CRC Handbook of Chromatography: Drugs

Volume VI

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CRC SERIES IN CHROMATOGRAPHY

SERIES PREFACE

The fat-soluble photosynthetic pigments present in plants and algae, including chlorophylls, carotenoids, and related pigments, comprise an important class of compounds with an extensive literature. Dr. Köst and his co-authors have done an admirable job in searching out and organizing much of the critical chromatographic data and methodology in the present volume.

Because of the chemical nature of these prenyllipid compounds, liquid chromatography is preferred for their isolation, separation, and determination. The most widely used methods include low-pressure column LC, paper chromatography, TLC, and, most recently, HPLC. All of these methods are covered by Dr. Köst.

Chromatography was "invented" in the early 1900s by Michael Tswett, a Russian botanist and plant physiologist who first applied liquid-solid chromatography on a column of chalk to resolution of the complex natural mixture of yellow and green chloroplast pigments in the extracts of leaves he was studying. On a personal note, I was fortunate to work with Dr. Harold Strain for five summers at the Argonne National Laboratory when I first began to teach. Dr. Strain was one of the first important American chromatography experts and used all variations of liquid chromatography extensively in his studies of photosynthetic pigments. My experience with Dr. Strain set the foundation for my lifelong career of research and writing in chromatography.

Readers of this Handbook are asked to contact the Series Editor if they find errors or omissions in coverage as well as with suggestions for future volumes and authors within the Handbook of Chromatography series.

Joseph Sherma

PREFACE

The phenomenal growth in the application of liquid chromatographic (LC) techniques for the determination of drugs in pharmaceutical preparations and in biological fluids has continued in the first half of this decade. In the mid to late 1970s, a large number of papers were published describing gas chromatographic (GC) procedures for the drug groups anticonvulsants and antidepressants. In the last few years, a large number of publications have appeared describing LC procedures for the same drugs.

There have been a number of improvements in the LC instrumentation and column technology. Variable wavelength absorbance detectors are now available which match fixed wavelength detectors in sensitivity. Some of these detectors allow monitoring of absorbance at multiple wavelengths. A number of manufacturers now market photo diode array absorbance detectors which allow instant absorbance scanning over a wide wavelength range of any eluting peak, check peak purity, and complement component separation by mathematical manipulation of absorbance data of incomplete chromatographic separation. There are also improvements in the design of electrochemical detectors. Multielectrode detectors are now available which require little maintenance and allow ultra-high sensitivity. Fluorescence detectors with monochromators and high energy power sources have also become available. However, use of lasers as power sources for fluorescence detectors is not yet common.

A number of manufacturers market on-line sample preparation systems, samplers allowing precolumn derivatization, and efficient postcolumn reactors. Although instrumentation for narrow bore LC is commercially available, this technique has not yet been commonly applied for the determination of drugs. There is improved quality control in the manufacture of columns. For convenience, cartridge-type columns and fittings requiring no tools have become available. Good quality silica-based columns can now be purchased at economical proces from general suppliers. However, polymer-based columns have failed to gain popularity and are relatively more expensive than silica-based columns because of limited sales.

In general, GC is now the preferred technique only when the required sensitivity is not available with an LC procedure for the determination of a particular drug. However, separation of widely different compounds is more efficiently accomplished with temperature programming GC analysis than by solvent programming LC analysis. Thus, considerable GC retention data of drugs with the use of capillary columns have been published for the identification of an unknown drug in a given matrix. However, the use of capillary columns for the determination of drugs has not been as widespread as was anticipated. The nitrogen detector is now the most widely used detector for the GC determination of drugs. Gas chromatography-mass spectrometry remains the ultimate standard to confirm the identification of an unknown drug.

There has been a further decline in the popularity of thin-layer chromatography in the past few years. In the majority of the laboratories, drugs of abuse are now screened by immunoassay for improved sensitivity and convenience.

The purpose of this handbook is to provide a reference source and summaries of different chromatographic techniques published in refereed journals during the past 6 years. When the number of publications of a given drug was numerous, only recent papers were selected, even if they described only the modification of the original key publications. Despite the size of this work, a number of publications or drugs might have been missed as the literature search was carried out manually. In some cases, copies of the required papers could not be obtained. A number of publications could not be included as they were either theoretical or did not provide information compatible with the format of this handbook.

There is a significant difference between the present volumes and Volumes I and II of this handbook. For a number of drugs, e.g., cyclosporine, the chromatographic parameters of a number of publications are identical. However, they differ in the sample preparation techniques.

Therefore, detailed summaries of extraction procedures have now been provided for comparison of the different publications for the determinations of a given drug.

I am grateful to Dr. Gillian Luxton, Head of the Clinical Chemistry Laboratory, St. Joseph's Hospital for her encouragement to accept this project and for providing all the required facilities.

Mrs. S. Rogers and Mrs. J. Maragno of this hospital library made a special effort to get copies of the published papers from different sources.

Mrs. D. Thompson, Director of the hospital pharmacy, arranged to get information from the Drug Information Center in Toronto.

Miss Maelly Lew went to different libraries to get the information in emergency situations when a paper under review would refer to earlier papers.

I thank Miss Elisa Capretta, Mrs. Mary Bruce, Miss Rhita Gilners, and Miss Abha Gupta for preparing this manuscript.

Mrs. Diane Kirshenblat provided moral support when there was a temptation to abandon the project.

I am grateful to Ms. Sandy Pearlman, Director of Editing and Mrs. Amy Skallerup, Senior Editor, CRC Press for their help during the early phases of manuscript preparation.

Mr. J. C. Richardson, Senior Coordinating Editor, had the difficult task of making this manuscript uniform within the constraints of space limitations. I thank him for his courteous response to my various suggestions and changes.

Finally, I thank my family members, who tolerated my absence for more than a year.

Ram N. Gupta
December, 1986

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 EDITOR

Ram N. Gupta, Ph.D., is Head of Toxicology in the Department of Laboratory Medicine at St. Joseph's Hospital and Professor in the Department of Pathology at McMaster University in Hamilton, Ontario, Canada.

Dr. Gupta received his M.Sc. degree in 1962 and Ph.D. degree in 1963 in Organic Chemistry from McMaster University. He continued working in the Chemistry Department of McMaster University as a Research Associate until 1971 when he moved to the Department of Pathology at the same university.

Dr. Gupta has been elected as a fellow of the Chemical Institute of Canada. He is a member of the American Chemical Society, American Association of Clinical Chemists, Canadian Society of Clinical Chemists, and the Association of Clinical Biochemists (U.K.). He is the author of more than 40 scientific publications.

His present research interests are the development of chromatographic procedures for the assay of drugs and other biochemicals in biological fluids.

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ORGANIZATION OF TABLES AND EXPLANATION OF ABBREVIATIONS

Gas Chromatography (GC)

Specimen: Cerebrospinal fluid (CSF); not available (NA). The number in parenthesis refers to milliliters of plasma or serum used for the preparation of sample extract unless stated otherwise. There is no indication when volumes of other specimens are different from that of plasma or serum.

Extraction: In this column, the extraction procedure is given a number and the corresponding procedure is described at the end of the table for the extraction of plasma or serum unless indicated otherwise. Any difference in the extraction procedure of another type of specimen is not indicated.

Column: Columns are made of glass or fused silica unless noted otherwise. Length is given in meters and inner diameter in millimeters.

Packing: The number in the parenthesis shows the mesh size of the support. The film thickness of the capillary columns is given in μm and indicated by a footnote.

Gas: Gas flow, if given in units other than milliliters per minute, has been indicated by a footnote.

DET: Detector. Flame ionization detector (FID); nitrogen phosphorous detector (NPD); also, alkali flame ionization detector; thermionic sensitive detector; or nitrogen specific detector; electron capture detector (ECD); electron-impact mass spectrometer (MS-EI); chemical ionization mass spectrometer (MS-CI); negative ion chemical ionization mass spectrometer (MS-NCI). Any other detector used and the reagent gas used for chemical ionization, if different from the carrier gas, are indicated by footnotes.

RT min: Retention time in minutes of the title drug. It may be the retention time of the parent drug or its derivative. A dash "—" indicates that the title drug is not determined in the procedure under review, whereas NA indicates that the retention time is not available.

Internal Standard: The names of the compounds used as internal standards are given in full. Any abbreviation used to describe the internal standard is explained by a footnote. A dash "—" indicates that no internal standard was used in the procedure. The retention time in minutes is given in parenthesis as it appears in the chromatogram. It may be of the parent compound or its derivative. The retention time when the internal standard is an isotropically labelled drug is considered the same as of the drug itself.

Deriv: Derivative. This column indicates the type of derivative formed at some stage of the sample preparation. The details of derivatization reagent and procedure are included in the corresponding extraction procedure. A dash "—" indicates that no derivative was prepared.

Other Compounds: Metabolites of the parent drug or other similar or unrelated drugs when determined simultaneously with the title drug are listed in this column. Their retention times are given in parenthesis.

Ref: Reference.

Liquid Chromatography (LC)

This includes column liquid chromatography, high pressure liquid chromatography, and high performance liquid chromatography (see under GC for the explanation of common columns).

Column: Columns are made of steel unless noted otherwise. Length is in centimeters and inner diameter in millimeters.

Packing: Packing is described by the trade names as used by the authors. Footnotes indicate if a precolumn, a guard column, or a temperature other than ambient were used.

Elution: The eluting solvent is given a number and the corresponding solvent is described at the end of the table. The procedure is isocratic unless indicated as gradient. The conditions for gradient elution are described with the description of the elution solvent.

Flow Rate: Flow rate given in other units has been changed to milliliter per minute; a footnote indicates that only the pump pressure is given. Detector (DET); absorbance (ABS). Wavelength (nm) for absorbance detection is given. Two numbers are given when the absorbance is monitored simultaneously at two different wavelengths. A footnote indicates a programmed change of absorbance wavelength. Fluorescence (FL). The first number in the parenthesis is the excitation wavelength (nm), and the second, the emission wavelength. Other detectors are described without the use of abbreviations. Potentials for electrochemical detectors and procedures involving post-column reactors are indicated by footnotes.

Thin-Layer Chromatography (TLC)

See under GC and LC for the explanation of common columns.

Plate: Unless otherwise noted, plates are made of glass. Laboratory indicates that the plates have been coated by the authors in their laboratory.

Layer: High performance thin-layer chromatography (HPTLC).

Solvent: Developing solvent is given a number which is described at the end of the table.

Post-Separation Treatment: (sp) The plate is spayed with the described reagent. (D) The plate is dipped in the described reagent. (E) The plate is exposed to the vapors of the described reagent.

Det: Detection. Qualitative detection is indicated as visual. Wavelength (nm) for short or long wave UV lamp is given when fluorescence or quenching of fluorescence is observed under UV light. When the plate is scanned with the densitometer for quantitative determination of drug concentration, the mode of scanning is indicated as reflectance, transmission or reflectance/transmission for simultaneous mode. Wavelength (nm) for scanning and for fluorescence scanning, the excitation (first) and emission (second) are given.

PANTOTHENIC ACID

Gas Chromatography

Specimen (mℓ)	Extraction	Column (m × mm)	Packing (mesh)	Oven temp (°C)	Gas (mℓ/min)	Det.	RT (min)	Internal standard (RT)	Deriv.	Other compounds (RT)	Ref.
Foodstuffs (2 g)	I-1	2.4 × 2	10% Carbowax 20M Chromaton (100/120)	T.P.[a]	N_2 (30)	FID	17.1	Ethyl laurate (13.5)	—	—	1

Liquid Chromatography

Specimen (mℓ)	Extraction	Column (cm × mm)	Packing (μm)	Elution	Flow (mℓ/min)	Det. (nm)	RT (min)	Internal standard (RT)	Other compounds (RT)	Ref.
Dosage	I-2	25 × 4.6	Zorbax C8 (10)[b]	E-1	1.5	ABS (214)	8	Adipic acid (13)	—	2

[a] Initial temperature = 120°C; rate = 5°C/minute; final temperature = 220°C.
[b] A Brownlee 3-cm guard cartridge packed with Spheri-5 RP18 was used.

Extraction — I-1. The sample was incubated with 20 mℓ of 25% HCl for 5 hr at 100°C. After cooling, the hydrolysate was neutralized with 40°C sodium hydroxide and filtered. The filtrate was extracted five times with 60-mℓ portions of dichloremethane. The combined organic extract was mixed with 1 mℓ of the internal standard solution (50 μg/mℓ in dichloremethane) and concentrated to about 100 μℓ in a current of nitrogen. Aliquots of 1 μℓ of the residue were injected.
I-2. Accurately weighed tablets corresponding to 55 to 80 mg of calcium pantothenate was blended with 250 mℓ of the internal standard solution (1 g/ℓ in 25% methanol). The resulting slurry was centrifuged, filtered, and aliquots of the filtrate were injected. After 4 min of injection, the guard column was switched off line and back flushed with an auxiliary pump using the same mobile phase.

Elution — E-1. Methanol-0.25 *M* phosphate buffer, pH 3.5 (12:88).

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2. **Franks, T. J. and Stodola, J. D.,** A reverse phase HPLC assay for the determination of calcium pantothenate utilizing column switching, *J. Liq. Chromatogr.*, 7, 823, 1984.

PAPAVERINE

Gas Chromatography

Specimen (mℓ)	Extraction	Column (m × mm)	Packing (mesh)	Oven temp (°C)	Gas (mℓ/min)	Det.	RT (min)	Internal standard (RT)	Deriv.	Other compounds (RT)	Ref.
Plasma	I-1	25 × 0.23	SE-30	295	He (1)	MS-EI	3	Strychnine (4)	—	—	1

Liquid Chromatography

Specimen (mℓ)	Extraction	Column (cm × mm)	Packing (μm)	Elution	Flow (mℓ/min)	Det. (nm)	RT (min)	Internal standard (RT)	Other compounds (RT)	Ref.
Plasma (1)	I-2	25 × 4.6	Partisil ODS (10)[a]	E-1	2.0	ABS (254)	5	Chlorpheniramine (7.2)	—	2
Plasma, urine (1)	I-3	25 × 4.6	Brownlee C8 (10)	E-2	2.7	ABS (239)	5	Laudanosine (9.5)	—	3
Serum (1)	I-4	30 × 3.9	μ-Bondapak C_{18} (10)	E-3	1.6	ABS (254)	4	Diphenhydramine (7.5)	—	4
Blood (4)	I-5	30 × 4	Micropak CN (10)	E-4	1.0	ABS (254)	3.4	Mepyramine (4)	—	5

[a] A Whatman guard column packed with Co:Pell ODS was used.

Extraction — I-1. The sample was mixed with 1 mℓ of 10 *N* KOH, the internal standard solution, and extracted three times with 5-mℓ portions of toluene. The combined organic phase was back extracted into 3 mℓ of 1 *N* HCl. The aqueous phase was made alkaline with 1 mℓ of 10 *N* KOH and extracted twice with 5-mℓ portions of ether. The combined organic extracts were dried over anlydrous sodium sulfate, evaporated, the residue dissolved in 100 μℓ of chloroform and 1-μℓ aliquots of the resulting solution were injected.

I-2. The sample was mixed with 100 μℓ of the internal standard solution (20 μg/mℓ in water), 0.2 mℓ of 7 *N* sodium hydroxide, and extracted with 10 mℓ of ether. The organic layer was back extracted into 0.2 mℓ of 0.3 *N* HCl and a 100-μℓ aliquot of the aqueous phase was injected.

I-3. The sample was mixed with 0.3 mℓ of the internal standard solution (0.2 μg/mℓ) and extracted with 10 mℓ of chloroform-isopropanol (95:5). The organic phase was evaporated with an air stream at warm temperature and the residue reconstituted with 100 μℓ of methanol. A 20-μℓ aliquot of the resulting solution was injected.

I-4. The sample was mixed with 100 μℓ of the internal standard solution (6 μg/mℓ in water), 0.5 mℓ of 5 *N* sodium hydroxide, and extracted with 25 mℓ of *n*-heptane. A 20-mℓ aliquot of the organic layer was back extracted into 0.3 mℓ of 0.1 *N* HCl. Aliquots of 10 to 50 μℓ of the aqueous phase were injected.
I-5. The sample was mixed with 100 μℓ of the internal standard solution (8 μg/mℓ in water), 10 mℓ of 0.4 *M* phosphate buffer, and extracted with chloroform-hexane (2:3). A 3-mℓ aliquot of the organic phase was evaporated at 45°C under nitrogen, the residue reconstituted with 250 μℓ of dichloromethane, and an aliquot of 100 μℓ was injected.

Elution — E-1. Methanol-1% acetic acid (55:45) containing 0.005 *M* 1-heptanesulfonic acid.
E-2. Methanol-0.015 *M* sodium borate, pH 8.5 (58:42).
E-3. Acetonitrile-0.05 *M* phosphate buffer, pH 3 (33:67).
E-4. *n*-Hexane-dichloromethane-acetonitrile-propylamine (50:25:25:01).

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PARABENS

Liquid Chromatography

Specimen (mℓ)	Extraction	Column (cm × mm)	Packing (μm)	Elution	Flow (mℓ/min)	Det. (nm)	RT (min)	Internal standard (RT)	Other compounds (RT)	Ref.
Dosage (0.3—1 g)	I-1	10 × 4.6	Perkin-Elmer C18 (3)	E-1	2.5	ABS (254)	Methyl P[a] = 0.55 Ethyl P = 0.62 Propyl P = 0.72	Butylparaben (0.88)	—	1

[a] P = Paraben.

PARABENS (continued)

Extraction — I-1. The sample was emulsified with 5 mℓ of tetrahydrofuran, mixed with 15 μℓ of the internal standard solution (94 μg/mℓ in 95% ethanol) and two drops of 10% HCl. An aliquot of the mixture was filtered through a 2-μm filter into the autosampler vial for injection.

Elution — E-1. Acetonitrile-water (65:35).

REFERENCE

1. **Dong, M. W. and DiCesare, J. L.,** Very high-speed liquid chromatography. III. Quantitative analysis of parabens in cosmetic products, *J. Chromatogr. Sci.*, 20, 49, 1982.

PARGYLINE

Gas Chromatography

Specimen (mℓ)	Extraction	Column (m × mm)	Packing (mesh)	Oven temp (°C)	Gas (mℓ/min)	Det.	RT (min)	Internal standard (RT)	Deriv.	Other compounds (RT)	Ref.
Microsomal incubation	I-1	1.2 × 2	10% SP-1000 Supelcoport (80/100)	130	H_2:8-He:92 (30)	NPD	2.5	Isobutyl-N-butyl-N-methyl carbamate (1.4)	Isobutyl chloro-formate	a	1

[a] Conditions for the extraction and chromatographic determination of different metabolites are described.

Extraction — I-1. The incubation mixture was treated with 0.5 mℓ of a 20% zinc sulfate solution. The supernatant was collected and the protein button rinsed with 2 mℓ of 1.15% KCl. The pH of the combined supernatant and washings was adjusted to 6 and extracted with an equal volume of dichloromethane. The organic phase was mixed with an appropriate amount of the internal standard and treated with isbutyl chloroformate in the presence of 6 mℓ of an aqueous solution containing 0.1 *M* sodium chloride in phosphate buffer, pH 6 for 10 min. Aliquots of 4 to 8 μℓ of the organic phase were injected.

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PEFLOXACIN

Liquid Chromatography

Specimen (mℓ)	Extraction	Column (cm × mm)	Packing (μm)	Elution	Flow (mℓ/min)	Det. (nm)	RT (min)	Internal standard (RT)	Other compounds (RT)	Ref.
Plasma, tissue (0.5)	I-1	10 × 5	Nucleosil C18 (10)	E-1	2.0	Fl (330,440)	4.8	1-Allyl-1-desethyl-pefloxacin (6.6)	Norpefloxacin (2.8)	1

Extraction — I-1. The sample was mixed with 0.1 mℓ of the internal standard solution (1 mg/mℓ in 0.01 *M* sodium hydroxide), 1 mℓ of 0.5 *M* sodium phosphate buffer, pH 7, and extracted twice with 10-mℓ portions of chloroform-isopentanol (9:1). The combined organic layers were evaporated at 60°C under a stream of air, the residue dissolved in 100 μℓ of 1% ammonia, and an aliquot of 25 μℓ of the resulting solution was injected.

Elution — E-1. Acetonitrile-water (150:850) containing 2 g sodium acetate trihydrate, 2 g citric acid monohydrate, and 1 mℓ triethylamine.

REFERENCE

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PEMOLINE

Gas Chromatography

Specimen (mℓ)	Extraction	Column (m × mm)	Packing (mesh)	Oven temp (°C)	Gas (mℓ/min)	Det.	RT (min)	Internal standard (RT)	Deriv.	Other compounds (RT)	Ref.
Plasma, urine (1)	I-1	1.6 × 2	3% OV-101 Chromosorb W (100/120)	240	N_2 (20)	ECD	0.6	Methyl-pemoline (0.8)	Hydro-lysis; methy-lation	—	1
Urine (5)	I-2	0.8 × 1	5% OV-17 Chromosorb 750 (80/100)	T.P.[a]	He (40)	NPD	2.4	N-Methylphthalimide (1.5)	Hydro-lysis; methy-lation	—	2