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Chromatographic Separation of Enantiomers of Chiral Amines or Amino Alcohols as 9-Anthraldimine Derivatives Using Polysaccharide-Derived Chiral Columns

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In a previous study, a convenient chromatographic separation for the enantiomers of amino acid methyl esters as 9-anthraldimine Schiff base derivatives on polysaccharide-derived chiral stationary phases (CSPs) was reported.[1] For derivatization of the amino acid methyl esters, 9-anthryl moiety of an aromatic group was introduced. The aldimine Schiff base derivatives prepared from aldehydes and amines have often been used as amine protecting groups.[2] Chromatographic enantiomer separation of the chiral 1,2-diamines prepared as the corresponding aldimine derivatives obtained after amine protection on a Chiralcel OD column has been reported by Duchateau *et al*.[3] A series of substituted benzaldehydes reacted with chiral 1,2-diamines for benzaldimine Schiff base derivatives were investigated but 9-anthraldehyde was not used. In this study, we extended liquid chromatographic enantiomer separation of 9-anthraldehyde Schiff base derivatives of chiral amines and amino alcohols using coated and covalently bonded polysaccharide-derived CSPs.[4-11] In particular, the aliphatic primary amines and amino alcohols show low UV absorption and therefore, an indirect detection method for analysis of these compounds is desirable because the 9-anthraldimine moiety has strong UV absorption to aid detection of those analytes. Also, in general, the enantioselectivities of aromatic compounds on polysaccharide-derived CSPs are superior to those of non-aromatic compounds.[4] Therefore, it is expected that the aromatic 9-anthraldimine moiety of the analyte serves as a good interaction site for enantiomeric resolution with the chiral selectors of the polysaccharide-derived CSPs. For the introduction of aromatic anthryl auxiliary group to amines or amino alcohols, the corresponding 9-anthraldimine derivatives of the analytes was readily prepared by stirring 9-anthraldehyde with amines or amino alcohols in the presence of excess $MgSO_4$ in 2-propanol (Figure 1).[2] The use of 2-propanol as a reaction solvent for the derivatization step is quite practical because 0.5-15% 2-propanol in hexane (V/V) as the mobile phases are used for HPLC analysis in this study. The derivatization process is very mild and simple, compared to the harsh and complicated method reported by Duchateau group.[3] They used 1 M NaOH solution (for pH 12) in methanol for derivatization of the chiral 1,2-diamines with benzaldehyde and then extracted the resulting aldimine derivatives with hexane.

Tables 1 and 2 show the chromatographic data for the

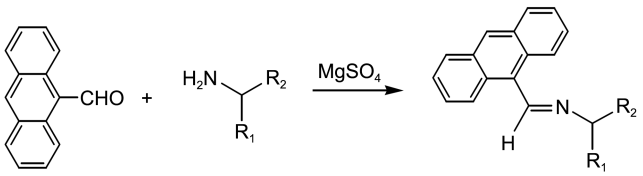


Figure 1. Preparation of 9-anthraldimine derivatives of amines or amino alcohols.

Table 1. Separation of the enantiomers of amines or amino alcohols as 9-anthraldimine derivatives on Chiralcel OD, Chiralcel OD-H and Chiralpak AD

Analyte	Chiralcel OD			Chiralcel OD-H			Chiralpak AD		
	α^a	$k'_1{}^b$	Rs[c]	α^a	$k'_1{}^b$	Rs[c]	α^a	$k'_1{}^b$	Rs[c]
1,3-Dimethylbutylamine	1.57	4.23	3.82	1.58	5.06	6.81	1.12	1.07	0.50
1,2-Dimethylpropylamine	1.51	2.92	3.16	1.56	3.41	5.40	1.18	1.11	1.02
α-Methylbenzylamine	1.22	8.40	1.73	1.25	10.47	3.15	1.16	3.27	1.12
1-Methylheptylamine	1.21	5.05	1.40	1.22	6.32	2.96	1.00	1.10	-
2-Amino-1-butanol	1.81	1.40[d]	3.96	1.85	1.34[d]	5.79	1.05	2.47[e]	0.26
2-Amino-4-methyl-1-pentanol	2.87	1.79[d]	6.72	2.89	0.93[d]	8.23	1.17	1.09[e]	0.65
1-Amino-2-propanol	1.29	3.36[d]	1.64	1.30	3.26[d]	2.85	1.22	3.25[e]	1.20
2-Amino-1-propanol	1.61	1.72[d]	3.23	1.64	1.62[d]	4.19	1.12	2.50[e]	0.84

Mobile phase; 0.5% 2-propanol in hexane (V/V); Flow rate = 1 mL/min; Detection UV 254 nm. [a]Separation factor. [b]Retention factor for the first eluted enantiomer. [c]Resolution factor. [d]15% 2-propanol in hexane (V/V). [e]10% 2-propanol in hexane (V/V).

Table 2. Separation of the enantiomers of amines or amino alcohols as 9-anthraldimine derivatives on Chiralpak IA, Chiralpak IB and Chiralpak IC

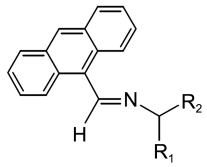
Analyte	Chiralpak IA			Chiralpak IB			Chiralpak IC		
	α^a	$k'_1{}^b$	Rs[c]	α^a	$k'_1{}^b$	Rs[c]	α^a	$k'_1{}^b$	Rs[c]
1,3-Dimethylbutylamine	1.00	5.41[d]	-	1.40	2.47	1.35	2.37	1.15	7.52
1,2-Dimethylpropylamine	1.00	6.10[d]	-	1.33	1.85	0.76	1.91	0.88	5.96
α-Methylbenzylamine	1.00	4.08[d]	-	1.21	4.03	1.56	1.84	2.45	5.65
1-Methylheptylamine	1.00	3.24[d]	-	1.18	3.00	0.82	1.84	1.53	6.33
2-Amino-1-butanol	1.00	1.56[e]	-	1.17	2.83[e]	0.35	1.28	1.44[e]	3.24
2-Amino-4-methyl-1-pentanol	1.10	1.42[e]	0.26	1.36	2.54[e]	0.77	1.29	1.32[e]	1.94
1-Amino-2-propanol	1.21	1.91[e]	1.18	1.00	6.14[e]	-	1.10	3.08[e]	1.05
2-Amino-1-propanol	1.10	1.54[e]	0.72	1.00	4.78[e]	-	1.41	1.78[e]	3.10

Mobile phase; 0.5% 2-propanol in hexane (V/V); Flow rate = 1 mL/min; Detection UV 254 nm. [a]Separation factor. [b]Retention factor for the first eluted enantiomer. [c]Resolution factor. [d]1% 2-propanol in hexane (V/V). [e]10% 2-propanol in hexane (V/V).

separation of the enantiomers of several chiral amines or amino alcohols as 9-anthraldimine Schiff base derivatives on typical coated polysaccharide-derived CSPs (Chiralcel OD, Chiralcel OD-H and Chiralpak AD) and covalently bonded polysaccharide-derived CSPs (Chiralpak IA, Chiralpak IB and Chiralpak IC), respectively.[4-11] Among the examined CSPs, Chiralcel OD (or OD-H) and Chiralpak IC showed very good enantioselectivity with base-line separation for all analytes, while Chiralpak IA showed the worst enantioseparation. Also, the enantioselectivities on Chiralcel OD-H packed with 5 μm silica gel were slightly greater than those on Chiralcel OD packed with 10 μm silica gel (Table 1). On the other hand, the resolution factors on Chiralcel OD-H were much greater than those on Chiralcel OD.[4] Unlike the resolution for the amino acid ester derivatives,[1] interestingly, Chiralpak IC showed the highest enantioselectivity for amine derivatives, while Chiralcel OD (or OD-H) showed greater enantioselectivity than Chiralpak IC for the resolution of amino alcohol derivatives. The recently developed Chiralpak IA and Chiralpak IB columns are covalently immobilized CSPs derived from the same chiral selectors as the coated Chiralpak AD and Chiralcel OD (OD-H) columns, respectively.[9] The separation factors and resolution factors of all analytes on the coated CSPs [Chiralcel OD (OD-H) and Chiralpak AD] were higher than those on the covalently bonded CSPs [Chiralpak IB and Chiralpak IA], respectively.[7,9] The lowered enantioselectivity on bonded CSPs might be responsible for the lack of ordered arrangement of the chiral selector bonded to the matrix.[12]

Table 3 summarizes the separation of the enantiomers of several amines including amino acid esters as 9-anthraldimine derivatives on Chiralcel OD-H. A consistent elution order of the 9-anthraldimine derivatives of amino acid esters (entries 1-6) is shown and the S-isomers are preferentially retained for all analytes on Chiralcel OD-H. As the (*S*)-enantiomers for 9-anthraldimine derivatives of leucine methyl and ethyl esters (entries 3,4) were preferentially retained, the same elution order of the 9-anthraldimine derivative of 2-amino-4-methyl-1-pentanol (lucinol: entry 7) was observed. However, the apparent inversion of the elution order of the enantiomers of the α-methylbenzylamine derivative (entry 8) is not real but arises from the Cahn-Prelog-Ingold priority sequence for assignment of absolute configuration. Consequently, the intrinsically similar elution order of the enantiomers of several amines including amino acid esters prepared as 9-anthraldimine derivatives in Table 3 would suggest that all these analytes were resolved by a consistent chiral recognition mechanism.

Table 3. Separation of the enantiomers of several amines including amino acid esters as 9-anthraldimine derivatives on Chiralcel OD-H



Entry	R_1	R_2	α^a	$k'_1{}^b$	Conf.[c]
1	Me	CO_2Me	1.25	4.10	S
2	Me	CO_2Et	1.24	3.61	S
3	*i*-Bu	CO_2Me	3.22	1.36	S
4	*i*-Bu	CO_2Et	3.10	1.15	S
5	$PhCH_2$	CO_2Me	1.38	3.19	S
6	Ph	CO_2Me	5.47	3.24	S
7	*i*-Bu	CH_2OH	2.89	0.93[d]	S
8	Ph	Me	1.25	10.47[e]	R

Mobile phase; 10% 2-propanol in hexane (V/V); Flow rate = 1 mL/min; UV 254 nm; [a]Separation factor. [b]Retention factor for the first eluted enantiomer. [c]Absolute configuration of the second eluted enantiomer. [d]15% 2-propanol in hexane (V/V). [e]0.5% 2-propanol in hexane (V/V).

For validation of the analytical method, the accuracy for both intra- and inter-day as well as the precision for the analytical method at three enantiomeric purities of (*S*)-2-amino-4-methyl-1-pentanol as 9-anthraldimine derivatives on Chiralcel OD-H are listed in Table 4. The accuracy for intra- and inter-day assay was determined to be 100.06-100.27% and 99.97-100.13%, respectively. The precision for intra- and inter-day assay expressed in % RSD was 0.41-1.47% and 0.82-2.12%, respectively. The results for accuracy and precision indicate that this validated method is highly suitable. As shown in Table 5, the developed analytical method was applied for the determination of enantiomeric

Table 4. Intra-day and inter-day precision and accuracy of the analytical method for (*S*)-2-amino-4-methyl-1-pentanol as 9-anthraldimine derivative

Enantiomeric purity of (*S*)-2-amino-4-methyl-1-pentanol (%)	Intra-day (n=5)		Inter-day (n=5)	
	Accuracy (%)	Precision (%)	Accuracy (%)	Precision (%)
98.9	100.06	0.94	99.97	2.12
96.9	100.27	0.41	100.13	1.73
94.9	100.09	1.47	100.06	0.82

Mobile phase; 7% 2-propanol/hexane (V/V); Flow rate = 1 mL/min; UV 254 nm.

Table 5. Determination of the enantiomeric purity of some commercially available amines or amino alcohols as 9-anthraldimine derivatives on Chiralcel OD-H or Chiralpak IC

Entry	Analyte	Company	R:S ratio[a]	RSD[b]
1	(*R*)-2-Amino-4-methyl-1-pentanol	Sigma	99.4:0.6	0.87%
2	(*S*)-2-Amino-4-methyl-1-pentanol	Aldrich	0.1:99.9	0.10%
3	(*R*)-α-Methylbenzylamine	Acros	99.4:0.6	0.56%

See Tables 1 and 2 for chromatographic conditions. [a]Average value of three determinations. [b]Relative standard deviation.

purity of some commercially available chiral amines and amino alcohols. The enantiomeric impurities of < 0.1-0.6% for 2-amino-4-methyl-pentanol and α-methylbenzylamine after derivatization with 9-anthraldehyde were determined. Typical chromatograms for determination of the enantiomeric purity of (*S*)-2-amino-4-methyl-pentanol (Aldrich reagent) as 9-anthraldimine derivative on Chiralcel OD-H are presented in Figure 2.

In conclusion, the liquid chromatographic separation of the enantiomers of 9-anthraldimine derivatives of chiral amines or amino alcohols was investigated on typical coated and covalently bonded polysaccharide-derived CSPs. The performance of Chiralcel OD (or OD-H) was superior to the other CSPs for resolution of 9-anthraldimine derivatives of amino alcohols. On the other hand, Chiralpak IC showed the best enantiomer separation of chiral primary amines as 9-anthraldimine derivatives. It should be noted that the 9-anthraldimine moiety has the advantage of strong UV absorption to aid detection of the aliphatic primary amines or amino alcohols. The analytical method was applied to measure the enantiomeric purity of some commercially available chiral amines and amino alcohols. Therefore, this convenient analytical method should be very efficient for determination of enantiomeric purity of amines or amino alcohols as 9-anthraldimine Schiff base derivatives with strong UV absorption.

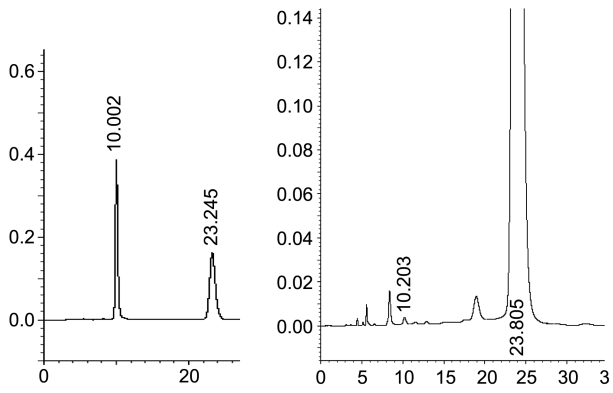


Figure 2. Chromatograms for enantiomer separation of 9-anthraldimine derivatives of racemic 2-amino-4-methyl-pentanol (left) and (*S*)-2-amino-4-methyl-pentanol (Aldrich reagent) (right, R:S = 0.1:99.9) on Chiralcel OD-H. Mobile phase; 7% 2-propanol/hexane (V/V); Flow rate = 1 mL/min; UV 254 nm; Injected amount, 3 μg (left), 8 μg (right).

Experimental Section

Chromatography was performed at room temperature using an HPLC Breeze system consisting of a Waters model 1525 binary pump, a Rheodyne model 7125 injector with a 20 μL loop and a dual absorbance detector (Waters 2487 detector). The coated-type CSPs [Chiralcel OD, Chiralpak AD (250 mm L × 4.6 mm I.D., 10 μm) and Chiralcel OD-H (250 mm L × 4.6 mm I.D., 5 μm)] and the covalently bonded type CSPs [Chiralpak IA, Chiralpak IB and Chiralpak IC (250 mm L × 4.6 mm I.D., 5 μm)] were purchased from Daicel Chemical Company (Tokyo, Japan). HPLC-grade hexane and 2-propanol were obtained from J. T. Baker (Phillipsburg, NJ). 9-Anthraldehyde, amines and amino alcohols are commercially available from Aldrich (Milwaukee, WI), Sigma (St. Louis, MO) and Acros (Belgium). All analytes of 9-anthraldimine derivatives were prepared as shown in Figure 1. The general procedure for the preparation of the corresponding 9-anthraldimine derivatives was as follows: 0.5 mmol of the amines or amino alcohols, an equimolar amount of 9-anthraldehyde and excess $MgSO_4$ (5-10 eq.) in 10 mL of 2-propanol were stirred at room temperature for 12 hr.[2] The reaction mixture was filtered to remove the solid and the resulting solution was directly injected on the HPLC. The intra-day precision and accuracy of the method were evaluated by analyzing samples in five replicates, performed by one operator within a day at three different enantiomeric purities of (*S*)-2-amino-4-methyl-pentanol as 9-anthraldimine derivative. The inter-day precision and accuracy were assessed by replicating the analysis of samples on 5 days at three different enantiomeric purities of (*S*)-2-amino-4-methyl-pentanol as 9-anthraldimine derivative. Precision was expressed as the intra-day and inter-day percent relative standard deviation.

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