_S	Temperature (˚C)
mandelic acid	10.2	15.1	0.78	15
	9.6	14.9	1.80	20
	9.1	12.7	1.07	25
	8.9	11.3	0.89	30
4-methoxymandelic acid	12.7	18.4	1.27	15
	9.9	12.5	1.33	20
	10.1	14.3	1.44	25
	10.2	13.5	1.11	30
2-chloromandelic acid	16.8	24.1	1.16	15
	14.9	19.3	1.35	20
	14.4	19.5	1.43	25
	14.1	18.5	0.90	30
3,4,5-trimethoxymandelic acid	12.8	14.6	0.78	15
	11.3	12.5	0.98	20
	11.7	13.5	1.02	25
	10.6	12.1	0.81	30

Chromatographic condition: CH_3OH-H_2O (20/80, v/v); the content of isoleucine ionic liquid: 1.25 mmol/L; the ratio of isoleucine ionic liquid and Cu (II): 2.5/1.0 (c/c); flow rate: 0.7 mL/min.

As the column temperature increased, a corresponding decrease in retention was observed, and the resolution increased first and then decreased. Usually, the faster the drug eluted from the column, the lower the resolution of the drug was. In this study, when the temperature increased, the resolution increased first, and if continue to rise the temperature, the resolution would decrease because of the elution capacity.

The results displayed that the temperature should be carefully controlled and 25˚C was closer to room temperature, so the other parameters were optimized at 25˚C.

The chromatogram of 4-methyloxymandelic acid was shown in Fig.2.

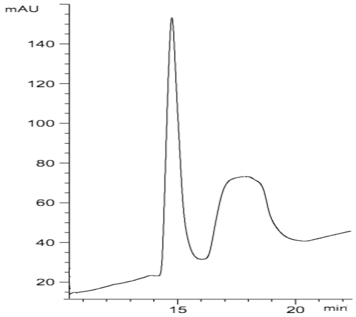


Fig.2. The chromatogram of 4-methoxymandelic acid

Chromatographic condition: CH_3OH-H_2O (20/80, v/v); content of isoleucine Ionic Liquid: 1.25 mmol/L; the ratio of isoleucine ionic liquid and Cu (II): 2.5/1.0(c/c); flow rate: 0.7 mL/min; temperature: 25˚C.

4. Conclusions

The enantiomers of mandelic acid and their derivatives (2-chloromandelic acid, 4-methoxymandelic acid and 3, 4, 5-trismethoxymandelic acid) were first separated with isoleucine ionic liquid and copper ion as chiral selector in HPLC. The optimum chromatographic conditions of four compounds as follows: the ratio of isoleucine ionic liquid and copper ion was in the range of 1.0/1.0~1.0/0.5 (c/c), the column temperature was at 25˚C, and the flow rate was 0.7 $mL·min^{-1}$.

A specific and sensitive HPLC method was developed for the separation of the enantiomers of mandelic acid, 2-chloromandelic acid, 4-methoxymandelic acid and 3, 4, 5-trismethoxymandelic acid.

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