Arch Pharm Res Vol 35, No 6, 1015-1019, 2012
DOI 10.1007/s12272-012-0609-6

Archives of Pharmacal Research
www.springer.com/12272

A Convenient Method for the Enantiomeric Separation of α-Amino Acid Esters as Benzophenone Imine Schiff Base Derivatives

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(Received January 28, 2012/Revised February 14, 2012/Accepted February 20, 2012)

A convenient liquid chromatographic method for the separation of α-amino acid esters as benzophenone Schiff base derivatives on coated chiral stationary phases (CSPs) (Chiralcel OD-H, Chiralcel OD, Chiralpak AD-H, Chiralpak AD, and Chiralpak AS) or covalently immobilized CSPs (Chiralpak IA, Chiralpak IB, and Chiralpak IC) derived from polysaccharide derivatives is described. Benzophenone imine derivatives of α-amino acid esters were readily prepared by stirring benzophenone imine and the hydrochloride salts of α-amino acid esters in 2-propanol. The chromatographic separations were conducted at a flow rate 1.0 mL/min and a detection wavelength of 254 nm; 0.5% 2-propanol/hexane (v/v) was used on CSPs. In general, the resolution of Chiralpak IC was superior to those of the other CSPs. In addition, the resolutions of other arylimine derivatives of α-amino acid esters and the effects of different mobile phases on the enantiomeric separation of α-amino acid esters as benzophenone imine derivatives on Chiralpak IC were investigated.

Key words: Chiral stationary phase, Benzophenone imine derivative, Enantiomer separation

INTRODUCTION

Several methods have been developed to determine the enantiomeric purities and/or configurations of α-amino acids in the pharmaceutical field (Subramanian, 2001; Francotte and Lindner, 2006). Of these techniques, liquid chromatographic enantiomer separation on chiral stationary phase (CSPs) has been known to be one of the most convenient and versatile methods. In a previous study, a liquid chromatographic method was described for the separation of enantiomers of α-amino acid esters and chiral amines as 9-anthraldimine Schiff base derivatives on polysaccharide-derived CSPs (Huang et al., 2011). Of the CSPs studied, Chiralcel OD (OD-H) provided greatest resolution of the enantiomers of several α-amino acid esters and chiral amines as 9-anthraldimine derivatives. Subsequently, we attempted to develop a convenient method for the derivatization of α-amino acid esters using aromatic Schiff base imines for enantiomer separation, and this work resulted in the selection of the benzophenone imine moiety used as an amine protecting group (O'Donnell and Polt, 1982; Greene and Wuts, 1999). The benzophenone imine group is expected to function as an aromatic auxiliary group for enantiomer resolution by the chiral selectors of CSPs (Duchateau et al., 1994). Benzophenone Schiff base derivatives have been previously used for the enantioselective synthesis of α-amino acid esters by phase-transfer alkylation (O'Donnell and Polt, 1982). Especially, the benzophenone imine derivatives of α-amino acid esters are readily prepared by simply stirring benzophenone imine and the hydrochloride salts of α-amino acid esters in 2-propanol at room temperature (Fig. 1) (O'Donnell and Polt, 1982; Greene and Wuts, 1999). In this study, we describe a convenient chromatographic separation of the enantiomers of α-amino

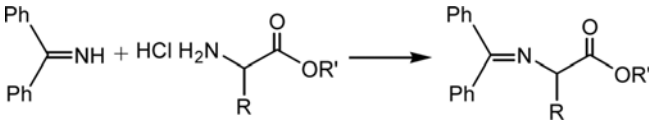

Fig. 1. Preparation of the benzophenone imine derivatives of α-amino acid esters.

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acid esters as their benzophenone imine derivatives on coated and on covalently bonded polysaccharide-derived CSPs.

MATERIALS AND METHODS

Instruments and reagents

Chromatography was performed at room temperature using an HPLC Breeze system (Waters) equipped with a Waters model 1525 binary pump, an autosampler, and a dual absorbance detector (Waters 2487 detector). HPLC-grade hexane and 2-propanol were obtained from J. T. Baker. Benzophenone imine and all α-amino acid esters were obtained from Aldrich or Sigma or Advanced ChemTech. The racemic and D- or L-analytes used were prepared, as shown in Fig. 1, by stirring 0.5 mmol of α-amino acid ester hydrochloride and an equimolar amount of benzophenone imine in 10 mL of 2-propanol at room temperature for 12 h. Reaction mixtures were filtered to remove ammonium chloride and resulting solutions were directly injected into the chromatograph.

Chromatographic conditions

All polysaccharide-derived CSPs, that is, Chiralcel OD, Chiralpak AD and Chiralpak AS (250 mm L × 4.6 mm I.D., 10 μm), and Chiralcel OD-H, Chiralpak AD-H, Chiralpak IA, Chiralpak IB, and Chiralpak IC (250 mm L × 4.6 mm I.D., 5 μm) were purchased from the Daicel Chemical Company. Chromatography was performed using a flow rate 1 mL/min, a detection wavelength of UV 254 nm, and 0.5% 2-propanol/hexane (v/v) as mobile phase for all CSPs. In addition, other solvents, such as, tetrahydrofuran, ethyl acetate, and dichloromethane in hexane were added when the covalently immobilized CSP, Chiralpak IC was examined.

RESULTS AND DISCUSSION

Enantiomeric separation of α-amino acid esters as benzophenone imine Schiff base derivatives

Tables I and II summarize chromatographic data for the separation of the enantiomers of several α-amino acid esters as benzophenone imine Schiff base derivatives on coated polysaccharide-derived CSPs (Chiralcel OD-H, Chiralcel OD, Chiralpak AD-H, Chiralpak AD and Chiralpak AS) and on covalently bonded polysaccharide-derived CSPs (Chiralpak IA, Chiralpak IB, and Chiralpak IC) using 0.5% 2-propanol/hexane (v/v) as mobile phase. Of the commonly used coated type CSPs (Chiralcel

Table I. Separation of the enantiomers of α-amino acid methyl esters as benzophenone imine derivatives on Chiralcel OD-H, Chiralcel OD, Chiralpak AD-H, Chiralpak AD, and Chiralpak AS

Analyte	Chiralcel OD-H				Chiralcel OD				Chiralpak AD-H				Chiralpak AD				Chiralpak AS			
	α^a	k'^b_1	Rs[c]	Conf.[d]	α^a	k'^b_1	Rs[c]	Conf.[d]	α^a	k'^b_1	Rs[c]	Conf.[d]	α^a	k'^b_1	Rs[c]	Conf.[d]	α^a	k'^b_1	Rs[c]	Conf.[d]
alanine	1.07	2.45	0.87	D	1.00	2.98	-		1.12	2.33	1.08	D	1.00	1.53	-		1.00	2.69	-	
asparagine	1.00	16.38	-		1.00	14.50	-		1.00	9.59	-		1.00	6.83	-		1.00	4.71	-	
aspartic acid	1.47	11.31	6.40	L	1.37	10.60	3.61	L	1.31	8.30	5.78	D	2.27	3.05	5.24	D	1.00	5.50	-	
leucine	1.00	1.42	-		1.00	1.43	-		1.00	1.32	-		1.00	1.16	-		1.87	0.78	0.96	D
phenylalanine	3.79	3.32	19.29	L	3.65	3.96	9.70	L	1.23	3.16	1.25	D	1.00	2.25	-		1.83	2.14	1.63	D
phenylglycine	1.38	3.24	4.78	L	1.42	3.09	2.77	L	1.35	4.71	2.23	D	1.16	3.27	0.91	D	1.39	2.79	1.25	D
valine	1.00	1.25	-		1.00	1.18	-		1.00	1.51	-		1.00	1.14	-		1.79	0.75	0.97	D

Mobile phase: 0.5% 2-propanol/hexane (v/v); Flow rate = 1.0 mL/min; Detection UV 254 nm. [a]Separation factor; [b]Capacity factor for the first enantiomer eluted; [c]Resolution factor; [d]Absolute configuration of the second enantiomer eluted.

Table II. Separation of the enantiomers of α-amino acid methyl esters as benzophenone imine derivatives on Chiralpak IA, Chiralpak IB, and Chiralpak IC

Analyte	Chiralpak IA				Chiralpak IB				Chiralpak IC			
	α^a	k'^b_1	Rs[c]	Conf.[d]	α^a	k'^b_1	Rs[c]	Conf.[d]	α^a	k'^b_1	Rs[c]	Conf.[d]
alanine	1.00	1.36	-		1.00	1.26	-		1.48	4.92	8.77	L
asparagine	1.16	5.95	3.01	D	1.55	4.40	6.19	L	1.00	5.87	-	
aspartic acid	1.12	4.99	2.20	D	1.49	4.23	5.70	L	1.00	5.88	-	
leucine	1.05	1.30	1.08	D	1.00	0.61	-		1.58	2.26	7.03	L
phenylalanine	1.00	2.05	-		2.62	1.58	8.39	L	1.12	8.48	2.08	L
phenylglycine	1.23	3.32	2.16	D	1.17	1.63	2.04	L	1.75	5.50	8.46	D
valine	1.06	1.14	0.17	L	1.00	0.56	-		1.98	2.41	12.94	L

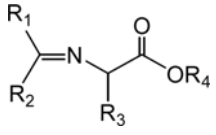
Mobile phase: 0.5% 2-propanol/hexane (v/v); Flow rate = 1.0 mL/min; Detection UV 254 nm. [a]Separation factor; [b]Capacity factor for the first enantiomer eluted; [c]Resolution factor; [d]Absolute configuration of the second enantiomer eluted.

OD-H, Chiralcel OD, Chiralpak AD-H, Chiralpak AD, and Chiralpak AS) listed in Table I, Chiralcel OD-H showed greatest enantioselectivity, and Chiralpak AD the least. Also, it is natural that the separation factors and resolution factors on Chiralcel OD-H and Chiralpak AD-H with the silica particle size 5 μm showed higher than those on Chiralcel OD and Chiralpak AD with the silica particle size 10 μm, respectively. Of the CSPs examined (Tables I and II), Chiralpak IC provided best enantioseparation, although it did not resolve the benzophenone imine derivatives of two polar analytes (asparagine and aspartic acid). It was interesting to find that the performances of Chiralpak IB and Chiralpak IC were complementary, for whereas Chiralpak IC failed to separate these two polar analytes, they were well separated by Chiralpak IB. Orders of enantioselectivity were OD-H > AD-H > AS~OD > AD for the coated CSPs and IC > IB~IA for the covalently bonded CSPs. In a previous study on the enantiomer separation of 9-anthraldehyde Schiff base derivatives of α-amino acid esters, it was found that among the CSPs investigated, Chiralcel OD (or OD-H) had greatest enantioselectivity, and Chiralpak IC least (Huang et al., 2011). The degree of enantiomer separation of benzophenone imine derivatives on Chiralpak IC in this study was lower than that of their 9-anthraldimine derivatives on Chiralcel OD (or OD-H) (Huang et al., 2011). However, the derivatization process required to produce benzophenone imine derivatives of amino acid esters is much more straightforward. Table III contains the chromatographic data of different aromatic imine derivatives of several α-amino acid esters on Chiralpak IC. Benzophenone imine derivatives were better enantioseparated than benzaldimine, 1-naphthaldimine, and 9-anthraldimine derivatives. Also, as the analyte is sterically hindered, the enantioselectivity increases (entries 1-3 and 8-10). The resolution of benzophenone imine derivatives of amino acid methyl esters was greater than that of the corresponding amino acid ethyl esters. In addition, the elution orders of all benzaldimine and 1-naphthaldimine derivatives were consistent with those of the corresponding benzophenone imine derivatives. However, the observed reversal of the elution orders of π-basic 9-anthraldimine derivative implies the different chiral recognition processes (entries 7 and 14).

Effects of mobile phase on the separation of α-amino acid esters as benzophenone imine derivatives and the application to measure the enantiomeric purity

In normal phase, coated type CSPs are not compatible with all solvents and for example, the use of ethyl acetate, tetrahydrofuran, and halogenated solvents as mobile phases or solvents for analytes is prohibited (Jin et al., 2006, 2009; Thunberg et al., 2008; Zhang et al., 2008). On the other hand, the solvent versatility of covalently bonded Chiralpak IC for the separation of

Table III. Separation of the enantiomers of α-amino acid esters as arylimine derivatives on Chiralpak IC



Entry	R_1	R_2	R_3	R_4	α[a]	k'_1[b]	Rs[c]	Conf.[d]
1	Ph	Ph	Me	Me	1.32	3.03	3.67	L
2	Ph	Ph	i-Bu	Me	1.40	1.55	4.18	L
3	Ph	Ph	i-Pr	Me	1.47	1.56	4.80	L
4	Ph	H	i-Pr	Me	1.37	2.61	6.12	L
5	4-MeO-Ph	H	i-Pr	Me	1.17	5.40	3.32	L
6	1-Naphthyl	H	i-Pr	Me	1.10	3.28	1.51	L
7	9-Anthryl	H	i-Pr	Me	1.23	4.07	2.89	D
8	Ph	Ph	Me	Et	1.29	3.01	1.75	L
9	Ph	Ph	i-Bu	Et	1.38	1.45	2.13	L
10	Ph	Ph	i-Pr	Et	1.43	1.52	4.20	L
11	Ph	H	i-Pr	Et	1.29	2.25	3.45	L
12	4-MeO-Ph	H	i-Pr	Et	1.15	5.34	1.81	L
13	1-Naphthyl	H	i-Pr	Et	1.05	3.05	0.44	L
14	9-Anthryl	H	i-Pr	Et	1.22	3.40	3.09	D

Mobile phase = 1% 2-propanol/hexane (v/v); Flow rate = 1.0 mL/min; Detection UV 254 nm. [a]Separation factor; [b]Capacity factor for the first enantiomer eluted; [c]Resolution factor; [d]Absolute configuration of the second enantiomer eluted.

Table IV. Effect of mobile phase on the enantiomeric separation of the benzophenone imine derivatives of α-amino acid methyl esters on Chiralpak IC

Mobile phase	0.5% 2-propanol/hexane				1% tetrahydrofuran/ hexane				2% ethyl acetate/hexane				30% dichloromethane/ hexane			
Analyte	α[a]	k'_1[b]	Rs[c]	Conf.[d]	α[a]	k'_1[b]	Rs[c]	Conf.[d]	α[a]	k'_1[b]	Rs[c]	Conf.[d]	α[a]	k'_1[b]	Rs[c]	Conf.[d]
alanine	1.48	4.92	8.77	L	1.40	4.60	2.73	L	1.27	4.70	4.89	L	1.25	2.17	2.68	L
asparagine	1.00	5.87	-		1.00	8.52	-		1.00	5.60	-		1.00	2.39	-	
aspartic acid	1.00	5.88	-		1.00	8.76	-		1.00	5.63	-		1.00	2.41	-	
leucine	1.58	2.26	7.03	L	1.50	2.42	5.92	L	1.44	3.11	2.93	L	1.33	1.58	2.91	L
phenylalanine	1.12	8.48	2.08	L	1.11	16.25	1.33	L	1.10	10.12	0.88	L	1.10	3.51	1.20	D
phenylglycine	1.75	5.50	8.46	D	1.64	6.64	5.01	D	1.51	7.35	3.71	D	1.04	2.14	0.47	D
valine	1.98	2.41	12.94	L	1.68	2.56	7.36	L	1.51	3.32	6.10	L	1.19	1.60	3.07	L

Chromatographic conditions; Flow rate = 1.0 mL/min; Detection UV 254 nm. [a]Separation factor; [b]Capacity factor for the first enantiomer eluted; [c]Resolution factor; [d]Absolute configuration of the second enantiomer eluted.

α-amino acid methyl esters as benzophenone imine derivatives was examined (Table IV). Enantioselectivities and resolutions were found to be greatly influenced by the nature of the mobile phase (Jin et al., 2006, 2009). Of the mobile phases investigated, 0.5% 2-propanol/hexane (v/v) best resolved the benzophenone imine derivatives of α-amino acid methyl esters, whereas the resolution provided by 30% dichloromethane/hexane (v/v) was poorest. Also, optical purity results of the stability test for the benzophenone imine derivative of L-aspartic acid dimethyl ester (Aldrich) stored at 4°C after derivatization with benzophenone imine in 2-propanol are shown in Table V. After 30 days of storage at 4°C, its optical purity was almost unaffected. Furthermore, stabilities of benzophenone imine derivatives of amino acid esters were found to be considerably more stable than those of the corresponding 9-anthraldehyde derivatives (Huang et al., 2011). We also used our developed chromatographic methods to determine the enantiomeric purities of several commercially available D- and L-amino acid methyl esters. As shown in Table VI, enantiomeric impurity levels of <0.01-0.56% were found after benzophenone imine derivatization. Typical chromatograms of the benzophenone imine derivative of commercially available L-phenylglycine methyl ester on Chiralpak IC are presented in Fig. 2.

Table V. Optical purity results of the stability test for the benzophenone imine derivative of L-aspartic acid dimethyl ester (Aldrich) stored at 4°C

Storage period	L:D ratio[a]	RSD[b]
1 Day	99.91:0.09	1.2%
3 Days	99.91:0.09	0.9%
5 Days	99.89:0.11	3.1%
8 Days	99.89:0.11	2.1%
9 Days	99.88:0.12	1.0%
15 Days	99.85:0.15	0.9%
16 Days	99.84:0.16:	1.0%
22 Days	99.82:0.18	3.1%
30 Days	99.81:0.19	3.0%

[a]Averages of three determinations; [b]Relative standard deviation.

In conclusion, a convenient liquid chromatographic method was developed for the resolution α-amino acid esters as their benzophenone imine Schiff base deriva-

Table VI. Determination of the enantiomeric purities of the benzophenone imine derivatives of commercially available α-amino acid methyl esters

Entry	Analyte	Company	D:L ratio[a]	RSD[b]
1	D-alanine methyl ester	Sigma	99.99:0.01	1.0%
2	L-alanine methyl ester	Aldrich	< 0.01: > 99.99	0.9%
3	L-aspartic acid dimethyl ester	Aldrich	0.09:99.91	2.0%
4	L-leucine methyl ester	Aldrich	0.05:99.95	0.8%
5	D-phenylalanine methyl ester	Advanced ChemTech	99.99:0.01	1.0%
6	L-phenylalanine methyl ester	Advanced ChemTech	0.02:99.98	0.8%
7	D-phenylglycine methyl ester	Aldrich	99.44:0.56	3.6%
8	L-phenylglycine methyl ester	Aldrich	0.11:99.89	2.4%

Refer to the experimental section for chromatographic conditions. [a]Average value of three determinations; [b]Relative standard deviation.

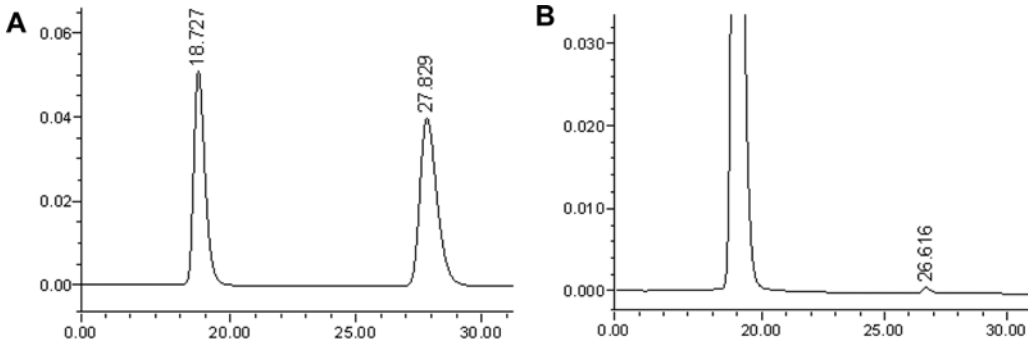


Fig. 2. Chromatograms of the enantiomeric resolution of the benzophenone imine derivative of racemic phenylglycine methyl ester (**A**) and L-phenylglycine methyl ester (Aldrich reagent) (**B**) (D:L = 0.11:99.89) on Chiralpak IC. Mobile phase: 1% 2-propanol/hexane(v/v); flow rate = 1.0 mL/min; detection wavelength: UV 254 nm; injection amount: 5 μg.

tives using several coated and covalently bonded polysaccharide-derived CSPs. Of the CSPs studied, in general, the covalently immobilized CSP, Chiralpak IC, provided excellent resolution of the benzophenone imine derivatives of α-amino acid esters, and greater solvent choice. The developed methods were also used to measure the enantiomeric purities of several commercially available α-amino acid methyl esters. It is expected that the described liquid chromatographic method will provide a useful means of resolving α-amino acid esters.

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