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Liquid chromatographic enantioseparation of several amino acids as nitrobenzoxadiazole derivatives on polysaccharide trisphenylcarbamate derived chiral stationary phases

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Abstract: Considering the greater role of α-amino acids in our daily lives, the enantiomer resolution of seven α-amino acids derivatized with fluorogenic reagent (4-fluoro-7-nitro-2,1,3-benzoxadiazole, NBD-F) by chiral HPLC on amylose or cellulose trisphenylcarbamate derived chiral stationary phases (CSPs) under simultaneous ultraviolet (UV) and fluorescence (FL) detection was performed. The degree of enantioseparation and resolution was affected by nature and selector backbones of the CSPs as well as the kind of amino acids. Baseline enantiomer separation and resolutions were observed for the enantiomers of all analytes as NBD derivatives especially on coated type amylose tris(3,5-dimethylphenylcarbamate) derived CSPs (Chiralpak AD-H and Lux Amylose-1). The other CSPs also showed good enantioselectivity except for the CSPs (Chiralpak IB, Chiralcel OD-H and Lux Cellulose-1) having cellulose tris(3,5-dimethylphenylcarbamate) as chiral selectors. The developed analytical chiral method was applied to determine the enantiomeric purity of seven commercially available L-α-amino acids and the impurities as D-forms were found to be in the range 0.08-0.87 %, respectively. The intra- and interday accuracy and precision assays showed high accuracy and precision of the developed analytical method. This chiral HPLC method for the enantiomer resolution of amino acids using fluorescent derivatization could be useful for the determination of enantiomeric purity of pharmaceuticals and biological study for amino acid type compounds among chiral drugs.

Key words: α-amino acids, chiral stationary phase, enantiomer separation, fluorescence, nitrobenzoxadiazole derivative

1. Introduction

The distinct interactions of each enantiomeric forms of a chiral compound in the stereoselective chiral environment of living systems have led a strong demand in the life and pharmaceutical sciences to develop and optimize the selective analytical and preparative separation techniques for the discrimination and purification of the individual enantiomers.[1,2] Chiral amino acids, the chiral building blocks of peptides and proteins, are the vital organic compounds needed for the optimal metabolism and proper functioning

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of the body.[3,4] They act as important chiral precursors for the formation of many important biological and physiological molecules which are used in various fields ranging from pharmaceutical, biochemistry, agrochemicals, as well as food chemistry.[4,5] Owing to the growing role and functions of stereoisomeric configuration of amino acids, the separation and resolution of amino acid enantiomers with a highly sensitive and selective chiral detection method has immense interest in the field of biomedical and pharmaceutical sciences.[6,7] Also, the determination of enantiomeric composition is essential for the preparation of enantiomerically pure analogs and diagnosis or treatment.[7] Among a wide range of analytical techniques, normal phase chiral high-performance liquid chromatography (HPLC) using chiral stationary phases (CSPs) has been found to be the most reliable, selective and widely used technique for the stereoselective enantiomer separation and analysis of chiral compounds.[8-10] Polysaccharide derived CSPs, especially trisphenylcarbamates derivatives of cellulose and amylose, have demonstrated higher enantioselective abilities, sensitivities and reproducibilities to make them most successful and widely applicable CSPs for the analytical and preparative scale enantiomeric resolution of structurally diverse chiral compounds and have been commercialized mostly under trade names Chiralpak, Chiralcel, and Lux.[9-13] However, as reported, specific groups of chiral analytes such as small nonaromatic carboxylic acids and native amino acids have exhibited inadequate chiral recognition ability towards these chiral selectors.[14] So, the derivatization of amino acids with strong chromophores and/or fluorophores has become a popular strategy in the field of chirotechnology to improve the detection sensitivity and enantioselectivity (improved peak shape and additional intermolecular interactions).[10,14-16] Common derivatizing reagents such as ninhydrin, o-phthalaldehyde (OPA), fluorenylmethyloxycarbonylchloride (FMOC-Cl), and 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) have been employed for the fluorescent derivatization of amino acids.[14,15,17,18] Among them, NBD-F is a highly effective and sensitive achiral derivatization reagent which readily reacts with amino acids to produce a relatively stable and highly fluorescent NBD-product.[15] Previous reports of the resolution of amino acids have been performed on the reversed phase mode employing monolithic ODS, Kromasil SIL, crown ether, macrocyclic glycopeptides and Pirkle type columns especially by LC-MS/MS or CE.[14,19,20] Therefore, in this present study, we aim to develop a simple, convenient and highly sensitive normal chiral HPLC for the enantiomeric resolution of seven α-amino acid analytes as NBD derivatives on ten amylose or cellulose trisphenylcarbamate derived CSPs under simultaneous ultraviolet (UV) and fluorescence (FL) detection. Also, the evaluation in terms of separation and recognition ability of these ten amylose and cellulose derived CSPs with coated or covalently immobilized chiral selectors during the enantiomer separation process of NBD-labeled α-amino acids is described.

2. Experimental

2.1. Enantioselective chromatography

All enantioselective separations and detection of seven α-amino acids were performed on an Agilent 1100 HPLC system (Palo Alto, CA, USA). The chromatographic apparatus consisted of a G1322A solvent vacuum degasser, a G1310A isocratic pump, an autosampler, a thermostatic column compartment, a multiwavelength G1315A simultaneous UV and a HP1046A programmed FL detector. The signal was acquired and processed by the Hewlett-Packard (HP) ChemStation software. Among the ten amylose or cellulose trisphenylcarbamates derived columns, the six covalently bonded Chiralpak IA [amylose tris(3,5-dimethylphenylcarbamate)], Chiralpak IB [cellulose tris(3,5-dimethylphenylcarbamate)], Chiralpak IC [cellulose tris(3,5-dichlorophenylcarbamate)], Chiralpak ID [amylose tris(3-chlorophenylcarbamate)], Chiralpak IE [amylose tris(3,5-dichlorophenylcarbamate)], and Chiralpak IF [amylose tris(3-chloro-4-methylphenylcarbamate)] (250 mm × 4.6 mm, I.D., 5 μm) were obtained from Daicel Company (Tokyo, Japan). The four coated type Chiralcel OD-H [cellulose tris(3,5-dimethylphenylcarbamate)] and Chiralpak AD-H [amylose tris(3,5-dimethylphenylcarbamate)]; Lux

Cellulose-1 [cellulose tris(3,5-dimethylphenylcarbamate)] and Lux Amylose-1 [amylose tris(3,5-dimethylphenylcarbamate)] (250 mm × 4.6 mm, I.D., 5 μm) columns were purchased from Daicel Company (Tokyo, Japan) or Phenomenex (Torrance, CA, USA).

2.2. Chemicals and reagents

4-Fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), a sensitive achiral derivatization reagent which reacts well with amino acids to produce relatively stable highly fluorescent NBD-product, was purchased from Tokyo Chemical Industry Co. Ltd. (Chuo-ku, Tokyo, Japan). All seven racemic and enantiomerically pure α-amino acid analytes used in the study were obtained from Junsei Chemical Co. Ltd. (Chuo-ku, Tokyo, Japan) or Katayama Chemical Industries Co. Ltd. (Chuo ward, Osaka, Japan) or Acros organics (Fair lawn, NJ, USA) or Sigma-Aldrich (St. Louis, MO, USA). HPLC grades of hexane, 2-propanol, trifluoroacetic acid (TFA) and acetonitrile for the mobile phase and sample preparation was procured from Burdick & Jackson (Morristown, NJ, USA) or Acros organics (Fair lawn, NJ, USA). Sodium tetraborate decahydrate, ethylenediaminetetraacetic acid disodium salt dehydrate (EDTA) and boric acid used for preparing buffer solution was obtained from Daejung Chemicals (Siheung, South Korea). Deionized purified water was generated using Milli-Q system from Merck Millipore (Burlington, MA, USA).

2.3. Derivatization of amino acids with NBD-F for sample preparation

Derivatization of α-amino acids was performed according to the modified derivatized procedure[21,22] by dissolving them on 50mM borate buffer (pH 8.0) with 20mM EDTA. After that, 300 μL of the sample solution was taken and mixed with 100 μL of 100 mM NBD-F in acetonitrile in a reaction vial. The vial was heated at 60 °C for 1 min and cooled it on an ice bath and then 40 μL of 50 mM HCl aqueous solution was added to the reaction mixture. This resultant sample was further diluted to a proper concentration for the injection in normal HPLC to determine NBD-labeled compounds.

2.4. Chromatographic conditions and analytical parameters

Separation of the enantiomers of α-amino acids as NBD derivatives using chiral HPLC was performed at room temperature (25 °C) with an injection volume of 1 μL and flow rate of 1.0 mL/min. Each sample was injected twice to ensure reproducibility and precision of the results. The optimized isocratic mobile phase used for the separation procedure was 10-20 % 2-propanol/hexane (V/V) with 0.1 % TFA. A simultaneous detection of UV 337 nm and FL (excitation 470 nm and emission 530 nm) were employed for the enantiomeric separation. The performance of the chromatographic system was evaluated in terms of the chromatographic parameters of retention factor (k), separation factor (α), and resolution (Rs). The retention factor k was calculated from $(t_R-t_0)/t_0$ where t_R and t_0 are the retention time of the analyte and hold up time, respectively. The separation factor α was calculated from k'_2/k'_1 whereas k_1 and k_2 are the retention factors of the both eluted enantiomers. The resolution factor Rs of the analytes was calculated using the equation: $2(t_2-t_1)/(W_1+W_2)$, where t_1, W_1, t_2, and W_2 are the retention times and respective baseline peak widths of the first and second eluting isomers, respectively.

3. Results and Discussion

Tables 1 and 2 show the chromatographic performances of each CSP for the enantiomeric separation of seven α-amino acids as NBD derivatives using normal phase HPLC under simultaneous UV and FL detection. Commercially available six covalently bonded and four coated type polysaccharide derived CSPs containing amylose or cellulose trisphenylcarbamates as chiral selectors were employed for the entire separation process. Several conditions such as pH, temperature, isocratic mobile phase and flow rate which influence the derivatization and enantiomer separation process were optimized to obtain a suitable new method protocol for the higher enantioselectivity and detection sensitivity. Most of the analytes under consideration demonstrated high baseline resolution

Table 1. Enantiomeric separation of several α-amino acids as NBD derivatives on six covalently bonded amylose and cellulose trisphenylcarbamates derived CSPs

Entry	Analytes	Chiralpak IA			Chiralpak IB			Chiralpak IC		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	2-Aminobutyric acid	1.66	3.06	6.68(L)[a]	1.00	2.70	-	1.05	17.10[b]	1.05(D)
2	Alanine	1.50	3.86	3.98(L)	1.00	3.75	-	1.08	6.66	1.24(L)
3	Leucine	1.43	3.71	5.30(L)	1.03	1.74	0.31(D)	1.00	4.49	-
4	Methionine	2.15	5.01	10.71(L)	1.00	3.97	-	1.12	8.06	1.72(D)
5	Phenylalanine	2.69	3.68	13.54(L)	1.04	3.73	0.42(D)	1.09	6.47	1.16(D)
6	Serine	1.13	10.15	0.78(L)	1.00	8.05	-	1.13	5.37	1.47(D)
7	Valine	1.33	3.30	4.00(L)	1.06	1.95	0.60(D)	1.00	5.58	-
Entry	Analytes	Chiralpak ID			Chiralpak IE			Chiralpak IF		
		α	k'_1	R_s	α	k'_1	R_s	α	k'_1	R_s
1	2-Aminobutyric acid	1.00	2.92	-	1.09	7.42	0.81(L)	1.13	3.18	1.40(D)
2	Alanine	1.09	3.70	1.24(D)	1.72	6.46	4.88(D)	1.18	4.39	1.55(D)
3	Leucine	1.03	1.94	0.51(L)	1.21	3.28	1.78(D)	1.00	2.14	-
4	Methionine	1.06	4.39	0.73(D)	1.36	6.82	2.78(D)	1.44	4.74	3.72(D)
5	Phenylalanine	1.00	3.55	-	1.45	6.01	3.09(D)	1.14	3.38	1.42(L)
6	Serine	2.30	4.82	6.99(D)	1.74	8.49	3.29(D)	2.15	5.64	3.43(D)
7	Valine	1.05	2.31	0.70(L)	1.14	4.95	1.25(D)	1.04	2.51	0.41(D)

Mobile phase: 20 % 2-propanol/hexane (V/V) with 0.1 % TFA, Flow rate: 1 mL/min, Detection: UV 310 nm, fluorescence 470 nm excitation, 530 nm emission, k'_1: retention factor of the first eluted enantiomer, α: separation factor, Rs: resolution factor, [a]the absolute configuration of the second eluted enantiomer, [b]10 % 2-propanol/hexane (V/V).

Table 2. Enantiomeric separation of several α-amino acids as NBD derivatives on four coated type amylose and cellulose trisphenylcarbamates derived CSPs

Entry	Analytes	Chiralcel OD-H			Lux Cellulose-1		
		α	k'_1	R_s	α	k'_1	R_s
1	2-Aminobutyric acid	1.08	3.31	0.59(D)[a]	1.06	3.90	0.76(D)
2	Alanine	1.04	5.41	0.36(D)	1.00	4.92	-
3	Leucine	1.00	2.22	-	1.00	2.41	-
4	Methionine	1.13	4.80	0.94(D)	1.05	5.39	0.62(D)
5	Phenylalanine	1.28	5.43	1.96(D)	1.16	5.92	1.76(D)
6	Serine	1.12	7.15	0.47(D)	1.09	7.11	1.04(D)
7	Valine	1.00	2.81	-	1.03	5.50	0.63(D)
Entry	Analytes	Chiralpak AD-H			Lux Amylose-1		
		α	k'_1	R_s	α	k'_1	R_s
1	2-Aminobutyric acid	2.10	3.34	9.87(L)	2.03	3.53	11.17(L)
2	Alanine	1.99	3.41	8.73(L)	1.92	3.92	10.67(L)
3	Leucine	1.54	4.47	5.72(L)	1.51	4.78	5.98(L)
4	Methionine	2.47	5.65	11.48(L)	2.43	6.30	12.54(L)
5	Phenylalanine	3.56	4.05	15.07(L)	3.23	4.50	15.74(L)
6	Serine	1.90	6.90	7.59(L)	1.79	8.25	8.54(L)
7	Valine	1.60	3.73	6.56(L)	1.54	4.22	6.93(L)

Mobile phase: 20 % 2-propanol/hexane (V/V) with 0.1 % TFA, Flow rate: 1 mL/min; Detection: UV 310 nm, fluorescence 470 nm excitation, 530 nm emission, k'_1: retention factor of the first eluted enantiomer, α: separation factor, Rs: resolution factor, [a]the absolute configuration of the second eluted enantiomer.

and symmetric peak shape. In general, among the used CSPs (*Table* 1 and 2), the best chiral recognition ability was shown by Chiralpak AD-H and Lux Amylose-1 (*Table* 2) on amylose tris (3,5-dimethylphenylcarbamate) as chiral selector by providing the complete separation and baseline resolutions of all studied seven α-amino acids as NBD derivatives. On the other hand, the enantiodiscrimination on cellulose tris (3,5-dimethylphenylcarbamate) derived CSPs (Chiralpak IB, Chiralcel OD-H and Lux Cellulose-1, *Table* 1 and 2) was comparatively low as only few analytes as NBD derivatives were partial or enantiomerically resolved. Especially, covalently bonded Chiralpak IB (*Table* 1) showed the worst enantiorecognition and separation of α-amino acids as NBD derivatives as most of studied the analytes were not resolved. The highest enantioseparation with enhanced retention and excellent reproducible resolution factors was shown by aromatic α-amino acid analyte (entry 5, phenylalanine) on amylose tris(3,5-dimethylphenylcarbamate) derived CSPs (α = 3.56 and 3.23, Rs = 15.07 and 15.74 on Chiralpak AD-H and Lux Amylose-1, *Table* 2). The aromatic moiety present in the phenylalanine might have impact on different chiral interaction with chiral selector of CSP and found to favor coated type Chiralpak AD-H or Lux Amylose-1 for the best separation and resolution.[10] When the enantiomeric resolution of alanine and serine derivatives is compared, the enantioselectivity of alanine derivatives was a bit superior than that of serine derivatives on Chiralpak IA, Chiralpak AD-H and Lux Amylose-1 CSPs with the same amylose tris (3,5-dimethylphenylcarbamate) chiral selector (*Table* 1 and 2). Oppositely, the enantioselectivity of serine derivatives on Chiralpak ID and Chiralpak IF in *Table* 1 was much greater than that of alanine derivatives. Compared to alanine derivatives, it is considered that the presence of hydroxyl group of serine derivatives might affect the chiral recognition mechanism positively or negatively. The enhanced enantioselectivities of serine derivatives on Chiralpak ID and Chiralpak IF in *Table* 1 might be related to favorable hydrogen bonding interaction by hydroxyl moiety of serine.[16,19] Additionally, among the six covalently bonded CSPs on *Table* 1 only, the enantioselectivities observed on Chiralpak IA [amylose tris(3,5-dimethylphenylcarbamate) selector] was superior and followed by the chlorine substituted amylose trisphenylcarbamates derived CSPs (Chiralpak IE and Chiralpak IF). Similar trend as Chiralpak AD-H and Lux Amylose-1 having amylose tris(3,5-dimethylphenylcarbamate) selector showed greater enantioselectivities than Chiralcel OD-H and Lux Cellulose-1 [cellulose tris(3,5-dimethylphenylcarbamate) selector] when compared between the four coated CSPs in *Table* 2. From the *Table* 1 and 2, we rationalized that the resolving powers were significantly affected by the nature and structural differences between amylose type cellulose based CSPs and also the position of electron donating or withdrawing substituents of phenylcarbamate moieties.[13,16,23,24] In our study, it appeared that the investigated α-amino acids analytes as NBD derivatives fit better to the amylose derived CSPs than to the cellulose derived CSPs.

Accordingly, from *Table* 1 and 2, the enantiomeric discrimination ability of CSPs having same chiral selector but of different nature were evaluated and compared. The performances in enantiomer separation of three CSPs, i.e. Chiralpak IA, Chiralpak AD-H, and Lux Amylose-1 with amylose tris(3,5-dimethylphenylcarbamate) as the same chiral selector were examined. All analytes were discriminated well with good separation and resolution factors in both types of CSPs, but in general the coated type Chiralpak AD-H and Lux Amylose-1 (*Table* 2) showed greater enantioselectivity and separation of seven α-amino acids as NBD derivatives than the covalently bonded Chiralpak IA (*Table* 1). The low potential of chiral recognition on immobilized polysaccharide CSPs is due to the modification of stereospecific conformation that can occur during the chemical bonding of chiral selectors with silica.[13,25] *Fig.* 1 shows the representative chromatograms of phenylalanine and serine as NBD derivatives on Chiralpak AD-H and Lux Amylose-1 under simultaneous UV and FL detection. The unreacted NBD-F peak was observed under UV 337 nm, but it did not appear at FL detection. It results in affording simple chromatograms under highly sensitive FL detection, which is an additional advantage of

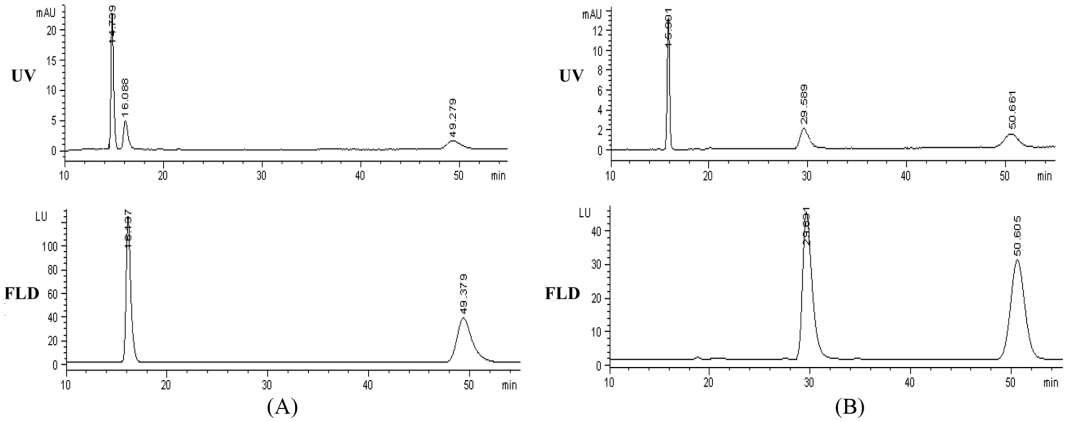

Fig. 1. Typical chromatograms for enantiomeric resolution of (A) phenylalanine and (B) serine as NBD derivatives on coated type Chiralpak AD-H and Lux Amylose-1, respectively under simultaneous UV and FL detection, Mobile phase: 20 % 2-propanol/hexane (V/V) with 0.1 % TFA, Flow Rate: 1 mL/min, Detection: UV 337 nm, FLD: excitation; 470 nm, emission; 530 nm, The unreacted NBD-F peak appeared around 15 min under UV 337 nm.

this developed analytical method. The basic polysaccharide backbone structure, amylose or cellulose, significantly influenced the elution order of enantiomers of NBD-α-amino acids.[24,26,27] Consistent trends in elution order patterns were observed for all seven α-amino acids as NBD derivatives on six CSPs having the same chiral selectors. The L-enantiomers were selectively retained on the amylose derived CSPs (Chiralpak IA, Chiralpak AD-H, and Lux Amylose-1) with amylose tris(3,5-dimethylphenylcarbamate) selector, whereas the elution order observed was inverted as D-enantiomers were secondly eluted for Chiralpak IB, Chiralcel OD-H and Lux Cellulose-1 with cellulose tris(3,5-dimethylphenylcarbamate). However, the elution orders were not always observed or consistent on four chlorine substituted amylose or cellulose trisphenylcarbamates derived CSPs (Chiralpak IC, ID, IE and IF). Unlike amylose or cellulose tris(3,5-dimethylphenylcarbamate), it can be assumed that the introduction of electron withdrawing chloro group in phenylcarbamate moiety might influence for the different chiral recognition mechanism and

Table 3. Intra- and interday accuracy and precision of the developed chiral HPLC method using three D- and L-phenylalanines as NBD derivatives on Chiralpak AD-H

Enantiomeric purity (%) of D-phenylalanine	Intraday (n=6)			Interday (n=6)		
	Observed purity (%)	Accuracy (%)	Precision (% RSD)	Observed purity (%)	Accuracy (%)	Precision (% RSD)
98.4	101.06	102.70	0.91	99.87	101.50	2.05
96.4	97.22	100.85	1.30	98.41	102.09	1.18
94.4	97.67	103.46	1.86	95.27	100.92	1.07

Enantiomeric purity (%) of L-phenylalanine	Intraday (n=6)			Interday (n=6)		
	Observed purity (%)	Accuracy (%)	Precision (% RSD)	Observed purity (%)	Accuracy (%)	Precision (% RSD)
98.4	100.56	102.20	1.70	101.15	102.80	1.21
96.4	97.70	101.35	0.97	96.10	99.69	1.64
94.4	94.37	99.97	0.49	94.92	100.55	2.13

See *Table 2* for the chromatographic conditions. RSD is the relative standard deviation.

results in different elution order.[13,24]

Intra- and interday assay for accuracy and precision were performed using D- and L-phenylalanine as NBD derivatives on Chiralpak AD-H using isocratic mobile phase of 20 % 2-propanol/hexane (V/V) with 0.1 % TFA, in accordance with the guidelines set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).[28] *Table* 3 shows the data for the estimation of accuracy and precision at three D- and L-phenylalanines (enantiomeric purities of 98.4, 96.4 and 94.4 %) as NBD derivatives under FL detection. The intra- and interday accuracy for D-phenylalanine was determined to be 100.85-103.46 and 100.92-102.09 %, respectively, while intra- and interday precision calculated in terms of relative standard deviation (% RSD) was 0.91-1.86 and 1.07-2.05 %, respectively. On the other hand, in case of L-phenylalanine, the intra- and interday accuracy was found to be 99.97-102.20 and 99.69-102.80 %, respectively, while intra- and interday precision was 0.49-1.70 and 1.21-2.13 %, respectively. The results of high accuracy and precision were within the acceptance criteria of the ICH guidelines and provided the evidence that the developed method is suitable and sensitive with acceptable reproducibility and accuracy to be applicable for enantiomer separation of α-amino acids as NBD derivatives and further purposes.[29] The accuracy of an analytical method is the closeness of agreement between the obtained value through the proposed calibration curve to the assumed or accepted true value and is determined as % relative recovery.[28] In this developed method, for the intra- and interday accuracy, enantiomeric purity at three levels, 98.4, 96.4 and 94.4 %, of D- and L-phenylalanine in the expected range was obtained by spiking the other enantiomer (D or L) and accuracy was determined as the ratio of found result to the expected or accepted value expressed as %. The obtained accuracy value of 100 % means that the found value is equal to accepted or true value. However, some of the accuracy in this study was observed above 100 %, which indicated that the found % relative recovery values in terms of purity were higher than the expected or accepted value of the spike. The accuracy of the developed method may have been overestimated due to the potential interference of enantiomeric impurity of the other enantiomer during the spiking to obtain the required purity, as well as the instability of the derivatized analyte. Enantiomeric purity analysis of amino acid is crucial during the preparation of pharmaceuticals and biochemicals.[7] This developed chiral HPLC method was applied for the determination of the enantiomeric purities of seven commercially available L-form amino acids from different suppliers as a test for the applicability of the proposed method. The averages of four measurements of enantiomeric impurities as D-forms of the seven analytes as NBD derivatives were found to be 0.08-0.87 % and shown in *Table* 4. The FL representative chromatograms for the determination of the enantiomeric purity of L-alanine and L-valine as NBD derivatives are shown in *Fig.* 2.

Table 4. Determination of enantiomeric purities of seven different commercially available L-form amino acids as NBD derivatives on Chiralpak AD-H

Entry	Analyte	Company	D : L ratio[a]	RSD (%)
1	2-Aminobutyric acid	Sigma-Aldrich	0.34 : 99.66	1.39
2	Alanine	Acros Organics	0.18 : 99.82	0.62
3	Leucine	Sigma-Aldrich	0.87 : 99.13	1.78
4	Methionine	Sigma-Aldrich	0.61 : 99.39	1.41
5	Phenylalanine	Acros Organics	0.08 : 99.92	0.31
6	Serine	Acros Organics	0.31 : 99.69	0.95
7	Valine	Junsei	0.65 : 99.35	1.01

[a]Average value of four replicates, see experimental section for chromatographic conditions, RSD is the relative standard deviation.

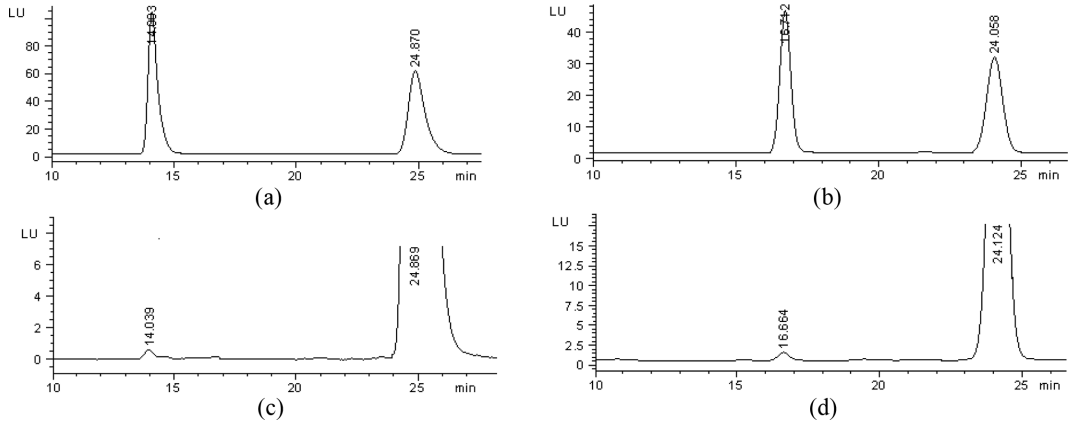

Fig. 2. Chromatograms for enantiomeric purity assay of (a) racemic alanine, (b) L-alanine (D:L=0.18:99.82), (c) racemic valine, and (d) L-valine (D:L=0.65:99.35) as NBD derivatives on coated type Chiralpak AD-H under FL detection, Mobile phase: 20 % 2-propanol/hexane (V/V) with 0.1 % TFA, Flow Rate: 1 mL/min, Detection: FLD: excitation; 470 nm, emission; 530 nm.

4. Conclusions

This study showed that the polysaccharide derived CSPs can successfully be used for the separation of enantiomers of α-amino acids as NBD derivatives. The advantages of this developed chiral HPLC method under FL detection are simple derivatization, and rapid analysis with enhanced selectivity and sensitivity. Among studied CSPs, Chiralpak AD-H and Lux Amylose-1 having the same amylose tris(3,5-dimethylphenylcarbamate) as the chiral selector exhibited most wide and superior enantiomer resolving ability towards studied seven α-amino acids as NBD derivatives. Based on the overall chromatographic results for the enantiomer resolution of amino acids using NBD fluorescent derivatization, this chiral HPLC method could be useful for the determination of the enantiomeric purity of pharmaceuticals and biological study for amino acid type compounds among chiral drugs.

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