Journal of Chromatography, 368 (1986) 413–422
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 987

Note

Resolution of chiral amines by high-performance liquid chromatography of their mixed chelate complexes with Cu(II)-L-proline*

STANLEY LAM* and GALINA MALIKIN
Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461 (U.S.A.)
(Received July 29th, 1986)

Resolution of optical isomers of amino acids is effected by complexation of the analyte acids with a chiral ligand and a metal ion introduced to the mobile phase. The mixed chelate copper complexes formed between the chiral ligand and the optical isomers are diastereomeric with different complex stability, and chromatographic property.

$$\text{Cu(L-Ax)}_2 + \text{L-Ay} \rightleftharpoons \text{Cu(L-Ax)(L-Ay)} + \text{L-Ax}$$
$$\text{Cu(L-Ax)}_2 + \text{D-Ay} \rightleftharpoons \text{Cu(L-Ax)(D-Ay)} + \text{L-Ax}$$

The optical isomers are chromatographed on a reversed-phase column and resolved as the diastereomeric metal complexes of the chiral ligand added to the mobile phase. This approach, was previously applied to the separation of dansyl derivatives of D- and L-amino acids[1–7]. In a continuing effort to resolve enantiomers by the mixed chelation approach, we achieved resolution of primary amino acids by reacting with *ortho*-phthalaldehyde (OPA) in the presence of N-acetyl-L-cysteine (NAC), followed by analysis of these derivatives by complexing with L-proline and Cu(II) on a reversed-phase column[8]. In this study, we report the separation of chiral amino alcohols with the same approach.

OPA derivatization is a favorite method for detecting amines and amino acids emerging from high-performance liquid chromatographic (HPLC) columns for many years[9]. Recently, OPA was also used as a pre-column derivatization agent for the analysis of amino acids by reversed-phase chromatography[10,11]. Primary amine reacts stoichiometrically with OPA in alkaline medium in the presence of a thiol[12]. The thiol commonly used is mercaptoethanol. However, chiral amines derivatized with OPA and mercaptoethanol are not isomerically resolved by HPLC on a reversed-phase column. Aswad[13] in an attempt to resolve optical isomers of the OPA derivatives substituted NAC for mercaptoethanol in the derivatization reaction, transformed the OPA derivative from enantiomer to diastereomer, and resolved D- and L-aspartic acid on a reversed-phase column.

HPLC of enantiomeric amines, especially those of pharmaceutical interest, has

* Presented at the *10th International Symposium on Column Liquid Chromatography, San Francisco, CA, May 18–23, 1986.*

0021-9673/86/$03.50

been done by a variety of approaches. Chiral amines and amino alcohols have been resolved by chromatography using organic mobile phases on a host of chiral stationary phases based on π–π solute sorbent interaction[14–17]. Karger and co-workers[18] also separated isomers of primary amino alcohols by derivatization with salicylaldehydes, followed by metal complexation and ligand-exchange chromatography. Amines were also converted from the enantiomers to diastereomers by reacting with chiral derivatization agents and separated as the diastereomeric amides of 1-[(4-nitrophenyl)sulfonyl]proline[19] and thioureas of 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate[20].

EXPERIMENTAL

Reagents

Acetonitrile and methanol, distilled in glass, were bought from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.), and *o*-phthalaldehyde and N-acetyl-L-cysteine were from Sigma (St. Louis, MO, U.S.A.). The free amino acids and amino

TABLE I

CHEMICAL STRUCTURES OF AROMATIC ALCOHOLS, ALIPHATIC ALCOHOLS AND AMINO ACIDS STUDIED

Compound	*R*	*R'*
OH R' | *Aromatic alcohols:* R-CH-CH-NH_2		
Phenylpropanolamine	C_6H_5	CH_3
Normetanephrine	$HO(CH_3OH)C_6H_3$	H
1-Amino-2-propanol	CH_3	H
NH_2 | *Aliphatic alcohols:* R-CH-CH_2OH		
Serinol	$HO\text{-}CH_2$	
Alaninol	CH_3	
Valinol	$(CH_3)_2\text{-}CH$	
Methioninol	$CH_3\text{-}S\text{-}(CH_2)_2$	
Norvalinol	$CH_3\text{-}(CH_2)_2$	
NH_2 | *Amino acids:* R-CH-COOH		
Serine	$HO\text{-}CH_2$	
Alanine	CH_3	
Valine	$(CH_3)_2\text{-}CH$	
Methionine	$CH_3\text{-}S\text{-}(CH_2)_2$	
Norvaline	$CH_3\text{-}(CH_2)$	
2-Amino-4-pentenoic acid	$H_2C{=}CH\text{-}CH_2$	
Leucine	$(CH_3)_2\text{-}CH\text{-}CH_2$	
β-Phenylserine	$C_6H_5\text{-}CH(OH)$	
α-Aminopimelic acid	$HO_2C(CH_2)_4$	
Phenylalanine	$C_6H_5\text{-}CH_2$	

alcohols were from Sigma or Fluka (Hauppauge, NY, U.S.A.). The chemical structures of the amines studied are shown in Table I. The mobile phases generally contained various concentrations of acetonitrile in a buffer that was 5.0 m*M* of L-proline, 2.5 m*M* of copper sulfate and 5.0 g/l of ammonium acetate. The buffer was adjusted to pH 7.0 with ammonium hydroxide.

Instrumentation

The HPLC system consisted of two Altex 110A pumps and a Model 420 gradient micropressor (Altex Scientific, Berkeley, CA, U.S.A.). The analytical column, 15 × 0.42 cm I.D., was packed with Nucleosil 5 C_{18} by the downward slurry technique. Samples were introduced by a Rheodyne 7105 injection valve. The OPA amino acids were detected with a Fluoro-tec filter fluorimeter (American Research Products, Kensington, MD, U.S.A.). The amplified detector signals were read out on a Model 4416 data system (Nelson Analytical, Cupertino, CA, U.S.A.) and a Model 56 chart recorder (Perkin Elmer, Norwalk, CT, U.S.A.).

Derivatization procedure

The OPA–NAC reagent was 25 m*M* of *ortho*-phthalaldehyde and N-acetyl-L-cysteine in equal molar concentrations dissolved in methanol–water (50:50). Derivatization of the free amino acids and amino alcohols was accomplished in a vial by dispensing 300 μl of 1.0 *M* sodium borate buffer pH, 9.4, 50 μl of the OPA–NAC reagent and 5 μl of a stock solution of the amino acid that was 5 m*M*. After mixing and standing at room temperature for 2 min, a 50-μl aliquot of the reaction mixture was injected into the HPLC system.

RESULTS

The enantiomers of amino acids and amino alcohols derivatized with OPA–NAC were resolved as the mixed complexes of Cu(II)–L-proline on a reversed-phase column (Tables II and III). The chromatograms of aromatic and aliphatic amines are shown with the D-isomers more retained (Figs. 1 and 2). The retention behavior of amino acids and amino alcohols are different (Fig. 3, Tables II and III). The amino

TABLE II

CAPACITY RATIO (*k'*), SELECTIVITY (α) AND RESOLUTION (R_s) OF OPA–NAC DERIVATIVES OF AROMATIC AMINO ALCOHOLS AND AMINO ACIDS

Mobile phase: 15% acetonitrile in a buffer containing 5 m*M* L-proline, 2.5 m*M* copper sulfate pentahydrate and 5.0 g/l ammonium acetate, pH 7.0. Flow-rate 2.0 ml/min.

Amino compound	*k' (1)*	*k' (2)*	α	R_s	*Config. (2)*
β-phenylserine (*threo*)	5.71	7.40	1.30	2.80	D, [1*R*,2*S*]
Normetanephrine	17.67	19.04	1.08	1.00	D, [*R*(+)]
Phenylpropanolamine	52.98	61.98	1.17	3.00	D, [1*S*,2*R*(+)]
Tyrosine	2.89	3.58	1.24	1.73	D, [*R*(+)]
Phenylalanine*	21.69	21.69	1.00	1.10	D, [*R*(+)]

* Configuration and resolution determined at 12.5% acetonitrile.

TABLE III

CAPACITY RATIO (k'), SELECTIVITY (α) AND RESOLUTION (R_s) OF OPA–NAC DERIVATIVES OF ALIPHATIC AMINO ALCOHOLS AND AMINO ACIDS

Mobile phase: 12.5% acetonitrile in a buffer containing 5 mM L-proline, 2.5 mM copper sulfate pentahydrate and 5.0 g/l ammonium acetate, pH 7.0. Flow-rate 2.0 ml/min.

Amino compound	k' (L)	k' (D)	α	(R_s)
Serinol*	3.90	3.90	1.00	–
1-Amino-2-propanol	10.60	11.08	1.05	0.60
Alaninol	14.54	17.11	1.18	2.70
Methioninol	63.01	73.61	1.17	1.70
Valinol	87.23	96.25	1.10	2.00
Norvalinol	98.40	117.95	1.20	3.70
2-Amino-4-pentenoic acid	7.88	9.45	1.20	1.80
Alanine	6.83	8.12	1.19	2.30
Valine	8.19	12.82	1.56	4.30
Methionine	12.65	14.63	1.16	1.80
Norvaline	14.18	17.07	1.20	2.40
Ethionine	29.33	33.49	1.14	2.60
Leucine	38.84	47.31	1.22	2.40
Norleucine	38.16	45.54	1.19	2.40
Tryptophan	30.17	40.29	1.34	4.90

* Not optically active.

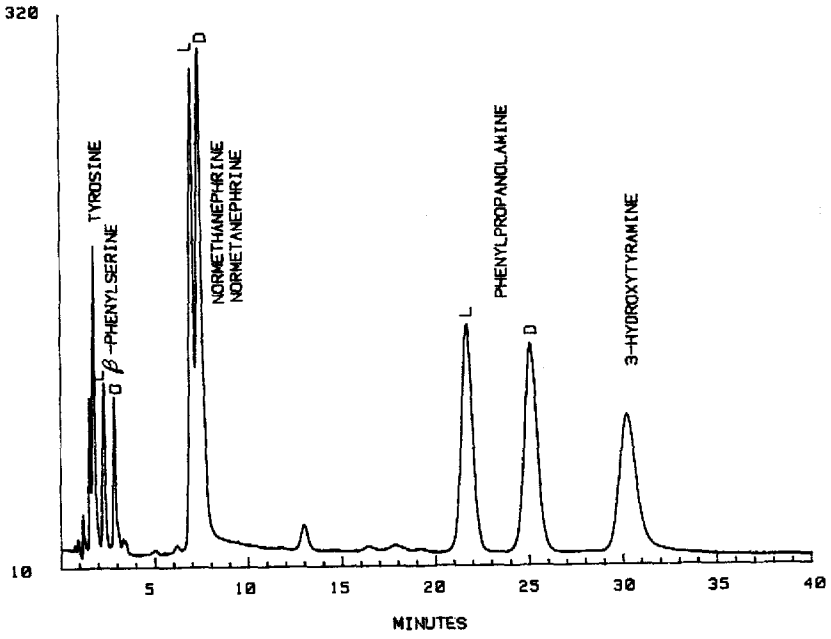

Fig. 1. Chiral separation of OPA–NAC derivatives of D,L-tyrosine, D,L-β-phenylserine, D,L-normetanephrine and D,L-phenylpropanolamine. Mobile phase: 17.5% acetonitrile in a buffer containing 5 mM L-proline, 2.5 mM copper sulfate pentahydrate and 5.0 g/l ammonium acetate, pH 7.0. Flow-rate: 2.0 ml/min.

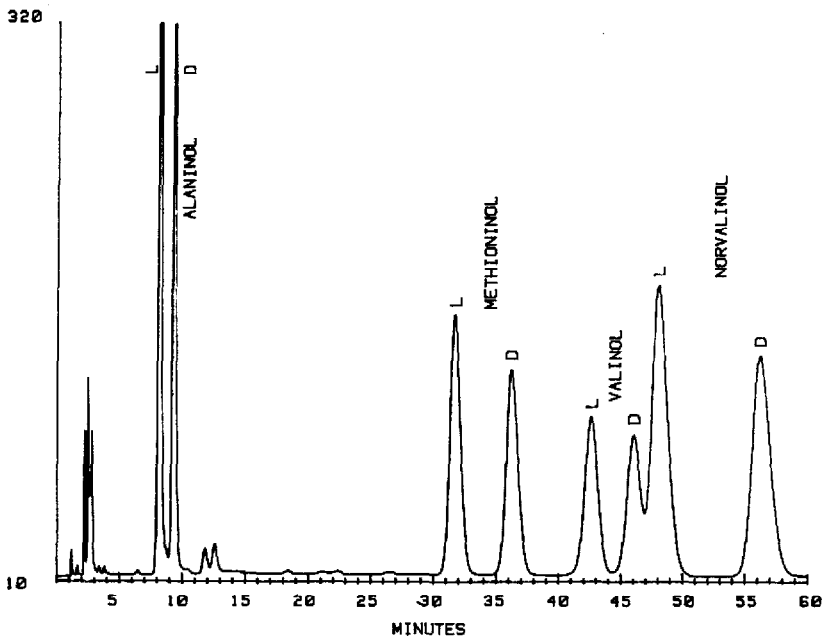

Fig. 2. Chiral separation of OPA–NAC derivatives of D,L-alaninol, D,L-methioninol, D,L-valinol, D,L-norvalinol. Mobile phase: 15.0% acetonitrile in a buffer containing 5 m*M* L-proline, 2.5 m*M* copper sulphate pentahydrate and 5.0 g/l ammonium acetate, pH 7.0. Flow-rate: 2.0 ml/min.

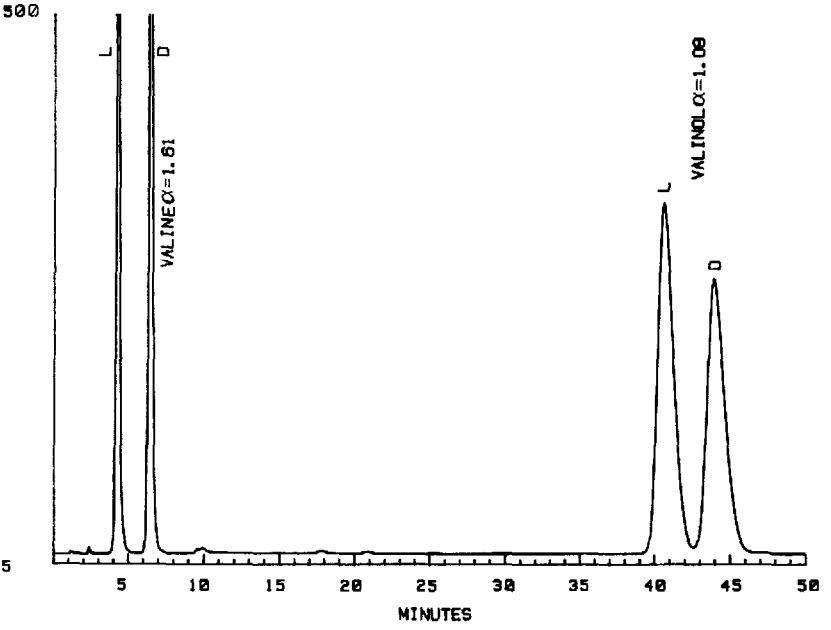

Fig. 3. Chiral separation of D,L-valine and D,L-valinol. Conditions as in Fig. 1. The valinol was strongly retained but the chiral separation was less.

TABLE IV

EFFECT OF ACETONITRILE CONCENTRATION ON THE RETENTION OF AROMATIC AMINO ALCOHOLS AND AMINO ACIDS

Mobile phase: specified percent of acetonitrile in a buffer containing 5 m*M* L-proline, 2.5 m*M* copper sulfate pentahydrate and 5.0 g/l ammonium acetate, pH 7.0 flow-rate 2.0 ml/min.

Amino compound	*12.5% Acetonitrile*			*15.0% Acetonitrile*			*17.5% Acetonitrile*		
	k' (L)	*k'* (D)	α	*k'* (L)	*k'* (D)	α	*k'* (L)	*k'* (D)	α
β-Phenylserine (*threo*)	7.83	9.93	1.27	5.71	7.40	1.30	1.89	2.58	1.36
Normetanephrine	30.33	32.89	1.08	17.67	19.04	1.08	7.52	8.00	1.06
Phenylpropanolamine	99.69	117.87	1.18	52.98	61.98	1.17	25.27	29.33	1.16
Tyrosine	4.82	6.59	1.37	2.89	3.58	1.24	1.25	1.25	1.30
Phenylalanine	33.78	35.30	1.04	21.69	21.69	1.00	9.00	9.00	1.00

TABLE V

EFFECT OF ACETONITRILE CONCENTRATION ON THE RETENTION OF ALIPHATIC AMINO ALCOHOLS AND AMINO ACIDS

Mobile phase, see Table IV

	12.5% Acetonitrile			*15.0% Acetonitrile*			*17.5% Acetonitrile*		
	k' (L)	*k'* (D)	α	*k'* (L)	*k'* (D)	α	*k'* (L)	*k'* (D)	α
Serinol	3.90	3.90	1.00	2.10	2.10	1.00	–	–	–
1-Amino-2-propanol	10.60	11.08	1.05	6.55	6.87	1.05	3.42	3.42	1.00
Alaninol	14.54	17.11	1.18	8.88	10.24	1.15	4.66	5.39	1.16
Methioninol	63.01	73.61	1.17	37.11	42.58	1.15	17.92	20.17	1.13
Valinol	87.23	96.25	1.10	50.24	54.30	1.08	23.66	25.35	1.07
Norvalinol	98.40	117.95	1.20	56.47	66.19	1.17	26.67	30.48	1.14
2-Amino-4-pentenoic acid	7.88	9.45	1.20	4.27	4.87	1.14	–	–	–
Alanine	6.83	8.12	1.19	1.33	1.33	1.00	0.65	0.65	1.00
Valine	8.19	12.82	1.56	4.27	6.87	1.61	1.73	2.77	1.60
Methionine	12.65	14.63	1.16	6.87	7.71	1.12	2.89	3.18	1.10
Norvaline	14.18	17.07	1.20	8.55	9.81	1.15	3.22	3.54	1.10
Ethionine	29.33	33.49	1.14	17.19	18.96	1.10	7.11	7.71	1.16
Leucine	38.84	47.31	1.22	20.72	24.06	1.16	9.33	10.45	1.12
Norleucine	38.16	45.54	1.19	21.98	27.76	1.26	9.33	10.29	1.10
Tryptophan	30.17	40.29	1.34	22.82	22.82	1.00	–	–	–

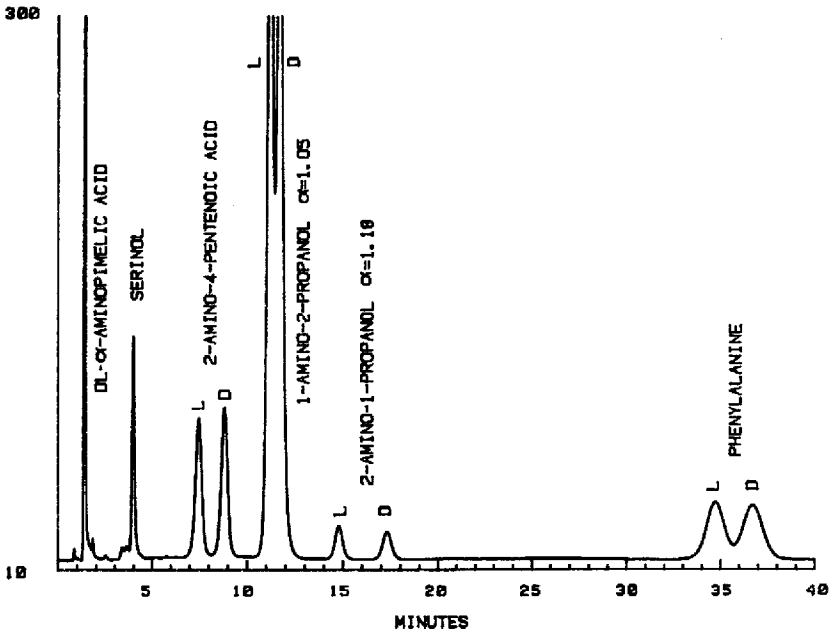

Fig. 4. HPLC of OPA–NAC derivatives of D,L-α-aminopimelic acid (not resolved here); serinol (not optically active); D,L-2-amino-4-pentenoic acid; D,L-amino-2-propanol; D,L-2-amino-1-propanol; D,L-phenylalanine. Mobile phase: 12.5% acetonitrile in a buffer containing 5 m*M* L-proline, 2.5 m*M* copper sulfate pentahydrate and 5.0 g/l ammonium acetate, pH 7.0. Flow-rate; 2.0 ml/min. Note the effect of the position of the amino group in amino-propanol on retention and chiral separation of aminopropanol.

alcohols are more hydrophobic and are strongly retained. The retention behavior is also dependent on the immediate substituents on the chiral carbon as the four isomers of aminopropanol demonstrated (Fig. 4). As in other reversed-phase systems, lowering the acetonitrile concentration usually resulted in longer retention of the solutes and better resolution of the optical isomers (Tables IV and V).

DISCUSSION

In our previous works, we have extended the mixed chelation approach for the separation of enantiomers of dansyl derivatives to the OPA–NAC derivatives of amino acids[8]. D- and L-isomers of all common amino acids after pre-column derivatization with OPA in the presence of NAC were separated on a reversed-phase column. Since the isoindole obtained by the reaction of OPA and NAC and an amino acid suggested that the carboxylate and the nitrogen of the N-acetylamino group of cysteine could form bi-dentate Cu(II) complexes, this technique for the isomeric resolution of amino acids is also applicable to amines.

The separation of the OPA–NAC amines generally follows the pattern that we observed previously with the OPA–NAC amino acids. The order of elution conforms to that of reversed-phase chromatography: the higher the carbon content, the bulkier the alkyl substituent on the asymmetric carbon, the longer the retention (Tables II

and III). Since the stereoselectivity is dependent on the retention of the diastereomeric metal complex on the C_{18} hydrocarbon stationary phase, the acetonitrile concentration of the mobile phase would thus affect both the capacity ratio (k') and the selectivity (α) of the OPA derivatives of amines. Lowering the acetonitrile content of the mobile phase promotes hydrophobic interaction of the solute and sorbent, resulting in longer retention and improved resolution of the optical isomers (Tables IV and V). Phenylalanine is one of the many amines which reverted from co-elution at 17.5% acetonitrile to resolution of the enantiomers at lower acetonitrile in the mobile phase.

Amino alcohols, being more hydrophobic, were generally more retained than their corresponding amino acids (Table II and Fig. 3). Nevertheless, optical isomers of amino acids are slightly better resolved than those of amino alcohols, probably because of the influence of the large carboxyl substituent on the chiral center, suggesting that bulkiness of the alkyl substituents plays an important role in the resolution of enantiomers. The separation of the four isomers of 2-aminopropanol and 1-amino-2-propanol further illustrates the role of substituents on isomeric resolution. Diminution of resolution of 1-amino-2-propanol is apparently due to a less substituted optical center by a methylene carbon inserted between the amine and the chiral center.

In conclusion, the procedure described here for the separation of amino alcohols by pre-column derivatization with OPA is well known for its fast and clean reaction that completes in less than 1 min with virtually no detectable by-products. The derivative fluoresces strongly above 450 nm upon excitation at 360 nm. By substitution of the chiral NAC for the commonly used thiols, a diastereomeric derivative is obtained when a primary amine is reacted with OPA. The OPA derivatives are separated on a reversed-phase column with a mobile phase containing Cu(II)–L-proline, and the amines detected with high sensitivity by fluorescence. Reversed-phase chromatography without the addition of copper complexes also effected the separation of the diastereomeric OPA derivatives. However, the resolution is highly dependent on the buffer used, and the chromatographic selectivity is less than that of the mixed chelate complexation approach. The reversed-phase work will be published in a future communication. Baseline resolution of closely related optical isomers like the propanolamines further demonstrates the selectivity of the mixed chelation approach which based its separation mechanisms both on metal complexation and reversed-phase hydrophobic interaction.

REFERENCES

1 S. Lam and F. Chow, *J. Liq. Chromatogr.*, 3 (1980) 1579.
2 S. Lam, F. Chow and A. Karmen, *J. Chromatogr.*, 199 (1980) 295.
3 S. Lam and A. Karmen, *J. Chromatogr.*, 239 (1982) 451.
4 S. Lam and A. Karmen, *J. Chromatogr.*, 289 (1984) 339.
5 S. Lam, H. Azumaya and A. Karmen, *J. Chromatogr.*, 302 (1984) 21.
6 S. Lam, *J. Chromatogr. Sci.*, 22 (1984) 416.
7 S. Lam and A. Karmen, *J. Liq. Chromatogr.*, 9 (1986) 291.
8 S. Lam, *J. Chromatogr.*, 355 (1986) 157.
9 J. R. Cronin and P. E. Hare, *Anal. Biochem.*, 81 (1977) 151.

10 H. Umagat, P. Kucera and L.-F. Wen, *J. Chromatogr.*, 239 (1982) 463.
11 B. N. Jones and J. P. Gilligan, *J. Chromatogr.*, 266 (1983) 471.
12 S. S. Simons, Jr. and D. F. Johnson, *J. Org. Chem.*, 43 (1978) 2886.
13 D. W. Aswad, *Anal. Biochem.*, 137 (1984) 405.
14 W. H. Pirkle and D. W. House, *J. Org. Chem.*, 44 (1979) 1957.
15 W. H. Pirkle, G. Hahler and M. H. Hyun, *J. Liq. Chromatogr.*, 9 (1986) 443.
16 N. Ôi, M. Nagase, Y. Inda and T. Doi, *J. Chromatogr.*, 265 (1983) 111.
17 T. D. Doyle, W. M. Adams, F. S. Fry, Jr. and I. W. Wainer, *J. Liq. Chromatogr.*, 9 (1986) 455.
18 L. R. Gelber, B. Feibush, J. L. Neumeyer and B. L. Karger, *The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Marach 5–9, 1984, Atlantic City, NY,* Abstract 721.
19 C. R. Clark and J. M. Barksdale, *Anal. Chem.*, 56 (1984) 958.
20 J. Gal, *J. Liq. Chromatogr.*, 9 (1986) 673.