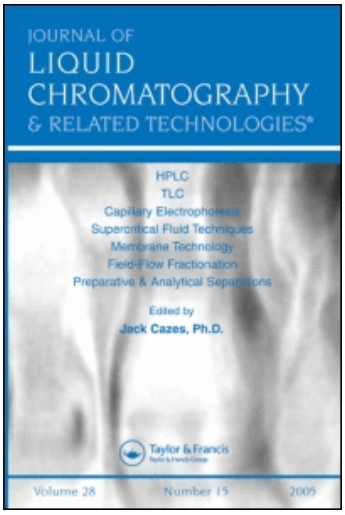

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INDIRECT HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ENANTIOSEPARATION OF RECEMIC AMINO ALCOHOLS WITH 1,3-DIACETOXY-1-(4-NITROPHENYL)-2-PROPYL ISOTHIOCYANATE AS DERIVATIZING AGENT

M. Péter[a]; F. Fülöp[a]
[a] Institute of Pharmaceutical Chemistry, University of Szeged, Szeged, Hungary

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INDIRECT HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ENANTIOSEPARATION OF RACEMIC AMINO ALCOHOLS WITH 1,3-DIACETOXY-1-(4-NITROPHENYL)-2-PROPYL ISOTHIOCYANATE AS DERIVATIZING AGENT

M. Péter, F. Fülöp

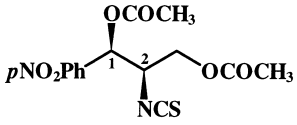
Institute of Pharmaceutical Chemistry
University of Szeged
P. O. Box 121
6701 Szeged, Hungary

ABSTRACT

Synthetically and pharmaceutically interesting racemic amino alcohols with two adjacent chiral centres were analysed by means of indirect high performance liquid chromatography. For resolution of the enantiomers, the recently developed chiral derivatizing agent (1*R*,2*R*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate ((*R*,*R*)-DANI) was applied. The diastereomeric thioureas produced after derivatization were separated under reversed-phase conditions. Of the organic modifiers applied in the eluent, methanol proved much more effective than acetonitrile.

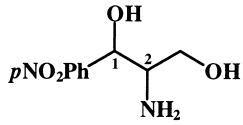
INTRODUCTION

The different chiral 1,2- and 1,3-amino alcohols with two adjacent chiral centres investigated in the present study are important compounds from both synthetic and pharmaceutical aspects. The (*S*,*S*) form of 2-amino-1-(4-nitrophenyl)-1,3-propanediol (**1**, Figure 1) is a side-product of chloramphenicol synthesis, while the (*R*,*R*) form is the effective intermediate. The (*S*,*S*) form was earlier successfully employed for the resolution of racemic chiral phosphoric acids and amino acids by diastereomeric salt formation.[1] Enantiomerically pure



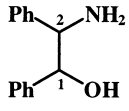
(*R*,*R*)-DANI

(1*R*,2*R*)-1,3-diacetoxy-1-(4-nitrophenyl)-
-2-propyl isothiocyanate



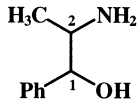
1

(1*R*,2*R*)- and (1*S*,2*S*)-
2-amino-1-(4-nitrophenyl)-1,3-propanediol



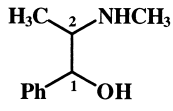
2

(1*R*,2*S*)- and (1*S*,2*R*)-
2-amino-1,2-diphenylethanol



3

(1*R*,2*S*)- and (1*S*,2*R*)-
norephedrine



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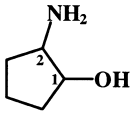
(1*R*,2*S*)- and (1*S*,2*R*)-
ephedrine



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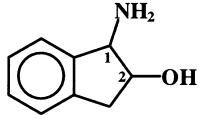
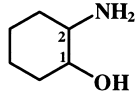
(1*R*,2*S*)- and (1*S*,2*R*)-
2-aminocyclopentanol



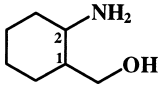
6

(1*R*,2*S*)- and (1*S*,2*R*)-
1-amino-2-indanol



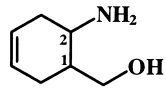
7

(1*R*,2*S*)- and (1*S*,2*R*)-
2-aminocyclohexanol



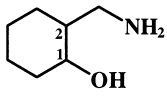
8 (*cis*) and **9** (*trans*)

(1*R*,2*S*)- and (1*S*,2*R*)- (**8**) and
(1*R*,2*R*)- and (1*S*,2*S*)- (**9**)
2-amino-1-hydroxymethylcyclohexane



10 (*cis*) and **11** (*trans*)

(1*R*,2*S*)- and (1*S*,2*R*)- (**10**) and
(1*R*,2*R*)- and (1*S*,2*S*)- (**11**)
2-amino-1-hydroxymethyl-4-cyclohexene



12 (*cis*) and **13** (*trans*)

(1*R*,2*S*)- and (1*S*,2*R*)- (**12**) and
(1*R*,2*R*)- and (1*S*,2*S*)- (**13**)
2-aminomethylcyclohexanol

Figure 1. Structures of the chiral derivatizing agent and the investigated compounds.

2-amino-1,2-diphenylethanol (**2**, Figure 1) can be used as a resolving agent for the resolution of sclareolide, which is a key intermediate for the synthesis of Ambrox(R).[2] Various *N*-substituted amino alcohol ligands derived from (1*S*,2*R*)-**2** have been applied in the synthesis of Ti(IV)-amino alcohol complexes.[3] (1*S*,2*R*)-**2** was also used as a highly effective chiral auxiliary in the asymmetric syntheses of β-lactams.[4] Norephedrine and ephedrine (**3** and **4**, Figure 1) are naturally-occurring alkaloids that have been used as medicinals for hundreds of years. They are, additionally, widely-used chiral synthons or catalysts for asymmetric syntheses.[5-8] The recently-designed scytalone dehydratase inhibitors, which behave as rice blast fungicides, are norephedrine derivatives.[9] Molecularly imprinted polymers of (1*R*,2*S*)-**3**, prepared as chiral stationary phases in thin-layer chromatography,[10] display versatile applicability. The applications of *cis*-1-amino-2-indanol (**6**, Figure 1) in asymmetric syntheses have been reviewed.[11] Further, (1*S*,2*R*)-**6** is a key component of an HIV protease inhibitor, indinavir.[12] Enantiomers of **5** and **7**-**13** can serve as building blocks of pharmacologically active fused saturated heterocycles.[13] Their synthetic applicability too is noteworthy.[14, 15]

In accordance with the importance of chiral amino compounds, many attempts have been made to separate their optical isomers by liquid chromatography (LC). Although several successful separations have been already carried out for **3** and **4**, there is still a steady interest in these compounds.[16-22] **2** has been resolved on a novel crown ether CSP.[23] The chloramphenicol base **1** was separated with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (TAGIT)[24] as chiral derivatizing agent (CDA), and compounds **8**-**11** with Marfey's reagent.[25] To the best of our knowledge, the separability of the enantiomers of **5**-**7** and **12**, **13** by LC has not yet been investigated.

Covalent diastereomer formation using CDAs offers an indirect mode of enantioseparation which is still popular despite the rapidly growing number of highly effective direct methods.[26-29] For amino compounds, the chemically most selective CDAs are isothiocyanates. Some examples applied for the separation of amino alcohols are TAGIT,[30-32] *N*-[(2-isothiocyanato)cyclohexyl]-3,5-dinitrobenzoyl amide [(*R*,*R*)- and (*S*,*S*)-DDITC],[33] *N*-[(2-isothiocyanato)cyclohexyl]pivalinoyl amide [(*R*,*R*)- and (*S*,*S*)-PDITC],[34] 4-(3-isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole [(*R*)- and (*S*)-NBD-PyNCS], 4-(3-isothiocyanatopyrrolidin-1-yl)-7-(*N,N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole [(*R*)- and (*S*)-DBD-PyNCS],[35] and 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate (NAP-IT).[36]

The aim of the present work was to develop a simple chromatographic method for the separation and identification of the above-mentioned amino alcohol enantiomers using a novel CDA, (1*R*,2*R*)-1,3-diacetoxy-1-(4-nitrophenyl)-2-propyl isothiocyanate [(*R*,*R*)-DANI, Figure 1], which is also accessible in the (*S,S*) enantiomeric form. The thiourea diastereomers formed were

analysed on a reversed-phase (C_{18}) column, mixtures of 0.1% aqueous trifluoroacetic acid (TFA) and methanol (MeOH) or acetonitrile (MeCN) being used for elution. The chromatographic features of the diastereomers were compared. The effects of these organic modifiers on the separation, and the sequence of elution of the thioureas were investigated.

EXPERIMENTAL

Chemicals and Reagents

TFA and triethylamine (TEA) of analytical reagent grade, and MeOH and MeCN of HPLC grade were obtained from Merck. 0.1% aqueous TFA was prepared with triply distilled water and purified by filtration through a 0.45 μm Millipore filter, type HV (Molsheim, France). Racemic or enantiomerically pure amino alcohols were commercial products of Fluka BioChemika (Buchs, Switzerland) or were synthesized according to refs. 37-39. (1*R*,2*R*)-**1** was a generous gift from Egis Pharmaceuticals (Budapest, Hungary). (*R*,*R*)-DANI was prepared according to ref. 40. Briefly, starting from the (1*R*,2*R*)-2-amino-1-(4-nitrophenyl)-1,3-propanediol, the first reaction step was performed in glacial acetic acid with acetyl chloride as acylating reagent. The resulting hydrochloride of the 1,3-diacetoxy analogue of the starting compound was treated with thiophosgene in a biphasic system in the presence of sodium hydrogencarbonate, to furnish the corresponding (*R*,*R*)-DANI.

Apparatus

The HPLC system consisted of an M-600 low-pressure gradient pump equipped with an M-486 tunable absorbance detector, and Millenium software version 2.1 (Waters Chromatography, Milford, MA, USA). The injector with a 20 μL loop was from Rheodyne (Cotati, CA, USA). Reversed-phase analyses were performed on a Nova-Pak C_{18} column, 150x3.9 mm I.D., 4 μm particle size (Waters Chromatography).

Derivatization Procedure

A 5 mM solution of the analyte was prepared with 0.4% TEA (in MeCN-water 1:1, pH~11). To 100 μL of this solution, 100 μL of the reagent (10 mM in MeCN) was added; the ratio of the reagent to the amino alcohol was 2:1. The mixture was thermostated at 60°C for 2 h. Then, 50 μL of glycine (50 mM in H_2O) was added to a 20 μL aliquot of the reaction mixture, and the mixture was thermostated for a further 30 min at 60°C in order to remove the excess of the reagent. The derivatives produced were injected onto a reversed-phase HPLC column after a 2-fold dilution with the eluent.

The conditions of derivatization (pH, reagent excess, temperature, reaction time) were investigated in detail in our previous studies.[40-42] The optimized conditions found proved well applicable for the amino alcohols studied here. No kinetic discrimination, nor racemization of either the CDA or the analytes were observed under the reaction conditions applied, and the peak areas of the corresponding diastereomers were practically equal.

RESULTS AND DISCUSSION

Separation of Diastereomers

The diastereomeric thioureas were analysed under reversed-phase conditions, with mixtures of 0.1% TFA and MeOH or MeCN as eluents. All of the samples were treated with glycine after derivatization with the chiral reagent (see Experimental). The reason for this was to remove the excess of reagent in order to shorten the analysis time (the reagent peak eluted later, especially with MeCN-containing eluents), or in some cases to avoid overlapping of the diastereomer and the reagent peaks (Figure 2).

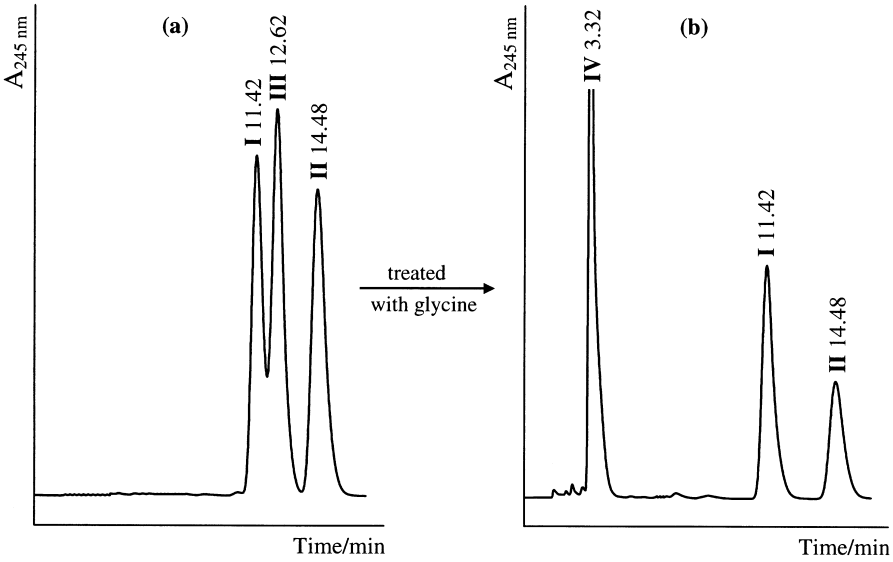


Figure 2. Chromatograms of norephedrine (3) diastereomers formed with (*R*,*R*)-DANI. Column, NovaPak C_{18}; flow rate, 0.8 mL min^{-1}; detection, 245 nm; eluent (*v*/*v*), TFA-MeOH=45:55. **I** (1*R*,2*S*)-3/(*R*,*R*)-DANI, **II** (1*S*,2*R*)-3/(*R*,*R*)-DANI, **III** unreacted (*R*,*R*)-DANI, **IV** glycine/(*R*,*R*)-DANI. (a) **I**:**II**=1:1, (b) **I**:**II**=2:1.

Table 1

Chromatographic Data on (*R,R*)-DANI-Derivatized Amino Alcohols with MeOH as Organic Modifier

Analyte	TFA-MeOH (*v/v*)	k_I[a]	k_{II}[a]	α[b]	R_S[c]	e.s.[d]
1	55:45	7.74	9.16	1.18	1.31	*S<R*
2	45:55	9.35	11.89	1.27	2.12	*S<R*
3	45:55	6.46	8.46	1.31	2.70	*S<R*
4	55:45	22.35	22.35	1.00	0.00	–
5	45:55	3.04	3.56	1.17	0.86	
	50:50	4.62	5.57	1.21	1.25	
	55:45	8.08	9.99	1.24	1.81	
6	45:55	5.88	7.06	1.20	1.35	*S<R*
	50:50	11.94	14.87	1.25	1.99	*S<R*
7	45:55	4.31	5.12	1.19	1.15	
	50:50	6.93	8.44	1.22	1.54	
8	45:55	3.25	4.18	1.29	1.61	*S<R*
9	45:55	3.66	4.75	1.31	1.64	*S<R*
10	45:55	2.71	3.53	1.30	1.65	*S<R*
11	45:55	3.01	3.77	1.26	1.42	*S<R*
12	45:55	5.22	5.73	1.10	0.63	
	50:50	8.10	9.02	1.11	0.91	
	55:45	15.83	17.97	1.14	1.31	
13	55:45	14.65	15.45	1.05	0.40	

Column, NovaPak C_{18} 150x3.9 mm I. D. (4 μm); t_0=1.53 min; flow rate, 0.8 mL min^{-1}; detection, 245 nm. I, first-eluting diastereomer; II, second-eluting diasteromer. [a]$k=(t_R-t_0)/t_0$. [b]$\alpha=k_{II}/k_I$. [c]$R_S=2(t_{II}-t_I)/(w_I+w_{II})$. [d]Elution sequence (refers to the configuration of the chiral centre bearing the amino group in the analyte).

Chromatographic data are summarized in Tables 1 and 2. MeOH (Table 1) proved much more effective as an organic modifier than MeCN (Table 2). Not only were the retention times significantly shorter, but the resolutions were better. Extreme examples are **2**, **6** and **7**, where excellent R_S values (R_S>1.5) were

Table 2

Chromatographic Data on (*R*,*R*)-DANI-Derivatized Amino Alcohols with MeCN as Organic Modifier

Analyte	TFA-MeCN (*v/v*)	k_I[a]	k_{II}[a]	α[b]	R_S[c]	e.s.[d]
1	75:25	32.25	36.56	1.13	1.46	*S*<*R*
2	70:30	50.55	50.55	1.00	0.00	–
3	70:30	33.77	40.16	1.19	2.01	*S*<*R*
4	70:30	50.55	50.55	1.00	0.00	–
5	75:25	20.47	22.69	1.11	1.20	
6	70:30	29.44	29.44	1.00	0.00	–
7	70:30	14.58	14.58	1.00	0.00	
	75:25	31.92	34.87	1.09	1.03	
8	70:30	13.33	14.08	1.06	0.50	*S*<*R*
	75:25	34.44	40.20	1.17	1.85	*S*<*R*
12	75:25	40.37	40.37	1.00	0.00	

Column, NovaPak C_{18} 150x3.9 mm I. D. (4 μm); t_0=1.53 min; flow rate, 0.8 mL min^{-1}; detection, 245 nm. I, first-eluting diastereomer; II, second-eluting diasteromer. [a]$k=(t_R-t_0)/t_0$. [b]$\alpha=k_{II}/k_I$. [c]$R_S=2(t_{II}-t_I)/(w_I+w_{II})$. [d]Elution sequence (refers to the configuration of the chiral centre bearing the amino group in the analyte).

readily achieved with MeOH, whereas, with MeCN-containing eluents, total coelution of the diastereomer peaks occurred (Figure 3). The chromatographic features of the diastereomers with MeOH-containing eluents will, therefore, be compared below.

Since the amino alcohols investigated in this study contain two adjacent chiral centres, four stereoisomers exist. Accordingly, for aliphatic compounds, *erythro* and *threo* isomers and, for cyclic compounds, *cis* and *trans* isomers, can be distinguished. All of the aliphatic compounds were 1,2-amino alcohols; *threo* enantiomers for **1**, and *erythro* enantiomers for the structurally closely related **2**-**4** were analysed. For **2** and **3**, very good resolutions (R_S>2) were achieved within reasonable times. However, **4** could not be separated at all. The difference between **3** and **4** is that **3** contains a primary, while **4** contains a secondary amino function due to the methyl group. The methyl group did not

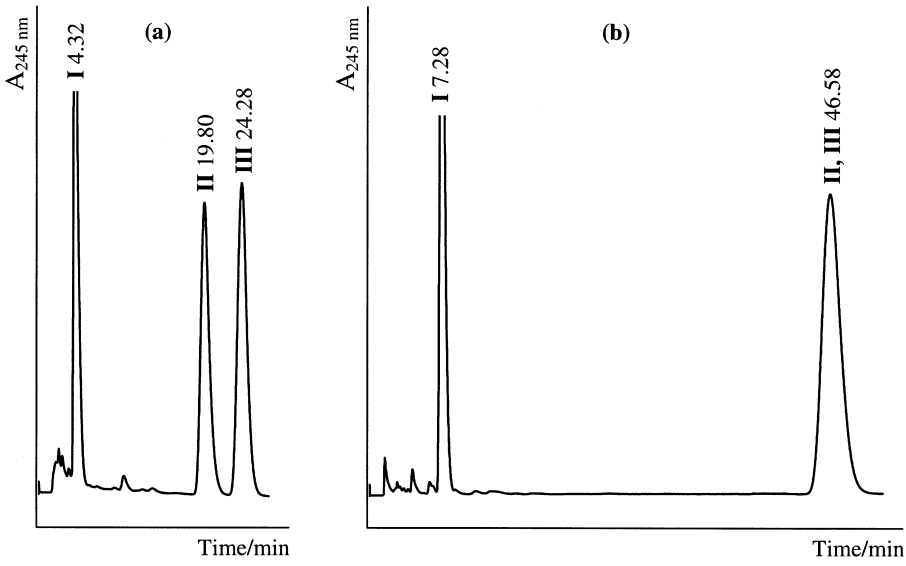


Figure 3. Chromatograms of 1-amino-2-indanol (6) diastereomers formed with (*R*,*R*)-DANI. Column, NovaPak C_{18}; flow rate, 0.8 mL min^{-1}; detection, 245 nm. **I** glycine/(*R*,*R*)-DANI, **II** (1*S*,2*R*)-6/(*R*,*R*)-DANI, **III** (1*R*,2*S*)-6/(*R*,*R*)-DANI. Eluent (*v*/*v*), (a) TFA-MeOH=50:50, **II:III**=5:6; (b) TFA-MeCN=70:30.

restrict the formation of thiourea derivatives, but its effect on the separability was striking and resulted in coelution of the diastereomer peaks.

As concerns the retention times of **1**-**4** at the same eluent strength, the derivatives of **1** eluted significantly faster. This is probably due to the presence of the second alcohol function in the molecule, which decreased the hydrophobicity of the diastereomers. Decrease of the eluent strength from 55% to 45% MeOH content allowed satisfactory separation (R_S=1.31).

Among the cyclic compounds, **5**-**7** contain the amino and alcohol functions in the *cis*-1,2 position. 1-Amino-2-indanol (**6**) and 2-aminocyclohexanol (**7**) can be derived from 2-aminocyclopentanol (**5**), the former as a benzene-fused derivative, the latter by expanding the ring size. As expected, both **6** and **7** eluted later than **5** from a reversed-phase column at the same mobile phase composition, because both are more hydrophobic. The R_S values relating to similar retention factors were similar for these three compounds.

Cis- and *trans*-2-amino-1-hydroxymethylcyclohexane (**8** and **9**) and *cis*- and *trans*-2-amino-1-hydroxymethyl-4-cyclohexene (**10** and **11**) are 1,3-amino alcohols and contain an amino group connected directly to the asymmetric centre. For these compounds, good resolutions of the diastereomers were detected within short analysis times. The unsaturated isomers eluted faster than the corresponding saturated derivatives, the former being less hydrophobic in consequence of the double bond. The resolution achieved for the *trans* alcohols **9** was slightly better than that for the *cis* compounds **8**; for the unsaturated alcohols (**10** and **11**), the R_S value was smaller for the *trans* isomers **11**. Efforts were made to separate all four stereoisomers of **8**, **9** and **10**, **11**. The resulting chromatograms are depicted in Figure 4. Although, the diastereomers derived from the enantiomeric pairs were well separated at TFA-MeOH=45:55 (*v*/*v*) (Table 1), the peaks of the *cis* and *trans* isomers partially overlapped in this eluent. A decrease in eluent strength was, therefore, necessary.

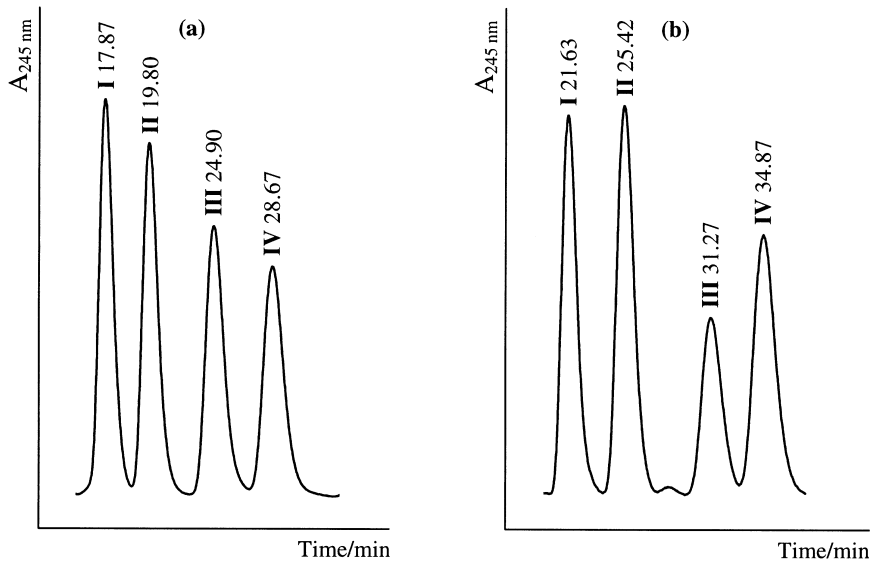


Figure 4. Separation of all four stereoisomers of 2-amino-1-hydroxymethylcyclohexane (**8** and **9**) and 2-amino-1-hydroxymethyl-4-cyclohexene (**10** and **11**). Column, NovaPak C_{18}; flow rate, 0.8 mL min^{-1}; detection, 245 nm. Eluent, TFA-MeOH=55:45; **I** (1*R*,2*S*)-**8**/(*R*,*R*)-DANI, **II** (1*S*,2*S*)-**9**/(*R*,*R*)-DANI, **III** (1*S*,2*R*)-**8**/(*R*,*R*)-DANI, **IV** (1*R*,2*R*)-**9**/(*R*,*R*)-DANI, **I**:**II**:**III**:**IV**=28:28:23:21. Eluent, TFA-MeOH=58:42; **I** (1*R*,2*S*)-**10**/(*R*,*R*)-DANI, **II** (1*S*,2*S*)-**11**/(*R*,*R*)-DANI, **III** (1*S*,2*R*)-**10**/(*R*,*R*)-DANI, **IV** (1*R*,2*R*)-**11**/(*R*,*R*)-DANI, **I**:**II**:**III**:**IV**=28:31:16:25.

Table 3

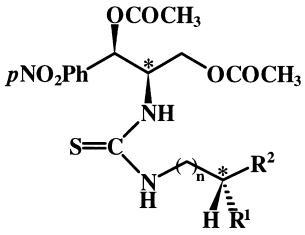
Chromatographic Data on (*R*,*R*)-DANI-Derivatized 2-Amino-1-Hydroxymethylcyclohexane (8 and 9) and 2-Amino-1-Hydroxymethyl-4-Cyclohexene (10 and 11)

Analyte	TFA-MeOH (*v*/*v*)	k_I	k_{II}	k_{III}	k_{IV}		
8, 9	55:45	10.68	12.55	15.27	17.74		
		$\alpha_{I,II}$	$\alpha_{II,III}$	$\alpha_{III,IV}$	$R_{S;I,II}$	$R_{S;II,III}$	$R_{S;III,IV}$
		1.18	1.22	1.16	1.35	1.81	1.49
Analyte	**TFA-MeOH (*v*/*v*)**	k_I	k_{II}	k_{III}	k_{IV}		
10, 11	55:45	8.64	10.10	12.41	13.74	1.17	1.23
	58:42	13.14	15.61	19.44	21.79	1.19	1.25
		$\alpha_{I,II}$	$\alpha_{II,III}$	$\alpha_{III,IV}$	$R_{S;I,II}$	$R_{S;II,III}$	$R_{S;III,IV}$
	55:45	1.17	1.23	1.11	1.34	2.00	1.02
	58:42	1.19	1.25	1.12	1.64	2.29	1.27

Column, NovaPak C_{18} 150x3.9 mm I. D. (4 μm); t_0=1.53 min; flow rate, 0.8 mL min^{-1}; detection, 245 nm. I, (1*S*,2*R*); II, (1*S*,2*S*); III, (1*S*,2*R*); IV, (1*R*,2*R*) isomer (refers to the configuration of the chiral centres in the analyte).

The four stereoisomers of **8**, **9** could be satisfactorily resolved in one chromatographic run by using TFA-MeOH=50:50 (*v*/*v*) for elution. For **10**, **11**, a further decrease in MeOH content was needed to obtain appropriate R_S values for all four diastereomer peaks. Retention factors (k), separation factors (α) and resolutions (R_S) are given in Table 3. For both saturated and unsaturated compounds, the first *trans* isomer eluted between the *cis* derivatives, the sequence of elution being (1*R*,2*S*)<(1*S*,2*S*)<(1*S*,2*R*)<(1*R*,2*R*).

Cis- and *trans*-2-aminomethylcyclohexanol (**12** and **13**) are also 1,3-amino alcohols, but the amino function, which is the reacting group during derivatiza-



Analyte to be derivatized	n
8 and **9**	0
12 and **13**	1

Figure 5. Structure of a thiourea diastereomer formed with (*R*,*R*)-DANI. The distance between the chiral centres marked with asterisks is a factor determining the separability.

tion, is in a remote position. Its effect is clear if we consider that **8** and **12** or **9** and **13** are positional isomers (Figure 1). The most important difference between their thiourea derivatives is that the distance between the asymmetric centres in the reagent and in the analyte is one atom longer for the aminomethyl-containing diastereomers (Figure 5). This results in significantly lower resolutions. At TFA-MeOH=45:55 (*v*/*v*), for the *cis* isomers **8** R_S=1.61, while for the *cis* isomers **12,** only R_S=0.61 was attained. Similarly, for the *trans* derivatives **9**, the resolution was R_S=1.64, whereas for the *trans* isomers **13,** no separation was observed. At a decreased MeOH content (45%), the resolution achieved for the *cis* **12** derivatives was appropriate (R_S=1.31), but for the *trans* compounds **13** it still remained under an acceptable limit (R_S=0.40). This confirms the fact that the optimum distance between the chiral centres is an essential feature for separability.[26]

Sequence of Elution

The sequence of elution of the diastereomers was determined by the addition of standards. (*R*,*R*)-DANI was used for derivatization. The sequence of elution was found to be *S*<*R* (the letter refers to the configuration of the chiral centre bearing the amino group in the analyte); this was true in all cases, for both organic modifiers, when examination was possible. As expected, the elution sequence was the reverse when (*S*,*S*)-DANI was applied as CDA. For some compounds (**5**, **7**, **12** and **13**), standard enantiomers were not available.

Detection Limit

The limit of detection for **3** at 245 nm at a signal to noise ratio of 3:1 was determined to be 0.16 nmol mL^{-1}. This is the same value as obtained previously for valine.[41] Since the peak areas for diastereomers injected after the same

preparation procedure were very similar, this level can be accepted as valid for the other amino alcohols too.

CONCLUSIONS

An indirect high performance liquid chromatographic method was developed for the separation of enantiomers of 1,2- and 1,3-amino alcohols possessing two adjacent chiral centres. (*R*,*R*)-DANI was used as a chiral derivatizing agent. Significant differences were found between the selectivities of organic modifiers applied to elute diastereomers from a reversed-phase column. MeOH proved much more effective than MeCN with respect to both retention times and resolutions. The results obtained for 1,3 positional isomers highlighted the role of the optimum distance between the chiral centres as a determining factor governing the achievement of resolution.

Of the 13 amino alcohols, ephedrine (**4**) could not be separated at all, which is a limitation of DANI as compared with TAGIT, as an example. However, compounds **8-11** were well resolved with DANI, whereas they could not be separated at all with TAGIT.

The applied reagent is available in both enantiomeric forms, giving the possibility for appropriate selection of the elution sequence. Further, the detection limit seems to be essentially independent of the nature of the analyte, in consequence of the good UV absorbance of the thiourea group present in each derivative. (*R*,*R*)- or (*S*,*S*)-DANI combines these advantages and is considered to be well applicable for the determination of enantiomeric impurities. In combination with the addition of standards, the developed method can be used to identify absolute configurations.

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Free samples of (*R*,*R*)-DANI are available upon request.

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