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CRC Handbook of Chromatography

Amino Acids and Amines

Volume II

Stanley Blackburn

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CRC Handbook of Chromatography

Amino Acids and Amines

Volume II

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CRC SERIES IN CHROMATOGRAPHY

SERIES PREFACE

This is the third volume in this series written by Dr. Stanley Blackburn. In 1983, the first volume on amino acids and amines was published, and in 1986, Volume I on peptides appeared. The current volume updates the coverage of methods and data for the gas and liquid chromatography of amino acids and amines. It includes a large amount of material on the resolution of optical isomers, which has been one of the most important areas of research for many compound types in recent years.

It is hoped that Dr. Blackburn will produce Volume II on peptides when this is justified by the amount of available new data.

I would appreciate hearing from readers who can offer corrections or comments on the present volume as well as suggestions for topics and authors of future volumes in the CRC Handbook of Chromatography series.

Joseph Sherma, Ph.D.
Easton, PA

PREFACE

The phenomenal rapid increase in the growth of chemical and biochemical research and information is now a matter of common knowledge. This increase in research has been accompanied by a comparable increase in the field of amino acid analysis. The original *Handbook of Chromatography* provided tables relating to the chromatography of a very wide range of chemical compounds. In the early 1970s such a compilation in one volume was no longer feasible and individual volumes dealing with specific classes of compounds including amino acids and amines (Volume I) were necessary. The continuing escalation of research in the amino acid and amine field is shown by the publication of *Amino Acids and Amines, Volume II* after a relatively short interval. During this period the ion exchange method pioneered by Moore and Stein, and its later development, is still the method of choice for the majority of research workers wishing to determine the amino acid composition of proteins and peptides. Alternative techniques involving high performance liquid chromatography of amino acid derivatives, however, are being intensively studied and may well be increasingly used in the future. The material in the present volume represents a continuation of that presented in *Amino Acids and Amines, Volume I*, and principally covers the literature since 1981. In addition to the extensive tables of chromatographic data, methods of sample preparation and derivatization and methods of detection are described. A further section of the volume reviews techniques used for the chromatographic separation of free amino acids and their enantiomers, gas chromatographic separations, and the separation of amino acid derivatives. Specific methods for the determination of proline and hydroxyproline are described. A section listing chromatographic materials draws the reader's attention to commercial sources of supply, especially for materials relevant to separations described in the present volume.

S. Blackburn

THE EDITORS-IN-CHIEF

Gunter Zweig, Ph.D., received his undergraduate training at the University of Maryland, College Park, where he was awarded the Ph.D. in biochemistry in 1952. Two years following his graduation, Dr. Zweig was affiliated with the late R. J. Block, pioneer in paper chromatography of amino acids. Zweig, Block, and Le Strange wrote one of the first books on paper chromatography, which was published in 1952 by Academic Press and went into three editions, the last one authored by Gunter Zweig and Dr. Joe Sherma, the co-Editor-in-Chief of this series. *Paper Chromatography* (1952) was also translated into Russian.

From 1953 to 1957, Dr. Zweig was research biochemist at the C. F. Kettering Foundation, Antioch College, Yellow Springs, Ohio, where he pursued research on the path of carbon and sulfur in plants, using the then newly developed techniques of autoradiography and paper chromatography. From 1957 to 1965, Dr. Zweig served as lecturer and chemist, University of California, Davis and worked on analytical methods for pesticide residues, mainly by chromatographic techniques. In 1965, Dr. Zweig became Director of Life Sciences, Syracuse University Research Corporation, New York (research on environmental pollution), and in 1973 he became Chief, Environmental Fate Branch, Environmental Protection Agency (EPA) in Washington, D.C. From 1980 to 1984 Dr. Zweig was Visiting Research Chemist in the School of Public Health, University of California, Berkeley, where he was doing research on farmworker safety as related to pesticide exposure.

During his government career, Dr. Zweig continued his scientific writing and editing. Among his works are (many in collaboration with Dr. Sherma) the now 11-volume series on *Analytical Methods for Pesticides and Plant Growth Regulators* (published by Academic Press); the pesticide book series for CRC Press; co-editor of *Journal of Toxicology and Environmental Health;* co-author of basic review on paper and thin-layer chromatography for *Analytical Chemistry* from 1968 to 1980; co-author of applied chromatography review on pesticide analysis for *Analytical Chemistry,* beginning in 1981.

Among the scientific honors awarded to Dr. Zweig during his distinguished career were the Wiley Award in 1977, the Rothschild Fellowship to the Weizmann Institute in 1963/64; and the Bronze Medal by the EPA in 1980.

Dr. Zweig authored or co-authored over 80 scientific papers on diverse subjects in chromatography and biochemistry, besides being the holder of three U.S. patents. In 1985, Dr. Zweig became president of Zweig Associates, Consultants in Arlington, Va.

Following his death on January 27, 1987, the Agrochemicals Section of the American Chemical Society posthumously elected him a Fellow and established the Gunther Zweig Award for Young Chemists in his honor.

Joseph Sherma, Ph.D., received a B.S. in Chemistry from Upsala College, East Orange, N.J., in 1955 and a Ph.D. in Analytical Chemistry from Rutgers University in 1958, carrying on his thesis research in ion exchange chromatography under the direction of the late William Rieman III. Dr. Sherma joined the faculty of Lafayette College in September, 1958, and is presently Charles A. Dana Professor and Head of the Chemistry Department.

Dr. Sherma, independently and with others, has written over 300 research papers, chapters, books, and reviews involving chromatography and other analytical methodology. He is editor for residues and trace elements of the *Journal of the Association of Official Analytical Chemists* and a member of the advisory board of the *Journal of Planar Chromatography.* He is a consultant on analytical methodology for many companies and government agencies.

Dr. Sherma has received two awards for superior teaching at Lafayette College and the 1979 Distinguished Alumnus Award from Upsala College for outstanding achievements as an educator, researcher, author, and editor. He is a member of the ACS, Sigma Xi, Phi Lambda Upsilon, SAS, AIC, and AOAC. Dr. Sherma's current interests are in quantitative TLC, mainly applied to clinical analysis, pesticide residues, and food additives.

THE AUTHOR

Dr. Stanley Blackburn gained his Honours B.Sc. degree in chemistry and his Ph.D. in organic chemistry at the University of Leeds, England. He is a former research scientist at the Wool Industries Research Association, Leeds, where his research interests included the development of chromatographic techniques, the structure and amino acid sequence of the proteins of wool keratin, and the end group determination of peptides and proteins.

Dr. Blackburn is a Chartered Chemist, a Fellow of the Royal Society of Chemistry, and a member of the Biochemical Society and the American Chemical Society. His current work is centered on scientific writing and documentation. He has written more than 30 scientific papers dealing with protein analysis and structure and is the author of several texts, including *Amino Acid Determination: Methods and Techniques, Protein Sequence Determination: Methods and Techniques*, and *Enzyme Strucutre and Function*, all published by Marcel Dekker, Inc. He is also author of two volumes of the *CRC Handbook of Chromatography, Amino Acids Amines, Volume I* and *Peptides, Volume I*.

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Section I

Tables

I.I. Gas Chromatography Tables
I.II. Liquid Chromatography Tables
I.III. Thin Layer Chromatography Tables

Section I

TABLES

Wherever possible tables are arranged according to classes of chemical compounds. This was not always possible when different types of compounds were chromatographed under the same experimental conditions. The reader is referred to the compound index for specific compounds which may appear in different tables.

Table GC 1
THE INFLUENCE OF THE ESTER GROUP ON THE SEPARATION OF *N*-TRIFLUOROACETYL AMINO ACID ESTERS

Column packing	P1	P1	P1	P1
Temperature	T1	T1	T1	T1
Gas Flow rate (mℓ/min)	He, 30	He, 30	He, 30	He, 30
Column				
Length (ft)	6	6	6	6
Diameter (mm, I.D.)	2	2	2	2
Material	G	G	G	G
Detector	FID	FID	FID	FID

	t_r(min)			
Amino acid	**Isopropyl ester**	***n*-Propyl ester**	**Isobutyl ester**	***n*-Butyl ester**
Alanine	0.90	1.11	1.38	1.60
Arginine	5.82	6.18	6.48	6.72
Glutamic acid	4.29	4.87	5.50	5.97
Leucine	1.82	2.22	2.60	2.87
Methionine	3.48	3.92	4.28	4.54
Norleucine	2.13	2.54	2.93	3.20
Phenylalanine	4.08	4.67	5.00	5.25
Proline	2.52	2.98	3.40	3.66
Serine	1.29	1.56	1.87	2.12
Tryptophan-1	8.45	9.20	9.81	10.44
Tryptophan-2	7.09	7.55	7.90	8.31

Note: The incomplete diacylation of tryptophan resulted in two derivatives.

Column packing: P1 = 2% OV-17/1% OV-210 on Supelcoport®, 100-120 mesh
Temperature: T1 = the temperature was raised from 125—135°C at 10°C/min, thereafter at 20°C/min to 220°C where it was held constant for 10 min

REFERENCE

1. **Gamerith, G.,** *J. Chromatogr.*, 268, 403, 1983.

Table GC 2
THE INFLUENCE OF THE ESTER GROUP ON THE SEPARATION OF *N*-HEPTAFLUOROBUTYRYL AMINO ACID ESTERS

Column packing	P1	P1	P1	P1
Temperature	T1	T1	T1	T1
Gas Flow rate (mℓ/min)	He, 30	He, 30	He, 30	He, 30
Column				
Length (ft)	6	6	6	6
Diameter (mm, I.D.)	2	2	2	2
Material	G	G	G	G
Detector	FID	FID	FID	FID

Table GC 2 (continued)
THE INFLUENCE OF THE ESTER GROUP ON THE SEPARATION OF *N*-HEPTAFLUOROBUTYRYL AMINO ACID ESTERS

Amino acid	t_r(min) Isopropyl ester	*n*-Propyl ester	Isobutyl ester	*n*-Butyl ester
Alanine	1.19	1.52	1.94	2.30
Arginine		9.31	9.50	9.74
Glutamic acid	6.50	7.51	8.27	8.86
Leucine	2.64	3.32	3.98	4.51
Methionine	5.56	6.25	6.70	7.08
Norleucine	3.20	3.94	4.61	5.11
Phenylalanine	6.74	7.25	7.63	7.98
Proline	3.79	4.64	5.24	5.68
Serine	2.35	2.91	3.44	3.97
Tryptophan-1	11.16	11.77	12.22	12.73
Tryptophan-2	10.05	10.44	10.70	11.06

Note: The incomplete diacylation of tryptophan resulted in two derivatives.

Column packing: P1 = 2% OV-1/1% OV-210 on Supelcoport®, 100-120 mesh
Temperature: T1 = 120°C for 0.5 min, then raised to 135°C at 5°C/min, then to 220°C at 15°C/min, the final hold time being 13 min

REFERENCE

1. **Gamerith, G.,** *J. Chromatogr.*, 268, 403, 1983.

Table GC 3
THE INFLUENCE OF THE ESTER GROUP ON THE SEPARATION OF *N*-HEPTAFLUOROBUTYRYL AMINO ACID ESTERS

Column packing	P1	P1	P1	P1
Temperature	T1	T1	T1	T1
Gas Flow rate (mℓ/min)	He, 30	He, 30	He, 30	He, 30
Column				
Length (ft)	6	6	6	6
Diameter (mm, I.D.)	2	2	2	2
Material	G	G	G	G
Detector	FID	FID	FID	FID

Amino acid	t_r(min) Isopropyl ester	*n*-Propyl ester	Isobutyl ester	*n*-Butyl ester
Alanine	1.44	1.94	2.49	2.88
Glutamic acid	7.20	7.95	8.56	8.87
Leucine	3.05	3.62	4.43	4.84
Methionine	6.21	6.70	7.06	7.36
Norleucine	3.23	4.02	4.76	5.07
Phenylalanine	6.82	7.32	7.50	7.81
Proline	4.18	4.89	5.45	5.82
Serine	0.91	0.64	1.44	1.66
Tryptophan-2	13.86	15.00	15.90	16.59

Table GC 3 (continued)
THE INFLUENCE OF THE ESTER GROUP ON THE SEPARATION OF *N*-HEPTAFLUOROBUTYRYL AMINO ACID ESTERS

Note: The incomplete diacylation of tryptophan resulted in two derivatives.

Column packing: P1 = 0.31% Carbowax® 20M/0.28% Silar® 5 CP/0.06% Lexan® on Chromosorb® W AW, 120-140 mesh
Temperature: T1 = 120°C for 0.5 min, raised at 5°C/min to 135°C and then at 15°C/min to 220°C and held constant for 10 min

REFERENCE

1. **Gamerith, G.,** *J. Chromatogr.*, 268, 403, 1983.

Table GC 4
THE INFLUENCE OF THE ACYL GROUP ON THE SEPARATION OF *N*-ACYL AMINO ACID *n*-PROPYL ESTERS

Column packing	P1	P1
Temperature	T1	T1
Gas Flow rate (mℓ/min)	He, 30	He, 30
Column		
Length (ft)	6	6
Diameter (mm, I.D.)	2	2
Material	G	G
Detector	FID	FID

	t_r(min)	
Amino acid	**TFA**	**HFB**
Alanine	1.22	1.52
Glutamic acid	7.30	7.51
Leucine	2.84	3.32
Methionine	5.86	6.25
Norleucine	3.38	3.94
Phenylalanine	6.96	7.25
Proline	4.19	4.64
Serine	1.86	2.91

Abbreviations: TFA = trifluoroacetyl; HFB = heptafluorobutyryl

Column packing: P1 = 2% OV-17/1% OV-210 on Supelcoport®, 100-120 mesh
Temperature: T1 = 120°C for 0.5 min, then raised to 135°C at 5°C/min, then to 220°C at 15°C/min, the final hold time being 13 min

REFERENCE

1. **Gamerith, G.,** *J. Chromatogr.*, 268, 403, 1983.

Table GC 5
THE INFLUENCE OF THE ACYL GROUP ON THE SEPARATION OF *N*-ACYL AMINO ACID ISOPROPYL ESTERS

Column packing	P1	P1
Temperature	T1	T1
Gas Flow rate (mℓ/min)	He, 30	He, 30
Column		
Length (ft)	6	6
Diameter (mm, I.D.)	2	2
Material	G	G
Detector	FID	FID

	t_r(min)	
Amino acid	**TFA**	**HFB**
Alanine	1.58	2.05
Glutamic acid	7.89	8.12
Leucine	3.75	4.46
Methionine	6.95	7.37
Norleucine	4.49	5.19
Phenylalanine	8.10	8.41
Proline	5.25	5.77
Serine	2.52	4.08

Abbreviations: TFA = trifluoroacetyl; HFB = heptafluorobutyryl

Column packing: P1 = 0.65% EGA on Chromosorb® W AW, 80-100 mesh

Temperature: T1 = held at 115°C for 1 min, then raised to 118°C at 1.5°C/min and to 210°C at 15°C/min, then held at this temperature for 15 min

REFERENCE

1. **Gamerith, G.,** *J. Chromatogr.*, 268, 403, 1983.

Table GC 6
THE INFLUENCE OF THE ACYL GROUP ON THE SEPARATION OF *N*-ACYL AMINO ACID *n*-BUTYL ESTERS

Column packing	P1	P1	P2	P2
Temperature	T1	T1	T2	T2
Gas Flow rate (mℓ/min)	He, 30	He, 30	He, 30	He, 30
Column				
Length (ft)	6	6	6	6
Diameter (mm, I.D.)	2	2	2	2
Material	G	G	G	G
Detector	FID	FID	FID	FID

Table GC 6 (continued)
THE INFLUENCE OF THE ACYL GROUP ON THE SEPARATION OF *N*-ACYL AMINO ACID *n*-BUTYL ESTERS

Amino acid	t_r(min)			
	TFA	HFB	TFA	HFB
Alanine	1.12	1.58	3.82	2.55
Glutamic acid	7.42	7.69	9.42	7.97
Leucine	2.62	3.39	4.44	3.16
Methionine	5.40	5.94	7.93	6.72
Norleucine	3.19	4.00	4.81	3.56
Phenylalanine	6.39	6.83	8.53	7.30
Proline	3.99	4.59	6.41	5.24
Serine	1.66	2.95	8.84	7.63

Abbreviations: TFA = trifluoroacetyl; HFB = heptafluorobutyryl

Column packing: P1 = 3% SP 2100 on Supelcoport®, 100-120 mesh
P2 = 5% Carbowax® 20M on Supelcoport®, 100-120 mesh
Temperature: T1 = 120°C increased to 130°C at 5°C/min and to 220°C at 15°C/min, then held at this temperature for 15 min
T2 = 130°C for 0.5 min, then raised to 135°C at 5°C/min and to 220°C at 15°C/min and held at this temperature for 13 min

REFERENCE

1. **Gamerith, G.,** *J. Chromatogr.*, 268, 403, 1983.

Table GC 7
ENANTIOMERS OF *N*-TRIFLUOROACETYL ISOPROPYL ESTERS OF α- AND γ-AMINO ACIDS ON *N*-LAUROYL-L-VALINE *TERT*-OCTYLAMIDE

Column packing	P1	P1	
Gas Flow rate (mℓ/min)	He, 3	He, 0.5—1.5	
Column			
Length (m)	50	9	
Diameter (mm, I.D.)	0.5	0.35	
Material	SS[a]	G[b]	
Detector	FID	FID	

Amino acid	Isomer	t_r(min)	T(°C)	t_r(min)	T(°C)
Protein α-Amino Acids					
Ala	D	5.50	130	6.96	115
	L	6.20		7.98	
Thr	D	8.70	130	11.34	115
	L	9.50		12.60	
Val	D	8.68	130	11.43	115
	L	9.38		12.48	
Gly		10.80	130	14.22	115
allo-Ile	D	12.50	130	16.59	115
	L	13.80		18.48	
Ile	D	13.80	130	18.48	115
	L	15.04		20.46	
Ser	D	18.28	130	23.94	115
	L	19.92		26.31	

Table GC 7 (continued)
ENANTIOMERS OF *N*-TRIFLUOROACETYL ISOPROPYL ESTERS OF α- AND γ-AMINO ACIDS ON *N*-LAUROYL-L-VALINE *TERT*-OCTYLAMIDE

Amino acid	Isomer	t_r(min)	T(°C)	t_r(min)	T(°C)
Protein α-Amino Acids					
Leu	D	18.04	130	25.68	115
	L	21.40		31.62	
Pro	D	19.30	130	26.13	115
	L	20.00		26.73	
Asp	D	55.70	130	84.15	115
	L	57.36		86.85	
Met	D	21.18	170	31.26	150
	L	23.06		34.38	
Glu	D	28.08	170	44.28	150
	L	30.10		47.73	
Phe	D	29.80	170	44.70	150
	L	31.80		48.37	
Orn	D	104.80	180		
	L	114.80			
Lys	D	150.00	180		
	L	161.50			
Nonprotein α-Amino Acids					
α-Aminobutanoic acid	D	7.90	130	9.90	115
	L	8.74		11.28	
α-Aminopentanoic acid	D	12.70	130	17.01	115
	L	14.40		19.86	
α-Aminohexanoic acid	D	21.28	130	28.56	115
	L	24.30		33.69	
α-Aminoheptanoic acid	D	27.10	140	51.36	115
	L	30.40		60.36	
α-Aminooctanoic acid	D	45.90	140	93.24	115
	L	51.32		109.71	
tert-Leucine	D	22.00	100		
	L	22.84			
Phenylglycine	D	33.90	150	23.40	150
	L	35.50		24.30	
γ-Amino Acids					
γ-Aminopentanoic acid	L	30.64	130	40.86	115
	D	31.90		42.45	
γ-Amino-δ-methylhexanoic acid	L	62.86	130	28.02	142
	D	66.20		29.82	
γ-Amino-ε-methylheptanoic acid	L	108.50	130	46.80	142
	D	115.88		49.59	

[a] Capillary column coated by the plug method with a 5% solution of the stationary phase in chloroform.

[b] Whisker-walled capillary washed with chloroform and dichloromethane and dried with nitrogen gas. The capillary was then coated using a 10% solution of the stationary phase in dichloromethane and was preconditioned for 1 day at 170°C.

Column packing: P1 = *N*-lauroyl-L-valine *tert*-octylamide

Table GC 7 (continued)
ENANTIOMERS OF *N*-TRIFLUOROACETYL ISOPROPYL ESTERS OF α- AND γ-AMINO ACIDS ON *N*-LAUROYL-L-VALINE *TERT*-OCTYLAMIDE

REFERENCE

1. **Charles, R. and Watabe, K.,** *J. Chromatogr.*, 298, 253, 1984.

Table GC 8
ENANTIOMERS OF *N*-TRIFLUOROACETYL ISOPROPYL ESTERS OF α- AND γ-AMINO ACIDS ON *N*-DOCOSANOYL-L-LEUCINE *TERT*-OCTYLAMIDE

Column packing	P1	P1
Gas Flow rate (mℓ/min)	He, 3	He, 0.5—1.5
Column		
Length (m)	50	9
Diameter (mm, I.D.)	0.5	0.35
Material	SS[a]	G[b]
Detector	FID	FID

Amino acid	Isomer	t_r(min)	T(°C)	t_r(min)	T(°C)
Protein α-Amino Acids					
Ala	D	4.30	120	3.18	130
	L	5.10		3.63	
Thr	D	4.84	120	4.20	130
	L	5.40		4.52	
Val	D	5.40	120	5.19	130
	L	6.04		5.70	
Gly		7.30	120	5.97	130
allo-Ile	D	8.20	120	7.68	130
	L	9.10		8.46	
Ile	D	9.10	120	8.46	130
	L	10.10		9.18	
Ser	D	9.50	120	8.78	130
	L	10.30		9.31	
Leu	D	11.30	120	10.32	130
	L	13.70		12.12	
Pro	D	14.00	120	13.29	130
	L	14.00		13.29	
Asp	D	36.84	120	46.14	130
	L	38.24		48.18	
Met	D	11.60	170	22.32	160
	L	12.60		24.18	
Glu	D	16.06	170	30.00	160
	L	17.16		32.22	
Phe	D	18.24	170	32.88	160
	L	19.30		35.19	
Tyr	D	24.20	170	57.00	160
	L	26.00		62.46	
Orn	D	22.44	200	32.49	195
	L	23.80		33.87	
Lys	D	31.26	200	47.55	195
	L	32.68		48.81	

Table GC 8 (continued)
ENANTIOMERS OF *N*-TRIFLUOROACETYL ISOPROPYL ESTERS OF α- AND γ-AMINO ACIDS ON *N*-DOCOSANOYL-L-LEUCINE *TERT*-OCTYLAMIDE

Amino acid	Isomer	t_r(min)	T(°C)	t_r(min)	T(°C)
Protein α-Amino Acids					
Nonprotein α-Amino Acids					
α-Aminobutanoic acid	D	5.02	120	4.68	130
	L	5.74		5.25	
α-Aminopentanoic acid	D	8.10	120	7.62	130
	L	9.60		8.76	
α-Aminohexanoic acid	D	13.10	120	12.45	130
	L	15.70		14.28	
α-Aminoheptanoic acid	D	15.00	130	21.69	130
	L	17.30		24.87	
α-Aminooctanoic acid	D	25.90	130	37.80	130
	L	29.86		43.44	
tert-Leucine	D	9.30	100		
	L	10.06			
Phenylglycine	D	18.26	150	17.70	160
	L	19.16		18.36	
γ-Amino Acids					
γ-Aminopentanoic acid	L	12.60	130	18.00	130
	D	12.90		18.54	
γ-Amino-δ-methylhexanoic acid	L	24.30	130	36.45	130
	D	26.10		39.45	
γ-Amino-ε-methylheptanoic acid	L	41.40	130	61.83	130
	D	44.50		66.24	

[a] Capillary column coated by the plug method with a 5% solution of the stationary phase in chloroform.

[b] Whisker-walled capillary washed with chloroform and dichloromethane and dried with nitrogen gas. The inner surface of the capillary was deactivated with benzyltriphosphonium chloride at 350°C for 3 hr and coated using a 10% solution of the stationary phase in dichloromethane. It was then preconditioned for 1 day at 200°C.

Column packing: P1 = *N*-docosanoyl-L-leucine *tert*-octylamide

REFERENCE

1. **Charles, R. and Watabe, K.,** *J. Chromatogr.*, , 298, 253, 1984.

Reproduced from Charles, R. and Watabe, K., *J. Chromatogr.*, 298, 253, 1984. With permission.

Table GC 9
ENANTIOMERS OF *N*-TRIFLUOROACETYL ISOPROPYL ESTERS OF α-AMINO ACIDS ON *N*-LAUROYL-L-VALINE *TERT*-OCTYLAMIDE AT 115°C

Column packing	P1	P1
Temperature (°C)	115	115
Gas Flow rate (mℓ/min)	He, 3	He, 0.5—1.5

Table GC 9 (continued)
ENANTIOMERS OF *N*-TRIFLUOROACETYL ISOPROPYL ESTERS OF α-AMINO ACIDS ON *N*-LAUROYL-L-VALINE *TERT*-OCTYLAMIDE AT 115°C

Column		
Length (m)	50	9
Diameter (mm, I.D.)	0.5	0.35
Material	SS[a]	G[b]
Detector	FID	FID
Amino acid	**t_r(min)**	
D-Alanine	8.70	6.96
L-Alanine	10.10	7.98
D-Allo-isoleucine	21.20	16.59
L-Allo-isoleucine	23.60	18.48
D-Isoleucine	23.60	18.48
L-Isoleucine	26.26	20.46
D-Leucine	32.40	25.68
L-Leucine	40.20	31.62
D-Proline	31.00	26.13
L-Proline	32.40	26.73
D-Valine	14.40	11.43
L-Valine	15.80	12.48

[a] Capillary column coated by the plug method with a 5% solution of the stationary phase in chloroform.

[b] Whisker-walled capillary washed with chloroform and dichloromethane and dried with nitrogen gas. The capillary was then coated using a 10% solution of the stationary phase in dichloromethane and was preconditioned for 1 day at 170°C.

Column packing: P1 = *N*-lauroyl-L-valine *tert*-octylamide

REFERENCE

1. **Charles, R. and Watabe, K.,** *J. Chromatogr.*, 298, 253, 1984.

Table GC 10
(R)- AND (*S*)-*N*-TRIFLUOROACETYL AMINO ACID ISOPROPYL ESTERS

Column packing	P1
Temperature (°C)	120
Gas Flow rate (mℓ/min)	N_2, 20
Column	
Length (m)	4
Diameter (mm, I.D.)	2

Table GC 10 (continued)
(R)- AND (*S*)-*N*-TRIFLUOROACETYL AMINO ACID ISOPROPYL ESTERS

Amino acid	t_r(min) R	S
Alanine	22	26
Leucine	78	99
Phenylalanine[a]	290	345
Valine	34	40

[a] Temperature = 130°C, flow rate = 30 mℓ/min.

Column packing: P1 = 10% optically active SP-300 on Supelcoport® 100-120 mesh

REFERENCE

1. **Deschenaux, R. and Bernauer, K.,** *Helv. Chim. Acta*, 67, 373, 1984.

Table GC 11
TRIFLUOROACETYL AMINO ACID *N*-BUTYL ESTERS

Column packing	P1
Temperature	T1
Gas Flow rate (mℓ/min)	H_2, 2
Column	
Length (m)	30
Diameter (mm)	0.33
Form	Capillary
Detector	FID

Amino acid	I
N-α-Acetyllysine	2019
Alanine	1159
2-Aminoadipic acid	1868
α-Aminoisobutyric acid	1235
β-Aminoisobutyric acid	1282
Aspartic acid + asparagine	1676
Creatinine	1440
Glutamic acid + glutamine	1807
Glycine	1173
Glycylproline	1931
Histidine (monoacyl)	1983
Hydroxyproline	1489
Isoleucine	1358
Leucine	1349
Lysine	1811
Methionine	1568
1-Methylhistidine + 3-methylhistidine	1888

Table GC 11 (continued)
TRIFLUOROACETYL AMINO ACID *N*-BUTYL ESTERS

Amino acid	I
N-α-Methyllysine	1851
N-Methylproline	1328
Nicotinic acid	1384
Norleucine[a]	1389
Ornithine	1690
Phenylalanine	1678
Phenylglycine	1584
Prolylhydroxyproline	2058
Pyroglutamic acid	1505
Serine	1257
Threonine	1246
N-TFA-Tyrosine	1976
N(*O*)-TFA-tyrosine	1769
Valine	1280

[a] Internal standard.

Column packing: P1 = OV-101 coated capillary column
Temperature: T1 = temperature programmed from 80—280°C at 3°C/min

REFERENCE

1. **Schneider, K., Neupert, M., Spiteller, G., Henning, H. V., Matthaei, D., and Scheler, F.,** *J. Chromatogr.*, 345, 19, 1985.

Table GC 12
N(*O*)-HEPTAFLUOROBUTYRYL AMINO ACID *n*-BUTYL ESTERS

Column packing	P1
Temperature	T1
Gas Flow rate (mℓ/min)	H_2, 2
Column	
Length (m)	50
Diameter (mm)	0.22
Form	Capillary
Material	Silica
Detector	FID

Amino acid	t_r(min)
Aca	43.03
Alanine	14.01
β-Aminoisobutyric acid	19.50
Arginine	43.21
Aspartic acid + asparagine	32.99
Creatinine + phenylalanine	34.00
Cycloleucine	23.56
Cysteine	25.95
Glutamic acid + glutamine	38.10
Glycine	16.76

Table GC 12 (continued)
N(O)-HEPTAFLUOROBUTYRYL AMINO ACID *n*-BUTYL ESTERS

Amino acid	t_r(min)
Histidine	53.20
Isoleucine	20.11
Leucine	19.85
Lysine	43.52
Methionine	31.13
1-Methylhistidine	44.49
3-Methylhistidine	46.66
Ornithine	41.51
Proline	24.99
Serine	19.27
Threonine	17.06
Tyrosine	39.03
Valine	16.57

Abbreviations: Aca = *trans*-4-(aminomethylcyclohexane) carboxylic acid used as an internal standard

Column packing: P1 = OV-1701 chemically bonded fused-silica capillary column

Temperature: T1 = temperature programmed from 130—260°C at 3°C/min

REFERENCE

1. **Schneider, K., Neupert, M., Spiteller, G., Henning, M. V., Matthaei, D., and Scheler, F.,** *J. Chromatogr.*, 345, 19,1985.

Table GC 13
N(O,S)-PENTAFLUOROBENZOYL AMINO ACID ISOBUTYL ESTERS

Column packing	P1
Temperature	T1
Gas Flow rate (mℓ/min)	He, 2
Column	
Length (m)	15
Form	Narrow bore Capillary
Detector	ECD

Amino acid	t_r(min)
Alanine	5.53
β-Alanine	7.00
γ-Aminobutyric acid	9.17
Aspartic acid	12.64
Citrulline	18.50
Cysteine	16.58
Glutamic acid	14.14
Glycine	6.00

Table GC 13 (continued)
N(O,S)-PENTAFLUOROBENZOYL AMINO ACID ISOBUTYL ESTERS

Amino acid	t_r(min)
Histidine	18.90
Hydroxyproline	11.78
Isoleucine	8.45
Leucine	8.31
Lysine	20.25
Methionine	12.23
α-Methyl-*p*-tyrosine (internal standard)	18.12
Ornithine	19.26
Phenylalanine	13.57
Serine	14.51
Threonine	8.93
Tryptophan	19.81
Tyrosine	21.08
Valine	7.22

Column packing: P1 = SE-54 (1% vinyl, 5% phenylmethyl polysiloxane)
Temperature: T1 = initial temperature of 140°C for 0.5 min, increasing at a rate of 5°C/min to 180°C, held for 0.1 min and increasing at 8°C/min to 310°C where it is maintained for 3 min

REFERENCE

1. **Yeung, J. M., Baker, G. B., and Coutts, R. T.,** *J. Chromatogr.*, 378, 293, 1986.

Table GC 14
N-TRIFLUOROACETYL ISOPROPYL ESTERS OF α-, β- AND γ-AMINO ACIDS ON DIAMIDE STATIONARY PHASES

Column packing		P1	P2
Temperature (°C)		140	130
Gas		He	He
Column			
Length (ft)		150	150
Diameter (in.)		0.02	0.02
Form		Capillary	Capillary
Material		SS	SS
Detector		FID	FID
Amino acid	**r[a]**	**$r^b_{D/L}$**	**$r_{D/L}$**
1[c]	D 0.17	0.924	0.797
	L 0.18		
2	L 0.41	1.027	0.958[d]
	D 0.42		
3	L 1.02	1.053	1.094[e]
	D 1.07		
4	D 0.29	1.00	0.816
	L 0.29		

Table GC 14 (continued)
N-TRIFLUOROACETYL ISOPROPYL ESTERS OF α-, β- AND γ-AMINO ACIDS ON DIAMIDE STATIONARY PHASES

Amino acid	r[a]	$r^{b}_{D/L}$	$r_{D/L}$
5	L 0.84 D 0.86	1.023	0.928[d]
6	L 1.89 D 2.04	1.080	1.049[d]
7	D 0.54 L 0.62	0.870	0.737
8	L 1.16 D 1.20	1.030	0.945[d]
9	L 3.37 D 3.62	1.077	1.047[d]

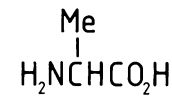
[a] Corrected relative time with respect to decyl acetate, which had a corrected retention time of 12 min at 140°C and 21 min at 130°C.

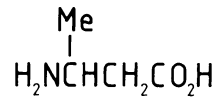
[b] $r_{D/L}$ = resolution factor = ratio of the corrected retention time of the D- over that of the L-enantiomer.

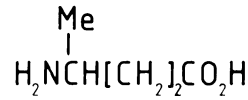
[c] The formulas of the amino acids are shown below.

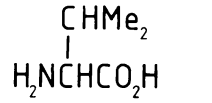
[d] Column 250 ft × 0.02 in.

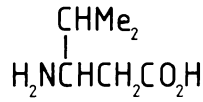
[e] Temperature = 120°C.

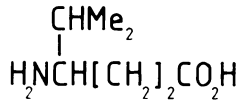
1 

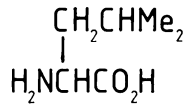
2 

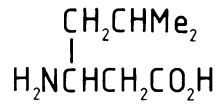
3 

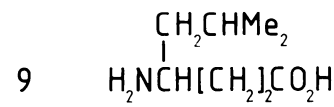
4 

5 

6 

7 

8 

9 

Packing: P1 = *N*-dodecanoyl-L-valine 6-undecylamide
P2 = *N*-dodecanoyl-L-valine *t*-butylamide

REFERENCE

1. **Feibush, B., Balan, A., Altman, B., and Gil-Av, E.,** *J. Chem. Soc. Perkin Trans.*, 2, 1230, 1979.

Table GC 15
ENANTIOMERS OF *N*-TRIFLUOROACETYL AMINO ACID ISOPROPYL ESTERS ON DIAMIDE STATIONARY PHASES

Column packing		P1		P2		P3		P4	
Amino acid	**Isomer**	**t_r(min)**	**T(°C)[a]**	**t_r(min)**	**T(°C)[a]**	**t_r(min)**	**t(°C)[a]**	**t_r(min)**	**T(°C)[a]**
Alanine	D	5.94	120	12.80	130	14.60	120	4.82	130
	L	6.48		15.76		12.60		5.84	
	D	5.00	130	8.46	150	9.80	130		
	L	5.40		9.84		8.60			
α-Aminobutyric acid	D	8.46	120	11.00	130	12.80	130	10.00	120
	L	9.44		13.90		11.00		7.92	
	D	8.60	130					9.14	130
	L	9.20						7.36	
β-Aminobutyric acid	D			21.88	130	22.80	130	13.52	120
	L	No separation		22.50		21.80		14.60	
α-Aminoheptanoic acid	D	42.88	120	53.60	130	58.80	130	43.92	120
	L	46.74		68.20		49.60		56.48	
	D	29.70	130					34.68	130
	L	31.80						43.92	
α-Aminohexanoic acid	D	24.64	120	30.90	130	34.40	130	25.08	120
	L	27.14		39.60		29.00		32.32	
	D	17.40	130					20.70	130
	L	18.70						26.02	
β-Amino-δ-methyl-hexanoic acid	D			63.90	130	60.40	130	41.40	120
	L			67.4		56.80		46.40	
γ-Amino-δ-methylhexanoic acid	D	52.90	130	97.90	130				
	L	51.90		92.68		No separation		No separation	
γ-Amino-ε-methylheptanoic acid	D	98.20	130	166.64	130				
	L	94.70		159.20		No separation		No separation	
β-Amino-γ-methylpentamoic acid	D			44.84	130	43.00	130	29.00	120
	L	No separation		46.96		40.00		33.40	
α-Aminooctanoic acid	D	75.26	120	93.20	130	101.20	130	75.76	120
	L	82.02		119.00		85.20		96.52	
	D	51.50	130					59.58	130
	L	55.10						75.76	

α-Aminopentanoic acid	D	14.56	120	18.20	130	21.80	130	13.84	120
	L	16.28		23.60		18.60		18.64	
	D	10.64	130					12.04	130
	L	11.50						15.34	
γ-Aminovaleric acid	D	28.20	130	56.60	130				
	L	28.20		56.60		No separation		No separation	
Aspartic acid	D	29.64	140	52.28	160	38.00	150	26.86	160
	L	30.30		54.46		36.80		27.88	
	D	15.10	160	41.08	170	26.60	160		
	L	15.30		42.50		26.00			
Glutamic acid	D	71.34	140	114.14	160	96.00	150	58.14	160
	L	75.48		126.64		87.60		64.98	
	D	34.80	160	85.70	170	63.60	160		
	L	36.20		95.50		59.80			
	D			61.14	180				
	L			66.64					
	D			40.28	200				
	L			43.20					
Glycine		13.40	120	24.36	130	19.20	130	9.94	130
		10.70	130						
Isoleucine	D	13.10	120	30.24	130	33.00	120	11.72	130
	L	14.16		36.40		28.40		14.42	
	D	9.86	130	19.14	150	21.60	130		
	L	10.66				18.88			
Allo-isoleucine	D	11.86	120	27.50	130	30.20	120	10.70	130
	L	13.10		33.44		25.80		13.00	
	D	9.24	17.58	150		19.68	130		
	L	10.10		20.08		17.20			
Leucine	D	19.60	120	38.80	130	47.92	120	17.20	120
	L	21.76		52.06		38.68		23.24	
	D	15.70	130	24.30	150	30.20	130	15.64	130
	L	17.20		29.54		25.20		20.74	
	D			10.70	170	12.60	150	6.50	150
	L			12.30		11.00		7.90	
	D							5.00	160
	L							5.88	
Methionine	D	51.12	140	81.76	160	69.60	150	42.96	160

Table GC 15 (continued)
ENANTIOMERS OF *N*-TRIFLUOROACETYL AMINO ACID ISOPROPYL ESTERS ON DIAMIDE STATIONARY PHASES

Amino acid	Isomer	t_r(min)	T(°C)[a]	t_r(min)	T(°C)[a]	t_r(min)	t(°C)[a]	t_r(min)	T(°C)[a]
	L	54.32		93.63		62.80		49.06	
	D	24.90	160	63.14	170	47.20	160		
	L	26.00		70.56		43.20			
	D			30.84	200	95.20	150	64.34	160
	L			32.86		90.40		72.06	
Phenylalanine	D	76.64	140	118.06	160	95.20	150	64.34	160
	L	81.94		133.52		90.40		72.06	
	D	39.00	160	90.06	170	66.40	160		
	L	40.70		99.44		60.80			
Phenylglycine	D	37.08	140	64.96	160	51.40	150	33.16	160
	L	37.74		69.90		47.20		35.96	
	D	19.70	160	50.08	170	35.40	160		
	L	19.70		53.08		33.00			
Proline	D	20.46	120	46.04	130	40.40	130	22.42	130
	L	21.20		47.92		40.40		22.42	
	D	17.70	130	29.02	150				
	L	17.70		30.06					
Serine	D	15.80	130	38.64	130	24.20	130	14.24	130
	L	16.80		45.40		22.08		16.04	
tert-Leucine	D	8.30	120	12.70	130	11.60	130	7.20	130
	L	8.80		13.90		11.00		8.02	
	D	5.90	130						
	L	6.10		20.12	130	12.40	130	7.16	130
Threonine	D	10.00	120	23.64		11.60		8.26	
	L	11.20		11.64	150				
	D	6.90	130	13.04					
	L	7.56		20.50	130	20.60	120	7.42	130
Valine	D	8.06	120	24.34		17.80		8.96	
	L	8.76		12.50	150	13.40	130		
	D	6.60	130	14.22		12.00			
	L	7.00							

[a] Column temperature.

Conditions	Packings:	P1, P2, P3, and P4 are chiral diamide phases of formula $C_{11}H_{23}$ CONHCH(R″)CONH-*tert*-C_4H_9 derived from L-alanine (P1,R″=CH_3), L-leucine (P2,R″=$CH_2CH[CH_3]_2$), D-phenylglycine (P3,R″=C_6H_5), and L-phenylalanine (P4,R″=$CH_2C_6H_5$)
	Columns:	Stainless steel capillary columns 100 ft × 0.02 in. I.D. were coated with P1 and P2, and columns 150 ft × 0.02 in. I.D. were coated with P3 and P4
	Gas:	He, flow rate 2.8—3.0 mℓ/min
	Detector:	FID

REFERENCE

1. **Chang, S.-C., Charles, R., and Gil-Av, E.,** *J. Chromatogr.*, 235, 87, 1982.

Table GC 16
N-TRIFLUOROACETYL AMINO ACID ISOPROPYL ESTERS ON DIAMIDE STATIONARY PHASES

Column packing	P1	P2	P3
Temperature (°C)	130	130	130
Gas Flow rate (mℓ/min)	He, 2.8—3.0	He, 2.8—3.0	He, 2.8—3.0
Column			
Length (ft)	150	100	150
Diameter (in., I.D.)	0.02	0.02	0.02
Material	SS	SS	SS
Detector	FID	FID	FID
Amino acid	$r_{D/L}$	$4_{L/D}$	$r_{L/D}$
Alanine	1.224	1.231	1.212
Allo-isoleucine	1.188	1.216	1.215
α-Aminopentanoic acid	1.244	1.297	1.274
Isoleucine	1.175	1.204	1.230
Leucine	1.288	1.342	1.326
tert-Leucine	1.094	1.095	1.114
Valine	1.172	1.187	1.207

Note: $r_{L/D}$ (for L-stationary phases) and $r_{D/L}$ (for D-stationary phases) = resolution factor = ratio of the corrected retention time of the second over that of the first emerging enantiomer.

Column packings: P1, P2, and P3 are *N*-lauroyl-*tert*-butylamide stationary phases derived from D-valine, L-leucine, and L-phenylalanine, respectively.

REFERENCE

1. **Chang, S.-C., Charles, R., and Gil-Av, Ed.,** *J. Chromatogr.*, 235, 87, 1982.

Table GC 17
THE ELUTION ORDERS OF *N*-PENTAFLUOROPROPIONYL AMINO ACID *n*-PROPYL ESTERS ON DIFFERENT COLUMNS

	Elution order		
Derivative of	**OV-101**	**Chiral**	**OV-101 and chiral**
Alanine	1	1	1
allo Isoleucine	7	4	6
Arginine	18	19	18
Aspartic acid	12	10	11
Cystine	10	11	10
Glutamic acid	14	14	14
Glycine	2	5	2
Histidine	19	18	
Isoleucine	8	6	5
Leucine	6	8	8
Lysine	15	17	17
Methionine	11	12	12
Ornithine	17	16	16
Phenylalanine	13	13	13

Table GC 17 (continued)
THE ELUTION ORDERS OF *N*-PENTAFLUOROPROPIONYL AMINO ACID *n*-PROPYL ESTERS ON DIFFERENT COLUMNS

Derivative of	Elution order		
	OV-101	Chiral	OV-101 and chiral
Proline	9	7	9
Serine	5	9	7
Threonine	4	3	3
Tryptophan	20	20	
Tyrosine	16	15	15
Valine	3	2	4

Conditions: Columns: Glass capillary coated with OV-101
Glass capillary coated with Chirasil®-val stationary phase
Two-column system; precolumn 80°C, isothermal for 5 min, then raised at 10°C/min to 130°C, then at 15°C/min to 200°C: main column 75°C raised at 5°C/min to 180°C
Carrier gas: Hydrogen
Detector: Flame ionization

REFERENCE

1. **Chinghai, W., Frank, H., Guanghua, W., Liangmo, Z., Bayer, E., and Peichang, L.,** *J. Chromatogr.*, 262, 352, 1983.

Table GC 18
THE EFFECT OF THE RATE OF TEMPERATURE PROGRAMMING ON THE ELUTION ORDER OF *N*-PENTAFLUOROPROPIONYL AMINO ACID *n*-PROPYL ESTERS

Derivative of	Temperature programming rate	
	A	B
Alanine	1	1
Alloisoleucine	4	5
Glycine	5	4
Isoleucine	6	6
Leucine	8	7
Proline	7	8
Serine	9	9
Threonine	2	2
Valine	3	3

Conditions: In the two-column system, column 1 is a glass capillary coated with OV-101, column 2 is a glass capillary coated with Chirasil®-val stationary phase; in temperature program A, the temperature of column 1 is increased from 80—200°C at 10°C/min, that of column 2 from 75—100°C at 2.5°C/min, then at 7°C/min to 200°C; in program B, the

Table GC 18 (continued)
THE EFFECT OF THE RATE OF TEMPERATURE PROGRAMMING ON THE ELUTION ORDER OF *N*-PENTAFLUOROPROPIONYL AMINO ACID *n*-PROPYL ESTERS

temperature of column 1 is increased from 40—200°C at 8°C/min, that of column 2 from 75—120°C at 2.5°C/min, is isothermal for 0.1 min and then is increased at 3°C/min to 200°C

Detector: Flame ionization

REFERENCE

1. **Chinghai, W., Frank, H., Guanghua, W., Liangmo, Z., Bayer, E., and Peichang, L.,** *J. Chromatogr.*, 262, 352, 1983.

Table GC 19
N-TERT-BUTYL-UREIDO-DL-AMINO ACID ISOPROPYL ESTERS

Amino acid	α	Column temp (°C)
Alanine	1.09	180
Aminobutyric acid	1.08	180
Aspartic acid	1.08	180
Alloisoleucine	1.09	180
Allothreonine (OTMS)[a]	1.05	180
Glutamic acid	1.07	200
Isoleucine	1.08	180
Isovaline	1.02	170
Leucine	1.09	180
Methionine	1.07	200
Norleucine	1.09	180
Norvaline	1.09	180
Phenylalanine	1.07	200
Phenylglycine	1.02	200
Pipecolic acid	1.03	160
Proline	1.03	180
Pyroglutamic acid	1.02	160
Serine (OTMS)[a]	1.11	180
Threonine (OTMS)[a]	1.09	180
Valine	1.08	180

Note: The D-enantiomers are eluted first.

[a] Side chain hydroxy group was silylated with MSTFA (*N*-methyl-*N*-trimethylsilyltrifluoroacetamide).

Conditions: A 25-m glass capillary column coated with XE-60-L-valine-(S)-α-phenylethylamide
Carrier gas = hydrogen

REFERENCE

1. **König, W. A., Benecke, I., Lucht, N., Schmidt, E., Schulze, J., and Sievers, S.,** *J. Chromatogr.*, 279, 555, 1983.

Table GC 20
N-METHYL-*N*-*TERT*-BUTYL-UREIDO-D,L-AMINO ACID *TERT*-BUTYLAMIDES (A) AND *N*-ISOPROPYLUREIDO-*N*-METHYL-D,L-AMINO ACID ISOPROPYLAMIDES (B)

Amino acid	α	Column temp (°C)
	A	
N-Methylalanine	1.023	160
N-Methylalloisoleucine	1.033	160
N-Methylaminobutyric acid	1.031	160
N-Methylhomophenylalanine	1.008	160
N-Methylisoleucine	1.028	160
N-Methylleucine	1.037	160
N-Methylphenylalanine[a]	1.009	160
N-Methylvaline	1.037	160
	B	
N-Methylalanine	1.014	170
N-Methylalloisoleucine	1.037	170
N-Methylaminobutyric acid	1.032	170
N-Methylhomophenylalanine	1.007	180
N-Methylisoleucine	1.047	170
N-Methylleucine	1.049	170
N-Methylphenylalanine[a]	1.000	180
N-Methylvaline	1.038	170

Note: L-Enantiomers are eluted first.

[a] D-Enantiomer is eluted first.

Conditions: A 25-m Pyrex® glass capillary column coated with XE-60-L-valine-(R)-α-phenylethylamide
Carrier gas = hydrogen

REFERENCE

1. **König, W. A., Benecke, I., Lucht, N., Schmidt, E., Schulze, J., and Sievers, S.,** *J. Chromatogr.*, 279, 555, 1983.

Table GC 21
t-BUTYLDIMETHYLSILYL DERIVATIVES OF AMINO ACIDS

Amino acid	Mono/di/tri derivative	Column temp (°C)	t_r(min)
Alanine	Di	185[a]	5.1
(3,3,3-$^2H_3$) Alanine	Di	185[a]	5.1
Histidine	Tri	250[a]	11.9
(2,3,3-$^2H_3$) Histidine	Tri	250[a]	11.9
Leucine	Di	180	4.7
(5,5,5-$^2H_3$) Leucine	Di	180	4.7
(1-^{13}C) Leucine	Di	180	4.7
(4,5,5,5,6,6,6-$^2H_7$) Leucine	Di	180	4.6
1-Methylhistidine	Di	250[a]	7.0
3-Methylhistidine	Di	250[a]	5.4

Table GC 21 (continued)
t-BUTYLDIMETHYLSILYL DERIVATIVES OF AMINO ACIDS

[a] Column length of 2 m.

Conditions: Packing: 3% OV-11
Column: 4 m × 6 mm
Gas: He; flow rate = 30 mℓ/min
Detection: Mass spectrometry

REFERENCE

1. **Schwenk, W. F., Berg, P. J., Beaufrere, B., Miles, J. M., and Haymond, M. W.,** *Anal. Biochem.*, 141, 101, 1984.

Table GC 22
AMINO ACIDS AS THEIR *TERT*-BUTYLDIMETHYLSILYL DERIVATIVES

Column packing	P1	P2	P3	P4
Temperature	T1	T1	T2	T2
Gas Flow rate (mℓ/min)	N_2, 18	N_2, 18	He, 5	He, 5
Column				
Length	6 ft	6 ft	25 m	12 m
Diameter (I.D.)	1.8 mm	1.8 mm	0.32 mm	0.32 mm
Form			Fused silica capillary	WCOT capillary
Material	G	G		
Detector	FID	FID	FID	FID
Amino acid			**t_r(min)**	
Alanine	7.69	9.15	8.48	6.94
α-Aminobutyric acid	8.50	9.92	9.37	7.57
γ-Aminobutyric acid	11.48	14.55	11.47	9.25
Arginine I	24.28	28.30	18.45	16.32
Arginine II				20.52
Aspartic acid	18.92	22.14	15.47	13.40
Carboxymethylcysteine	24.48	29.08	18.98	16.65
Cysteine	19.69	23.21	16.06	13.90
Cystine I			23.89	21.69
Cystine II			25.72	
Glutamic acid	20.90	24.37	16.58	14.50
Glycine	7.33	9.92	8.99	6.68
Histidine	25.43	31.15	19.30	16.95
Isoleucine	11.08	12.98	10.77	9.02
Leucine	10.50	12.20	10.32	8.67
Lysine I	22.78	25.58	17.90	15.51
Lysine II		26.21	17.41	
Methionine	15.29	19.33	14.05	11.42
Norleucine	11.48	13.46	10.98	9.25
Phenylalanine	17.42	21.75	15.34	12.61
Proline	11.48	14.55	11.64	9.25
Serine	16.12	18.19	13.48	11.87
Threonine	16.67	18.56	13.72	12.18
Tryptophan I	26.41	33.23	20.11	17.53
Tryptophan II		36.78	22.10	
Valine	9.63	11.39	9.90	8.16

Table GC 22 (continued)
AMINO ACIDS AS THEIR *TERT*-BUTYLDIMETHYLSILYL DERIVATIVES

Packing: P1 = 3.0% SE-30 on Supelcoport®, 100-120 mesh
P2 = 3.0% SP-2250 on Supelcoport®, 100-120 mesh
P3 = fused silica capillary column with 0.25-μm bonded methylphenyl (50%) silicone
P4 = WCOT capillary column coated with 0.5 μm SE-30

Temperature: T1 = after an initial hold of 1 min at 110°C the column is temperature programmed at 5°C/min to 260°C
T2 = after an initial hold of 2 min at 100°C the column is temperature programmed at 10°C/min to 280°C

REFERENCE

1. **Mawhinney, T. P., Robinett, R. S. R., Atalay, A., and Madson, M. A.,** *J. Chromatogr.*, 358, 231, 1986.

Table GC 23
ENANTIOMERS OF *N*-TRIFLUOROACETYL DIISOPROPYL ESTER DERIVATIVES OF SUBSTITUTED GLUTAMIC ACIDS

Column packing	P1
Gas, inlet pressure	He, 1.5 bar
Column	
Length (m)	50
Diameter (mm, I.D.)	0.25
Material	Fused silica
Detector	FID

Racemate	Temp (°C)	First isomer eluted	t_r (min)[a]	α
Glutamic acid	165	R	11.7	1.082
2-Methylglutamic acid	160	—	7.8	1.000
	165	—	6.5	1.000
4-Methyleneglutamic acid	165	R	9.9	1.086
threo-4-Hydroxyglutamic acid	165	2R,4R	14.3	1.058
erythro-4-Hydroxyglutamic acid	165	2R,4S	12.7	1.071
threo-3-Methylglutamic acid	160	2R,3S	10.9	1.092
erythro-3-Methylglutamic acid	160	2R,3R	10.9	1.073
threo-4-Methylglutamic acid	160	2R,4R	11.8	1.076
erythro-4-Methylglutamic acid	160	2R,4S	12.4	1.105
threo-3-Fluoroglutamic acid[b]	175	2S,3S	30.2	1.065
erythro-3-Fluoroglutamic acid[b]	175	2S,3R	40.0	1.050
threo-4-Fluoroglutamic acid	165	2R,4R	15.9	1.063
erythro-4-Fluoroglutamic acid	165	2R,4S	15.0	1.053

[a] Retention time (min) from the solvent peak; retention time of the solvent peak 5.0 min.
[b] As *N*-acetyl *O,O'*-diisopropyl ester.

Packing: P1 = polysiloxane XE-60-(*S*)-valine-(*S*)-phenylethylamide coated on Chrompack® column

REFERENCE

1. **Maurs, M., Ducrocq, C., Righini-Tapie, A., and Azerad, R.,** *J. Chromatogr.*, 325, 444, 1985.

Table GC 24
THE EFFECT OF ELECTRON CAPTURE DETECTION (ECD) ON AMINO ACID RMRs

Amino acid	RMR_{ECD}/RMR_{FID}
Glutamic acid	3.9
Histidine	2.5
Hydroxyproline	1.8
Methionine	1.4
Serine	2.0
Tryptophan	6.0
Tyrosine	1.4

Note: RMR is the molar response relative to norleucine. The RMRs are calculated for each amino acid by peak-height measurement. The sensitivity of electron capture detection and flame ionization detection is compared by using the quotient between RMRs.

REFERENCE

1. **Bengtsson, G., Odham, G., and Westerdahl, G.,** *Anal. Biochem.*, 111, 163, 1981.

Table GC 25
VOLATILE AMINES

Column packing	P1	P2
Temperature (°C)	80	50
Gas Flow rate (mℓ/min)	He, 17.6[a]	He, 17.6[a]
Column		
Length (m)	2.10	2.10
Diameter (mm, O.D., I.D.)	6.35, 2.0	6.35, 2.0
Material	Glass[b]	Glass[b]
Detection	FID	FID

Amine	t_r(min:sec)	
Methylamine		0.54
Dimethylamine		2.00
Ethylamine		2.48
Trimethylamine	0.54	3.24
Isopropylamine	1.21	
n-Propylamine	1.54	
Isobutylamine	3.12	
n-Butylamine	4.09	
Pyrrolidine	6.30	
2-Methylbutylamine	6.45	
3-Methylbutylamine	7.00	
n-Pentylamine	8.30	
Piperidine	12.00	

[a] Helium loaded with ammonia.
[b] Borosilicate glass.

Column packing: P1 = 28% Pennwalt® 223 + 4% KOH on Gas-Chrom® R, 80-100 mesh

Table GC 25 (continued)
VOLATILE AMINES

P2 = 4.8% PEG 20M + 0.3% KOH on Carbopack® B, 100-120 mesh

REFERENCE

1. **Pons, J.-L., Rimbault, A., Darbord, J. C., and Leluan, B.,** *J. Chromatogr.*, 337, 213, 1985.

Table GC 26
ALIPHATIC AMINES

Column packing	P1
Temperature	T1
Gas Flow rate (mℓ/min)	N_2, 45
Column	
Length (m)	2
Diameter (mm, I.D.)	2
Material	G
Detection	D1

Amine	t_r(min)
Methylamine	1.08 ± 0.005
Dimethylamine	1.93 ± 0.004
Ethylamine	2.39 ± 0.003
Trimethylamine	2.71 ± 0.001
Isopropylamine	4.46 ± 0.007
n-Propylamine	5.63 ± 0.007
tert-Butylamine	6.54 ± 0.008
Diethylamine	7.02 ± 0.010
sec-Butylamine	8.22 ± 0.007
Isobutylamine	8.73 ± 0.005
n-Butylamine	9.93 ± 0.011

Column packing: P1 = SEPABEAD GHP-1 (GHP-1) 60-80 mesh, a spherical styrene-divinylbenzene copolymer (Mitsubishi Chemical, Tokyo); 10 g of GHP-1 is extracted with 100 mℓ of methanol for 3 hr in a Soxhlet® apparatus; it is then mixed with a solution of 1.0 g of KOH in 30 mℓ of methanol, and the mixture is dried under reduced pressure below 50°C in a rotary evaporator; the treated material is packed into the chromatographic column, which is conditioned at 200°C for 2 days; during the conditioning, 10 μℓ of water is injected 15—20 times to ensure a stable column; before use, 10 μℓ of 1 *N* KOH solution is injected twice at 170°C

Temperature: T1 = the column temperature is isothermal at 140°C for 3 min, is increased to 170°C at 10°C/min, and is maintained at this temperature for 10 min

Detection: D1 = nitrogen-phosphorus flame ionization detector

Table GC 26 (continued)
ALIPHATIC AMINES

REFERENCE

1. **Kuwata, K., Akiyama, E., Yamazaki, Y., Yamasaki, H., and Kuge, Y.,** *Anal. Chem.*, 55, 2199, 1983.

Table GC 27
LIGAND-EXCHANGE GAS CHROMATOGRAPHY OF LOWER ALIPHATIC AMINES

Amine	Adjusted retention time (min)[a] (NH_3 conc in mobile phase [μmol/mℓ])							
	1.04	**2.79**	**4.68**	**6.51**	**8.60**	**10.80**	**13.21**	**15.39**
Ethylamine	11.4	3.7	2.3	1.6	1.2	1.0	0.8	0.7
n-Propylamine	15.6	4.9	2.9	2.2	1.7	1.5	1.2	1.0
Isopropylamine	4.1	1.3	0.9	0.7	0.6	0.5	0.4	0.4
n-Butylamine	33.5	10.8	6.4	4.7	3.7	3.2	2.7	2.4
Isobutylamine	13.3	4.5	2.7	2.0	1.6	1.4	1.1	0.9
sec-Butylamine	6.5	2.3	1.5	1.2	1.0	0.8	0.7	0.6
tert-Butylamine	2.1	0.9	0.6	0.5	0.4	0.4	0.3	0.3
n-Amylamine	74.6	23.7	14.3	10.5	8.4	7.1	6.1	5.5
Isoamylamine	52.7	17.0	10.3	7.7	6.1	5.1	4.4	4.2
Neopentylamine	9.6	3.1	2.0	1.5	1.2	1.1	0.9	0.8
tert-Amylamine	4.4	1.7	1.1	0.9	0.8	0.7	0.6	0.5
n-Hexylamine		55.5	32.7	24.1	18.8	16.7	14.2	12.4
n-Heptylamine		127.0	74.8	54.7	43.2	37.7	32.6	28.5
2-Ethylhexylamine		83.2	48.2	35.9	29.0	24.8	21.5	19.1
1,1,3,3-Tetramethylbutylamine	16.0	5.3	3.9	3.3	3.0	2.7	2.6	2.4
Dimethylamine	5.2	1.4	0.9	0.6	0.5	0.5	0.4	0.3
Diethylamine	1.7	0.7	0.6	0.5	0.5	0.4	0.4	0.3
Di-*n*-propylamine	2.8	1.5	1.2	1.1	1.0	1.0	0.9	0.9
Diisopropylamine	0.7	0.5	0.5	0.4	0.4	0.4	0.4	0.3
N-Ethyl-*n*-butylamine	4.6	2.2	1.7	1.4	1.3	1.2	1.1	1.0
N-Ethyl-*tert*-butylamine	0.9	0.7	0.6	0.6	0.5	0.5	0.5	0.4
Di-*n*-butylamine	11.7	5.9	4.7	4.3	4.0	3.8	3.6	3.5
Diisobutylamine	2.5	1.8	1.7	1.6	1.6	1.5	1.5	1.5
Di-*n*-amylamine	58.8	26.7	20.7	18.4	17.0	16.0	15.4	15.4
Diisoamylamine	30.0	14.3	11.2	10.0	9.3	8.8	8.5	8.3
Trimethylamine	0.4	0.2	0.2	0.1	0.1	0.1	0.1	0.1
Triethylamine	0.7	0.5	0.5	0.5	0.5	0.5	0.4	0.4
Tri-*n*-propylamine	2.3	2.2	2.2	2.2	2.2	2.2	2.1	2.1
Tri-*n*-butylamine	15.0	14.6	14.4	14.4	14.3	14.0	14.0	13.9

[a] Retention time of methane was used as t_o.

Conditions:
Column: 3 m × 4 mm I.D.
Packing: 5% Cu(II) stearate coated on Chromosorb® G AW/DMCS, 80-100 mesh

Chromosorb® G AW/DMCS is added to a benzene solution of metal stearate and the solvent is evaporated with a rotary vacuum evaporator; the dried packing material is resieved to 80-100 mesh and equilibrated with ammonia vapor for 6 hr to form metalammine complex by spreading in a dish, which is placed in a desiccator containing concentrated aqueous ammonia at the bottom

Table GC 27 (continued)
LIGAND-EXCHANGE GAS CHROMATOGRAPHY OF LOWER ALIPHATIC AMINES

Glass spiral columns are prepared and conditioned for at least 12 hr by passing nitrogen containing ammonia and water vapor through them at a temperature 5°C or more higher than the operating temperature
Carrier gas: N_2, H_2O concentration in gas 0.75—112 μmol/mℓ
Flow rate: 20.0 mℓ/min
Temperature: 80°C
Detector: Hydrogen flame ionization detector

REFERENCE

1. **Fujimura, K., Kitanaka, M., Takayanagi, H., and Ando, T.,** *Anal. Chem.*, 54, 918, 1982.

Table GC 28
LOWER ALIPHATIC AMINES

Column packing	P1	P2	P3
Temperature (°C)	80	80	80
Gas Flow rate (mℓ/min)	N_2, 20	N_2, 20	N_2, 20
Column			
Length (m)	3	3	3
Diameter (mm, I.D.)	4	4	4
Form	Spiral	Spiral	Spiral
Material	G	G	G
Detector	D1	D1	D1
NH_3 concentration[a]	2.50	2.49	2.50
H_2O concentration[b]	0.35	0.38	0.53

Amine	Net retention volume V_N^c		
Ethylamine	82.6	36.7	82.2
n-Propylamine	92.9	39.3	90.0
Isopropylamine	13.7	18.4	41.7
n-Butylamine	188.2	81.3	183.9
Isobutylamine	92.9	35.4	71.7
sec-Butylamine	35.6	24.9	65.2
tert-Butylamine	6.9	13.1	31.3
n-Amylamine	408.6	171.7	327.3
Isoamylamine	298.4	128.5	237.2
tert-Amylamine	19.5	28.8	50.9
n-Hexylamine	929.5	397.2	596.0
Diethylamine	5.7	19.7	52.2
Di-*n*-propylamine	16.1	38.0	82.3
Diisopropylamine	4.6	13.1	28.7
Di-*n*-butylamine	101.0	173.0	279.1
Diisobutylamine	20.7	66.9	80.9
Di-*n*-amylamine	441.8	901.7	989.3
Diisoamylamine	219.2	464.0	530.8
Trimethylamine	1.1	3.9	7.8
Triethylamine	4.6	17.0	27.4
Tri-*n*-propylamine	24.1	104.9	75.6
Tri-*n*-butylamine	163.0	977.9	366.4

[a] NH_3 concentration in gas phase (μmol/mℓ).
[b] H_2O concentration in gas phase (μmol/mℓ).
[c] V_N values are calculated from $V_N = jV_{R'}$ where j is the pressure drop correction factor and $V_{R'}$ is the adjusted retention volume.

Table GC 28 (continued)
LOWER ALIPHATIC AMINES

Packing: P1 = 5% Cu(II) stearate on Chromosorb® G AW/DMCS, 80-100 mesh

P2 = 5% sodium stearate on Chromosorb® G AW/DMCS, 80-100 mesh

P3 = 5% PEG 20 M on Chromosorb® G AW/DMCS, 80-100 mesh

Packing materials are prepared by adding Chromosorb® G AW/DMCS to a benzene solution of Cu(II) stearate or an absolute methanol solution of sodium stearate; the solvent is evaporated using a rotary vacuum evaporator and resieving the dried packing materials to 80-100 mesh; the material is then equilibrated with ammonia vapor for 6 hr to form the metal-amine complex by spreading it in a dish which is placed in a desiccator containing concentrated aqueous ammonia at the bottom; the chromatography columns are prepared and conditioned for at least 12 hr by passing nitrogen-containing ammonia and water vapor through them at a temperature 5°C or more higher than the operating temperature

Detector: D1 = hydrogen flame ionization detector

REFERENCE

1. **Fujimura, K., Kitanaka, M., Takayanagi, H., and Ando, T.,** *Anal. Chem.*, 54, 918, 1982.

Table GC 29
THE EFFECT OF WATER VAPOR IN THE GAS PHASE OF THE RETENTION OF ALIPHATIC AMINES

Column packing	P1	P1
Temperature (°C)	80	80
Gas Flow rate (mℓ/min)	N_2, 20.0	N_2, 20.0
Column		
Length (m)	3	3
Diameter (mm, I.D.)	4	4
Form	Spiral	Spiral
Material	G	G
Detector	D1	D1
NH_3 concentration[a]	5.03	5.03
H_2O concentration[b]	0	0.80
Amine	**Adjusted retention time (min)[c]**	
Ethylamine	3.8	2.0
n-Propylamine	3.9	2.8
n-Butylamine	8.8	6.0
Isobutylamine	3.6	2.5
sec-Butylamine	1.7	1.4
tert-Butylamine	0.9	0.6
n-Amylamine	19.2	13.5
n-Hexylamine	43.6	30.2
n-Heptylamine	101.3	67.6
Di-*n*-propylamine	1.2	1.2
Di-*n*-butylamine	4.6	4.6
Di-*n*-amylamine	20.3	19.5
Triethylamine	0.4	0.5
Tri-*n*-propylamine	2.3	2.2
Tri-*n*-butylamine	15.1	14.5