GPC

COLUMN REGENERATION SERVICE

μSTYRAGEL™ TOYO SODA ULTRA STYRAGEL™
SHODEX μBONDAGEL™ μSPHEROGEL™

All of these GPC columns give excellent performance. However, when they fail for various reasons (many of which are beyond your control) you must replace them at great expense. The GPC gels themselves NEVER WEAR OUT and may be used over and over as often as necessary!

ASI can clean and repack any 10 micron GPC packing to ***exceed*** new column specifications. All columns are unconditionally guaranteed for ***in use*** performance. Regardless of responsibility, if an ASI repacked column fails ***for any reason*** within the warranty period—it will be repaired at no additional cost.

Compare the performance, warranty and price and you will see that ASI offers a truly remarkable value.

REPACKED BY ASI				REPLACEMENT COST		
PERFORMANCE	WARRANTY	PRICE	COLUMN TYPE	PERFORMANCE	WARRANTY	PRICE
>20,000 p/m	120 days	$250.	WATERS ASSOC. μSTYRAGEL™	9000-12000 p/m	?	$595.
>30,000	90	$350.	WATERS ASSOC. ULTRASTYRAGEL™	>46,000	?	$675.
>20,000	120	$250./500.	TOYO SODA (TSK)	20,000	?	$630./1050.
>20,000	120	$250./500.	SHODEX	20,000	30 DAYS	$750./1080.
>20,000	120	$250.	BECKMAN μSPHEROGEL™	20,000	30?	$620.
>15,000	90	$175.	WATERS ASSOC. μBONDAGEL™	?	?	$405/495

asi ANALYTICAL SCIENCES INCORPORATED
SUITE B-24, AIRPORT PARK • 1400 COLEMAN AVENUE • SANTA CLARA, CALIF 95050 • (408) 779-0131

.... THE WORLD'S FINEST LIQUID CHROMATOGRAPHY COLUMNS

Circle Reader Service Card No. 101

JOURNAL OF LIQUID CHROMATOGRAPHY

Editor: DR. JACK CAZES Editorial Secretary: ELEANOR CAZES

P. O. Box 1440-SMS
Fairfield, Connecticut 06430

Editorial Board

E. W. ALBAUGH, *Gulf Research and Development Company, Pittsburgh, Pennsylvania*
K. ALTGELT, *Chevron Research Company, Richmond, California*
A. ASZALOS, *U.S. Food and Drug Administration, Washington, D. C.*
H. BENOIT, *Centre des Recherches sur les Macromolecules, Strasbourg, France*
W. BERTSCH, *University of Alabama, University, Alabama*
B. BIDLINGMEYER, *Waters Associates, Inc., Milford, Massachusetts*
P. R. BROWN, *University of Rhode Island, Kingston, Rhode Island*
J. A. CAMERON, *University of Connecticut, Storrs, Connecticut*
J. V. DAWKINS, *Loughborough University of Technology, Loughborough, England*
J. E. FIGUERUELO, *University of Valencia, Burjasot, Spain*
D. H. FREEMAN, *University of Maryland, College Park, Maryland*
R. W. FREI, *The Free University, Amsterdam, The Netherlands*
J. C. GIDDINGS, *University of Utah, Salt Lake City, Utah*
R. L. GROB, *Villanova University, Villanova, Pennsylvania*
E. GRUSHKA, *The Hebrew University, Jerusalem, Israel*
G. GUIOCHON, *Ecole Polytechnique, Palaiseau, France*
M. GURKIN, *E-M Science, Inc., Gibbstown, New Jersey*
A. E. HAMIELEC, *McMaster University, Hamilton, Ontario, Canada*
S. HARA, *Tokyo College of Pharmacy, Tokyo, Japan*
D. J. HARMON, *B. F. Goodrich Research Center, Brecksville, Ohio*
G. L. HAWK, *Zymark Corporation, Hopkinton, Massachusetts*
M. T. W. HEARN, *St. Vincent's School of Medical Research, Victoria, Australia*
E. HEFTMANN, *U.S. Department of Agriculture, Berkeley, California*
A. HEYRAUD, *Centre National de la Recherche Scientifique, France*
P. Y. HOWARD, *Micromeritics Instrument Corp., Norcross, Georgia*
H. J. ISSAQ, *Frederick Cancer Research Facility, Frederick, Maryland*
J. JANCA, *Institute of Analytical Chemistry, Brno, Czechoslovakia*
J. F. JOHNSON, *Institute of Materials Science – U. Conn., Storrs, Connecticut*
B. L. KARGER, *Northeastern University, Boston, Massachusetts*
P. T. KISSINGER, *Purdue University, West Lafayette, Indiana*
J. KNOX, *The University of Edinburgh, Edinburgh, Scotland*
J. C. KRAAK, *University of Amsterdam, Amsterdam, The Netherlands*
J. LESEC, *Ecole Superieure de Physique et de Chemie, Paris, France*
B. MONRABAL, *Dow Chemical Iberica, S. A., Tarragona, Spain*
S. MORI, *Mie University, Tsu, Mie, Japan*
A. K. MUKHERJI, *Xerox Corporation, Webster, New York*
J. A. NELSON, *M. D. Anderson Hospital and Tumor Institute, Houston, Texas*
L. PAPAZIAN, *American Cyanamid Corporation, Stamford, Connecticut*
V. PRETORIUS, *University of Pretoria, Pretoria, South Africa*
QIAN RENYUAN, *Institute of Chemistry, Beijing, People's Republic of China*

(continued)

JOURNAL OF LIQUID CHROMATOGRAPHY

Editorial Board *continued*

C. QUIVORON, *Ecole Superieure de Physique et de Chemie, Paris, France*
F. M. RABEL, *Whatman, Inc., Clifton, New Jersey*
J. RIVIER, *The Salk Institute, San Diego, California*
C. G. SCOTT, *Hoffman-LaRoche, Inc., Nutley, New Jersey*
R. P. W. SCOTT, *Perkin-Elmer Corporation, Norwalk, Connecticut*
H. SMALL, *Dow Chemical Company, Midland, Michigan*
E. SOCZEWINSKI, *Medical Academy, Lubin, Poland*
E. STAHL, *Universitat des Saarlandes, Saarbrucken, West Germany*
B. STENLUND, *Abo Akademi, Abo, Finland*
J. C. TOUCHSTONE, *Hospital of University of Pennsylvania, Philadelphia, Pennsylvania*
S. H. WONG, *University of Connecticut School of Medicine, Farmington, Connecticut*

JOURNAL OF LIQUID CHROMATOGRAPHY

November 1983

Aims and Scope. The journal publishes papers involving the application of liquid chromatography to the solution of problems in all areas of science and technology, both analytical and preparative, as well as papers that deal specifically with liquid chromatography as a science within itself. Included will be thin-layer chromatography and all modes of liquid chromatography.

Indexing and Abstracting Services. Articles published in *Journal of Liquid Chromatography* are selectively indexed or abstracted in:

• Analytical Abstracts • ASCA • BioSciences Information Service of Biological Abstracts (BIOSIS) • Chemical Abstracts • Current Awareness in Biological Sciences • Current Contents/Life Sciences • Current Contents/Physical and Chemical Sciences • Engineering Index • Excerpta Medica • Journal of Abstracts of the All-Union Institute of Scientific and Technical Information of the USSR • Physikalische Berichte • Science Citation Index

Manuscript Preparation and Submission. See the last page of this issue.

Subscription Information. *Journal of Liquid Chromatography* is published in fourteen numbers and two supplements in January, February, March (3 numbers), April, May, June (2 numbers), July, August, September (2 numbers), October, November, and December by Marcel Dekker, Inc., 270 Madison Avenue, New York, New York 10016. The subscription rate for Volume 6 (1983), containing fourteen numbers and two supplements, is $298.00 per volume (prepaid). The special discounted rate for individual professionals and students is $149.00* per volume. To secure this special rate, your order must be prepaid by personal check or may be charged to MasterCard or VISA. Add $36.80 for surface postage outside the United States. For airmail to Europe, add $72.32; to Asia, add $91.52.

Mailing Address. *Please mail payment with order to:* Marcel Dekker Journals, P. O. Box 11305, Church Street Station, New York, New York 10249.

Copyright © 1983 by Marcel Dekker, Inc. All rights reserved. Neither this work nor any part may be reproduced or transmitted in any form or by any means, electronic or mechanical, microfilming and recording, or by any information storage and retrieval systems without permission in writing from the publisher.

Permission to photocopy for internal or personal use or the internal or personal use of specific clients is granted by Marcel Dekker, Inc. for libraries and other users registered with the Copyright Clearance Center (CCC), provided that the stated fee is paid directly (per copy) to the CCC, 21 Congress Street, Salem, MA 01970. Special requests should be addressed to Marcel Dekker, Inc., Permissions Dept., 270 Madison Avenue, New York, New York 10016.

Contributions to this journal are published free of charge. Second-class mailing permit pending at New York, New York and at additional mailing offices.

***THIS REFLECTS A 50% DISCOUNT GIVEN TO INDIVIDUAL SUBSCRIBERS.**

Analytichem introduces off-line Chromatographic Mode Sequencing.

Now there is a simple new bench technique that provides more efficient chemical isolation than any technology available today. In a fraction of the time. And at a fraction of the cost. It's called Chromatographic Mode Sequencing. The technique has been developed by Analytichem International for use with Bond Elut solid phase extraction columns.

Chromatographic Mode Sequencing (CMS) is a simple off-line procedure that yields remarkably selective results. CMS allows the chemist to manipulate the interaction of various mobile and stationary phases to produce an almost limitless number of interactive modes. The result is that CMS, using Bond Elut, can solve virtually any chemical isolation problem. Yet it is disarmingly simple to perform.

The selectivity of this new technique is unsurpassed even by the most sophisticated column switching procedures. And the simplicity of CMS means you can process a far greater volume of samples than with any other method available. CMS makes a totally new dimension in chemical isolation possible. And Bond Elut makes CMS possible. To learn how CMS can simplify your chemical isolation problems, call Analytichem today.

Analytichem International
24201 Frampton Ave., Harbor City, CA 90710, USA (800) 421-2825.
In California (213) 539-6490
Telex 664832 ANACHEM HRBO

Circle Reader Service Card No. 110

JOURNAL OF LIQUID CHROMATOGRAPHY

Volume 6, Number 13, 1983

Special Section – Liquid Chromatography Directory

CONTENTS

Practical Microbore Column HPLC: System Development and Drug Applications 2383
C. Eckers, K. K. Cuddy, and J. D. Henion

Instrumental Broadening Correction in Size Exclusion Chromatography through Fast Fourier Transform Techniques 2411
D. Alba and G. R. Meira

Analysis of Block and Statistical Copolymers by Gel Permeation Chromatography: Estimation of Mark-Houwink Constants 2433
J. M. Goldwasser and A. Rudin

Protein Separations on Octyl and Diphenyl Bonded Phases 2465
F. M. Rabel and D. A. Martin

Separation of the Proteins of Locust Neurosecretory Endings with HPLC 2501
J. Girardie, M. Allard, A. Faddoul, and E. Neuzil

High-Pressure Liquid Chromatographic Assay of Azlocillin and Mezlocillin with an Anion-Exchange Extraction Technique 2513
C. E. Fasching and L. R. Peterson

Development of a Standardized Analysis Strategy for Basic Drugs, Using Ion-Pair Extraction and High-Performance Liquid Chromatography. IV. Application to Solid Pharmaceutical Dosage Forms 2521
G. Hoogewijs and D. L. Massart

Ligand Exchange Chromatography of Cephalosporin C on Polystyrene Resins Containing Copper Complex of Lysine Derivatives 2543
D. Sacco and E. Dellacherie

A Rapid HPLC Analysis of Diazepam in Animal Feed 2557
A. P. Pakuts, R. H. Downie, and T. I. Matula

Degradation of High Purity Water on Storage 2565
R. Gabler, R. Hegde, and D. Hughes

Letter to the Editor 2571

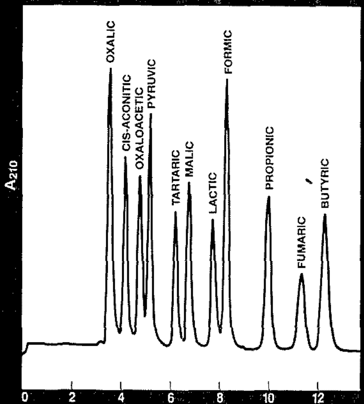
Liquid Chromatography Calendar 2573

Liquid Chromatography Directory 2579

Organic Acid Determination

Separate organic acids with an ORH-801 column. The polymeric packing material is stable from pH 0 to 14. Analyze foods, beverages, urines, cerebral spinal fluids and other complex samples. No elaborate sample preparation is required. Isocratic eluents permit high sensitivity analyses using UV, conductivity and electrochemical detectors.

Interaction Chemicals Inc.
1615 Plymouth St., Mt. View, CA 94043 USA
415-969-2400



Polymeric Columns for Liquid Chromatography

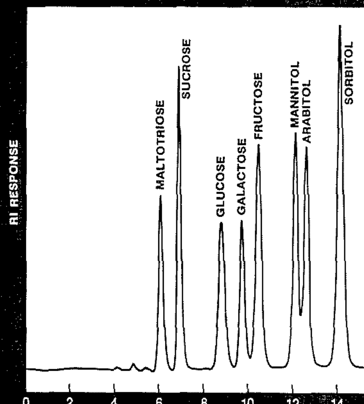
INTERACTION

Circle Reader Service Card No. 118

Carbohydrate Determination

Separate carbohydrates with a CHO-620 column. The polymeric packing material is not degraded by aqueous eluents. Analyze beverages such as beer, wine and fruit juice. Analyze dairy products, corn syrup and other complex samples without elaborate sample preparation. Only water is required as the eluent.

Interaction Chemicals Inc.
1615 Plymouth St., Mt. View, CA 94043 USA
415-969-2400

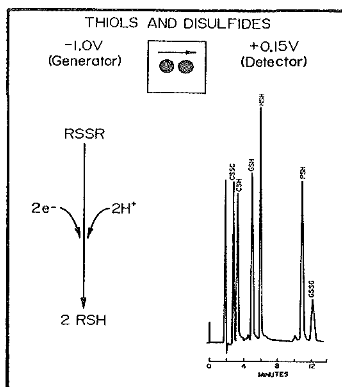


Polymeric Columns for Liquid Chromatography

INTERACTION

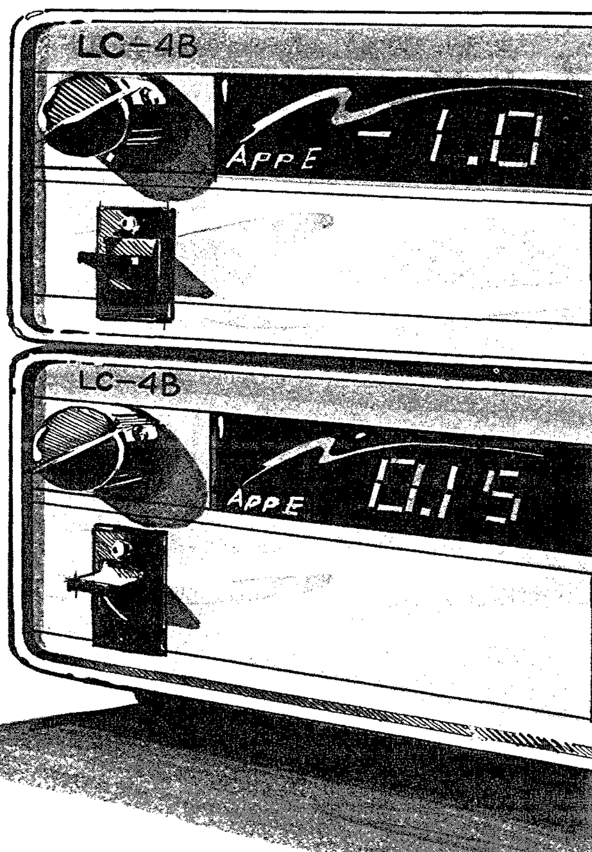
Circle Reader Service Card No. 119

Liquid Chromatography / Electrochemistry



Dual Electrode Detection: Standard mixture of Cystine, Cysteine, Glutathione, Homocysteine, Penicillamine, Glutathione Disulfide

In 1974 BAS introduced the first commercial LCEC system. With over 5000 units installed and the largest research team to support them, it's not surprising that our nearest competitors are several generations behind us. Our thin-layer transducers lead the way with a wide selection of electrode materials in a variety of interchangeable configurations. The latest member of the family is the new LC4B/17 dual electrode amperometry package. Parallel and series amperometry can enhance selectivity, detection limits, speed and gradient compatibility. Let us show you how it's done, no one else can.



BAS

Purdue Research Park,
2701 Kent Avenue,
West Lafayette, IN. 47906
317-463-4527 TLX 276141

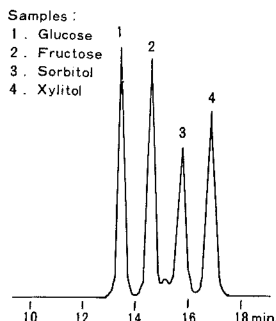
JAPAN: Tokai Irika Co. (Tokyo)
CANADA: Mandel Scientific
FRANCE: Biochrom s.a.r.l. (Vindelle)

Shodex® PAK

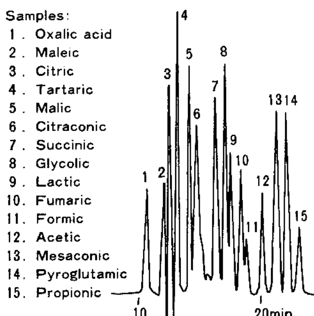
Packed Columns for High-Speed Chromatography

- ○ **Ionpak CH-801:** High-performance columns for analyses of saccharides and sugar alcohols. Analyzes saccharides and sugar alcohols present in solutions for fluid therapy in 20 minutes.
- ○ **Ionpak KC-811:** High-resolution columns for analyses of organic acids.
- ○ **Ionpak KS-801:** GPC columns for analyses of monosaccharides and disaccharides, with aqueous solutions as their mobile phase.
- ○ **Ionpak KS-802:** GPC columns for analyses of oligosaccharides, with aqueous solutions as their mobile phase.

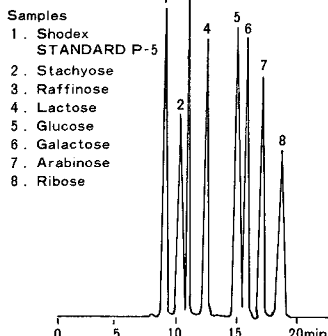
Separation of saccharides and sugar alcohols
Column : Ionpak CH-801×2
Mobile phase : Distilled water
Detector : Shodex RI.



Separation of carboxylic acids in soy sause
Column : Ionpak KC-811 ×2
Mobile phase : 0.003M $HClO_4/H_2O$
Analyzer : Organic acids analyzer Shodex LC ST-3



Separation of saccharides
Column : Ionpak KS-801×2
Mobile phase : Distilled water
Detector : Shodex RI.



Nomenclature	Specifications		
	Theoretical plates	Exclusion limit (Dextran)	Size
Ionpak CH-801	8,000 minimum	(1×10^3)	8mmϕ×300mm
Ionpak KC-811	15,000 //	(1×10^3)	//
Ionpak KS-801	15,000 //	1×10^3	//
Ionpak KS-802	15,000 //	1×10^4	//
Precolumns are available for each type of column.			

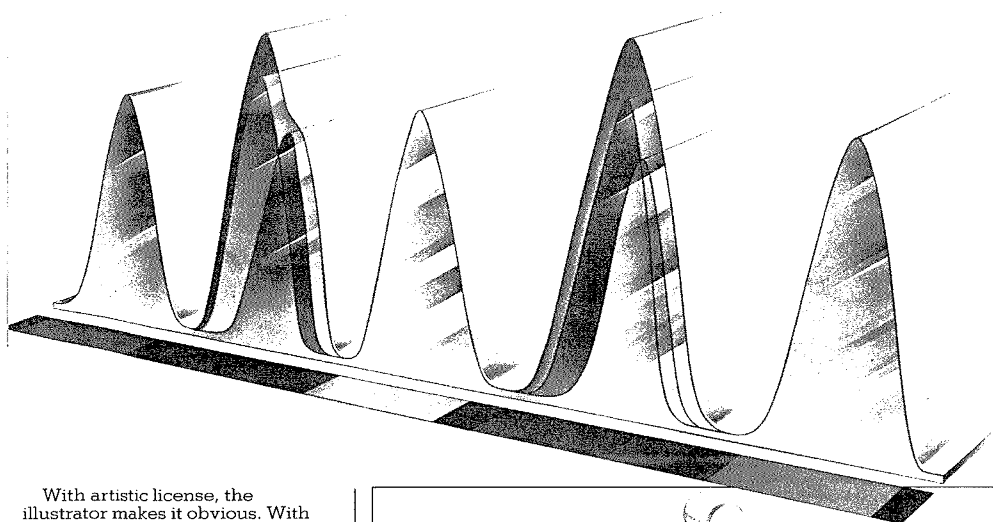
○ Combined use of Shodex® RI, a highly sensitive and stable refractive index detector, is recommended for high-speed liquid chromatography.
○ Other types of Shodex® PAK columns, such as the high-performance GPC column with 70,000 theoretical plates/m, are also available. Please contact us for further information, including applications data.

SHOWA DENKO K. K.
Instrument Products Department
13-9, Shiba Daimon 1-chome, Minato-ku, Tokyo 105, Japan
Telephone : (03) 432-5111 Telex : J26232
Cable : SECIC TOKYO

SHOWA DENKO AMERICA, INC.
280 Park Avenue
West Building, 27 th Floor
New York, N Y 10017, U S A
Telephone (212)687 0773
TLX : 423898
TWX : 710-581-2987

NEW HPLC DETECTION METHOD

DETECT AND ANALYZE UNRESOLVED COMPOUNDS.
QUICKLY. INEXPENSIVELY. RELIABLY.



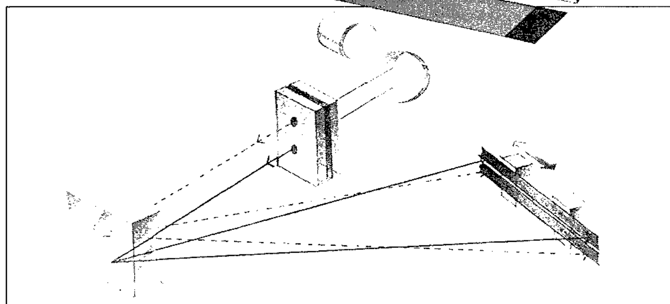
With artistic license, the illustrator makes it obvious. With innovative design, Micromeritics makes it *easy.*

Micromeritics Model 788 Dual Variable Wavelength Detector is the only HPLC detector that offers true simultaneous sample detection at two distinct wavelengths in a single flow cell. The 788 allows accurate quantitation of overlapped components—even those with identical retention times. It verifies the purity of the co-eluting components by absorbance ratio. And it provides complete data for dual characterization of chemical species.

Simplicity is the word for the 788 UV-VIS detector. Sample and reference information from a single flow cell are simultaneously projected in spectral form onto the focal plane of two photodiode pairs. A fixed pair accepts the dual information at the wavelength selected by changing the grating angle. The other is adjusted along the focal plane to accept information of another wavelength. So simple!

And the advantages are obvious:

- Provides real time information with *true simultaneous* detection at both selected wavelengths—unlike detectors using "rapid scanning" or two flow cells.
- Allows quantitation of unresolved components by selectively dialing out the interferent.

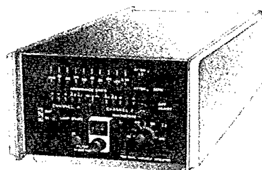


New HPLC detection method employs: Long life deuterium lamp. Single high pressure flow cell (tested to 4000 psi). Variable angle concave grating. Two photodiode pairs (one adjustable). Measurable wavelength 190 to 750 nm.

- Doubly characterizes chemical compounds using both absorbance ratio data and retention time.

Simplicity means savings! With its clean, reliable design, the 788 actually costs *thousands* of dollars less than competitive detectors with more complicated apparatus, scanning devices, and intricate, unnecessary variables.

For complete information on the 788 detector, contact Micromeritics Instrument Corporation, or call 404/448-8282 for the name of your nearest Micromeritics representative.



mi® micromeritics®

Micromeritics Instrument Corporation, 5680 Goshen Springs Road, Norcross, Georgia 30093 U.S.A. 404/448-8282.

Circle Reader Service Card No. 115

HPLC NEWS

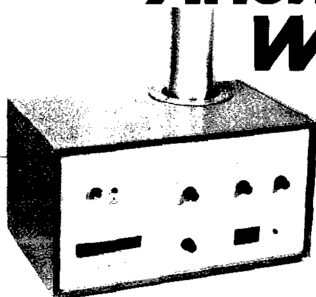
"Evaporative Analyzer As A Mass Detector"

Question: What is a viable alternative to isocratic refractive index detection for **POLYMERS, CARBOHYDRATES, LIPIDS**

Answer: The **ACS MODEL 750/14 "MASS DETECTOR"**

Why: Because the mass detector is

- **More sensitive**
- **Unaffected by ambient temperature changes**
- **Easily operated with solvent gradients**



Send for applications list and brochure

Applied Chromatography Systems (USA) Inc
315 S. ALLEN ST., SUITE 125, STATE COLLEGE, PA 16801, USA
TELEPHONE: (814) 237-1709
TELEX NO: 902623-ACS-USA-SCG.

Circle Reader Service Card No. 103

Catecholamines & Metabolites

or other neurochemically significant compounds

WITHIN

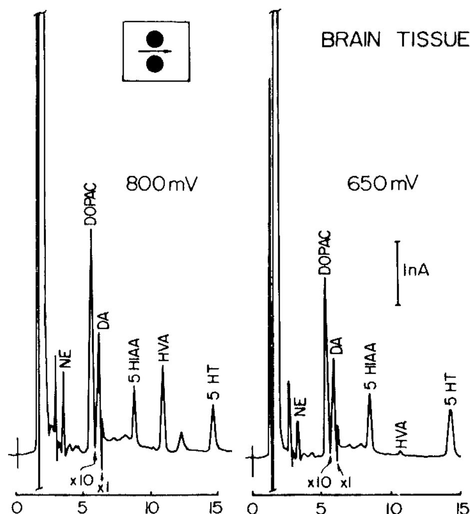
Brain Tissue
Adrenal Tissue
Body Fluids
Dosage Forms
Plant Matter
Insect Tissue

WITHOUT

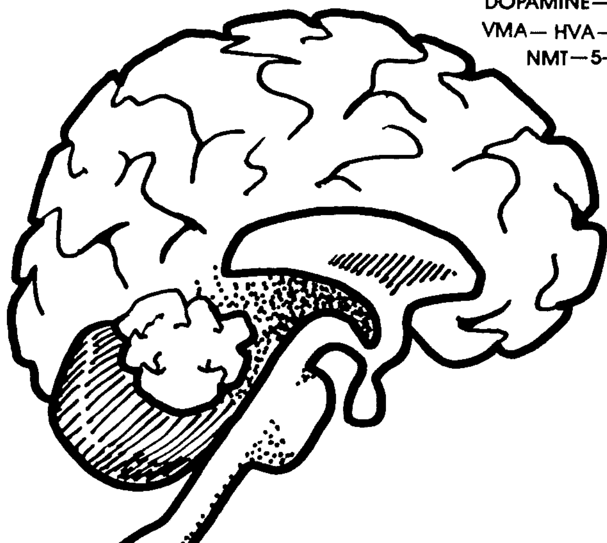
Derivatives
Radioisotopes
Fluorescence
Gas Chromatography
Mass Spectrometry
A Rich Uncle

BAS Analyzers based on liquid chromatography/electrochemistry (LCEC) have replaced radiochemical and fluorescence procedures in all of the above areas. Detailed procedures, sample preparation materials, and support from our own analytical laboratory compliment each complete instrument package. These systems are capable of monitoring many phenolic natural products and drugs. All of the catecholamine metabolites and many tryptophan metabolites can be determined. In addition, the activity of many neurologically important enzymes can be measured. Let us know your specific requirements.

DON'T CIRCLE THE NUMBER! Call or write today for a detailed reply!



DOPAMINE—NOREPINEPHRINE—EPINEPHRINE
VMA— HVA—DOPAC—MHPG—M—NM 3-MT
NMT—5-HT—5-HIAA — TH—DBH— COMT
PHENOTHIAZINE —MORPHINE
TRICYCLIC ANTIDEPRESSANTS
GLUTATHIONE—GABA



1205 KENT AVENUE
WEST LAFAYETTE, IN
47906
317 463 2505

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2383-2409 (1983)

PRACTICAL MICROBORE COLUMN HPLC: SYSTEM DEVELOPMENT AND DRUG APPLICATIONS

C. Eckers, K.K. Cuddy and J.D. Henion*
Diagnostic Laboratory
New York State College of Veterinary Medicine
Cornell University
Ithaca, NY 14853

ABSTRACT

Commercially available packed microbore columns have been used to demonstrate the potential of microbore high performance liquid chromatography (micro-LC) systems. The necessity of optimizing the chromatographic system for micro-LC is demonstrated using sulfa drugs and antibiotic standards, and some of the advantages of micro-LC such as high mass sensitivity are shown. Finally micro-LC has been used for the determination of two drugs, reserpine and trichlormethiazide in biological fluids.

INTRODUCTION

There is increasing interest in the use of reduced diameter (microbore) columns for HPLC, and there are a number of reported advantages from these systems [1]. Economy of both solvent and packing material is possible due to the reduced dimensions of the HPLC system. High mass sensitivity and high speed separations can be obtained, and high efficiencies achieved by packing long

Copyright © 1983 by Marcel Dekker, Inc.

0148-3919/83/0613-2383$3.50/0

columns or joining columns together [2]. The decreased mobile phase flow rate increases the compatability for interfacing a micro-LC system to a mass spectrometer [3], in addition to other mass sensitive detectors. Much work has been carried out using packed microbore 1mm i.d. columns [1,2].Columns of even smaller i.d. have also been used for HPLC eg open tubular [4] and packed [5,6] capillary columns.

In this work some of the practical considerations are shown for setting up a micro-LC system using commercially available 1mm i.d. packed columns. The apparatus used is based upon commercially available equipment designed for conventional HPLC, i.e. columns of 4.6 mm i.d. and flow rates of 1-2 mL min^{-1}. However, equipment used for micro-LC must be modified to incorporate low dead volumes. For example, if a flow rate of 40 µL min^{-1} is used a dead volume of 20 µL in the detector would have a marked effect on peak resolution totally independent of the separating power of the column. Thus the injection volume, detector cell volume and connecting tube volumes are all important and must be minimized.

A sulphonamide drug mixture was used to determine the effect of the detector cell volume on the micro-LC system and to demonstrate picogram on-column sensitivity. Mixtures of standard antibiotics were used to

demonstrate the effect of mobile phase flow rate and injection volume on the system. Finally, two biological applications are shown for the determination of reserpine and trichlormethiazide in equine urine.

EXPERIMENTAL

Apparatus

The basic chromatographic system used in this work is similar to that previously described [3,7]. It consists of a modified Waters M-660 solvent programmer [8] and two Waters M-6000A pumps (part no 98470; Waters Associates, Milford, MA), delivering flow rates of 10-999 μL min^{-1}. The output from the two pumps is delivered directly to the inlet port of a valve loop injector. Two valve loop injector designs have been used. The first injector utilized was a Valco submicroliter injector (Part No. AH-CFSV -4-UHPA-N60, Valco Inc., Houston, TX) that was manually actuated. Later improvements included the addition of an air actuator (Part No. AH3-60) and an electronic actuator (Part No. ECF5V.5). The Valco injector equipped with a 0.5 μL loop did not provide routine reliability with either manual, air or electronic actuation.

A Rheodyne Model 7410 submicroliter injector (Rheodyne Inc., Cotati, CA) equipped with a 0.5 μL loop and manually actuated was installed in the micro-LC system. This injector has performed reliably for

two years with only routine maintenance. Controlled experiments for reproducibility of injection volume and ghosting were very satisfactory.

A number of packed microbore (1 mm i.d.) reversed-phase HPLC columns were used in this work; a) Alltech Associates, C_{18}, 50 cm, Part No. 1740, Deerfield, IL; b) Chrompack, C_{18}, 50 cm, Part No. 25755, The Netherlands; c) CM Laboratories, C_{18}, 50 cm Nutley, NJ; d) Whatman, ODS-3, 25 cm Part No. 4240-128, Clifton, NJ; e) P. Kucera, Zorbax, 50 cm. Hoffman-LaRoche, Nutley, NJ. In order to minimize dead volume the micro-LC column is connected directly to the outlet port of the injector and detector for columns a,b,c, and e. However, due to incompatability of fittings, a small length of 0.004 in i.d. tubing (Part No. 3031; Alltech Associates, Deerfield, IL) was used for connecting column d to the injector and detector. In all cases 2 μm frits were positioned at the column inlet to prevent column particulates from entering the injector, and at the column outlet to prevent loss of packing material into the detector cell. Dirty or clogged frits cause a gradual increase in pressure in the system. This can be corrected by removing the frit and immersing it in methanol in an ultrasonic bath, or if the frit is part of the column, placing the end of the column in methanol in the ultrasonic bath. This can be

prevented to a certain extent by prefiltration of solvents and sample solution before use.

A Waters Model 440 fixed wavelength (254 nm) UV detector was used for this work equipped with either a regular detector cell (approximately 12 μL volume) or a "micro-cell" (approximately 1 μL volume: Part No. 97212 Waters Assoc.), similar to that previously described by Hermansson [9].

The formation of air bubbles in the micro-LC system can lead to troublesome problems in the UV detector, and they can arise in a number of ways. Any leaks in the HPLC hardware must be eliminated since anywhere that solvent leaks out air can leak in. Dissolved gases in the solvents themselves are minimized by continuous helium sparging [10[. Sometimes bubbles arise upon mixing pure methanol and pure water in the mixing chamber. If this is a problem it can often be overcome by pre-mixing, for example, 10% methanol in the water prior to use. A new microbore column or one which has not been used recently will often have air trapped within it although reversed phase columns should be stored in methanol. It has been found that the best method of eliminating this is to pump pure methanol through the column for a period of time. The pressure drop which occurs after the HPLC effluent leaves the detector is often sufficient to cause formation of bubbles in

the cell. In most cases it is advisable to have some source of back pressure on the cell. This can be accomplished in a number of ways. For example, an old HPLC column connected to the exit of the detector cell will provide sufficient back pressure to preclude outgassing of the eluent while it is passing through the detector cell.

Solvents and Samples

The solvents used in this work were distilled-in-glass methanol and acetronitrile (Burdick and Jackson, Muskegon, MI) and HPLC grade water (J.T. Baker Co., Phillipsburg, NJ). The solvents are suction filtered through a 0.2 µm pore size Millipore filter (Part No. GVMP 0270; Millipore Corp., Bedford, MA) and stored in clean, 500 mL glass bottles. Samples are dissolved in the HPLC eluent whenever possible and filtered through a 0.45 µm disposable filter (Part No. SLHVO25NS; Millipore Corp.) before use.

The sulfa drug standards were obtained from commercially available sources. The antibiotic samples were obtained from The Upjohn Co., (Kalamazoo, MI) Trichlormethiazide (Naquasone; Shering Corp., Bloomfield, NJ) was administered orally (400 mg) to a standardbred horse, and urine samples were collected as has been previously described [3]. Extraction and preliminary sample clean up of trichlormethiazide administration

and spiked urine samples were performed as previously described for hydrochlorothiazide [11]. Hydrochlorothiazide (Merck, Sharp and Dohme, West Point, PA) was used as the internal standard and standard trichlormethiazide was obtained by extraction from a pharmaceutical preparation (Naquasone).

Extraction of Reserpine from Equine Plasma

Samples of control equine plasma (4 mL) were spiked with 10-50 ng/mL reserpine (Aldrich Chem. Co., Milwaukee, WI) and extracted with 5 mL of trisolvent (Hexane:dichloromethane:ether, 1:1:1) for 5 min in screw cap test tubes on a Roto Rack at 60 rpm. After centrifugation the organic layer was transferred to a clean test tube and concentrated to dryness in a 65°C water bath under a gentle stream of nitrogen. The residue was redisolved in 50 μL of methanol for micro-LC.

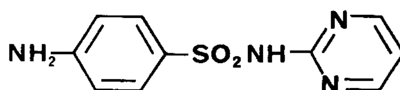
RESULTS AND DISCUSSION

a) Effect of Detector Cell Volume Upon Chromatographic Separations

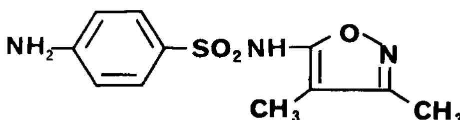
The conventional Waters UV detector is equipped with a detector cell volume of approximately 12 μL. The low flow rates used for micro-LC would generate considerable band broadening from this large cell volume. The results from this conventional LC cell has been compared to that of a "micro-cell" of approximately 1 μL cell volume.

Table 1 Sulfonamide sulfadrugs:sulfadiazine (1), sulfisoxazole (2) and sulfadimethoxine

1. Sulfadiazine



2. Sulfisoxazole



3. Sulfadimethoxine

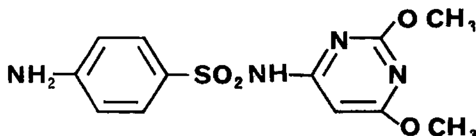


Figure 1 shows the separation of three sulfa drugs (Table 1) sulfadiazine (1), sulfisoxazole (2), and sulfadimethoxine (3), using the 12 μL volume cell (Fig. 1a) and the micro-cell (Fig. 1b). It can be seen that significantly better efficiency is obtained using the micro-cell. Table 2 summarizes the separation parameters obtained using both cells. The peak asymetry (A_s), resolution (R_s) and plate counts ($N_{\frac{1}{2}pkht}$ or N_5 are all increased by using the micro-cell. It was also found that at lower levels eg. approximately 20 pg of sulfa drug injected on column, the first peak, sulfadiazine (1) could not be distinguished from the solvent peaks using the conventional cell, although it was easily observed using the micro cell.

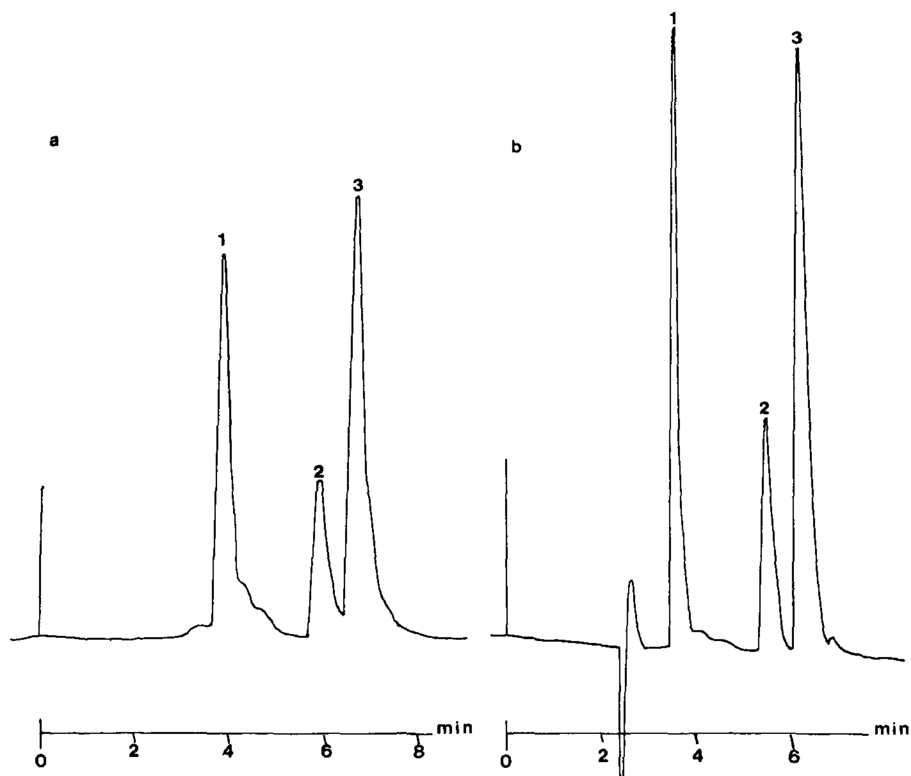


Fig. 1 The separation of three sulfa drugs (1, 2 and 3; 16.5 ng of each), using a) detector cell volume 12 μL and b) detector cell volume 1 μL (micro cell). Conditions: column, Whatman ODS-3 25 cm x 1 mm I.D.; mobile phase, acetonitrile: water, 4:6 (v:v); flow rate, 40 μL min^{-1}; injection volume, 0.5μL.

Table 2 Micro-LC separation parameters for the sulfadrugs in Table 1 using the conventional Waters Model 440 12 μL detector cell and a modified 1 μL micro-LC detector cell. The micro-LC column and conditions were as in Figure 1

	12 μL Cell				1 μL Cell			
	As	$N_{\frac{1}{2}}$	N_5	Rs	As	$N_{\frac{1}{2}}$	N_5	Rs
Sulfadiazine	2.75	1942	1892		2.14	2449	2970	
				2.50				4.04
Sulfisoxazole	2.09	2106	2916		1.40	3219	3540	
				1.09				1.46
Sulfadimethoxine	2.60	2116	2070		1.50	2959	2652	
Av. plates/meter		8467	9168			11503	12216	

As = assymmetry

$N_{\frac{1}{2}}$ = number of plates per column based on peak width measured at half peak height

$N_{5\sigma}$ = number of plates per column based on peak width masured at 4.4% of peak height

Rs = resolution

The plate counts shown in Table 2 are not as high as might be expected from micro-LC columns. This work was carried out on one of the earliest micro-LC columns available in the laboratory, which had already seen significant usage. Also, the sulfa drugs are polyfunctional molecules with a number of active sites and are more likely to have peak tailing than the aromatic mixtures supplied with the column which generate approximately 10,000-20,000 plates per column. Similar results to these have been previously shown using three different sulfadrugs, and these examples also illustrate the benefit of reducing the detector cell volume [9].

b) Sensitivity

High mass sensitivity is one of the advantages of micro-LC. The same sample concentration was used for both micro-LC and conventional LC eg. 1μ g μL^{-1}. The volume of the eluted peak by conventional LC was approximately 1 mL so that a mass of 0.5 μg can be detected-assuming the concentrations at peak maximum is twice the average peak concentration. If the peak volume eluted using micro-LC is 50 μL, then the mass to be placed on the column to produce a similar detector response need only be 25 ng [1]. It has been proposed by Scott and Kucera [2] that micro-LC is approximately 21 times more sensitive than conventional LC for columns packed under the same conditions. Since sensitivity

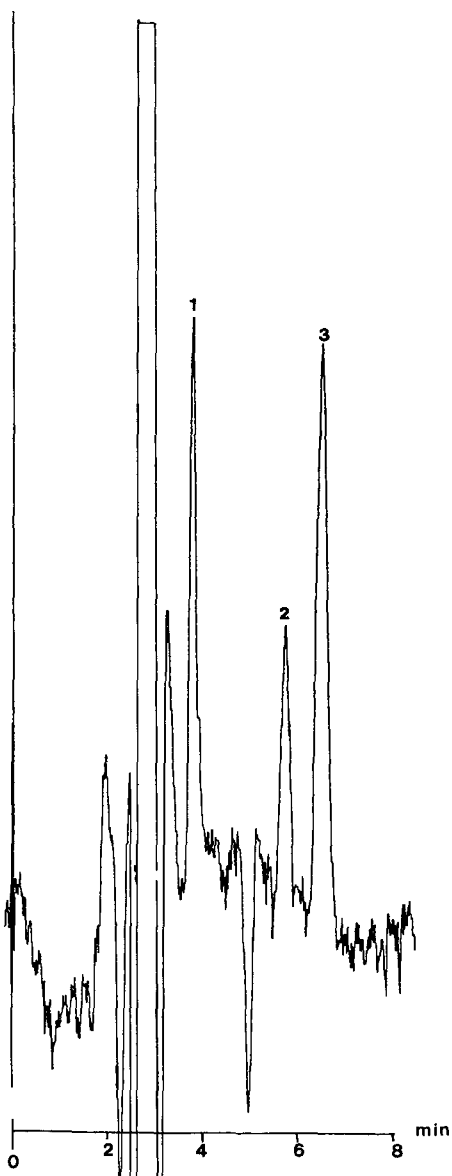


Fig. 2 The separation of 16.5 pg each of the three sulfa drugs (1, 2 and 3). HPLC conditions as Fig. 1; 1 μL volume microcell.

is dependent on the peak volume and thus the column efficiency, very high efficiency columns will provide decreased peak volumes resulting in higher sensitivity. Figure 2 shows the separation of three sulfa drugs with 16.5 pg of each injected on column with a signal to noise ratio of 7:1.

The amount of sample which can be injected onto a microbore column is a limiting factor in this work since sample size greatly affects the efficiency of a micro-LC separation (ref. section d). For example, in Figure 2 the injection volume was 0.5 μL of a 33 ppb solution. If a conventional LC column (4.6 mm i.d.) had been used an injection volume of eg. 10-25 μL could be used. Thus for the same sample 330-825 pg could be injected onto the column. Since at least 20 times more sample can be injected on to the column, this somewhat negates the sensitivity advantage obtained using micro-LC. However, for many applications eg. determination of drugs in biological fluids or tissues, often only a very small sample size is available.

If very dilute samples eg 1 ppb or less, are examined some method of sample pre-concentration is required. In one report using pre-concentration [7], 1 ppb of acetophenone was detected. However, a 10 mL sample was used and this is equivalent to 10 ng of acetophenone on column. Since we have seen appreciable

signal with less than 20 pg of the sulfa drugs injected on column, a similar sample pre-concentration system could provide ppt detection limits.

Another advantage of using some kind of sample pre-concentration system is that it can include a method of eliminating extraneous matter such as proteins and lipids from biological samples before they are introduced onto the microbore column. This eliminates an extraction procedure which should speed analysis time and reduce the possible sources of error in a system.

c) Effect of Flow Rate on Separation

The separation of the three standard antibiotics nodusmicin (4), nargenicin (5) and 18-deoxynargenicin (6), at flow rates of 30 $\mu Lmin^{-1}$ is shown in Figure 3. Although approximately equal amounts of the three antibiotics were injected on column, a significantly reduced UV response for (4) is seen presumably due to the absence of the large chromphoric R_2 group (ref. Table 3).

The flow rate of the HPLC eluent can markedly affect the plate count (N) of an HPLC column and it has been demonstrated for microbore columns that the HETP value decreases with decreasing mobile phase velocity [2]. Table 4 shows the values of N obtained for the last peak (6) in Figure 3 and the resolution R_s of peaks 5 and 6, obtained at flow rates of 10-110 $\mu Lmin^{-1}$. It can be seen that both plate count and reso-

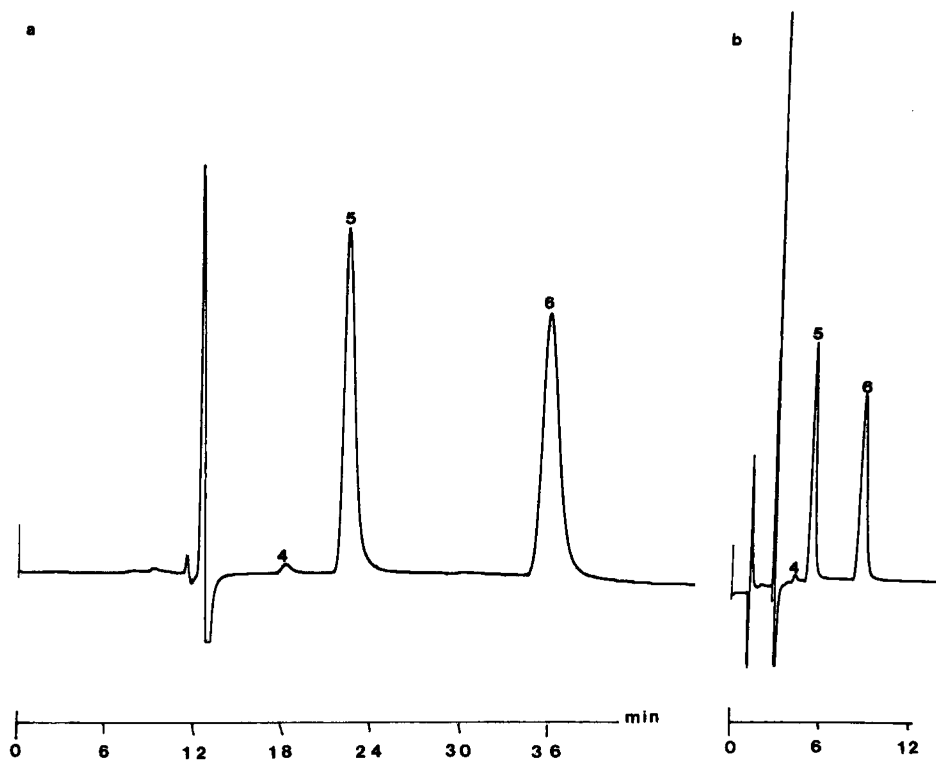
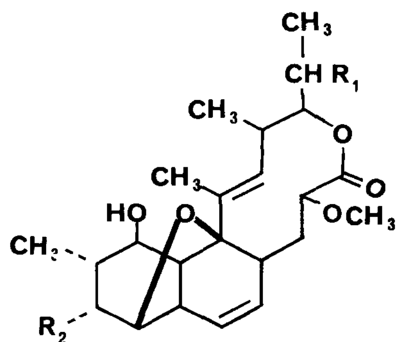


Fig. 3 The separation of three antibiotics (4, 5 and 6; 55 ng of each) at a) 30μL min^{-1} and b) 90μL min^{-1}
Conditions: column, Whatman ODS-3 25 cm x 1 mm I.D.; mobile phase, methanol: water, 7:3 (v:v); injection volume 0.5 μL; 1 μL volume micro-cell.

Table 3 Structures of nodusmicin (4), nargenicin (5) and 18-deoxynargenicin



	R_1	R_2
4. Nodusmicin	— OH	— OH
5. Nargenicin	— OH	—O—CO— NH
6. 18-dexoynargenicin	— H	—O—CO— NH

lution increase with decreasing flow rate, reaching maximum values at around 30 μLmin^{-1}. The results obtained at 10 μL min^{-1} show decreased N and R_s. This is possibly due to unreliability of the pump performance at these very slow flow rates rather than column performance.

Thus, for this micro-LC system the optimum flow rate for N and R_s is approximately 30 μLmin^{-1}. In general, 40 μLmin^{-1} has been used for the majority of the work shown in this paper since this appears to give acceptable plate counts, and HPLC separations generally within 30 min. Also, 40 μLmin^{-1} of eluent has been found to produce a tolerable pressure for interfacing

Table 4 Plate count (N) and resolution comparison for 18-deoxynargenicin (6) in the mixture shown in Figure 4 at varying eluent flow rates (10-110 μL/min) using a 0.5 μL injector loop volume (a) and at 40 μL/min eluent flow rate using the 5.0 μL injector loop volume (b)

Flow Rate ($\mu L\ min^{-1}$)	N Column (peak 6)	Rs (between 5 and 6)
10[a]	2500	6.71
30[a]	4700	6.14
50[a]	3700	5.2
50[b]	600	1.9
70[a]	2900	4.9
90[a]	2400	4.6
110[a]	1873	3.7

a. 0.5 μL injector loop, Rheodyne Model 7410

b. 5.0 μL injector loop, Rheodyne Model 7410

the micro-LC system to a mass spectrometer for micro LC/MS [3].

d) Effect of Injection Volume on Separations

Figure 4 shows a comparison of the separation of the three standard antibiotics 4, 5 and 6, using a 0.5 μL injection volume (4a) and a 5 μL injection volume (4b). The same mass of sample was injected on column in both cases.

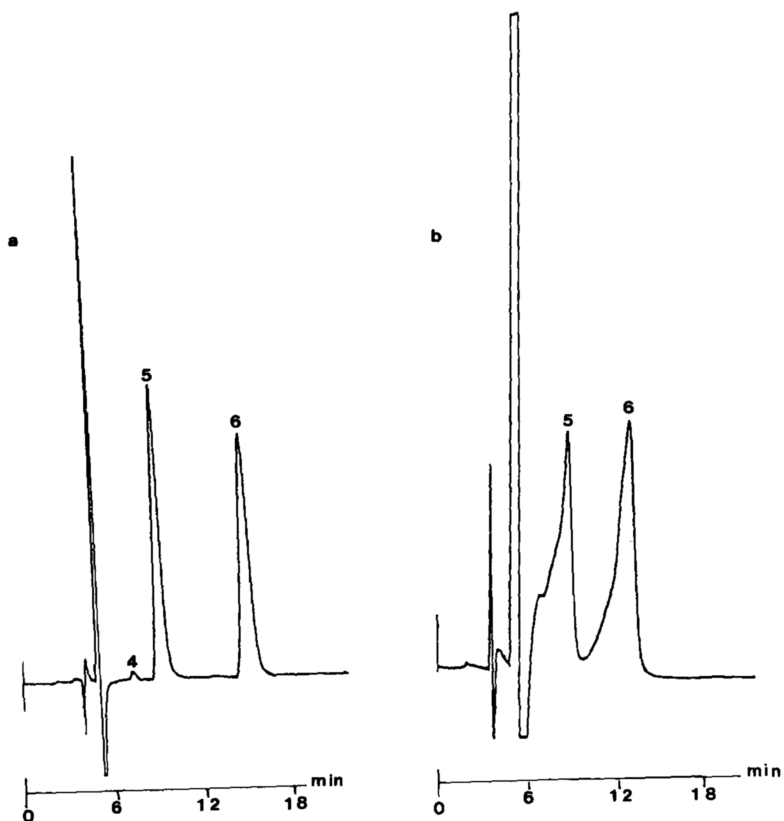
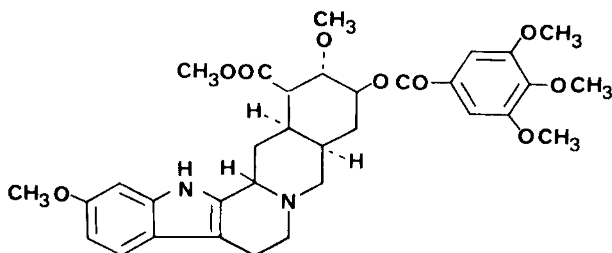


Fig. 4 The separation of three antibiotics (4,5 and 6 Using a) injection volume 0.5 μL of 110 ng/μL of 4,5 and 6, and b) injection volume of 5 μL of 11 ng/μL of 4,5 and 6. HPLC conditions as Fig. 3; flow rate 50 μL min^{-1}.

Table 4 shows a comparison of plate counts for the last peak in Figure 4, 18-deoxynargenicin (6), at flow rates ranging from 10-110 μL/min utilizing the antibiotic mixture and micro-LC conditions shown in Figure 4. Table 4 also shows the resolution of the last two components (5 and 6) as a function of micro-LC flow rate using the 0.5 μL injector loop for flow rates of 10, 30, 50, 70, 90 and 110 μL/min and the analogous data for the 5.0 L injector loop at 50 μ L/min. The corresponding plate count and resolution at 50 μL/min, for example, resulting from the use of the 0.5 and the 5.0 μL injector loop clearly documents the loss of chromatographic performance when the larger sample volume is injected onto the micro-LC column. The maximum sample which can be injected onto a chromatographic column without causing band broadening is proportional to the internal diameter of the column. It has been calculated that for a 1 mm x 250 mm i.d. column with approximately 10,000 plates this volume is approximately 1 μL [8].

e) <u>Determination of Reserpine in Equine Plasma</u>

Reserpine (7) is a potential drug of abuse in the horse because it produces a state of indifference to the environment and thus can be used to calm the animal [12]. Its determination in biological fluids is difficult because a sensitive method is not readily avail-



7. Reserpine

able. Using ion-pairing HPLC techniques with fluorescence monitoring, a detection limit of 100 pg of reserpine per mL of plasma was reported [13]. It was thought that the sensitivity afforded by micro-LC should be advantageous in the determination of reserpine.

Figure 5 shows preliminary micro-LC results for the determination of reserpine in equine plasma. Figure 5a is a UV chromatogram of an extract of a control equine plasma and Fig. 5b shows a UV chromatogram of an extract of the same plasma spiked with 50 ng of reserpine. This is equivalent to 500 pg injected on column. 10 ng of reserpine per mL of plasma (ie 400 pg on column) was easily detected. If the extract is taken up in a smaller sample volume, detection limits of the order of high picogram per mL of plasma should be attainable.

These measurements were made using UV detection at 254 nm which is not an absorbance maximum for re-

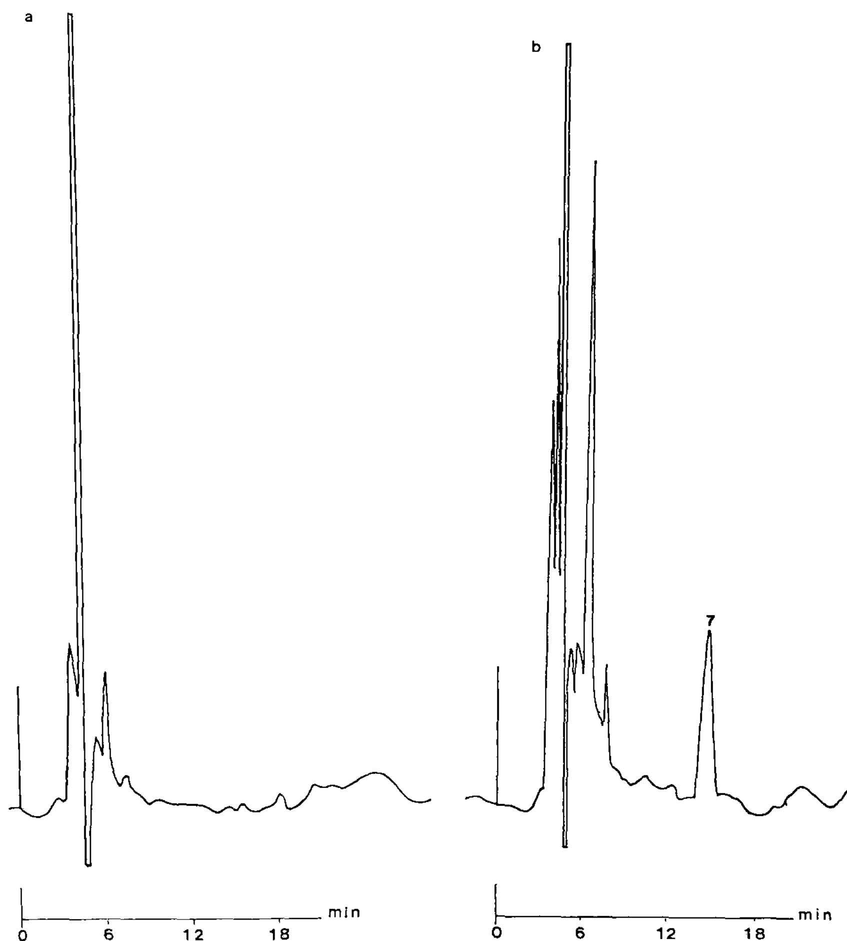
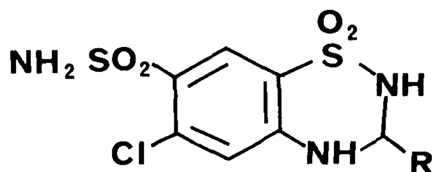


Fig. 5 Micro LC of equine plasma extracts; a) blank extract, and b) spiked with 50 ng reserpine (7) per mL of plasma. Conditions: column, Whatman ODS-3, 30 cm x 1 mm I.D.; mobile phase, methanol: water, 9:1 (v:v); flowrate, 40 μL min^{-1}; injection volume, 0.5μL; 1 μL volume micro-cell.

Table 5 Structures of the diuretics trichlormethiazide (8) and hydrochlorothiazide (9)



	R
8. Trichlormethiazide	– $CHCl_2$
9. Hydrochlorthiazide	– H

serpine (μ_{max} 216, 267, 295 nm; E 617,000; 17,000; 10,200). If a 267 nm filter was used the response would be increased. If a micro fluorescence detector was utilized it is likely that increased sensitivity would be available. These preliminary results demonstrate the potential of the technique and at the present time the detection limits for reserpine in plasma by micro-LC are being assessed.

f) Determination of Trichlormethiazide in Equine Urine

Trichlormethiazide (8, Table 5) is a potent thiazide diuretic. A method for its determination in human plasma and urine has been published [14], and also an HPLC assay of hydrochlorthiazide (9, Table 5), another thiazide diuretic found in equine plasma and urine,

using trichlormethiazide as internal standard [11]. In the latter studies trichlormethiazide was determined using hydrochlorthiazide as internal standard.

Figure 6 shows micro-LC UV chromatograms of equine urine extracts. Figure 6a is from an extract of control equine urine and Figure 6b an extract of control urine spiked with 2.5 μg/mL each of trichlormethiazide and hydrochlorthiazide. A linear cabibration of the trichlormethiazide/hydrochlorthiazide ratio based on peak heights ratios, was obtained over the range of 10 ng - 10 μg of trichlormethiazide per mL of urine. Figure 7 shows a preliminary result obtained from the administration of trichlormethiazide to a horse. The urine sample was collected eight hours after oral administration of 400 mg of trichlormethiazide (Naquasone) and the sample was spiked with 500 ng/mL of hydrochlorthiazide. This example was obtained using a different micro-LC column than that used in Figure 6.

CONCLUSIONS

The results shown illustrate that micro-LC using commercially available columns and equipment is a viable analytical tool. The potential of micro-LC for solving many analytical problems is obvious and there are certain advantages over conventional HPLC, particularly if solvent consumption is important or if one is sample limited. The implementation of these techniques to micro

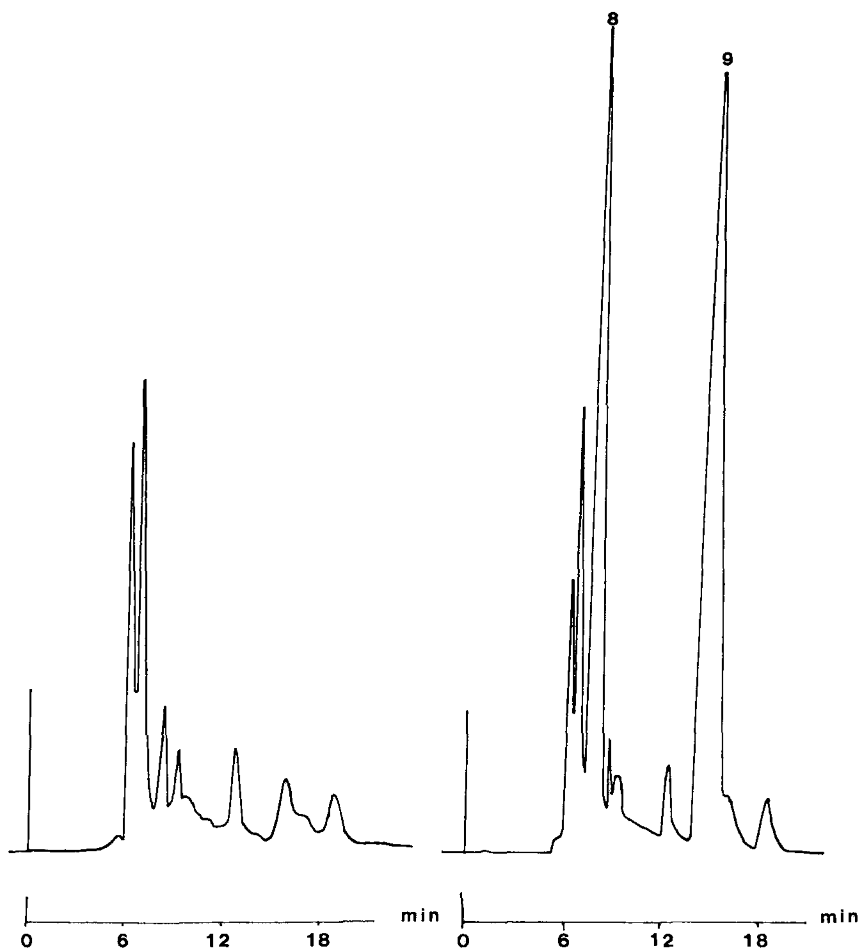


Fig. 6 Micro-LC of equine urine extracts; a) control urine extract and b) extract of control urine spiked with 2.5 μg each of trichlormethiazide (8) and hydrochlormethiazide (9) per mL of urine. Conditions: column, Alltech C_{18}, 50 cm x 1 mm; mobile phase, methanol: water, 4:6 (v:v); flow rate, 40 μL min^{-1}; injection volume, 0.5 μL; micro cell.

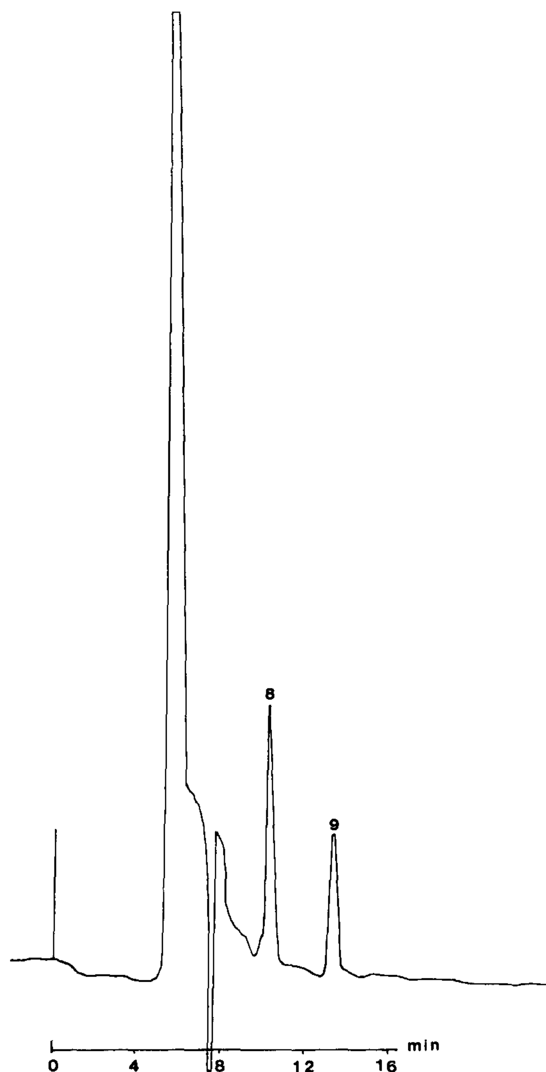


Fig. 7 Extract of equine urine taken 8 hrs after administration of 400 mg of trichlormethiazide (8) to a horse, spiked with 0.5 µg of hydrochlorthiazide per mL of urine. Conditions: column, CM Laboratories, C_{18}, 25 cm x 1 mm i.d.; mobile phase, acetonitrile: water, 35:65 (v:v); flowrate, 40 µL min^{-1}; injection volume, 0.5 µL; 1 µL volume micro-cell.

LC/MS offers the added opportunity for specificity and improved LC/MS sensitivity.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. S. Bakalyar of Rheodyne for the gift of the 7410 injector, Mr. M. Lally and Waters Assoc. for their help in setting up the micro-LC system, the column manufacturers and Dr. P. Kucera for a number of microbore columns, Dr. E. Dewey for administration of the Naquasone and supplying the plasma and urine samples and Dr. J.C. Greenfield (The Upjohn Co.) for supplying the antibiotic standards. We also thank the Zweig Memorial Fund and the New York State Racing and Wagering Board Drug Testing and Research Program for financial support of this work.

REFERENCES

1. Scott, R.P.W., J. Chromatogr. Sci., 18, 49 (1980).
2. Scott, R.P.W. and Kucera, P., J. Chromatogr., 169, 51 (1979).
3. Eckers, C., Skrabalak, D.S. and Henion, J.D., Clin. Chem., 28, 1882 (1982).
4. Yang, F.J., J. Chromatogr. Sci., 20, 241 (1982).
5. Novotny, M., J. Chromatogr. Sci., 18, 473 (1980).
6. Yang, F.J., J. Chromatogr., 236, 265 (1982).
7. Scott, R.P.W. and Kucera, P., J. Chromatogr., 185, 27 (1979).
8. Kucera, P., J. Chromatogr., 198, 93 (1980).
9. Hermansson, J., Chromatographia, 13, 741 (1980).

10. Bakalyar, S.R., Bradley, M.P.T. and Honganen, R., J. Chromatogr., 158, 277 (1978).

11. Henion, J.D. and Maylin, G.A., J. Anal. Toxicol., 4, 185 (1980).

12. American Horse Shows Association Drug Symposium, Washington, D.C., September 12, 1973.

13. Sams, R., Anal. Lett., 11, 697 (1978).

14. Meyer, M.C. and Hwans, P.T.R., J. Chromatogr., 223, 466 (1981).

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2411-2431 (1983)

INSTRUMENTAL BROADENING CORRECTION IN SIZE EXCLUSION CHROMATOGRAPHY THROUGH FAST FOURIER TRANSFORM TECHNIQUES

D. Alba and G.R. Meira*
INTEC (CONICET and Univ. Nac. del Litoral)
C.C. N° 91 - 3000 Santa Fe
Argentina

ABSTRACT

This paper shows the practicability of the use of the fast Fourier transform (FFT), with appropriate filtering in the frequency domain, as a means of deconvoluting Tung's integral formula (1). The method is limited to uniform instrumental spreading functions, but presents several important advantages: it is numerically efficient, no assumptions about the shape of the spreading function are made, it eliminates the high-frequency measurement noise components from the corrected chromatogram without modifying the original data, and provides a means of physically interpreting the results.

INTRODUCTION

Most of the methods of correction for instrumental broadening in size exclusion chromatography (SEC) are based on the integral equation proposed by Tung (1):

$$f(v) = \int_{-\infty}^{+\infty} w(y)\ g(v,y)\ dy \tag{1}$$

where v,y : both represent elution volume or elution time;

(*) To whom correspondence should be sent.

Copyright © 1983 by Marcel Dekker, Inc. 0148-3919/83/0613-2411$3.50/0

$f(v)$: is the baseline-corrected chromatogram;

$g(v,y)$: is the unit mass (or normalized) detector response for a truly monodisperse polymer species with mean retention volume y; and

$w(v)$: is the corrected chromatogram.

There are two main problems associated to Eqn. (1). The first is related to the calibration, i.e. the determination of $g(v,y)$. The second deals with its solution, i.e. the way of calculating $w(v)$.

With respect to the calibration, and due to the impossibility of fractioning perfectly monodisperse polymers, several techniques have been proposed, e.g.:

a) methods that utilize low polydispersity standards (2);
b) methods that employ standards of known molecular weigth distribution (3);
c) the reverse-flow technique (4); and
d) the recycle technique (5, 6).

In order to solve Eqn. (1), two important simplifications have been generally considered:

a) Assume the instrumental spreading function $g(v,y)$ to be uniform, i.e. independent of the mean retention volume y (1, 7, 8, 9, 10, 11). With the exception of (7), all these works adopt $g(v)$ Gaussian symmetric, and some of them suggest the possibility of correcting the chromatogram by sections, when the variation of $g(v,y)$ with y is significant.

b) Assume $g(v,y)$ Gaussian symmetric, with its variance dependent on elution volume (12, 13).

Instrumental broadening correction with non-symmetrical, non-uniform calibration functions have been attempted in the early work by Hess and Kratz (2), but as can be deduced from (14), that approach generally leads to ill-conditioned numerical problems. Later on, (15) and (16) propose general methods for solving Eqn. (1), but from the evaluation by (12) with regards to computing time, this last work suggests the convenience of simplifying the corrections methods in order to allow their implementation in relatively small computing systems.

In what follows, the assumption of uniform spreading will be made in which case Eqn. (1) may be written:

$$f(v) = \int_{-\infty}^{+\infty} w(y)\, g(v-y)\, dy \qquad (2)$$

Taking Fourier transformations of this convolution integral, one obtains

$$F(\nu) = W(\nu)\, G(\nu) \qquad (3)$$

where

$$F(\nu) = \int_{-\infty}^{+\infty} f(v) \exp\left[-\frac{j\,\nu v}{2\pi}\right] dv \qquad (4)$$

and so on.

Pierce and Armonas (8) and Tung (9) were the first to consider Eqn. (3) with the hypothesis of $g(v)$ Gaussian, and as a means of theoretically obtaining an expression for $w(v)$.

Vladimiroff (17) first suggested the possibility of obtaining w(v) through the FFT algorithms and a discrete equivalent of Eqn. (3); and a theoretical application of this idea was presented in (18). In this last work, a Gaussian spreading function was employed, and a synthetic two-peak distribution was utilized as the "corrected" chromatogram. The main problem exhibited was that the minimum tolerable signal to noise ratio in the chromatogram for good results, was somewhere around the relatively high value of 1000:1; even though the spreading curve was completely noise-free. A filtering procedure consistent in setting to zero all values of $F(\nu)$ smaller than 0,1% the maximum was used, and the possibility of processing chromatograms with signal to noise ratios greater than 200:1 was suggested. In the present work, a simple but slightly different filtering procedure is proposed; which produces satisfactory results even with experimental spreading curves and chromatograms with signal to noise ratios in the order of 20:1. The technique is particularly useful when the variation of g(v) with y is unimportant and when g(v) is obtained through direct measurement.

THEORY

For numerical work, discrete versions of f(v) and g(v) must be considered. When the sampling interval Δv is taken to be constant, then one can represent these functions by f(n) and g(n), where $v = n\,\Delta v$ and $n = 0,1,\ldots,N-1$. As should be apparent from what follows, it is convenient to consider the same total length N in both series, by adding the appropriate number of zeroes to the original data set. Furthermore, note that for the purposes of the

spreading correction, the sampling instant numbers n are displaced with respect to the original chromatogram sampling instants, which are proportional to elution volume or elution time.

The discrete Fourier transform (DFT) of, e.g., f(n) will be:

$$F(m) = \sum_{n=0}^{N-1} f(n) \exp \left[- j \frac{2\pi}{N} m\, n\right] \tag{5}$$

where $m = 0,1,\ldots,N-1$ is the discretized version of the frequency ν, such that $\nu = m\,\Delta\nu$ with $\Delta\nu$ also constant. Note that F(0) is the cumulative height of f(n). Due to the exponential term in (5), F(m) is periodic with period N. Furthermore, since f(n) is real, the modulus and phase (or the imaginary and real parts) of F(m) are all symmetric with respect to $m = N/2$. Thus, only a semi-period needs to be represented to provide the whole information. When antitransforming F(m) through:

$$f(n) = \frac{1}{N} \sum_{m=0}^{N-1} F(m) \exp \left[j \frac{2\pi}{N} m\, n\right] \tag{6}$$

the reconstructed curve will also be periodic.

A discrete counterpart of the convolution integral given by Eqn. (2) may be written:

$$f(n) = \sum_{k=0}^{N-1} w(k)\, g(n-k) \tag{7}$$

If w(n) and g(n) have N_1 and N_2 non-zero elements each, then the resulting function will have $N_3 = N_1 + N_2 - 1$ non-zero

values. The discrete version of Eqn. (3) is, on the other hand:

$$F(m) = W(m)\ G(m) \tag{8}$$

Note that when solving this expression, a sufficiently long period N will have to be taken in order to avoid the overlapping of successive periods.

The FFT algorithms, originally described by Cooley and Tuckey (19), but presently available in the major computer languages and in practically every computer library, is a highly efficient means of numerically solving Eqns. (5) and (6). When this algorithm is employed however, the period N must also verify:

$$N = 2^k \qquad (k = 1,2,...) \tag{9}$$

In practice, at least a value of k that will insure the condition

$$N > N_1 + N_2 - 1 \tag{10}$$

will have to be selected.

Eqn. (8) can be conveniently used to obtain $f(n)$ as follows:

1) calculate $F(m)$ and $G(m)$ by fast Fourier transforming $f(n)$ and $g(n)$;

2) calculate $W(m)$ through:

$$W(m) = F(m) \cdot \frac{1}{G(m)} \tag{11}$$

3) antitransform $W(m)$ via the FFT algorithm.

Note the following:

a) Due to practical reasons and for better graphical interpretation (see next section), we have found preferable to perform the product indicated in Eqn. (11) rather than the direct quotient.

b) Numerical problems will appear when $|G(m)|$ attains values close to zero.

c) Since $g(n)$ is normalized, then $G(0) = 1$ and therefore $W(0) = F(0)$; i.e. the method does not modify the chromatogram area.

d) Typical values for k and N are 7 (or 8) and 128 (or 256), respectively.

e) If $g(n)$ is symmetric with its mean at $n = 0$, then $\underline{/G(m)} = 0$ for all m, and $\underline{/W(m)} = \underline{/F(m)}$; i.e., $w(n)$ will not be translated with respect to $f(n)$. A deformation shift will be produced if $g(n)$ is asymmetric, and a pure translation will occur when its mean is not placed at $n = 0$. A practical way of dealing with this last bias, is to center the maximum of $g(n)$ at $n = 0$. Due to the periodicity of $g(n)$ when the DFT is applied, the values of $g(n)$ for $n < 0$ must be reproduced at the end of the first period, however. Physically, this implies a non-causal system with the unit mass impulse applied at $n=0$. This procedure is consistent with the fact that the experimental points of the calibration curve log M vs t obtained from

narrow-distributed standards are normally taken at the times of the maxima.

f) Even though the FFT operates with the real and imaginary parts of the transformed functions, useful information can be obtained from the observation of the corresponding modulus and phase.

APPLICATION

In this work, a series 3-B Perkin-Elmer liquid chromatograph linked to a PDP 11/40 process computer was utilized. Programs for the automatic chromatographic data acquisition and data treatment were written in FORTRAN IV. In particular, only those related to the instrumental broadening correction will be here discussed.

Three illustrative examples will be considered. Example 1 represents the ideal case where noiseless $g(n)$ and $f(n)$ functions are available, and where $|G(m)|$ does not attain values close to zero. In Example 2 noiseless chromatograms were also utilized, but a curve of $|G(m)|$ with near-zero values is considered. Finally, Example 3 illustrates the use of the technique with noisy chromatograms and with near-zero values in $|G(m)|$. In what follows, all functions considered are discrete, but shall be represented by continuous lines joining the individual points.

Example 1

Consider the spreading curve $g(n)$ and the uncorrected chromatogram $f(n)$, which are represented in Fig. 1. As previously

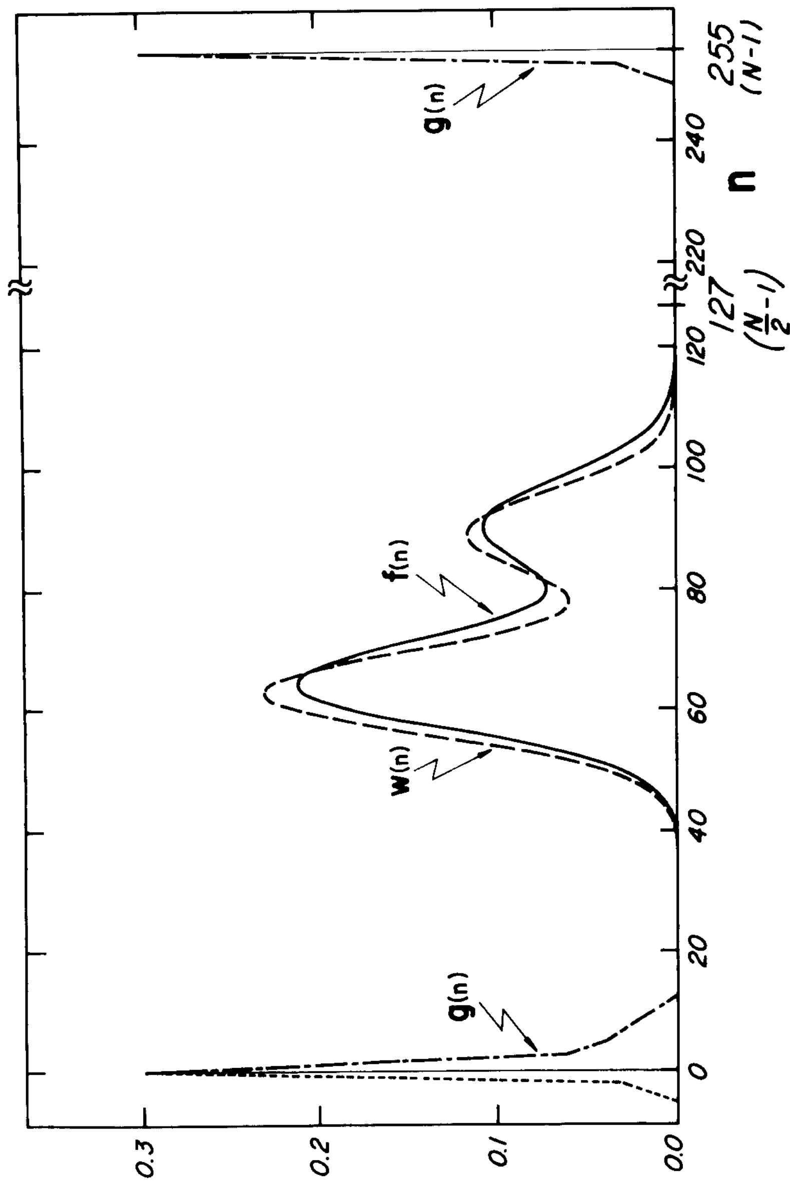


FIGURE 1: Example 1: measured chromatogram, calibration curve and corrected chromatogram.

explained, it is convenient to consider the maximum of $g(n)$ at $n=0$, with the left hand side of this curve at the end of the first period. Fig. 2 shows the magnitude and phase of all the transformed variables. The corrected chromatogram is also represented in Fig. 1. Note the following:

a) $F(m)$ and $G(m)$ both act as low-pass filters, but a necessary physical prerequisite to perform the deconvolution is that the cut-off frequency of $G(m)$ must be higher than that of $F(m)$.

b) The minimum of $|G(m)|$ is well above the limit below which the measurement and truncation errors normally produce intolerable relative errors in $1/|G(m)|$.

c) The shape of $1/|G(m)|$ indicates that $1/G(m)$ will act as a high-pass filter. This means that when multiplied by $F(m)$, $W(m)$ will have enhanced high frequency components.

d) The phase difference between $\angle F(m)$ and $\angle W(m)$ causes a distorsion shift in the corrected chromatogram with respect to the original curve.

Example 2

Consider $g(n)$ and $f(n)$ of Fig. 3. In Fig. 4, the amplitudes of the transformed functions are represented. Notice that because $|G(m)|$ adopts very small values (with high relative errors) after $m = 50$, its inverse shows very high spurious peaks at around $m = 100$. Therefore, when multiplying $|G(m)|$ by $|F(m)|$, $|W(m)|$

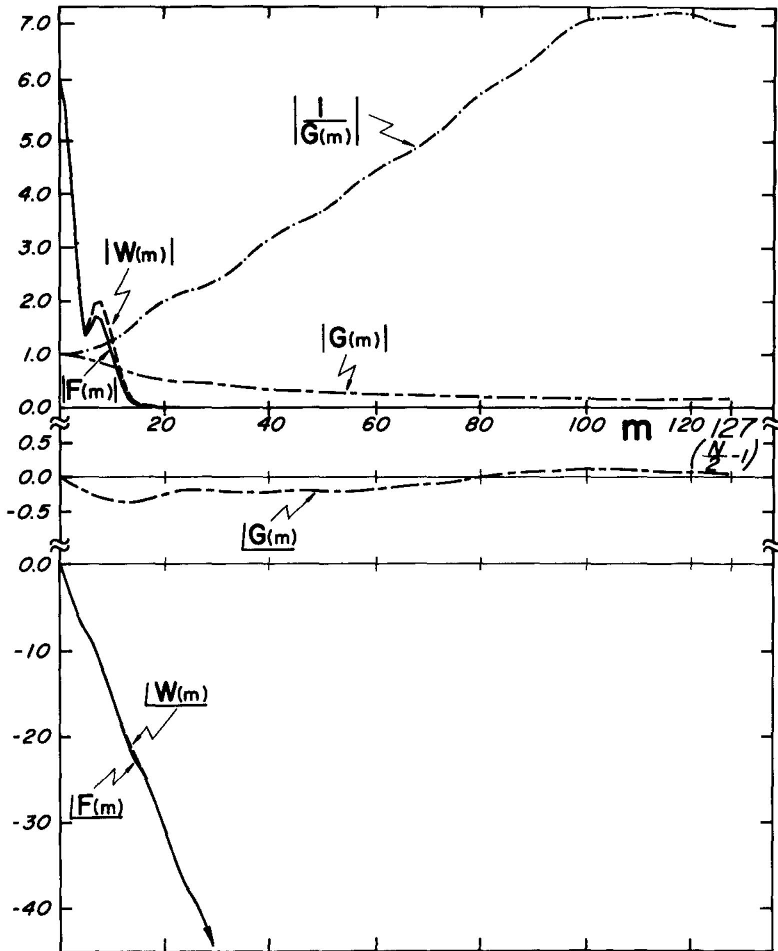


FIGURE 2: Example 1: modulus and phase of the transformed variables.

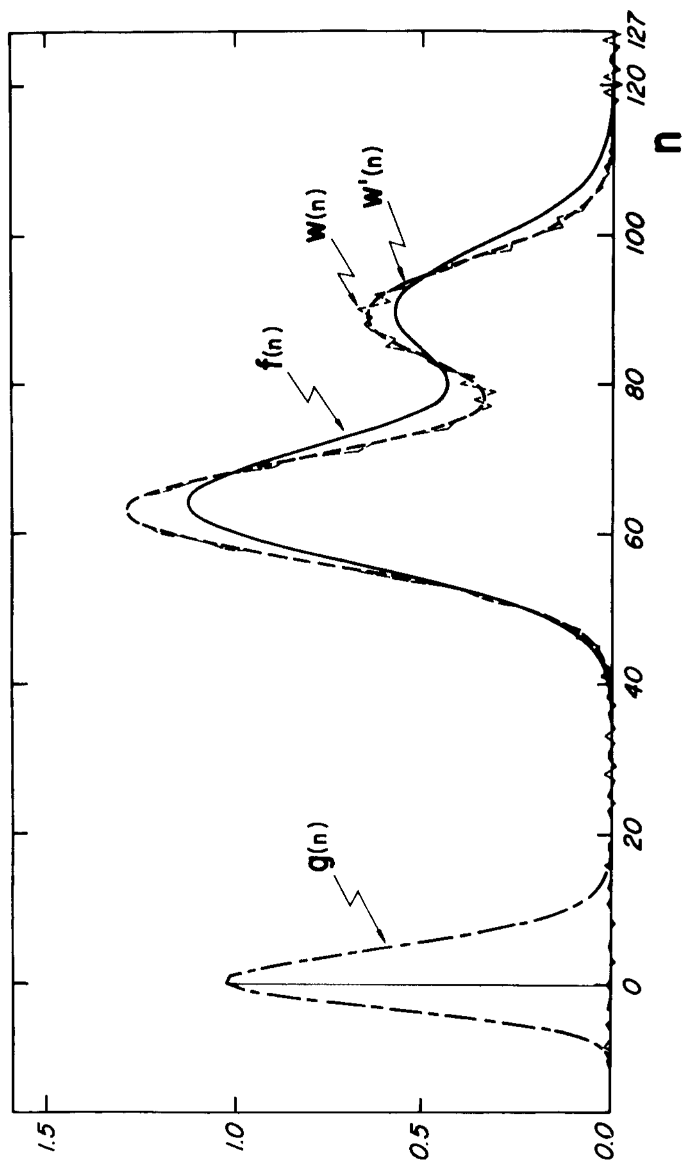


FIGURE 3: Example 2: measured chromatogram, calibration curve and corrected chromatograms (with and without filtering).

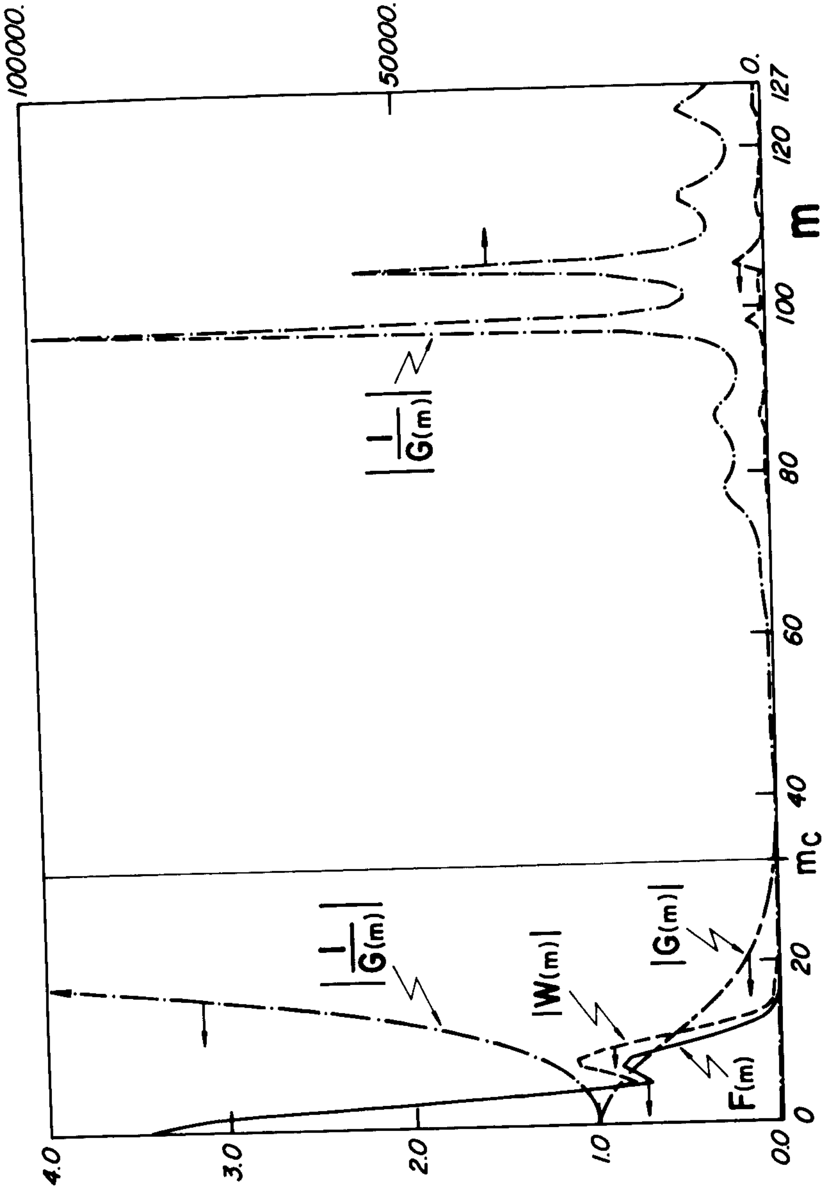


FIGURE 4: Example 2: modulus of the transformed functions, indicating the "effective" $1/|G(m)|$ function employed.

also results with relatively high values at those same frequencies; and consequently its antitransform will also be noisy (curve $w(n)$, Fig. 3). The procedure to smooth out this curve is simple; previous to antitransforming, and after a value of m where $|W(m)|$ is sufficiently low (e.g. $m_c = 32$) the amplitudes of $W(m)$ are set to zero and up to the value $m = N - m_c - 1$. The final result is function $w'(n)$ of Fig. 3. The process described is equivalent to having compensated $1/ G(m)$ with an ideal low-pass filter of unit magnitude and cut-off frequency at $m = 32$, previous to its multiplication with $F(m)$. This compensated transfer function is shown in Fig. 4 in the scale of the left axis. Note that at the frequencies of interest, and due to the right hand side scaling, the magnitude of the uncompensated of $1/G(m)$ is drawn over the horizontal axis.

For practical applications, the above mentioned compensation procedure may be alternatively performed as follows:

a) Eliminate the high-frequency components of $1/ |G(m)|$ after a frequency where the magnitude of $G(m)$ is very low and with high relative errors. This will reduce the computation time, by considering only a fraction of the total period.

b) If $w(n)$ is still noisy, then set to zero the high-frequency components of $|W(m)|$ after its first minimum, previous to its antitransformation. Note that this technique is possible because the first near zero valued minimum of $|W(m)|$ should appear at lower frequencies than those of the main components of

$G(m)$, and because the noise components of $|W(m)|$ are, in general, of much higher frequencies than those corresponding to the fractionation phenomena. If the first minimum of $|W(m)|$ did not have a near-zero value, then oscillatory corrected curves could be produced. Fortunately, this is not normally the case.

Example 3

Consider now the observed chromatogram of Fig. 5 a), (obtained by fractioning a PS standard of $M_w = 500$ through an A-803 Shodex column), which is already contaminated with high-frequency measurement noise. For correction, a typical asymmetric spreading function due to axial dispersion in capillaries, fittings, detectors, etc., was employed; which was obtained by injecting the same PS standard through the chromatograph fitted without the column. The effects of noisy chromatograms can be solved as in Example 2, by compensating $1/G(m)$ previous to its multiplication by $F(m)$. The corrected smooth chromatogram $w'(n)$ (in this case not corrected for axial dispersion in the column) is also represented in Fig. 5 a).

The selection of the appropriate cut-off frequency m_c for $W(m)$ is a trade-off between low values of m_c that eliminate all of the high frequency measurement and calculation noise but also part of the useful information (thus producing smooth and distorted corrected chromatograms with low frequency oscillatory components), and high values of m_c that minimize such distorsion but contaminate the corrected curve with zero-mean high frequency

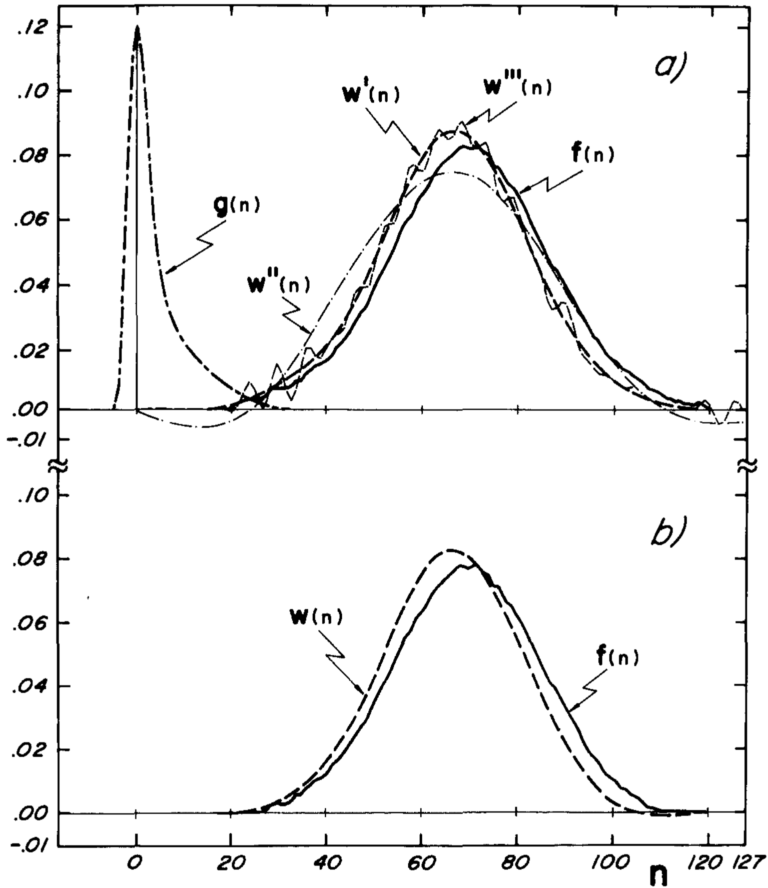


FIGURE 5: Example 3: a) calibration curve, measured and corrected chromatograms with various filterings, b) measured and corrected chromatograms with excessively high baseline correction.

noise. In the example being considered, an appropriate correction ($w'(n)$) is produced when $m_{c_2}=12$, excessive distorsion is observed with $m_{c_1}=4$ (curve $w''(n)$, Fig. 5a) , and poor filtering results if $m_{c_3}=50$ (curve $w'''(n)$, Fig. 5a). The amplitudes of the corresponding transformed functions are given in Fig. 6.

The leading and lagging edges of a chromatogram should rise slower than the corresponding spreading function. When this does not occur, then negative peaks are produced in the corrected curve. Fig. 5 b) is illustrative of this effect, due to a higher than appropriate selection of the chromatogram baseline.

DISCUSSION

The FFT technique is a powerful means of deconvoluting Tung's integral. Its main limitation is that the spreading curve $g(v)$ must be considered uniform, but on the other hand it is non-iterative, the shape of $g(v)$ can be arbitrary, and it is numerically efficient (in our computer, the calculation time for $N = 256$ was less than 3s).

When comparing alternative deconvolution methods, possibly the most important aspect is the ability of each method to cope with (and explain the reasons for) oscillatory or noisy results. There are two principal reasons for these oscillations: the high frequency measurement noise that is normally present in the original data, and the calculation noise introduced by truncation errors. With regards to the measurement noise, many authors state that an appropriate smoothening of the observed chromatograms is

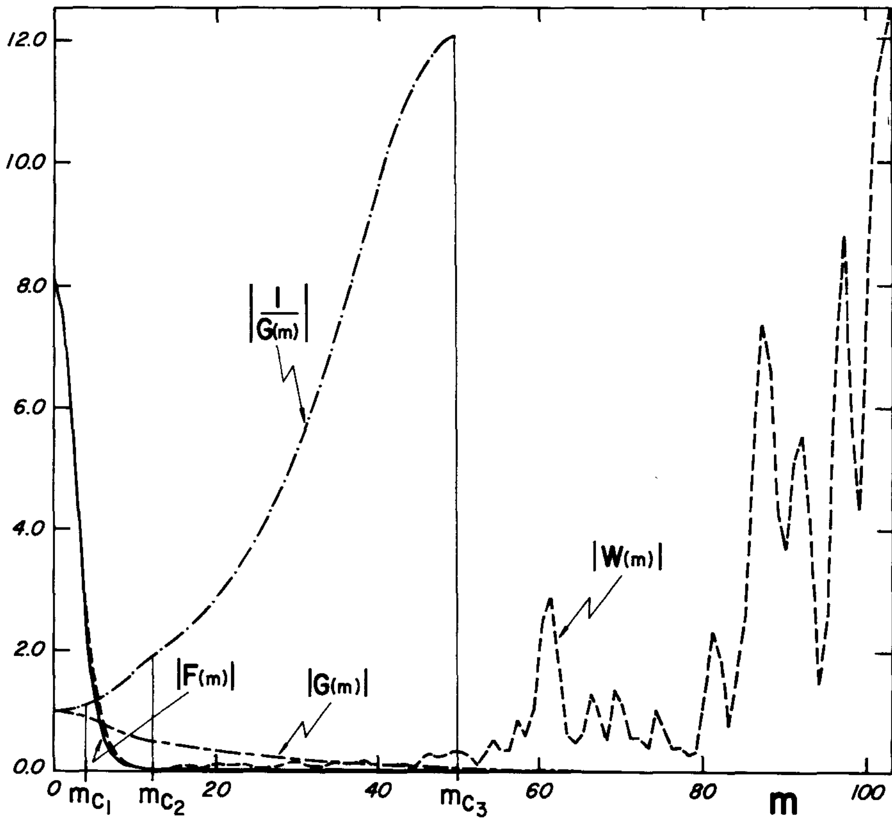


FIGURE 6: Example 3: modulus of the transformed functions.

necessary prior to the main calculations; and thus methods involving regression fits (1, 2), seven point cubic filtering (11), etc. have been proposed. In the work by Vladimiroff (18), a theoretical expression for $g(n)$ is assumed, and a preestimation of the signal to noise ratio is used to specify the cut-off frequency in the compensation of $F(m)$. As should be clear from example 3, both a too high or a too low value for the cut-off frequency may lead to completely erroneous results.

In the present work, the smoothening and/or fitting of either $g(n)$ or $f(n)$ is not required because the problems originated by the measurement and calculation noise, can be simultaneously eliminated in the last step, prior to antitransforming $W(m)$. In this way, the deconvolution is performed with all of the original data, without loss of information. The fact that the low frequency components due to the fractionation phenomena are normally well apart from the high frequency counterparts due to measurement and/or calculation noise, justifies this filtering procedure.

The program, which is available from the authors, has proven satisfactory even when the data acquisition and reduction is made fully automated, without operator intervention.

Finally, another potential advantage of the technique refers to the possibility of convoluting the individual calibrations of columns, tubings, detectors, etc., to obtain the total spreading function in accord to the configuration employed.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Mr. M. Brandolini for his help with the experimental work, to Dr. A.E. Cassano for his support and to Dr. J. Weisz for the revision of the manuscript.

REFERENCES

1. Tung, L.H., J. Applied Polymer Sci., 10, 375 (1966).
2. Hess, M. and Kratz, R.F., J. Polymer Sci. A-2, 4, 731 (1966).
3. Husain, A., Hamielec, A.E. and Vlachopoulos, J., J. Liquid Chromatography, 4, 459 (1981).
4. Tung, L.H., Moore, J.C. and Knight, G.W., J. Applied Polymer Sci., 10, 1261 (1966).
5. Waters, J.L., J. Polymer Sci. A-2, 8, 411 (1970).
6. Gruneberg, M. and Klein, J., J. Liquid Chromatography 3, 1593 (1980).
7. Balke, S.T. and Hamielec, A.E., J. Applied Polymer Sci., 13, 1381 (1969).
8. Pierce, P.E. and Armonas, J.E., J. Polymer Sci. C, 21, 23 (1968).
9. Tung, L.H., J. Applied Polymer Sci., 13, 775 (1969).
10. Tung, L.H., J. Applied Polymer Sci., 10, 1271 (1966).
11. Chan, K.S. and Huang, R.Y.M., J. Applied Polymer Sci., 13, 1459 (1969).
12. Vozka, S. and Kubin, M., J. of Chromatography, 139, 225 (1977).
13. Hamielec, A.E., J. of Liquid Chromatography, 4, (10), 1697-1707 (1981).
14. Demoment, G., "Algorithme de Deconvolution ...", Proc. of "Journees d'etude sur la deconvolution des signaux", CEN Cadarache (1982).

15. Ishige, T., Lee, S.I. and Hamielec, A.E., J. Applied Polymer Sci., 15, 1607 (1971).

16. Chang, V.S. and Huang, R.Y.M., J. Applied Polymer Sci., 16, 329-335 (1972).

17. Vladimiroff, T., J. Applied Polymer Sci., 14, 1397 (1970).

18. Vladimiroff, T., J. Chromatography, 55, 175 (1971).

19. Cooley, J.W. and Tukey, J.W., Math. Comput., 19, 297 (1965).

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2433-2463 (1983)

Analysis of Block and Statistical Copolymers by Gel Permeation Chromatography: Estimation of Mark-Houwink Constants

J. M. Goldwasser and A. Rudin*

Guelph-Waterloo Centre for Graduate Work in Chemistry
University of Waterloo
Waterloo, Ontario, Canada N2L 3G1

Abstract

A method is introduced for the estimation of Mark-Houwink constants of block and statistical copolymers. The homopolymer Mark-Houwink constants and copolymer composition are required. Use of estimated Mark-Houwink constants in GPC analyses gives results which agree well with those calculated with experimentally determined constants. Comparisons between this method and those of Runyon and coworkers (11) and Chang (12) are made for block copolymers. Chang's method was also extended to statistical copolymers, and where it compared closely with the one introduced here. The use of copolymer Mark-Houwink constants as a qualitative measure of polymer compatibilities in different solvents is also discussed.

*Address communications to this author.

Copyright © 1983 by Marcel Dekker, Inc. 0148-3919/83/0613-2433$3.50/0

Introduction

Gel permeation chromatography (GPC) has become a very powerful tool in the analysis of polymer samples. Fundamental to the interpretation of a chromatogram is the ability to translate the elution volume at which a polymer fraction appears into molecular weight. This was originally done by constructing a calibration curve of ln M vs. elution volume (ve) where M is the molecular weight of a narrow molecular weight version of the polymer being studied (1-3). The more recent universal calibration method relates ve to the hydrodynamic volume (Vh), which is a function of the product of intrinsic viscosity and molecular weight ($[\eta]M$) and permits the use of a single set of calibration standards for the GPC analysis of virtually any polymer system for which the Mark-Houwink constants are known in the GPC solvent (4-6).

The analysis of copolymers by GPC presents a very difficult problem because the Mark-Houwink constants are not generally known. Although it is possible to determine these constants either through the tedious method of fractionation or by other means (7-10), their values will change as a function of copolymer composition, and consequently would have to be redetermined for each composition of copolymer present. This problem becomes intractable in cases where the copolymer composition is heterogeneous, i.e. drifts with molecular weight. Runyon and coworkers (11) and Chang (12) have suggested methods for the calculation of the molecular weight distribution of copolymers which require the calibration curves or Mark-Houwink constants of the constituent homopolymers and the copolymer composition. These methods, however, have been applied only to diblock copolymers and not to multiblock systems or to statistical copolymers.

In this article, we propose a means for facilitating the interpretation of GPC chromatograms of copolymers of either block or statistical structures. This entails the calculation of the copolymer Mark-Houwink constants which can be accomplished with the knowledge of the copolymer composition and the Mark-Houwink constants of the contributing segments.

This method can be applied not only to binary but also to higher order copolymers provided that reliable composition analyses can be achieved.

Theory

The hydrodynamic volume of a solvated polymer coil can be defined as

$$Vh = \frac{4\pi}{3} r_H^3 \qquad (1)$$

where r_H is the hydrodynamic radius. It can be deduced from the Einstein equation (13) that

$$r_H^3 = \frac{3}{10\pi N_o} [\eta]M \qquad (2)$$

at infinite dilution, where N_o is Avogadro's constant. As a result, Vh can be expressed as:

$$Vh = \frac{4\pi}{3\phi''} [\eta]M \qquad (3)$$

where $\phi'' = 6.3067 \times 10^{24}$ (cgs units) and is related to Flory's universal constant ϕ' through the relationship between r_H and the radius of gyration, r_G, and the Flory-Fox equation (14,15).

The Mark-Houwink relation is:

$$[\eta] = K\ M^a \qquad (4)$$

where K and a are the Mark-Houwink constants. Therefore,

$$Vh = \frac{4\pi}{3\phi''} K\ M^{a+1} \qquad (5)$$

This is the widely accepted universal calibration expression, and is applicable at infinite dilution. Appropriate allowances can be made where necessary to account for concentration effects (6,16-18).

In general, one may express the hydrodynamic radius of any polymer coil according to Flory (19) as follows

$$\bar{r}_H^2 = \bar{r}_{Ho}^2\ \alpha^2 \qquad (6)$$

where the subscript zero refers to the unperturbed radius that exists under Theta conditions, and α is the chain expansion factor. This is a measure of the extent to which polymer-solvent "long range interactions" perturb the molecular dimensions of the polymer coil. One may express α according to the following relationship (20)

$$\alpha = \sigma\, M^{\varepsilon/2} \tag{7}$$

where σ and ε are constants characteristic of the polymer and solvent in question.

The mean square end-to-end radius, $\bar{r}^2$, and consequently $\bar{r}_G^2$ and $\bar{r}_H^2$ of a polymer are the sum of the contributions of all the constituent segments of the coil. A suitable functional form (see Appendix I) is:

$$\bar{r}_H^2 = \Sigma(\bar{r}_H^2)_i = \Sigma(\bar{r}_{Ho}^2)_i\, \alpha_i^2 \tag{8}$$

where the subscript, i, which refers to a segment, may represent any group of monomer units. For copolymers, segments are defined as the different moieties present whose contributions determine the coil size. For a binary copolymer these include runs of homopolymer units as well as hetero segments. The chain expansion factor of a copolymer, α_c, is a function of the molecular weight of the entire chain, M_c. The coefficients σ_i and ε_i of the relation in eq. (7) are, however, assumed to be composition dependent. Consequently, we recast eq. (8) as follows:

$$(\bar{r}_H^2)_c = \Sigma(\bar{r}_{Ho}^2)_i\, \sigma_i^2\, M_c^{\varepsilon_i} \tag{9}$$

where the subscript c refers to copolymer. The molecular weight of the ith segment of the copolymer is M_i where

$$M_i = w_i M_c \tag{10}$$

and w_i is the corresponding weight fraction of the whole copolymer. Then

$$(\bar{r}_H^2)_c = \Sigma w_i \left(\frac{\bar{r}_{Ho}^2}{M}\right)_i \sigma_i^2 M_c^{\varepsilon_i+1} \tag{11}$$

From Flory (19):

$$K_\theta = \phi''\left(\frac{\bar{r}^2_{Ho}}{M}\right)^{3/2} \tag{12}$$

and

$$K = K_\theta \sigma^3 \tag{13}$$

while

$$(a + 1) = 3/2(\varepsilon + 1) \tag{14}$$

where K_θ is the Mark-Houwink constant under Theta conditions. Then the following expression describes Vh:

$$Vh = \frac{4\pi}{3}(\bar{r}^2_H)^{3/2} = \frac{4\pi}{3\phi''}[\Sigma w_i(K_i M_c^{(a_i+1)})^{2/3}]^{3/2} \tag{15}$$

In the case of an A-B block copolymer, there would be only two segments, poly-A and poly-B, which represent the two types of interactions present, and characterize the expansion of their corresponding homopolymer coils. The influence of the A-B interactions have been shown to be minimal and may usually be ignored (21-25). However, for statistical copolymers, one must take into account the A-B heterointeractions which have been observed to contribute to coil size and consequently there would be three segments included in the calculation (25-29).

Results and Discussion

The Mark-Houwink constants for polystyrene (PS), poly(methyl methacrylate) (PMMA) and the alternating styrene-methyl methacrylate copolymer are recorded for several solvents in Table I. These constants were then used to calculate copolymer Mark-Houwink constants for both block and statistical copolymers of these components. This was done for several compositions and solvent systems as will be discussed.

Block Copolymers

The Mark-Houwink constants were determined for several compositions of styrene-methyl methacrylate block copolymers by calculating the Vh of those

Table I

Mark-Houwink Coefficients

Polymer	Solvent	K (x 10^3) (mL/g)	a	Reference
Polystyrene	tetrahydrofuran (THF)	6.82	0.766	30
	methyl ethyl ketone (MEK)	19.5	0.635	31
	toluene	7.5	0.750	32
	n-chlorobutane	15.1	0.659	33
Poly(methyl methacrylate)	THF	12.8	0.69	34
	MEK	6.80	0.72	35
	toluene	7.10	0.73	36
	n-chlorobutane	50.5	0.5	37
Alternating poly(styrene-co-methyl methacrylate)	THF	7.75	0.76	29
	MEK	11.5	0.69	29
	toluene	10.9	0.73	29
	n-chorobutane	10.8	0.70	29

copolymers at different molecular weights according to equation (15). A linear regression of the log Vh vs log M_c plot was then performed. The Mark-Houwink pre-exponential constant for the copolymer, K_c, was obtained from the intercept of the line and exponent, a_c, was estimated from the slope according to equation (5). These values are tabulated for four solvents in Table II. Because there are no heterointeractions involved in the coil expansion, the values obtained for both K_c and a_c lie between those for the two homopolymers in all cases. These constants are also composition dependent, with the values progressing linearly in a and with a slight curvature in K from near those of polystyrene to near PMMA as the methacrylate content increased in the copolymer. This is illustrated in Figures 1 to 4 for the tetrahydrofuran and n-chlorobutane solvent systems.

The Mark-Houwink constants for 1:1 styrene-methyl methacrylate block copolymer have been determined previously for the solvent systems reported in this study (29). Table III reports these values and a comparison of the molecular weights that one obtains upon conversion from hydrodynamic volume using the experimental and calculated values for the applicable molecular weight range for the experimental values, i.e. between 10^5 and 10^6 for the n-chlorobutane solvent system and between 10^4-10^6 for the others. Agreement in all cases is within 10%. This may be considered quite good when one considers the possible error in the reported values of the homopolymer Mark-Houwink constants and experimental error in the measured block copolymer Mark-Houwink constants. In addition, discrepancies may also have arisen because in some cases fractionation was incomplete, and $\bar{M}_w/\bar{M}_n$ was as high as 1.6.

Other workers have also suggested ways of relating the molecular weight of an eluting block copolymer to those of the homopolymer which comprise it. Runyon and coworkers have empirically postulated that

$$M_c = M_A^{w_A} M_B^{w_B} \tag{16}$$

where M_A and M_B are the molecular weights of the homopolymers A and B which elute at the same time as the copolymer with molecular weight, M_c (11).

Table II

Calculated Mark-Houwink Constants for Poly(styrene-co-methyl methacrylate) Block Copolymers at Several Compositions in Different Solvents

Copolymer Composition	THF		MEK		Toluene		n-Chlorobutane	
(wt. fraction styrene)	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a
0.2	11.239	0.705	8.647	0.700	8.850	0.719	38.313	0.537
0.3	10.541	0.714	9.689	0.691	9.789	0.715	53.637	0.554
0.4	9.891	0.721	10.814	0.682	10.771	0.710	29.671	0.570
0.5	9.287	0.729	12.026	0.674	11.795	0.706	26.283	0.586
0.6	8.723	0.736	13.327	0.665	12.858	0.703	23.371	0.602
0.7	8.197	0.744	14.722	0.657	13.961	0.699	20.857	0.617
0.8	7.707	0.751	16.214	0.650	15.103	0.696	18.673	0.631

Table III

Experimental Mark-Houwink Constants and Comparison of Molecular Weights Obtained from Hydrodynamic Volumes Using Experimental and Calculated Mark-Houwink Constants for 1:1 Block Poly(styrene-co-methyl methacrylate)

Solvent	K ($\times 10^3$ mL/g)[a]	a[a]	Molecular Weight[a]	Molecular Weight[b]	Hydrodynamic Volume
THF	6.41	0.76			
			1.0×10^4	0.952×10^4	4.668×10^{-20}
			1.0×10^5	0.942×10^5	2.686×10^{-18}
			1.0×10^6	1.034×10^6	1.546×10^{-16}
MEK	14.66	0.67			
			1.0×10^4	1.101×10^4	4.660×10^{-20}
			1.0×10^5	1.095×10^5	2.180×10^{-18}
			1.0×10^6	1.089×10^6	1.020×10^{-16}
Toluene	13.18	0.69			
			1.0×10^4	0.979×10^4	5.037×10^{-20}
			1.0×10^5	0.958×10^5	2.467×10^{-18}
			1.0×10^6	0.938×10^6	1.208×10^{-16}
n-Chlorobutane	44.46	0.55			
			1.0×10^5	1.073×10^5	1.661×10^{-18}
			1.0×10^6	1.018×10^6	5.892×10^{-16}

(a) reference (29) (b) this work

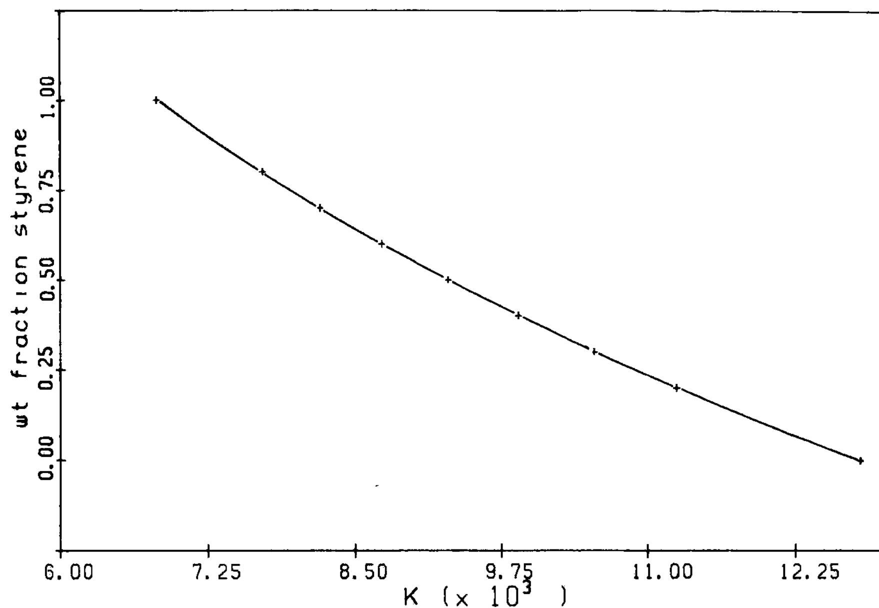


Figure 1. Styrene-Methyl Methacrylate Block Copolymer Composition vs. K in THF.

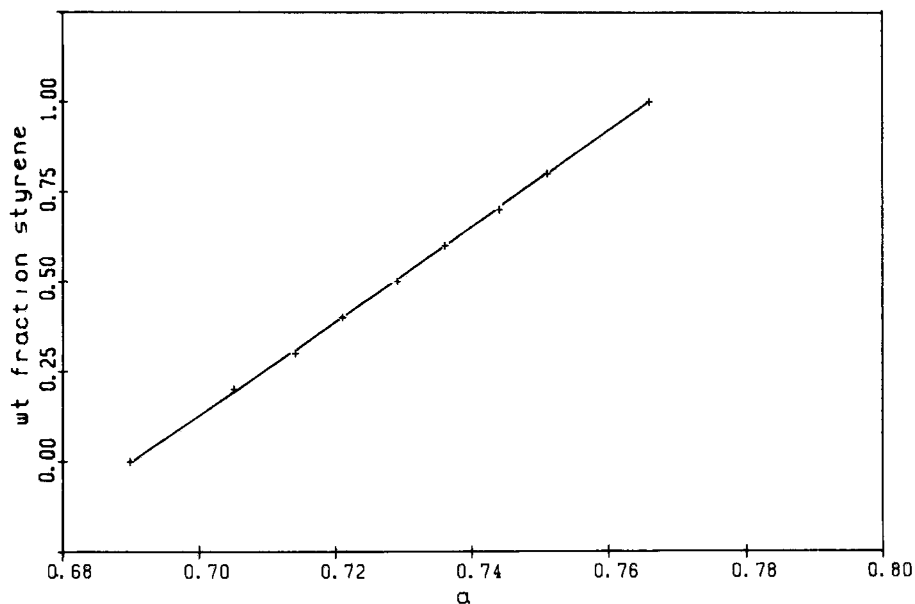


Figure 2. Styrene-Methyl Methacrylate Block Copolymer Composition vs. a in THF.

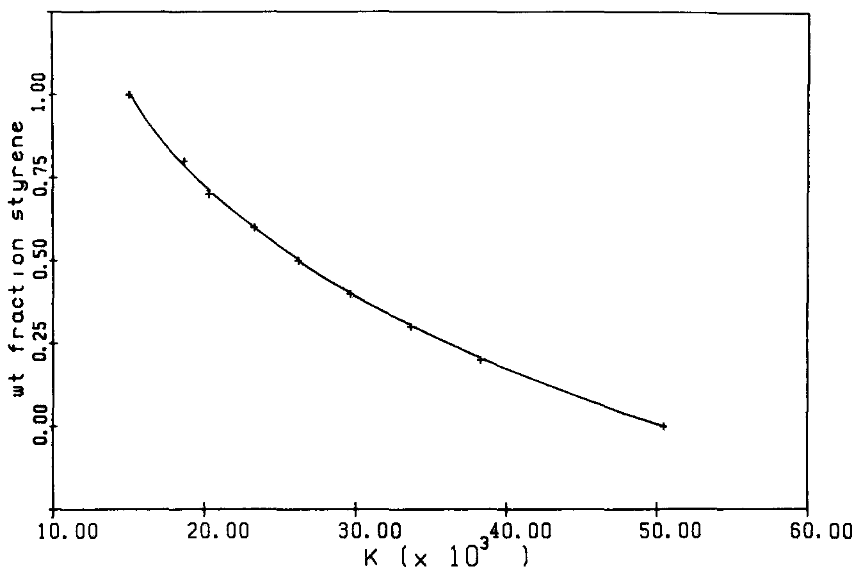


Figure 3. Styrene-Methyl Methacrylate Block Copolymer Composition vs. K in n-Chlorobutane.

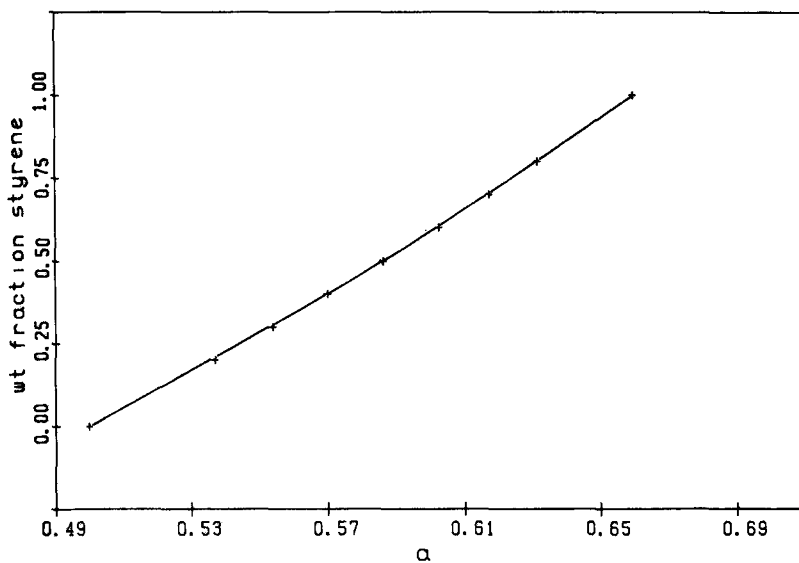


Figure 4. Styrene-Methyl Methacrylate Block Copolymer Composition vs. a in n-Chlorobutane.

Chang proposed (12) that since two homopolymers will elute at the same time when

$$M_1 = \left[\frac{K_2}{K_1} M_2^{(a_2+1)}\right]^{1/(a_1+1)} \tag{17}$$

where the subscripts 1 and 2 refer to different polymers, one may assume a block copolymer with molecular weight M_c will have the same elution volume as a homopolymer with molecular weight M_A when

$$M_c = M_1 + M_2 \tag{18}$$

and

$$M_A = M_1 + \left[\frac{K_2}{K_1} M_2^{(a_2+1)}\right]^{1/(a_1+1)} \tag{19}$$

Mark-Houwink constants were calculated for the different composition styrene-methyl methacrylate block copolymers as described before using these two methods and are presented in Tables IV and V. Comparison of these constants and those in Table II show excellent agreement for each of the solvent systems with the exception of n-chlorobutane. Molecular weights calculated from Vh's using the Mark-Houwink constants derived from these three methods are within 2% of each other for the range of 10^4-10^6. For the n-chlorobutane system, disparities between this work and Runyon's range up to 10% but only 5% between this work and Chang's. However, the disagreement between the values calculated according to Runyon and Chang range up to 15%. This probably results from the large difference in Mark-Houwink constants of the homopolymers in this solvent and is a manifestation of the difference of methods for determination of copolymer constants. It is in cases such as this that the weakness of the empirical method begins to appear. Even so, when applied to the GPC analysis of a broad distribution polymer of $\bar{M}_n \approx 178,000$ and $\bar{M}_w \approx 450,000$, the total discrepancy is reduced to about 6%.

Table IV

Calculated Mark-Houwink Constants for Poly(styrene-co-methyl methacrylate) Block Copolymers at Several Compositions in Different Solvents after the Method of Runyon et al (11)

Copolymer Composition	THF		MEK		Toluene		n-Chlorobutane	
(wt. fraction styrene)	K ($x10^3$ mL/g)	a	K ($x10^4$ mL/g)	a	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a
0.2	11.335	0.705	8.468	0.702	8.533	0.722	40.423	0.529
0.3	10.658	0.712	9.434	0.694	9.348	0.718	36.046	0.544
0.4	10.016	0.720	10.498	0.685	10.237	0.714	32.071	0.560
0.5	9.408	0.727	11.670	0.676	11.206	0.710	28.466	0.575
0.6	8.832	0.735	12.959	0.668	12.261	0.706	25.206	0.592
0.7	8.286	0.742	14.375	0.660	13.410	0.701	22.263	0.608
0.8	7.770	0.750	15.929	0.651	14.660	0.698	19.613	0.624

Table V

Calculated Mark-Houwink Constants for Poly(styrene-co-methyl methacrylate) Block Copolymers at Several Compositions in Different Solvents after the Method of Chang (12)

Copolymer Composition	THF		MEK		Toluene		n-Chlorobutane	
(wt. fraction styrene)	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a
0.2	11.412	0.706	8.513	0.700	8.752	0.720	39.737	0.535
0.3	10.774	0.713	9.496	0.691	9.652	0.715	35.482	0.552
0.4	10.169	0.721	10.572	0.682	10.601	0.711	31.771	0.568
0.5	9.590	0.728	11.748	0.673	11.602	0.707	28.488	0.583
0.6	9.033	0.736	13.031	0.665	12.656	0.703	25.542	0.598
0.7	8.493	0.743	14.430	0.657	13.765	0.699	22.853	0.613
0.8	7.962	0.750	15.957	0.649	14.932	0.696	20.340	0.627

Statistical Copolymers

The application of equation (15) to the estimation of Mark-Houwink constants of statistical copolymers is much more difficult than for block copolymers. Because heterointeractions play an important role in the determination of coil size in solution, it is necessary to know the fractions of homo and hetero diads present in the polymer chain as well as the homo and hetero Mark-Houwink constants.

The fractions of homo and heterodiads may be either determined spectroscopically (37) or calculated from the reactivity ratios and feed composition assuming a simple copolymer (39) or other model. If the Mayo-Lewis (39) copolymer equation applies then the mole fractions (N_{ii}) of the various diad types can be calculated from the following well-known expressions (40):

$$N_{12} = \frac{P_{12}P_{21}}{0.5(P_{12} + P_{21})} \tag{20}$$

$$N_{11} = 0.5N_{12}\left(\frac{1}{P_{12}} - 1\right) \tag{21}$$

$$N_{22} = 0.5N_{12}\left(\frac{1}{P_{21}} - 1\right) \tag{22}$$

where the subscripts 12 and 21 refer to the heterodiads, 11 refers to the homodiad of monomer 1, and 22 refers to the homodiad of monomer 2, and

$$P_{12} = \frac{1}{r_1 \frac{[M]_1}{[M]_2} + 1} \tag{23}$$

$$P_{21} = \frac{\frac{[M_1]}{[M_2]}}{\frac{[M_1]}{[M_2]} + r^2} \tag{24}$$

The term, P_{ij}, denotes the probability of occurrence of an M_iM_j sequence, $[M_1]/[M_2]$ denotes the ratio of concentration or mole fractions of monomers 1 and 2 in the feed, and r_1 and r_2 are the reactivity ratios. The mole

fractions of homo and heterodiads may then be converted into weight fractions for use in equation (15). These calculations were done for several compositions of styrene-methyl methacrylate statistical copolymers, where $r_1 = 0.449$, $r_2 = 0.480$ (41). The results are tabulated in Tabel VI. The importance of taking into account the contributions of the heterointeractions is well illustrated for this case. The proportion of heterodiads present ranges to nearly 70% for the 1:1 copolymer. Even for copolymers of high content in either styrene or methyl methacrylate the amount of heterodiads present exceeds 35%.

The Mark-Houwink constants for hetero segments may be estimated in two ways. An alternating copolymer may be prepared and constants determined either classically or as described by Dobbin et al (9, 10). These constants are to be considered as equivalent to those which determine the heterointeraction contribution in a statistical copolymer. Conversely, a statistical copolymer of one composition may be prepared and its Mark-Houwink constants determined. Equation (15) could then be employed to solve for the heteroconstants from the copolymer and contributing homopolymer Mark-Houwink constants, the weight fractions of homo and heterodiads present, and Vh at several molecular weights. The copolymer Mark-Houwink constants could then be determined for any composition of the statistical copolymer as described before.

The Mark-Houwink constants for alternating poly(styrene-co-methyl methacrylate) and those for polystyrene and poly(methyl methacrylate) which are listed in Table I were used with the weight fractions of homo and heterodiads from Table VI to calculate the copolymer Mark-Houwink constants for statistical copolymers at several compositions in different solvents. These values are recorded in Table VII.

A comparison of molecular weights obtained from hydrodynamic volume using the calculated Mark-Houwink constants and values obtained experimentally (28) are presented in Table VIII for three different compositions of statistical poly(styrene-co-methyl methacrylate) in toluene and n-chlorobutane. The

Table VI

Weight Fraction of Homo and Heterodiads Present in Statistical Poly(styrene-co-methyl methacrylate) of Various Compositions

Copolymer Composition (wt. fraction styrene)	wt. Fraction Homostyrene diads (w_{11})	wt. Fraction Homomethacrylate diads (w_{22})	wt. Fraction Hetero diads (w_{12})
0.20	0.012	0.619	0.369
0.30	0.033	0.444	0.523
0.40	0.076	0.288	0.636
0.50	0.152	0.165	0.683
0.60	0.271	0.083	0.646
0.70	0.426	0.036	0.537
0.80	0.606	0.013	0.381

Table VII

Calculated Mark-Houwink Constants for Poly(styrene-co-methyl methacrylate)

Statistical Copolymers at Several Compositions in Different Solvents

Copolymer Composition	THF		MEK		Toluene		n-Chlorobutane	
(wt. fraction styrene)	K (x10^3 mL/g)	a	K (x10^3 mL/g)	a	K (x10^3 mL/g)	a	K (x10^3 mL/g)	a
0.2	10.437	0.718	8.503	0.707	8.544	0.729	26.263	0.585
0.3	9.532	0.731	9.440	0.700	9.320	0.728	20.225	0.619
0.4	8.789	0.742	10.457	0.693	10.159	0.727	16.375	0.647
0.5	8.213	0.750	11.570	0.686	11.081	0.723	14.168	0.665
0.6	7.787	0.756	12.819	0.677	12.121	0.718	13.190	0.675
0.7	7.473	0.760	14.218	0.667	13.282	0.712	13.056	0.677
0.8	7.220	0.763	15.791	0.657	14.570	0.705	13.437	0.673

Table VIII

Experimental Mark-Houwink Constants and Comparison of Molecular Weights Obtained from Hydrodynamic Volumes Using Experimental and Calculated Mark-Houwink Constants for Different Composition Statistical Poly(styrene-co-methyl methacrylate) Samples

Copolymer Composition (wt. fraction styrene)	Solvent	K ($\times 10^3$ mL/g) (experimental)	a (experimental)	Molecular Weight (from experimental values)	Molecular Weight (from calculated values)	Hydrodynamic Volume
0.30	toluene	11.4	0.70	1×10^5	0.932×10^5	2.394×10^{-18}
				1×10^6	0.898×10^6	1.200×10^{-16}
	n-chlorobutane	26.5	0.60	1×10^5	1.032×10^5	1.760×10^{-18}
				1×10^6	1.005×10^6	7.007×10^{-17}
0.57	toluene	13.2	0.71	1×10^5	0.999×10^5 (a)	3.111×10^{-18}
				1×10^6	0.986×10^6 (a)	1.595×10^{-16}
	n-chlorobutane	24.9	0.63	1×10^5	1.078×10^5 (b)	2.336×10^{-18}
				1×10^6	1.016×10^6 (b)	9.965×10^{-17}
0.71	toluene	8.32	0.75	1×10^5	0.982×10^5	3.107×10^{-18}
				1×10^6	1.034×10^6	1.747×10^{-16}
	n-chlorobutane	17.6	0.67	1×10^5	1.151×10^5	2.617×10^{-18}
				1×10^6	1.146×10^6	1.224×10^{-16}

calculated values of (a) $K = 11.785 \times 10^{-3}$ $a = 0.720$

(b) $K = 13.384 \times 10^{-3}$ $a = 0.673$

applicable molecular weight range of the experimental constants is ~10^5-10^6, and consequently the comparison is restricted to that range. The agreement of molecular weights obtained from the calculated and experimental constants is excellent. Only in one case, the 7:3 styrene-methyl methacrylate copolymer in n-chlorobutane, is there deviation between the two values of about 15%; in all other cases agreement is within 10%. Again, the discrepancies may perhaps be ascribed to the experimental limitations in determining the Mark-Houwink constants as described before.

Although no other methods have been postulated for the correlation of hydrodynamic volume to molecular weight of statistical copolymer, Chang's hypothesis for block copolymers (12) may be extended towards application for statistical copolymer analysis. This can be done by inserting the heterodiad contributions into equations (18) and (19) as follows:

$$M_c = M_{11} + M_{22} + M_{12} \qquad (25)$$

$$M_A = M_{11} + \left[\frac{K_{22}}{K_{11}} M_{22}^{(a_{22}+1)}\right]^{1/(a_{11}+1)} + \left[\frac{K_{12}}{K_{11}} M_{12}^{(a_{12}+1)}\right]^{1/(a_{11}+1)} \qquad (26)$$

The copolymer Mark-Houwink constants may then be calculated from these equations as previously described. Table IX lists the results of these calculations for several compositions of statistical poly(styrene-co-methyl methacrylate) in four solvents. The values obtained according to this method are almost identical to those calculated according to equation (15) in all cases except for the n-chlorobutane solvent system. Even so, a comparison of molecular weights calculated from hydrodynamic volumes in the applicable range using the two methods show agreement within 5% in all cases. The method of Runyon and coworkers (11) does not lend itself for application in a statistical copolymer system, because of its empirical nature.

The values of both K and a, as determined from equation (19) for statistical copolymers are composition dependent as in the block copolymers

Table IX

Calculated Mark-Houwink Constants for Poly(styrene-co-methyl methacrylate) Statistical Copolymers at Several Compositions in Different Solvents after Chang (12)

Copolymer Composition	THF		MEK		Toluene		n-Chlorobutane	
(wt. fraction styrene)	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a	K ($x10^3$ mL/g)	a
0.2	10.740	0.718	8.107	0.707	8.295	0.729	28.050	0.581
0.3	9.877	0.730	9.003	0.700	9.052	0.728	22.037	0.614
0.4	9.113	0.741	10.038	0.693	9.904	0.727	17.937	0.641
0.5	8.473	0.749	11.199	0.685	10.850	0.723	15.331	0.660
0.6	7.970	0.756	12.489	0.676	11.906	0.718	13.922	0.671
0.7	7.584	0.760	13.906	0.667	13.068	0.712	13.416	0.674
0.8	7.284	0.763	15.496	0.656	14.360	0.705	13.521	0.672

case. However, because of the influence on coil size of the heterointeractions, both constants are weighted towards those of the heterodiads. This is especially true at compositions approaching 1:1 styrene-methyl methacrylate where the proportion of heterodiads is greatest. This manifests itself in a significant deviation in the plots of copolymer composition vs. both K and a from those obtained for the block copolymers. This is shown for both tetrahydrofuran and n-chlorobutane in Figures 5-8.

The values of the hetero Mark-Houwink constants, relative to those of the homopolymers, provide a comparative measure of the compatibility of the two components in a particular solvent system. The interaction of two components which are incompatible will expand the coil size relative to the non-interactive, i.e. block case, because of segmental repulsions. Conversely, attractive interactions will contract the coil size relative to the non-interactive case. The solvent, of course, plays a large role in these interactions. As a result, a solvent system which is of approximately the same quality for both homopolymer components would expand the copolymer coil (where repulsive interactions are present) to a substantially smaller extent than when it is a good solvent for one component and a poor solvent for the other. This is because the solvated segments of the heterodiad are of increased incompatibility in the latter case.

Table X shows the fraction of volume increase of the statistical poly(styrene-co-methyl methacrylate) over those of the block copolymers at different compositions in the four solvents studied. In all cases, the amount of volume increase was composition dependent, and reached a maximum where the proportion of heterodiads was greatest, at about 1:1 styrene-methyl methacrylate content. In addition, the maximum volume increase ranged from about 14% in methyl ethyl ketone, a solvent which is of approximately the same quality for polystyrene as poly(methyl methacrylate), to about 61% in n-chlorobutane which is a fairly good solvent for polystyrene but a Theta solvent for poly(methyl methacrylate). Maximum increases in volume for tetrahydrofuran and toluene were both about 19%.

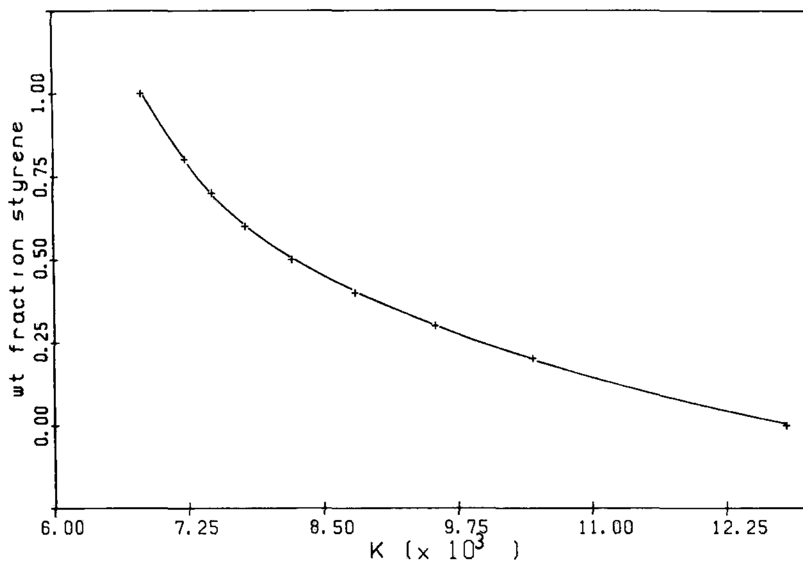


Figure 5. Statistical Copolymer Composition vs. K in THF.

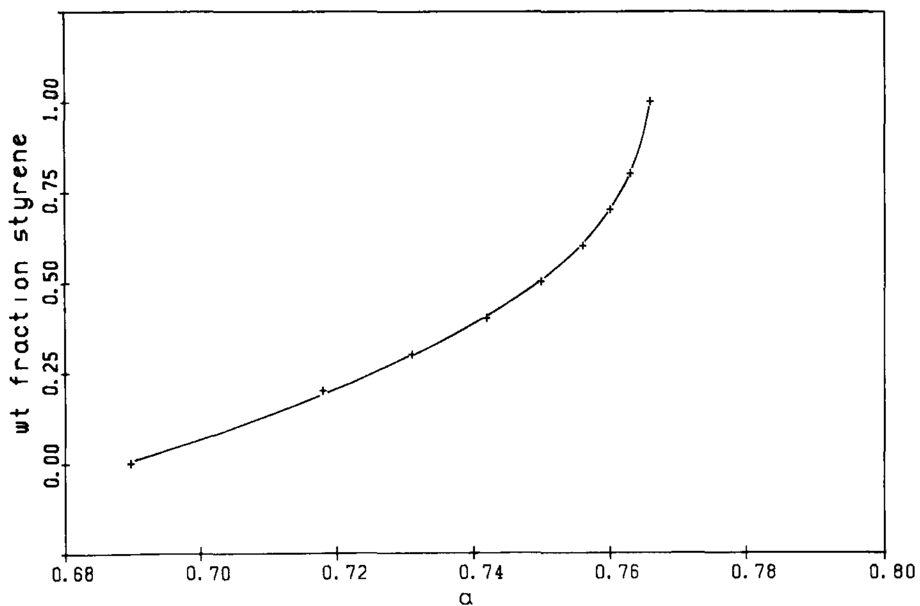


Figure 6. Statistical Copolymer Composition vs. a in THF.

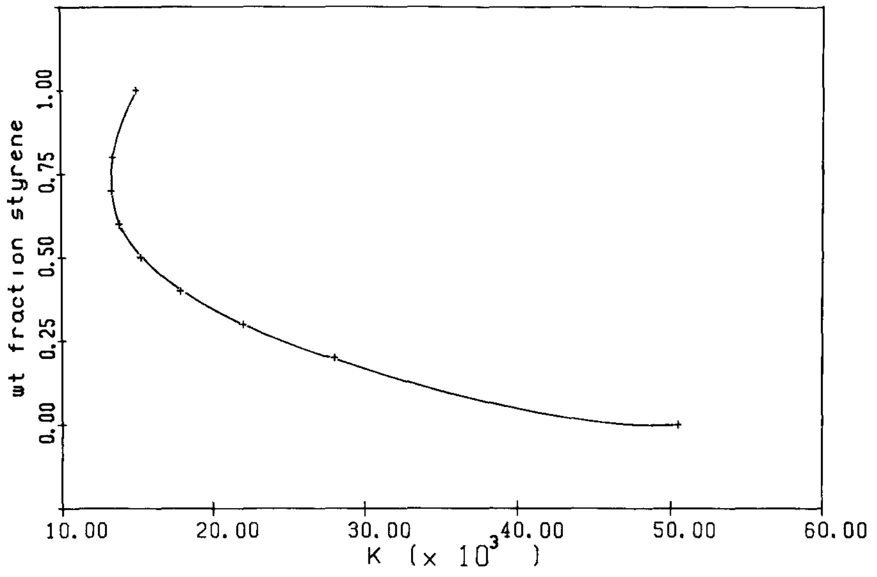


Figure 7. Statistical Copolymer Composition vs. K in n-Chlorobutane.

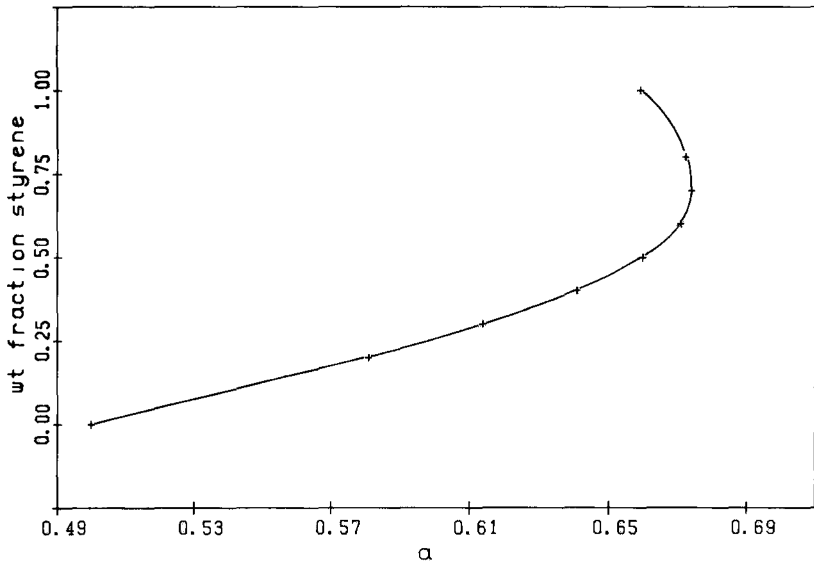


Figure 8. Statistical Copolymer Composition vs. a in n-Chlorobutane.

Table X

Comparison of Hydrodynamic Volumes Calculated from Mark-Houwink Constants of Block and Statistical Poly(styrene-co-methyl methacrylate) of Different Compositions in Several Solvents for Molecular Weight of 1 x 10^6

Solvent	Copolymer Composition (wt.frac. St.)	Hydrodynamic Volume Block Copolymers ($x10^{16}$)	Hydrodynamic Volume Statistical Copolymers ($x10^{16}$)	Fraction Volume Increase
THF	.2	1.268	1.409	.1113
	.3	1.347	1.540	.1436
	.4	1.392	1.653	.1876
	.5	1.460	1.726	.1820
	.6	1.510	1.778	.1768
	.7	1.585	1.803	.1372
	.8	1.642	1.815	.1057
MEK	.2	0.910	0.986	.0831
	.3	0.901	0.994	.1032
	.4	0.888	0.999	.1256
	.5	0.884	1.004	.1355
	.6	0.865	0.982	.1353
	.7	0.856	0.949	.1088
	.8	0.855	0.918	.0728
toluene	.2	1.212	1.343	.1084
	.3	1.268	1.445	.1394
	.4	1.302	1.553	.1928
	.5	1.349	1.603	.1881
	.6	1.411	1.637	.1597
	.7	1.450	1.651	.1385
	.8	1.505	1.644	.0924
n-chlorobutane	.2	0.424	0.564	.3304
	.3	0.471	0.695	.4759
	.4	0.518	0.829	.5990
	.5	0.573	0.920	.6055
	.6	0.635	0.983	.5472
	.7	0.697	1.000	.4340
	.8	0.758	0.974	.2855

It should be possible, therefore, to exploit this phenomenon to obtain a qualitative measure of relative compatibilities or incompatibilities of different polymers in different solvent systems. This may be done simply by estimating the Mark-Houwink constants of the statistical copolymers whose constitutive homopolymers are to be studied in the desired solvents. Plots of the copolymer composition vs. K or a are then constructed. A concave distortion from the block copolymer plot would indicate repulsive interactions and hence incompatibility and convex deviation would indicate attractive interactions and compatibility. The relative degree of deviation is an expression of the relative degree of compatibility or incompatibility between the two polymers. A comparison of Figures 5 and 6 with Figures 7 and 8 shows that for the styrene/methyl methacrylate system, incompatibility in n-chlorobutane is much more acute than in tetrahydrofuran.

GPC Analysis

The use of copolymer Mark-Houwink constants, determined as described above is most easily applied to the GPC analysis of samples with homogeneous composition, i.e. low conversion, constant feed or azeotropically produced copolymers. This is because only one detector and a single set of Mark-Houwink constants are then required. The situation becomes more complex for copolymers of heterogeneous composition. In that case, one requires a method for converting the GPC trace into weight fraction of eluting copolymer and following the composition change with elution volume. The first requirement may be fulfilled with the use of a densimeter detector whose response factor has been shown to be composition independent to a first approximation (42). The second requirement can be fulfilled if one uses a calibrated second detector such as infrared (IR).

GPC analysis of ternary and higher order copolymers may be accomplished even with heterogeneous samples provided that one can follow the copolymer composition--with e.g. a multiband or Fourier transform IR detector--and determine accurately the weight fractions of homo and heterodiads present at each weight fraction in a statistical system. This would entail expanding equations (19-23) to accommodate higher order copolyerizations.

Conclusions

A method for the calculation of Mark-Houwink constants for both block and statistical copolymers has been successfully applied to the styrene methyl methacrylate system in several solvents. Agreement of molecular weights calculated from Vh using these constants and experimental values is very good. Comparison between this and other predictive methods yield results which are also in good agreement. Plots of copolymer composition vs. K or a may be used as a qualitative measure of relative polymer compatibilities in solution. The ability to calculate Mark-Houwink constants at any copolymer composition enables GPC analysis of those copolymers provided that the weight fraction of eluting copolymer and copolymer composition can be followed. Application of GPC to the analysis of higher order copolymers is possible but becomes increasingly more complex as the number of components increases.

Acknowledgment

This research was supported by the Natural Science and Engineering Research Council of Canada.

References

(1) J. C. Moore, J. Polym. Sci., A2, 835 (1964).

(2) K. H. Altgelt and J. C. Moore, in "Polymer Fractionation", M. J. R. Cantow ed., Academic Press, New York, 1977, Chapter B4.

(3) A. C. Ouano, E. M. Barrell II, and J. F. Johnson, in "Polymer Molecular Weights", Part II, P. E. Slade Jr. ed., Marcel Dekker, New York, 1975, Chapter 6.

(4) Z. Grubisic, P. Rempp, and H. Benoit, J. Polym. Sci. B, 5, 753 (1967).

(5) E. Nichols, ACS Advances in Chem. Ser., 125, 148 (1973).

(6) A. Rudin and R. A. Wagner, J. Appl. Polym. Sci., 20, 1483 (1976).

(7) S. L. Terry, Ph.D. Thesis, Cornell University, 1969.

(8) A. R. Weiss and E. Cohn-Ginsberg, J. Polym. Sci. B, 7, 379 (1969).

(9) C. J. B. Dobbin, A. Rudin, and M. F. Tchir, J. Appl. Polym. Sci., 25, 2985 (1980).

(10) C. J. B. Dobbin, A. Rudin, and M. F. Tchir, J. Appl. Polym. Sci., 27, 1081 (1982).

(11) J. R. Runyon, D. E. Barnes, J. F. Rudd, and L. H. Tung, J. Appl. Polym. Sci., 13, 2359 (1969).

(12) F. S. C. Chang, J. Chromatog., 55, 67 (1971).

(13) A. Einstein, "Investigations on the Theory of Brownian Motion", Dover Publications, N.Y., 1954.

(14) C. M. Kok and A. Rudin, Makrom. Chemie, Rapid Commun., 2, 655 (1981).

(15) P. J. Flory and T. G. Fox, J. Amer. Chem. Soc., 73, 1904 (1951).

(16) H. Kh. Mahabadi and A. Rudin, Polym. J., 11, 123 (1979).

(17) A. Rudin, J. Polym. Sci. A-1, 9, 2587 (1971).

(18) W. L. Elsdon, J. M. Goldwasser, and A. Rudin, J. Polym. Sci. B, 19, 483 (1981).

(19) P. J. Flory, "Principles of Polymer Chemistry", Cornell University Press, Ithica, 1953.

(20) P. J. Flory and W. R. Krigbaum, J. Chem. Phys., 18, 1086 (1956).

(21) S. Krause, J. Phys. Chem., 68, 1948 (1964).

(22) A. Dondos, D. Froehlich, R. Rempp,and H. Benoit, J. Chem. Phys., 64, 1012 (1967).

(23) A. Dondos, P. Rempp, and H. Benoit, Polymer, 13, 97 (1972).

(24) A. Dondos, P. Rempp, and H. Benoit, Makrom. Chemie, 130, 233 (1964).

(25) A. Dondos, P. Rempp, and H. Benoit, Makrom. Chem., 175, 1659 (1974).

(26) D. Froelich and H. Benoit, Makrom. Chemie, 92, 224 (1966).

(27) A. Dondos and H. Benoit, Makrom. Chemie, 118, 165 (1968).

(28) T. Kotaka, Y. Murakami, and H. Inagaki, J. Phys. Chem., 72, 829 (1968).

(29) T. Kotaka, T. Tanaka, H. Onhumi, Y. Murakami, and H. Inagaki, Polym. J., 1, 245 (1970).

(30) K. A. Boni, F. A. Sliemers, and P. B. Stickney, J. Polym. Sci. A-2, 6, 1567 (1968).

(31) J. Oth and U. Desreux, Bull. Soc. Chim. Belges, 63, 285 (1954).

(32) P. Outer, C. I. Carr, and B. H. Zimm, J. Chem. Phys., 18, 830 (1950).

(33) H. Utiyama, Dissertation, Kyoto Univ., Kyoto, Japan, 1962.

(34) A. Rudin and H. L. W. Hoegy, J. Polym. Sci. A-1, 10, 217 (1972).

(35) J. Bischoff and V. Desreux, Bull. Soc. Chim. Belges, 61, 10 (1952).

(36) S. N. Chinai, J. D. Matlock, A. L. Resnick, and R. J. Samuels, J. Polym. Sci., 17, 391 (1955).

(37) H. Inagaki and S. Kawai, Makrom. Chem., 79, 42 (1964).

(38) H. J. Harwood, Angew. Chem. (Int. Ed.), 4, 1051 (1965).

(39) F. R. Mayo and F. M. Lewis, J. Amer. Chem. Soc., 66, 1594 (1944).

(40) A. Rudin, K. F. O'Driscoll, and M. S. Rumack, Polymer, 22, 740 (1981).

(41) J. M. Goldwasser and A. Rudin, J. Polym. Sci. A-1, 20, 1993 (1982).

(42) W. L. Elsdon, J. M. Goldwasser, and A. Rudin, J. Polym. Sci. A-1, in press.

(43) J. G. Kirkwood and J. Riseman, J. Chem. Phys., 16, 565 (1948).

(44) C. M. Kok and A. Rudin, Mackromol. Chem. Rapid Commun., 2, 655 (1981).

Appendix I

The mean squared end-to-end distance of a freely orienting chain consisting of x_a segments each of length ℓ_a is:

$$\bar{d}_a^2 = x_a \ell_a^2 \qquad (I-1)$$

where $d_a = 2r_a$.

Similarly, the mean squared end-to-end distance of a freely orienting macromolecule with x_b segments of length ℓ_b is:

$$\bar{d}_b^2 = x_b \ell_b^2 \qquad (I-2)$$

If one end of the first molecule were attached to an end of the second polymer the mean squared end-to-end distance of the combined chain would be:

$$\bar{d}_{a+b}^2 = x_a \ell_a^2 + x_b \ell_b^2 \qquad (I-3)$$

Similarly, for a macromolecule comprising n subsections, each consisting of x_i segments of length ℓ_i:

$$\bar{d}_n^2 = \sum_{i=1}^{n} x_i \ell_i^2 = \sum_{i=1}^{n} \bar{d}_i^2 \tag{I-4}$$

The relations quoted above apply also to copolymers if ℓ is taken to mean an average bond length.

Equation (I-4) shows that the squared end-to-end distance of a random coil macromolecule equals the sums of the squares of the end-to-end distances of its segments.

The mean squared end-to-end distance of a real polymer chain is given by the same formulas as those above with β_i^2 substituted for the ℓ_i^2 of a freely orienting chain. Here β_i^2 is a constant characteristic of the particular polymer to take account of restricted bond angles and preferred conformations.

In the unperturbed state the mean squared end-to-end distance is often represented as:

$$\bar{d}_o^2 = \sum_{i=1}^{n} x_i \beta_i^2 = \sum_{i=1}^{n} (\bar{d}_i^2)_o \tag{I-4a}$$

In solution the dimensions of the polymer coil are perturbed by the solvent and the relations operating are amended by including Flory's chain expansion factor α. In the case of interest here each segment may have a different expansion factor, α_i, with a common solvent. Considering solvent-perturbed dimensions:

$$\bar{d}^2 = \sum_{i=1}^{n} x_i \beta_i^2 \alpha_i^2 = \sum_{i=1}^{n} \bar{d}_i^2 \tag{I-5}$$

For random coil polymers the radius of gyration is related to the end-to-end distance by:

$$\bar{r}_G^2 = \frac{\bar{d}_o^2}{6} \tag{I-6}$$

It is also expected theoretically (43) and experimentally (44) that the hydrodynamic radius is related by a constant factor to the radius of gyration and hence to the hydrodynamic radius.

As a result, equation (8) of this paper follows directly from equation (I-5). Equation (8) applies rigorously to polymers in which the segmental dimensions are described by equation (I-1) (with β substituted for ℓ). This includes block and graft copolymers with long, flexible segments. In a more general sense, equation (8) provides a suitable functional form for describing copolymers. For a statistical copolymer, the equation serves as a framework for group contribution calculations, as in this article, although the individual segment sizes may have no physical meaning.

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2465-2499 (1983)

PROTEIN SEPARATIONS ON OCTYL AND DIPHENYL BONDED PHASES

Fredric M. Rabel and Debra A. Martin
Whatman Inc., 9 Bridewell Place
Clifton, New Jersey 07014

ABSTRACT

Two new packing materials specifically designed to handle high performance liquid chromatographic separations of proteins and peptides have been made. These are built on a 300 A pore silica gel to allow access of large molecular weight species, and are exhaustively bonded, first with an octyl or a diphenyl silane and final end-capping with trimethylsilyl groups. The media are called Protesil 300 Octyl and Protesil 300 Diphenyl. Their unique selectivity is shown with various samples of dipeptides and proteins. The Octyl phase has specific affinities for alkyl functionalities, the Diphenyl phase has specific affinities for aromatic functionalities. Loading and mass recovery studies have been done on these media to show their capabilities under the elution conditions shown in the various separations. Comments regarding their correct use have also been made.

INTRODUCTION

There is an ever increasing interest in separating moderate to large molecular weight biopolymers. For many years the literature has been filled with such separations on a variety of media, but the advent of high performance liquid chromatography (HPLC) has brought not only much increased speed, but more importantly, newer, more efficient and more selective media for such separations.

Copyright © 1983 by Marcel Dekker, Inc. 0148-3919/83/0613-2465$3.50/0

Two general groups of HPLC media now exist for separating biopolymers. Those whose mode of separation is based upon size or steric exclusion and those whose mode of separation is based upon varying interaction with a bonded phase. Almost all of both groups of packings are manufactured with a silica gel backbone because its rigid nature is able to stand up to the packing and operating pressures needed for HPLC. Silica gel does have a distinct disadvantage, however, when doing biopolymer separations. Many biopolymers bond irreversibly to the silanol groups. There are, however, references to biopolymer separations being done with fair success on bare 60 or 280 A pore silica gel with aqueous mobile phases(1,2). Such mobile phases would have deactivated most of the silanol sites, but irreversible adsorption still would have occurred although this fact was substantiated in only one of the two references cited (1). Regardless of the yield, pure protein was obtained. One of these references (1) also contains comments on the effects of the pore sizes of the silica gels regarding the penetration or exclusion of various size proteins. If the pores of the gel were too small to accommodate the protein or viruses, they passed through the column easily. If the pores of the gel were larger, the same protein or viruses penetrated into the pores and became adsorbed, eluting at a later time. Such information is similar to that known for years by workers in the gel permeation and gel filtration fields.

Classically ion exchange celluloses and gels have been used to separate many proteins. The ion exchange sites were usually weak and the backbones used prevented irreversible adsorption after gradient pH or salt conditions for the separation were established. To update these media for modern HPLC use, Regnier and his coworkers (3-6) bonded glyceryl-like phases and ion exchangers to various controlled porosity

supports. Thus a combination of size exclusion and/or ion exchange modes could be used for biopolymer separations. This media research laid much of the ground work for these types of columns and packings now available commercially from a number of sources. A review of these types of packings has been written (7), as well as an excellent evaluation procedure (8).

Some interesting work on biopolymer separations on silica gel bonded with hydroxyl, amino, or cyano phases has been done (9-13). Much work remains to be done in this area, however, to differentiate between the various separation mechanisms possible. The bulk of current HPLC work on biopolymers has been published using 60 to 100 A pore silica which has been bonded with hydrocarboneous phases (C_{18}, C8, phenyl) (13-21). The references cited are only a sample of what appears in the literature. The use of these bonded phases grew naturally since many biopolymers were soluble in the largely aqueous mobile phases which are frequently used with these media, and because the media were readily available in every HPLC laboratory. The results were very successful for the most part, and their use continues for probably 80% of the biopolymer work done today.

Since the backbone for most of this media is silica gel, after initial bonding it is best to use a secondary capping reaction (with trimethylchlorosilane and related compounds) to remove as many residual silanols as possible. Although the success of such capping is variable from manufacturer to manufacturer, the media with such capping will always give better yields of any biopolymer separated. When uncapped reversed phase packings are used for biopolymer separations, various additional components are usually required in the mobile phase as is done with such separations on pure silicas. Hancock and Sparrow (22) reported that an inital wash with methanol and operation with high concentrations

(0.17 M) of triethylammonium phosphate, pH 3.2, was necessary to get efficient protein and peptide separation on an uncapped medium. Still they observed poor efficiencies when strongly basic amino acid (arginine) containing solutes were separated unless a hydroxylic solvent (methanol) was used.

A great deal of research into the chemical factors and possible mechanism of biopolymer separations on bonded reversed phase packings has been done. These constitute some of the most interesting papers in the LC literature today because they lead to a better understanding of what to expect when doing such separations, as well as new directions for media research. Some of the pertinent results will be briefly discussed since they relate to the direction taken in the development and testing of the Protesil 300 media.

Researchers (15,23,24) have noted the decreased efficiency of protein separations as compared to small molecular weight solutes separated on reversed phase media. This is due to the slower mass transfer of the larger molecular weight species, the possible presence of isomers of the solute which elute close to one another, and the inability of the biopolymers to penetrate into the small pores of some of the reversed phase media now available. Although the first two of these reasons are due to the physicochemical or chemical nature of the species involved, the latter situation is at least minimized by using larger pore diameter supports.

As with any LC column, the faster the flow rate during a protein or peptide separation, the less the efficiency (9,11,23,24). For 4.0 to 4.6 mm ID columns the flow rate should be less than 1 ml/min. This allows

more equilibration time for the slower diffusing biopolymers, resulting in better resolution. If operating isocratically, most biopolymers are extremely sensitive to the mobile phase composition. As little as a 1% change in organic/water ratio can drastically change retention and resolution, or result in no elution at all (9,13,16,25). Gradients are also very useful because of this, but also must be run slowly for best results. Jones, et al (23) recommend 2 hour gradients at 20 ml/hr flow rates. Another reason for slower flow rates, aside from better efficiency, is that the limits of detection are greater (24). Thus trace proteins or peptides can be overlooked if fast flow rates are used.

The elution conditions for biopolymers from reversed phase columns can be varied considerably. Of primary concern is the selectivity to get the separation desired. To this end the literature is filled with attempts using water combined with acetonitrile, methanol, ethanol, tetrahydrofuran, or propanol as mobile phases (16,17,24,25). Rubinstein (11) pointed out the anomoly found when doing such separations - the larger the peptide the more organic needed for elution, but many proteins or larger peptides are precipitated out of solution by high concentrations of organics. He recommended gradient work with 1-propanol (0 to 40%) to do wide range molecular weight protein separations. The disadvantage of lower efficiency and high back pressure (due to viscosity) of this mobile phase combination is offset by its versatility.

Regarding the mobile phase composition, simple organic/water ratios suffice to separate smaller non-polar di- and tri- peptides. The addition of ion pairing salts or acids, however, are generally required for the separation of larger molecular weight biopolymers (9,11,13-15,20-22,26,27). These modifiers serve two functions: a) to solubilize the solutes in the mobile phase (changing the ionic forms or

configurations of the species), and b) to condition the bonded phase. Because they become a part of the separated components, care in selection of the salts or acids must be used. Will the modifier interfere with detection (UV or MS) (9,28)? Can it be easily removed or is it compatible with the biochemical system if future work on the species is to be done (9,16)? Will it (like any part of the mobile phase) denaturize or change the biopolymer?

One of the most important variables in the separation of biopolymers on bonded reversed phase media are the media themselves. A few groups (14,16,17) have commented on the similarities of elution order when going from a C8 to a C18 bonded phase. Assuming both packings are capped and both are made from the same type reagent (monofunctional silane), then such similarities are expected. Thus only more lipophilic character is found in the C18 as compared to the C8 column. Longer retentions of non-polar solutes are found in a C18 column. Faster elution from the C18 column requires that a higher percentage of the organic solvent be used in the mobile phase. For more water soluble species, like most biopolymers, it is better to use a C8 column since the compounds elute with less organic solvent in the mobile phase. As a rule, C8 columns also equilibrate more rapidly, an important factor if various mobile phases for optimization need to be tried.

An interesting study was done by Lewis and coworkers (13) in which octyl, cyanopropyl, and diphenyl bonded phases were compared. They found similarities in the C8 and cyanopropyl bonded phases for some protein mixtures, but neither of these columns would separate all the components (2α,1β,1γ) of denatured human Type I collagen except the diphenyl

bonded phase. This pointed out the potential utility of at least a combination of C8 and diphenyl bonded phases for protein separations.

A number of studies of small peptides ($\sim$ 20 residues or less) separations on reversed phase media (14,16,17,29,30) have shown a high correlation of the retention to the summing of individual lipophilic or lipophobic contributions of each amino acid (and end groups). This indicates that amino acid composition is a major factor determining retention. The small differences observed between elution orders or times points to the lesser but important role played by other factors such as polarity, sequence, size, charge distribution and conformation. Such information can be useful in looking into possible separation mechanisms when any similarities or differences in elution order or times are observed.

EXPERIMENTAL

Equipment

The liquid chromatograph used was a Perkin Elmer (Norwalk, CT) Series 3B with a Valco (Houston, TX) 7000 psig Universal inlet injector, a Laboratory Data Control (Riviera Beach, FL) Spectromonitor II (1202) and data handling was with a Hewlett Packard (Avondale, PA) Integrator 3380A and a Hewlett Packard HP2126BOEM disc based system with CIS (Computer Inquiry Systems, Englewood Cliffs, NJ) installed HP RTE2 software.

It is important that a dynamic mixing chamber be made for the gradient system since aqueous buffer and 1-propanol are being mixed.

Because of the great viscosity difference, no static mixer (T or coil) was found to work. A suitable mixing chamber was made from a 9.4 mm x 5 cm tube with appropriate fittings and a magnetic stir bar. The device was placed just before the injector and held to pressures of 3000 psi.

Reagents

Bovine serum albumin (fraction V powder), aldolase (type X from rabbit muscle), insulin (from bovine pancreas) and cytochrome C (from horse heart) were purchased from Sigma (St. Louis, MO); lysozyme (from hen egg white) and hemoglobin (human) from Calbiochem (La Jolla, CA); human serum (lyophilized) and all dipeptides from U.S. Biochemical Corp. (Cleveland, OH); and β-lactoglobulin from Polysciences (Warrington, PA).

The 1-propanol was reagent grade from Mallinckrodt (St. Louis, MO), the triethylamine and glacial acetic acid were reagent grade from J.T. Baker (Phillipsburg, NJ). The water was deionized.

Protesil 300 Octyl, Protesil 300 Diphenyl, Partisil 10 CCS/C_8, Partisil 10 ODS-3, Partisil 10 ODS-2 and CoPell ODS are available from Whatman Inc. (Clifton, NJ). All Protesil and Partisil columns used are 4.6 mm x 250 mm and were off the shelf. The various characteristics of all the reversed phase media mentioned in this work are given in Table I.

The initial work on the Protesil 300 columns was done using only methanol/water combinations. With a simple mobile phase such as this, explanation of observations regarding selectivity would be much easier.

TABLE I

MEDIA CHARACTERISTICS

(all are 10 μm, irregular)

	Pore Diam A	Surf Area m^2/g	Phase	%C [a)]	Reag. Funct. [b)]	End Cap	% SiOH Coverage [c)]
Protesil 300 Octyl	300	250	C8	7.5	mono-	Yes	95+
Protesil 300 Diphenyl	300	250	Ph_2	8.0	di-	Yes	95+
Partisil 10 CCS/C_8	80	375	C8	9.0	mono-	Yes	95+
Partisil 10 ODS-3	80	375	C18	10	tri-	Yes	95+
Partisil 10 ODS-2	80	375	C18	15	tri-	No	75

a) % C is the weight % carbon as determined after all bonding is completed. Usually 90-95% of this value is the weight % due to the initial bonding reagent, with the capping reagent accounting for the balance.

b) The functionality of the initial bonding reagent; the second bonding reagent (for end-capping) is always Me_3SiX, where may be halide, alkoxy, or amino.

c) Approximate % coverage of available silanols base upon retentions as measured with a nitrobenzene sample with heptane mobile phase, and/or an aniline/phenol sample with a methanol/water (60:40) mobile phase.

Additional modifiers in the mobile phase may help the separation, but complicate the explanation of the possible mechanism of such separations.

Many successful protein separations are done with gradients to allow the best peak capacity and speed. The gradient simplifies finding the right elution conditions since, as was pointed out, the correct ratio of organic/water is critical to any protein elution and the reproducibility of this elution time. High sensitivity using the triethylammonium acetate at 210 and 280 nm has been reported in the literature (9) and was the buffer of choice. The buffer solution was made by adding triethylamine to 0.5 M acetic acid until the desired pH is reached. After being made, the buffer was passed through a Partisil 10 ODS-2 (15%C, not end-capped) column to remove impurities that might elute to give baseline drift or spurious peaks during gradient runs. A treatment of similar buffers has been reported (22,31). The organic solvent used in the gradient runs was 1-propanol, as reported by Rubinstein (11), so that maximum solubility of the proteins throughout the separation could be maintained.

Protesil 300 Quality Control

To ensure reproducible columns, extensive quality control on the base silica gel, the bonded product, and the finished column is done. Before bonding, the silica is tested for pore size distribution, particle size distribution, surface area and exclusion limits. After bonding and after % carbon is assured to be within the specified range, the diphenyl or octyl chromatographic batch tests are performed. Several columns are packed and are given the standard column Q.C. test to check bed uniformity. A sample containing benzene and phenanthrene is separated

and capacity factors, efficiencies, and asymmetry ratios are measured. After assuring that the batch test columns are well packed, they are tested by polystyrene SEC for total pore volume and exclusion limits. Next the columns are tested for protein separation with cytochrome C and bovine serum albumin under gradient conditions after which capacity factors and resolution are calculated. The final batch test is a check for residual silanols with an aniline and phenol sample. If properly capped, the aniline will elute before phenol with a methanol/water mobile phase. A mass recovery test is performed using lysozyme because of its susceptability to surface effects (8,12,32). The procedure requires comparison of the area of lysozyme peaks with a packed and with an empty column under isocratic conditions. Generally the amount of lysozyme retained on the column is minimal and the percent recovery increases as the mass of lysozyme injected on the column is increased.

After a Protesil 300 batch has passed all the tests, it is released for packing of the commercial columns. Each of these columns is tested only for bed uniformity by measurement of capacity factors and efficiency with the benzene and phenanthrene sample. If the column passed this final test it is released for sale. It is important to note that any Protesil column sold has never been subjected to a protein which might interfere with subsequent work done on this new column.

RESULTS AND DISCUSSION

In an attempt to develop column media that would allow improved biopolymer separations, many of the findings mentioned previously were taken into account by the people in our research group. Thus, the media needed larger pores to accommodate the larger molecular weight species.

It also had to be bonded with different groups to get unique selectivities and be well end-capped to prevent irreversible adsorption. A low carbon percentage of the bonded phase was also important so that elution would be accomplished with high water content mobile phases, minimizing the possibility of biopolymer precipitation by high organic content mobile phases.

The initial result of these considerations are two new biopolymer separation columns called Protesil 300 Octyl and Protesil 300 Diphenyl. They are carefully sized 10um irregular silica with 300 A pores bonded and end-capped with 7.5% carbon for the octyl phase and with 8.0% carbon for the diphenyl phase.

The level of carbon on the Protesil 300 bonded phases is low to moderate as compared to other bonded reversed phases on the market (which range from 5 to 24% carbon). This, again, was to allow higher water containing mobile phases to be used, thus keeping increased solubility of the biopolymers. The carbon level was also kept about the same on each so that the same mobile phase composition as derived from one type of Protesil 300 column could be used on the second type of Protesil 300 column. Thus the different selectivities of the two bonded phases can be observed without reoptimization of the mobile phase. In other words, the same polarity mobile phase will elute components of the sample with either Protesil 300 column, yet the selectivities will be different on each.

The octyl and diphenyl phases were chosen to be bonded because these had been pointed out to have the most different selectivities by Lewis and his coworkers (13), and was substantiated by work in our laboratory.

As mentioned previously, a few research groups found that only elution time seemed to change from a C8 to a C18 bonded phase - assuming each was made on the same backbone gel and with the same bonding chemistry.

An investigation was begun with the Protesil 300 Octyl and Diphenyl columns to show their unique selectivities. The initial results are reported here. On occasion, Partisil 10 CCS/C_8 column (80 A pores, 9% carbon, end-capped) was used for comparison.

Selectivity Studies

The dipeptides seen in Figure 1 were used to investigate the selectivities of the Protesil 300 Octyl and Diphenyl media. These simplified structures allow the substituent and end groups to be more easily compared. The numbers in parentheses after each compound name are the Rekker lipophilic substituent summations (without correction for terminally located residue) (33). The compounds elute in the expected order on both columns based upon relative lipophilicities. Looking at the chromatograms of each, Figures 2 and 3, there are unique differences. The Protesil 300 Octyl (Figure 2) does separate the aliphatic substituent compounds better than the Diphenyl column. There is no separation of the aromatic substituent compounds, however, on the Octyl column. On the Protesil 300 Diphenyl column (Figure 3), however, separation of these aromatic containing compounds is easily accomplished. At the same time resolution of the aliphatic dipeptides is lost. Complete separation of the aliphatic species on the Octyl column and of the aromatic species on the Diphenyl is possible with a more water rich mobile phase or gradient. The same mixture of dipeptides is completely resolvable on the Partisil 10 CCS/C_8 (Figure 4). Thus short chain or low molecular weight species

DIPEPTIDE STRUCTURES AND ELUTION ORDER
(Rekker's constant)

PROTESIL 300 OCTYL | **PROTESIL 300 DIPHENYL**

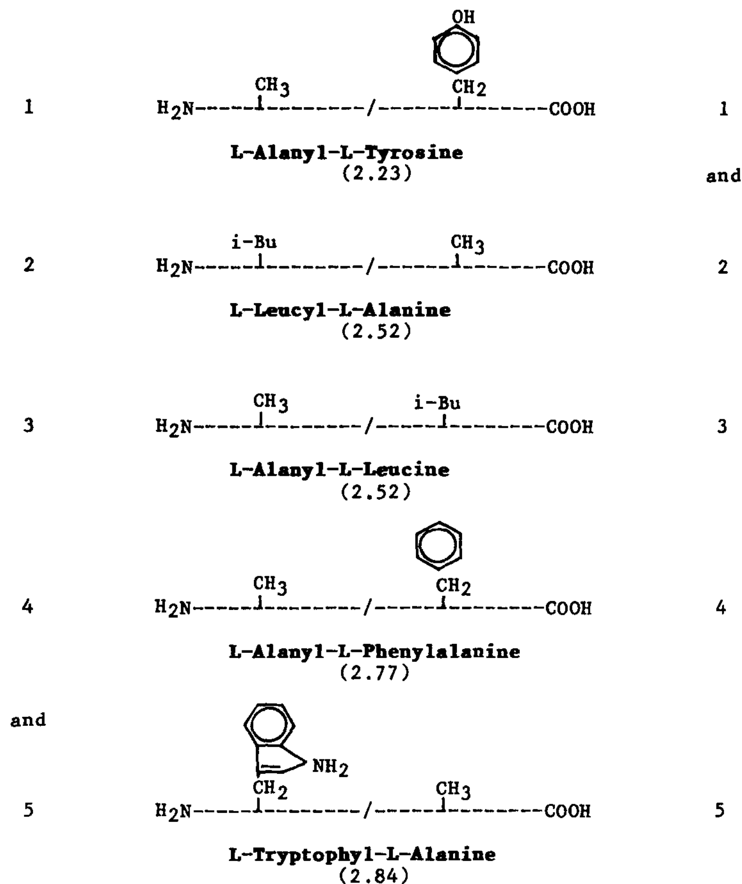


Figure 1 Structure and elution order of dipeptides on Protesil 300 Octyl and Protesil 300 Diphenyl. Drawings show substituents only. The numbers in parentheses are the summation figures from the Rekker (33) lipophilicities of each amino acid.

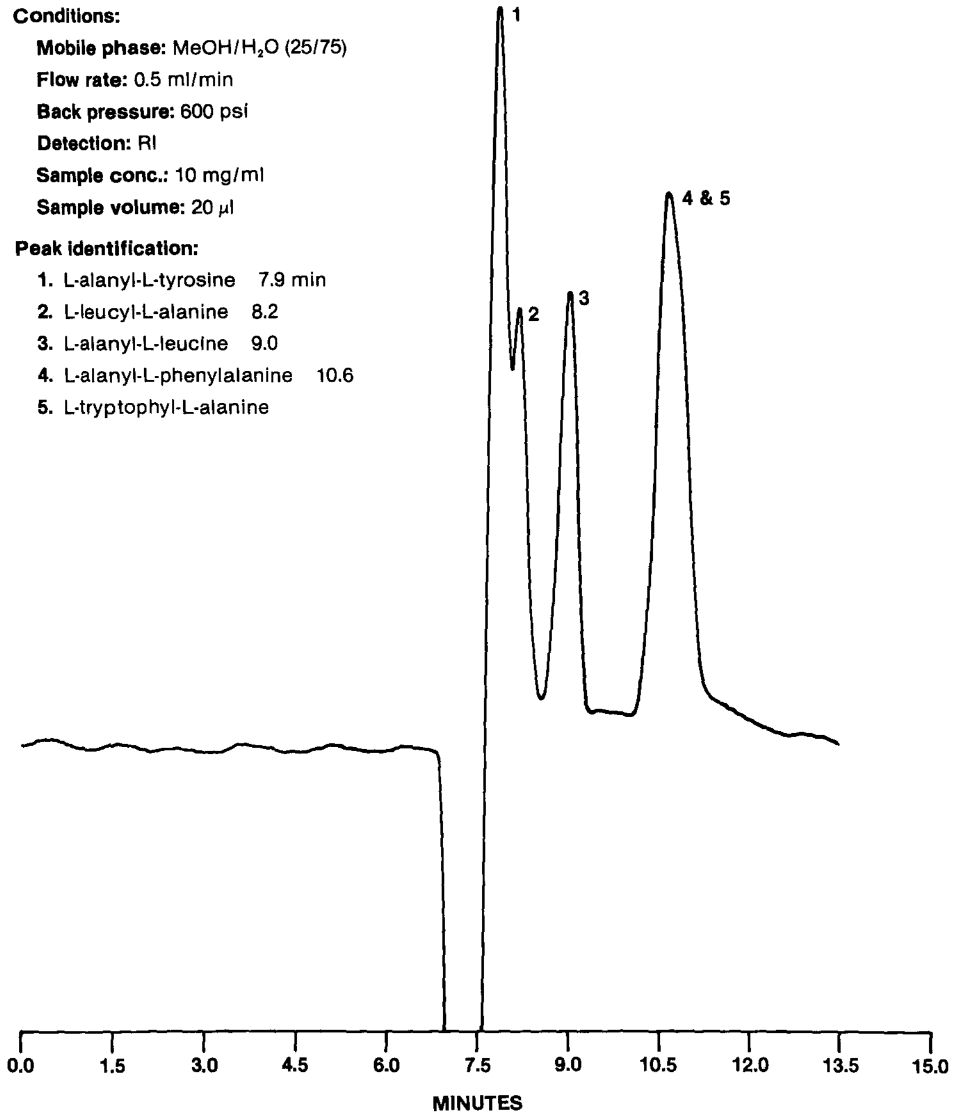


Figure 2

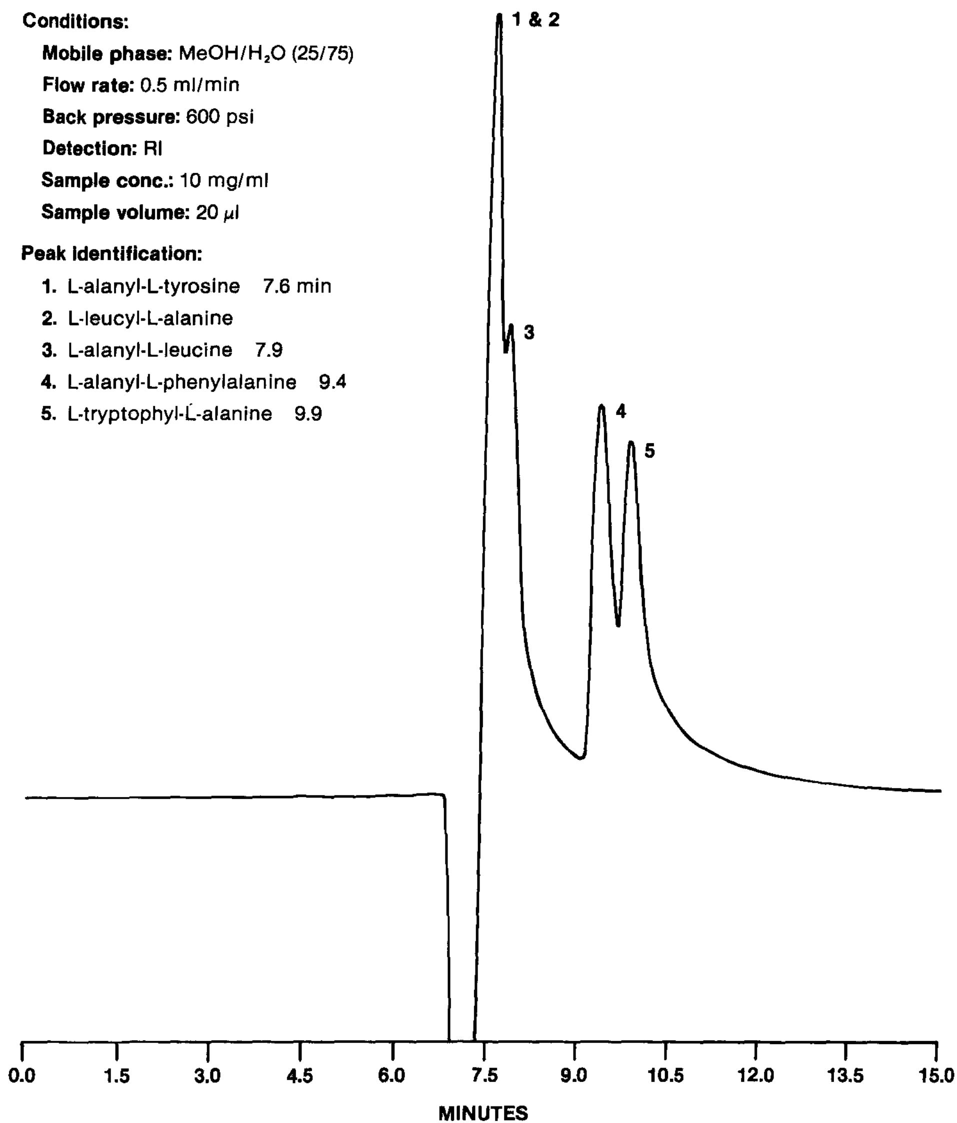


Figure 3

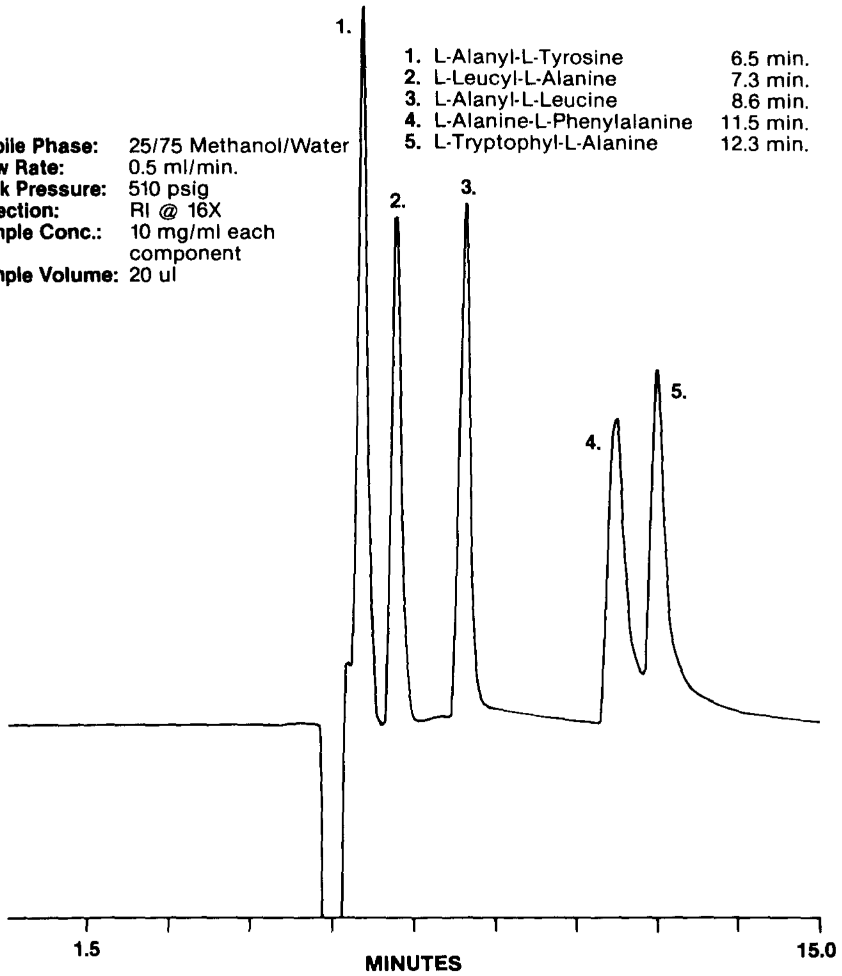


Figure 4

are able to interact with the 80 A, C8 bonded phase to get a better separation, probably because of the greater interaction with C8 due to the closer spacing of these groups on the silica. Larger molecular weight species, however, do not separate well on a small pore bonded phase, as has been mentioned, but are better separated on large pore media as will be shown.

A mixture of moderate molecular weight proteins was separated on these same three columns. These chromatograms are shown in Figures 5,6, and 7. On all columns these proteins seem to separate well, but with different relative retentions. On each column, the higher molecular weight BSA (bovine serum albumin) elutes before the β- lactoglobulin of 1/3 the molecular weight. Such anomolous elutions are often seen in biopolymer separations on reversed phase media where various lipophilic interactions are occurring. The conformation of each protein species, as well as its composition of amino acids, will determine its elution characteristics in each combination of column (type of bonded phase) and mobile phase. In each chromatogram all the peaks are fairly symmetrical except that of BSA which apparently is beginning to be separated into its dimeric and trimeric forms (34).

The Partisil 10 CCS/C_8 separation (Figure 7) is interesting in that even through this product has the largest percent carbon (9 versus 7.5 or 8) of the three columns, the elution time is the shortest. This is probably a result of the smaller pores not allowing much interaction with the bonded phase in these pores, thus giving faster elution of all the components.

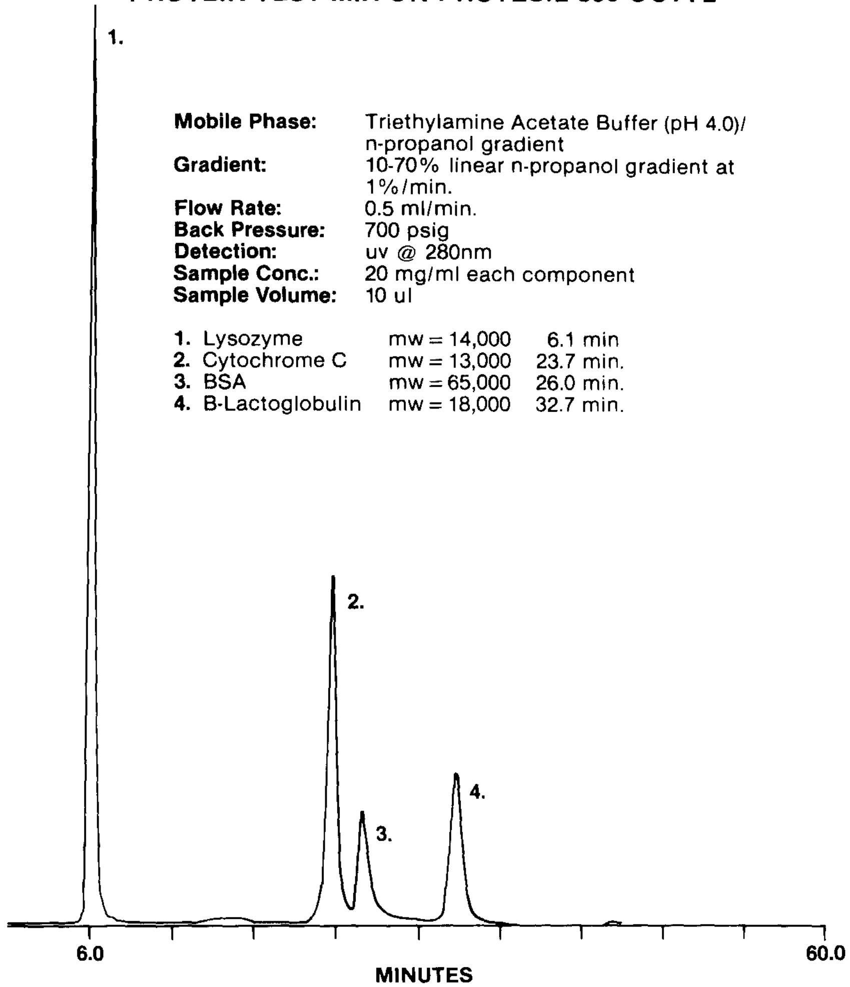


Figure 5

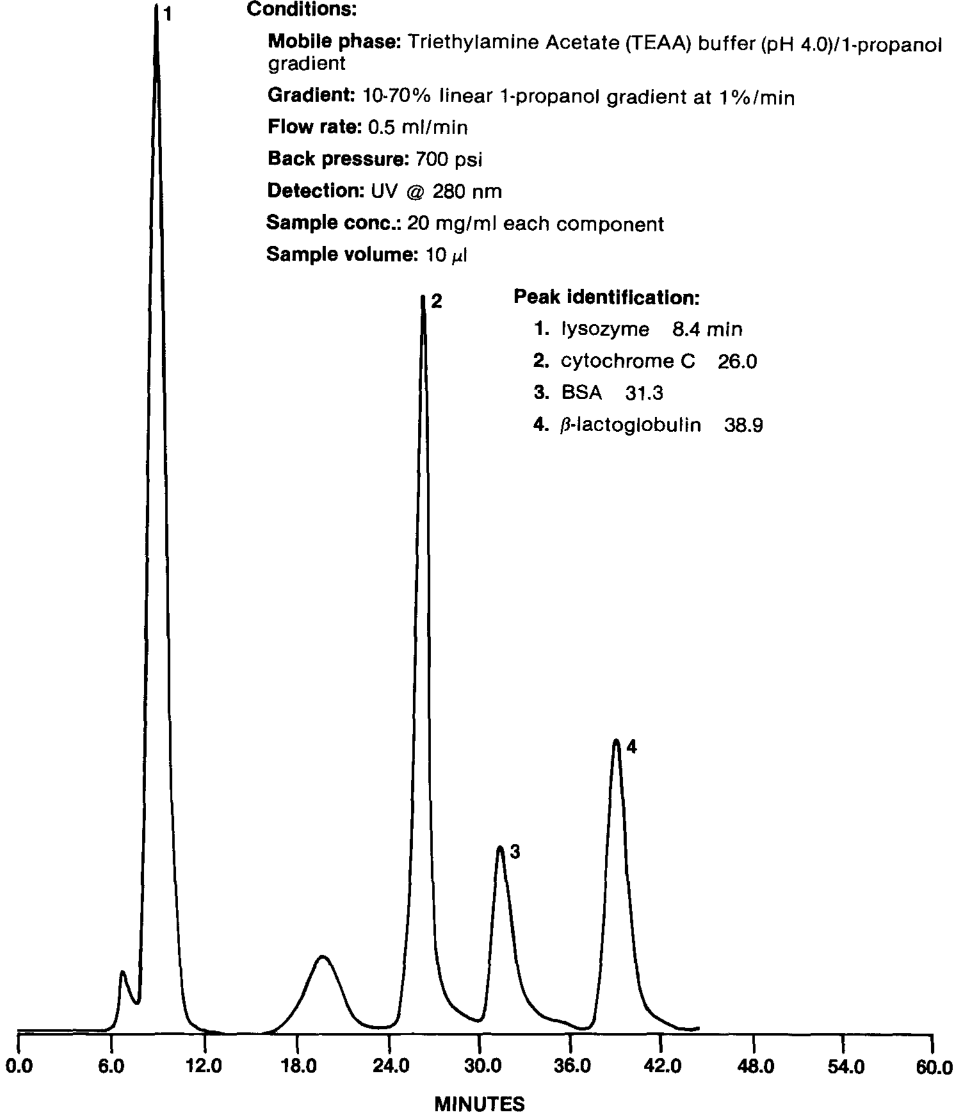


Figure 6

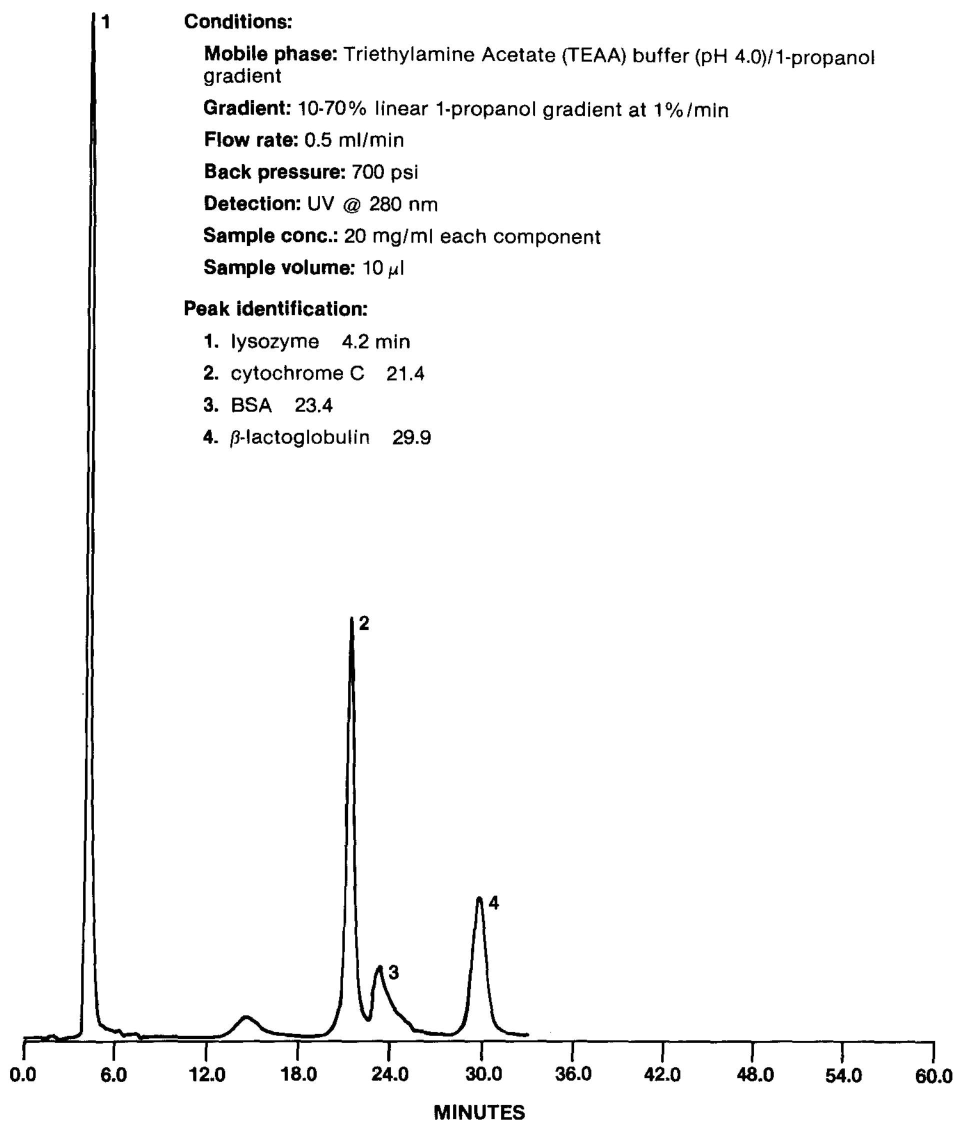


Figure 7

A second mixture of low to high molecular weight proteins was also separated on the Protesil 300 columns (Figures 8 and 9). These chromatograms are even more interesting. Even though the elution order for these proteins is the same on both columns, the Protesil 300 Octyl gives two peaks for hemoglobin (confirmed with a pure sample). An investigation as to the identity of these peaks is now being undertaken. The initial supposition is that perhaps the mobile phase is causing the heme and the globin to be separated and these are seen eluting as two peaks on the Octyl column. The same breakdown of hemoglobin would occur in the Diphenyl column because the mobile phase is the same, but the aromatic interaction of the diphenyl groups with the pyrrole rings of the heme are too strong to allow elution under the conditions employed. If such is the case, a different mobile phase (possibly close to neutrality) might be suggested to allow unmodified hemoglobin to be separated.

Since the conformations of these various proteins in the mobile phases used are not known, no definite comments on their elution order with regard to the bonded phase can be made. Work with smaller peptides whose structures are known and whose conformations would be less complex are now being done to confirm the unique selectivities found with the dipeptides.

An additional test of the selectivity differences on Protesil 300 Octyl and Diphenyl comes from samples of human serum separated on both. These profiles are seen in Figures 10 and 11. All peaks are unidentified at this time, but the largest peak is probably albumin. When the end point of the gradient is held for one hour, no further peaks eluted from either column.

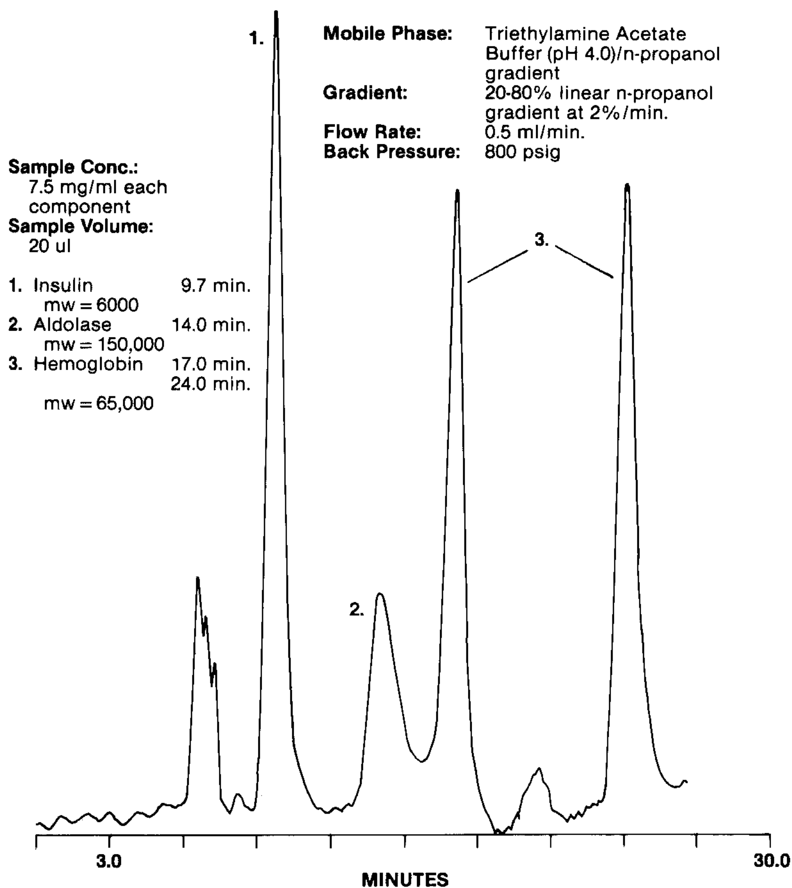


Figure 8

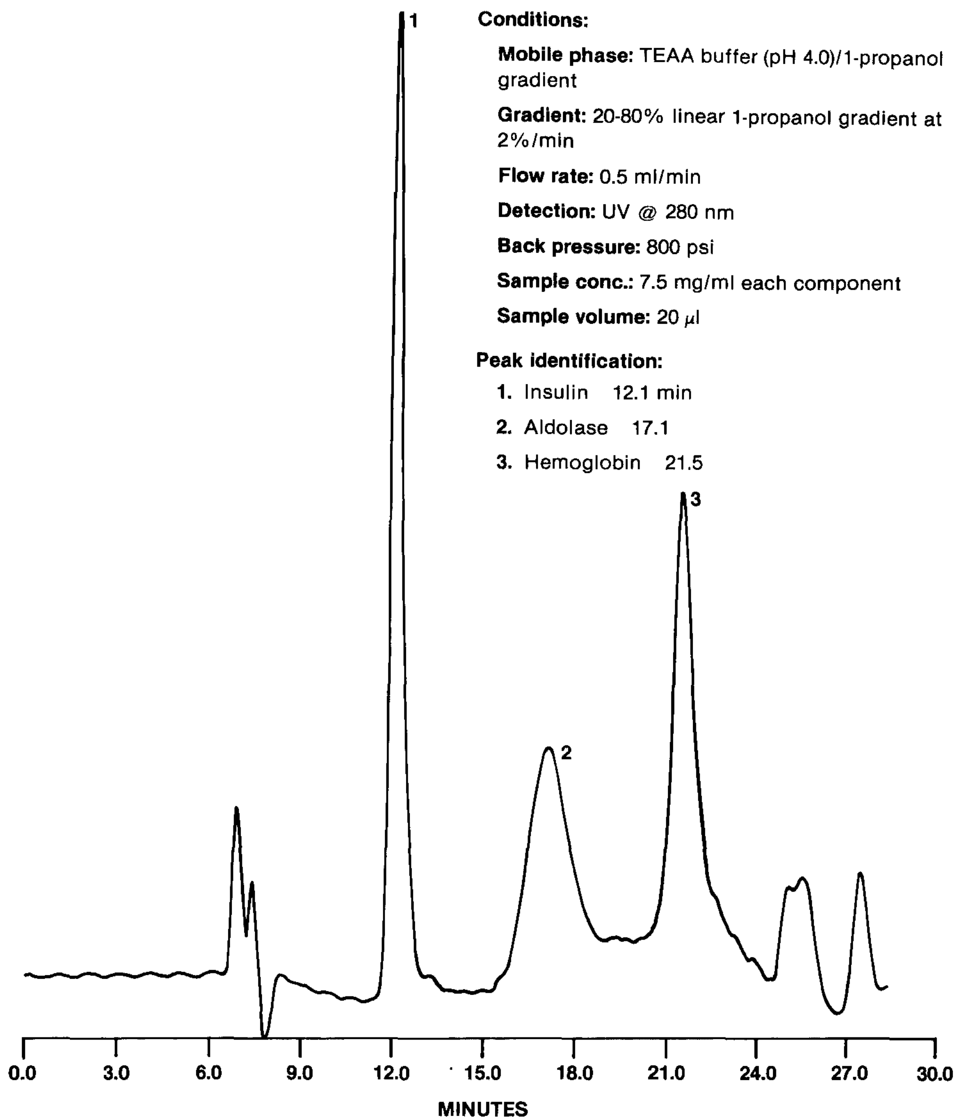


Figure 9

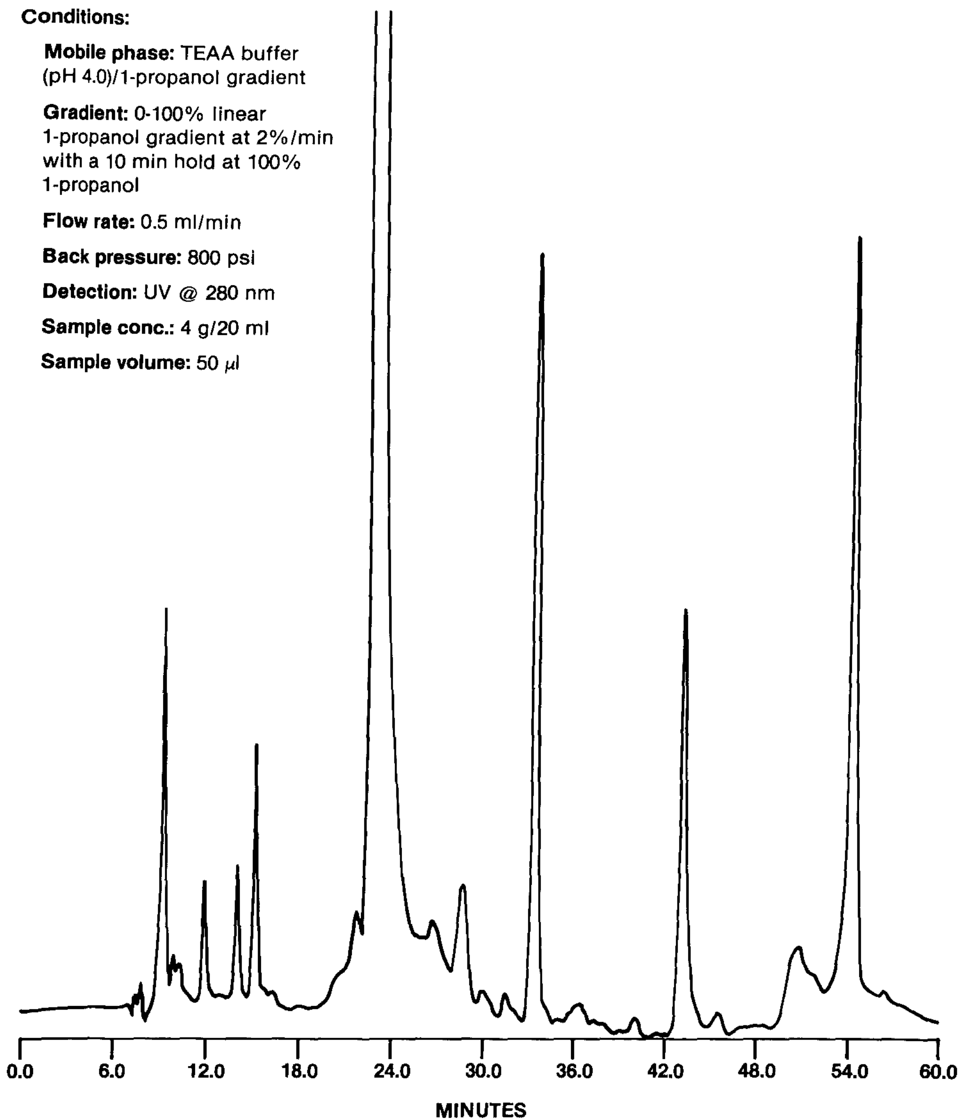


Figure 10

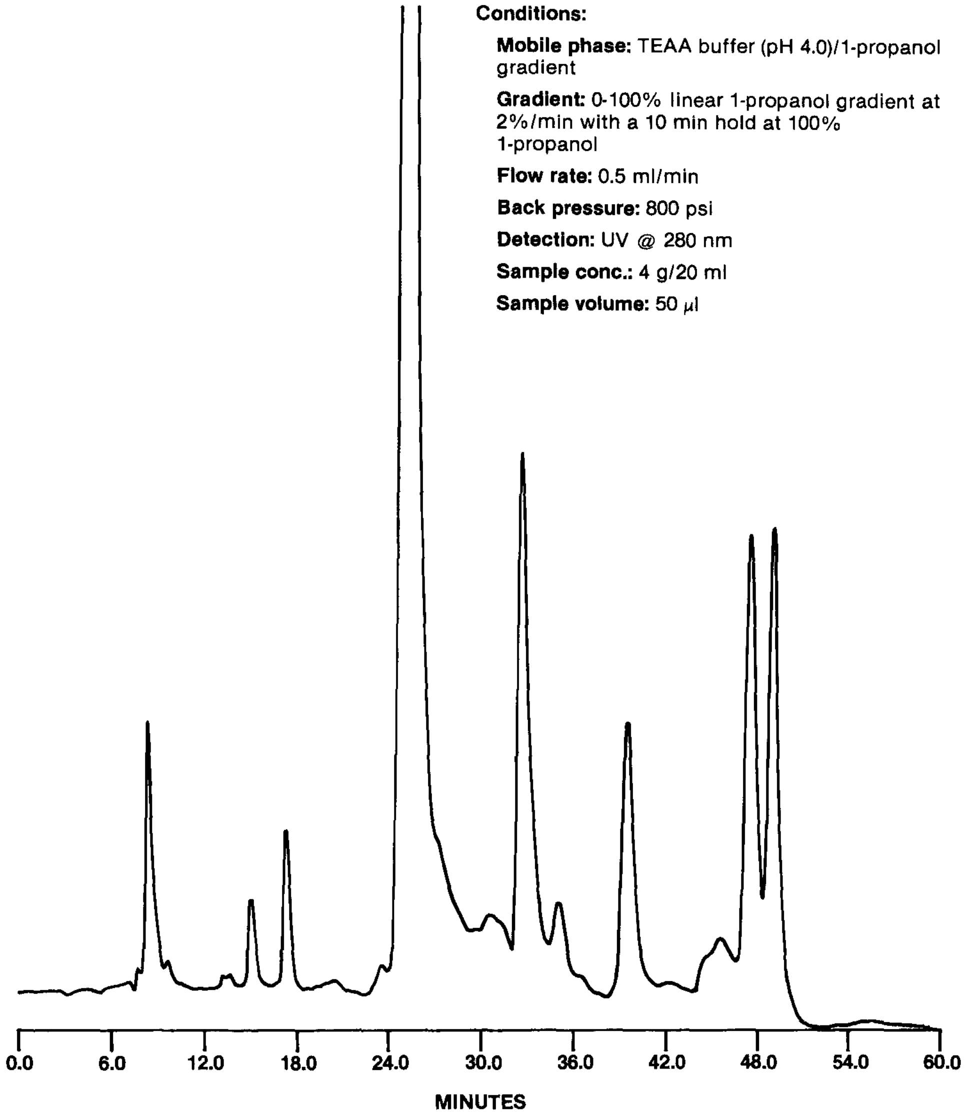


Figure 11

Loadability Studies

Since many protein separations will have to be scaled up to produce more of each species, a study into the loadability of these columns was done. The Partisil 10 CCS/C_8 was compared to the Protesil 300 Octyl and Diphenyl. Figure 12 shows tailing peaks and complete merging of the cytochrome C and BSA peaks in going from 2.5 mg of each protein in 50 ul to 3.75 mg in 75 ul injection on the Partisil 10 CCS/C_8. Figure 13 shows that the large sample is still able to be separated on the Protesil 300 Octyl column. Similarly, Figure 14 shows up to 5 mg each in 100 ul can be separated on the Protesil 300 Diphenyl column with no loss in resolution. These latter two chromatograms show that the maximum load is still higher because the peak retention is the same (indicating no mass overload) and the peak shape is symmetrical (indicating no volume overload) (35). The higher loadability of large pore bonded reversed phase media for biopolymers has been noted previously (13). Gradient elution of proteins has also been shown to give improved capacity (11).

Mass Recovery

Quantities of lysozyme were injected into Protesil 300 Octyl and Diphenyl and Partisil CCS/C_8 and ODS-3 columns and into an empty column to study mass recovery. A guard column (2.1 mm x 7 cm) filled with CoPell ODS was also put in line to see if additional lysozyme might be lost due to irreversible adsorption on the guard column media. The results are shown in Table II. As is usually found with such studies, the larger the quantity injected, the higher the recovery. In all of the columns the recovery is quite good as expected, since all of the media shown are end-capped.

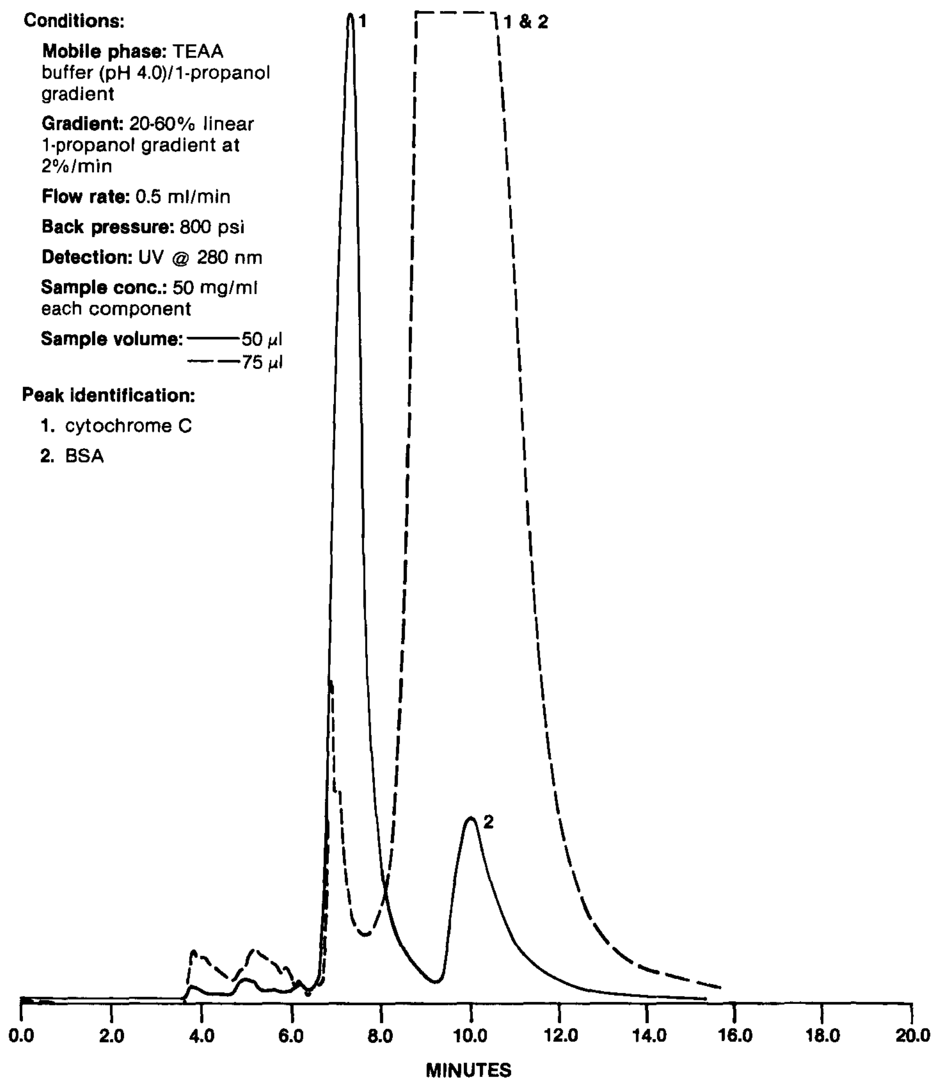


Figure 12

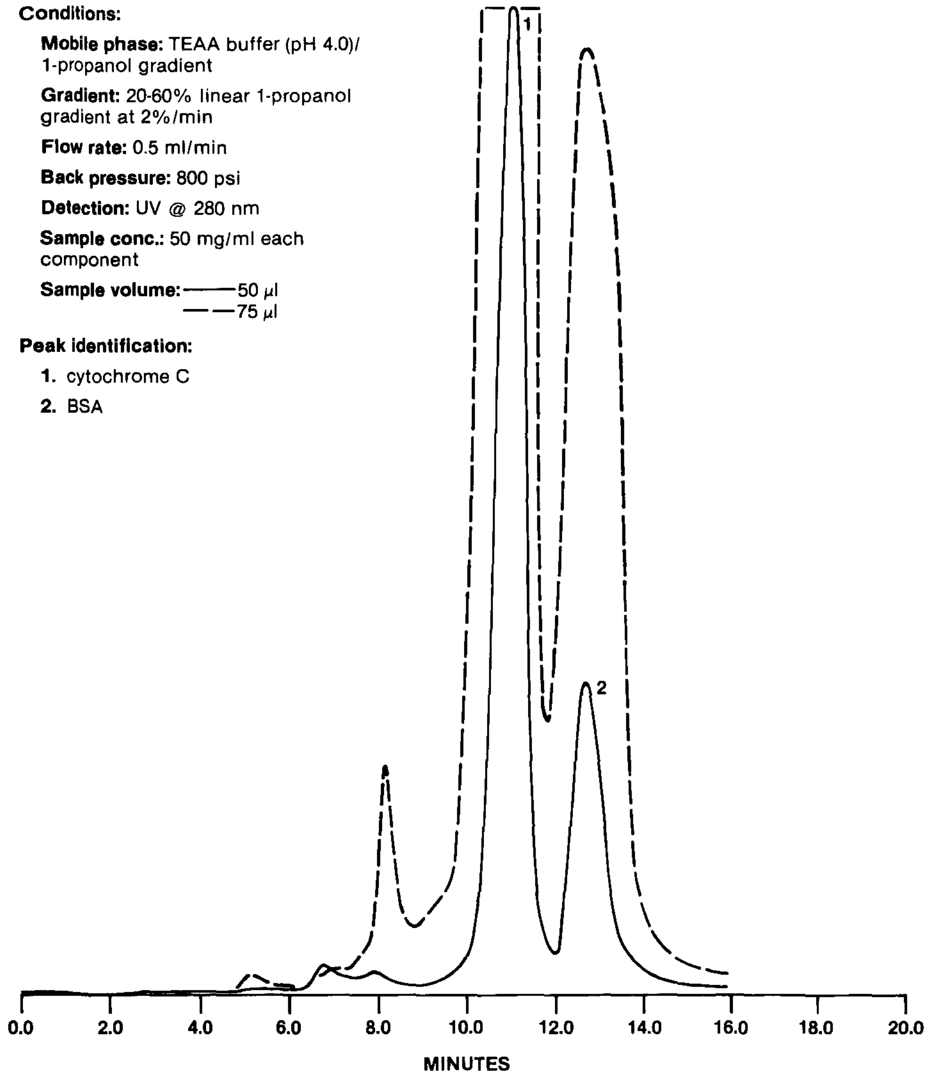


Figure 13

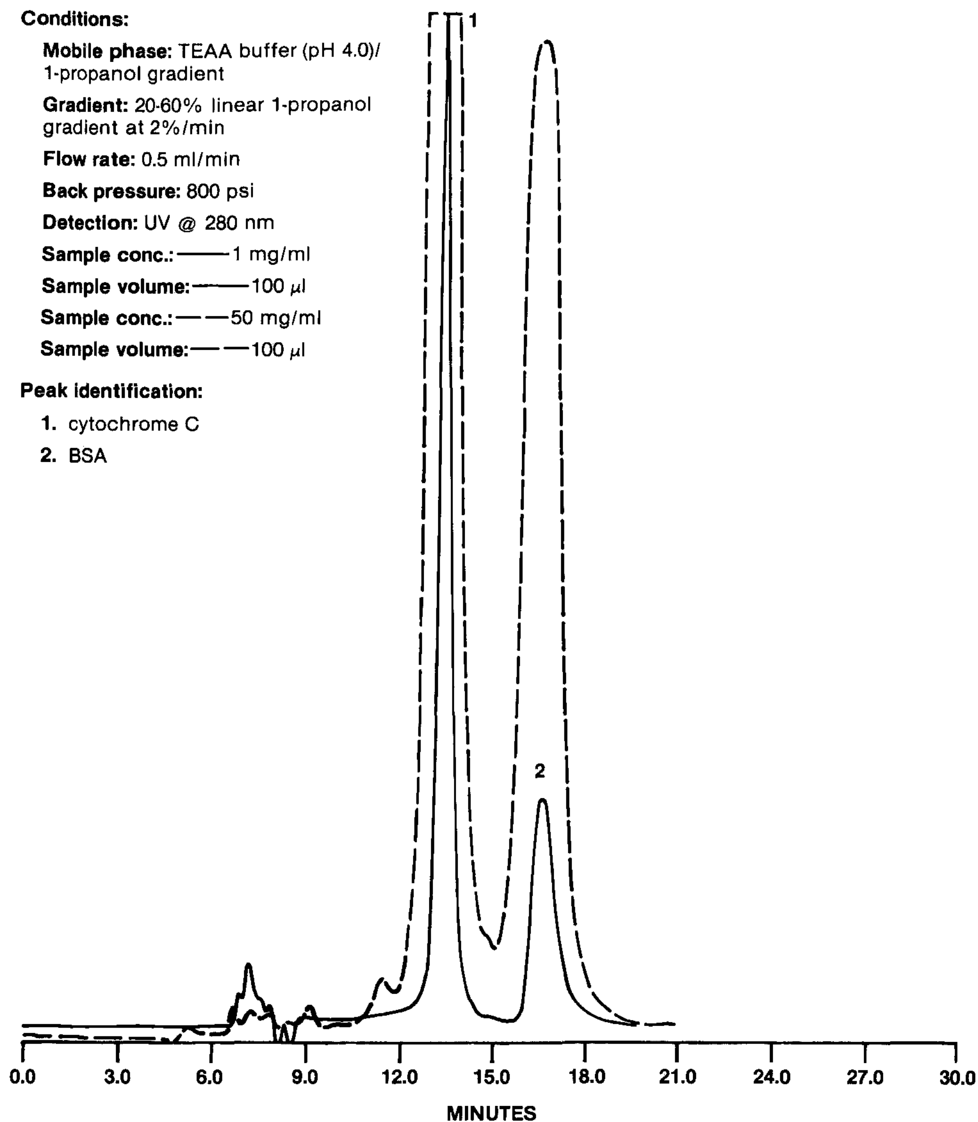


Figure 14

TABLE II

MASS RECOVERY OF LYSOZYME

Mass (ug)	Pr 300 Diphenyl	Pr 300 Octyl	Pa 10 CCS/C8	Pa 10 ODS-3	Gd Col (CoPell ODS) & Pr 300 Diphenyl
100	99	94	96	86	87
50	93	86	92	77	80
20	84	78	83	71	72
10	81	78	80	65	68

Abbreviations: Pr 300 = Protesil 300
Pa 10 = Partisil 10
Gd Col = Guard Column

With the guard column added to the system, there is some additional loss of protein. This is, however, a small price to pay for the extra life such guard columns often give to the analytical column.

The recovery studies based upon biological activities of proteins passed through the Protesil 300 columns are now underway and will be reported separately.

Comments

It is important for each company to provide as much information as possible on its columns and their use, so that the chromatographer gets the maximum potential from each column. Although a more thorough treatment of the general care of columns is available (36), a few more specific comments will be made here as a precautionary measure.

Any sample going onto a Protesil column should be cleaned up in some manner, if it is other than a standard. This may involve passing through mini-columns of ion exchange celluloses, size exclusion media or other. Filtration and/or a good centrifugation of the sample will also prevent particulates from getting into the LC system. Both Rubinstein (11) and Nice and his coworkers (15) have commented on sample preparation prior to HPLC separation.

Since the buffer salts or acids often used in biopolymer separations are not available usually as ′chromatographic grades′, they should be monitored carefully (UV scan of 1 M solutions possibly) and purified if necessary. Many such reagents can be purified easily by passing through columns of silica gel, alumina, polymer, or reversed phase bonded media.

When preparing buffer solutions, the pH of the aqueous portion is adjusted to that specified, then the organic is added. There is a shift to higher ′apparent pH′ (pH*) values on addition of organic (36). This apparent pH should not be allowed to become too high (no greater than 7 or 7.5) or the backbone of any bonded silica will be short lived.

Both pre-columns (before the injector) and guard columns (after the injector) are recommended for longest column life. Each serves a particular function and their use has been discussed (36,37).

It is critical that when any biopolymer separation is to be attempted that the sample solution be completely miscible with the isocratic mobile phase or the extremes of a gradient mobile phase. This will prevent high molecular weight species from being inadvertently precipitated at the head of the Protesil column.

If work with different biopolymers is to be done, it is recommended that 'dedicated' columns be considered to prevent cross contamination (15,16).

As was pointed out by Su and his coworkers (30), with the larger peptides the secondary and tertiary structures will become more important and 'the choice of mobile phase composition will have a profound effect on resolution and sample recovery.' Thus judicious and varied choices for mobile phase composition should be tried as well as the different Protesil 300 columns for optimum separation results.

<u>Summary</u>

Two new HPLC column packings have been designed for use as biopolymer separation media. Information derived from the work of many research groups has lead to what could be considered the optimal combination of bonded phase types, percent bonded phase, end-capping, and pore diameter. Various studies with dipeptide and protein mixtures have been done to show the distinct differences in selectivity and loadability of the Protesil 300 Octyl and Diphenyl in comparison with each other, and with Partisil 10 CCS/C_8, a similar bonded phase but on a smaller pore size silica gel. Each new Protesil packing appears to have unique selectivities, related to the differing hydrophobicities (or lipophilicities) of the bonded phases and their interaction with such sites on protein and peptide structures. Mass recovery has been shown to be excellent. With the usual column care, these columns will be long lived and will be a useful addition to the separation arsenal of the biochemist.

REFERENCES

1. Kielev, A.V., Khokhlova, T.D., and Eltekov, Y.A. in Column Chromatography, 5th International Symposium on Separation Methods, Lausanne, Oct. 7-10, 1969 (Kovats, E., editor), p. 124-125, Swiss Chemists Association, publisher, available from Saulerlander AG, CH-5001 Aarau, Switzerland.

2. Pickart, L.R. and Thaler, M.M., Prep. Biochem., 5, 397-412 (1975).

3. Chang, S.H., Gooding, K.M., and Regnier, F.E., J. Chromatogr., 125, 103-114 (1976).

4. Chang, S.H., Noel, R., and Regnier, F.E., Anal. Chem.,48, 1839-1845 (1976).

5. Schlabach, T.D., Chang, S.H., Gooding, K.M., and Regnier, F.E., J. Chromatogr., 134, 91-106 (1977).

6. Gooding, D.L., Chatfield, C., and Coffin, B., Am. Lab., Aug. 1980,48-61.

7. Barth, H.G., J. Chrom. Sci., 18, 409-429 (1980).

8. Pfannkoch, E., Lu, K.C., Regnier, F.E., and Barth, H.G., J. Chrom. Sci., 18, 430-441 (1980).

9. Rivier, J.E., J. Liq. Chrom., 1, 343-366 (1978).

10. Becker, N. and Unger, K.K., Chromatographia 12, 539-544 (1979).

11. Rubinstein, M., Anal. Biochem., 98, 1-7 (1979).

12. Schmidt, D.E., Giese, R.W., Connor,D., and Karger, B.L., Anal. Chem., 52, 177-182 (1980).

13. Lewis, R.V., Fallon, A., Stein, S., Gibson, K.D., and Udenfriend, S., Anal. Biochem., 104, 153-159 (1980).

14. Molnar, I. and Horvath, C., J. Chromatogr., 142, 623-640 (1977).

15. Nice, E.C., Capp, M., and O'Hare, M.J., J. Chromatogr., 185, 413-427 (1979).

16. O'Hare, M.J. and Nice, E.C., J. Chromatogr., 171, 209-226 (1979).

17. Blevins, D.D., Burke, M.F., and Hruby, V.J., Anal. Chem., 52, 420-424 (1980).

18. Margolis, S.A., and Longenback, P.J. in The Role of Peptides in Neuronal Function (Barker, J.L. and Smith, Jr., T.G., editors), Dekker, New York (1980).

19. Schroeder, W.A., Shelton, J.B., and Shelton, J.R., Hemoglobin 4, 551-559 (1980).

20. Bennett, H.R.J., Solomon, S., and Glotzman, D., Biochem. J., 197, 391-400 (1981).

21. Stricker, M.P., Gemski, M.J., and Doctor, B.P., J. Liq. Chrom., 4, 1765-1775 (1981).

22. Hancock, W.S. and Sparrow, J.T., J. Chromatogr., 206, 71-82 (1981).

23. Jones, B.N., Lewis, R.V., Paabo, S., Kojima, K., Kimura, S., and Stein, S., J. Liq. Chrom., 3, 1373-1383 (1980).

24. Meek, J.L. and Rossetti, Z.L., J. Chromatogr., 211, 15-28 (1981).

25. Stoklosa, J.T., Ayi, B.V., Shearer, C.M., and DeAngelis, N.J., Anal. Letters, B11, 889-899 (1978).

26. Hancock, W.S., Bishop, C.A., Prestidge, R.L., Harding, D.R.K., and Hearn, M.T.W., J. Chromatogr., 153, 391-398 (1978).

27. Hearn, M.T.W., Grego, B., and Hancock, W.S., J. Chromatogr., 185, 429-444 (1979).

28. Desiderio, D.M. and Cunningham, M.D., J. Liq. Chrom., 4, 721-733 (1981).

29. Meek, J.L., Proc. Nat´l. Acad. Sci. USA, 77, 1632-1636 (1980).

30. Su, S.J., Grego, B., Niven, B., and hearn, M.T.W., J. Liq. Chrom., 4, 1745-1764 (1981).

31. Gloor, R. and Johnson, E.L., J. Chrom. Sci., 15, 413-423 (1977).

32. Gruber, K.A., Whitaker, J.M., and Morris, M., Anal. Biochem., 97, 176-183 (1979).

33. Rekker, R. in The Hydrophobic Fragmental Constant, p. 301, Elsevier, New York (1977).

34. Welinder, B.S., J. Liq. Chrom. 3, 1399-1416 (1980).

35. Scott, R.P.W. and Kucera, P., J. Chromatogr., 119, 467-482 (1976).

36. Rabel, F.M., J. Chrom. Sci., 18, 394-408 (1980).

37. Atwood, J.G., Schmidt, G.J., and Slavin, W., J. Chromatogr., 171, 109-115 (1977).

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2501-2512 (1983)

SEPARATION OF THE PROTEINS OF LOCUST NEUROSECRETORY ENDINGS WITH HPLC

J. Girardie, M. Allard , A. Faddoul and E. Neuzil

Laboratoire de Neuroendocrinologie,
ERA 850 CNRS, Université de Bordeaux I,
Avenue des Facultés, 33405 Talence Cedex

Laboratoire de Biochimie Médicale,
Université de Bordeaux II,
146, rue Léo Saignat, 33076 Bordeaux,
France

ABSTRACT

High performance liquid chromatography on gel permeation columns was use to analyse the protein and peptide content of the neurosecretory corpora cardiaca of the locust (*Locusta migratoria*). A variety of extraction conditions and mobile phase compositions were tested for their separation efficiency.

A good resolution, showing 12 peaks distributed within 5 distinct groups, could be achieved in 32 min with the fresh homogenate from only two corpora cardiaca (0.5 M Tris buffer, 0.3 NaCl, 0.1 % SDS, pH = 7.4 ; flow rate : 0.5 ml/min). Similar HPLC patterns were obtained with three fractions previously separated on a Sephadex G-100 column as well as with isolated neurosecretory vesicles.

HPLC then appears as a promising technique in the field of insect neurohormone isolation.

Copyright © 1983 by Marcel Dekker, Inc.

0148-3919/83/0613-2501$3.50/0

INTRODUCTION

The minute quantity of available material is the main difficulty encountered in studying neurohormones. Contrasting with the numerous neurofactors isolated from 'higher' animals, the structures of which have been thoroughly determined, only two insect neuropeptides are well known (5) and their structure confirmed by synthesis. The proteins of the whole brain of locust (incorporating corpora cardiaca) have been studied using classical DEAE-cellulose and Sephadex chromatographic columns (1). In this paper, the HPLC technique performed on gel permeation columns has been used for analysing the proteins of dissected corpora cardiaca of the locust. HPLC appears to be a valuable technique in this new field, on account of its high sensitivity permiting a fast examination of very small quantities of biological material.

MATERIAL AND METHODS

Preparation of Samples

Mature adult locusts (*Locusta migratoria*), reared in crowd, were sacrificed by decapitation and the neurosecretory lobes of corpora cardiaca rapidly removed. Three types of neurosecretory corpora material were used :

a) Crude extracts of corpora cardiaca were obtained by ultrasonification (CIT Alcatel apparatus ; 3 x 20 sec) followed by centrifugation. The precise conditions vary with the different experiments and will be detailed in the next section. The supernatants were injected directly into the chromatographic column.

b) Corpora cardiaca (2,000 dissected samples) stored in cold acetone (4°C) were collected by decantation, ultrasonicated in 0.01 M sodium acetate buffer, 0,15 M NaCl, pH = 7.4 and centrifuged (10.000 g ; 20 min). The extract was submitted to gel filtration on Sephadex G-100 (900 x 15 mn column) and eluted with the same medium.

c) The technique of Nordmann *et al.* (4) using a sucrose/metrizamide gradient of an osmolality value of 0.46 was used for the isolation of the neurosecretory vesicles from 500 corpora cardiaca.

Techniques

Two models of HPLC apparatus were used :

a) a Waters LCIV system, with a SF 770 low wavelength absorbance detector and a gel permeation column (Waters I 125, 7.8 mm x 30 cm).

b) a Beckman basic isocratic system with a 254 nanometers analyser optical unit, and a permeation column (Altex TSK, gel 2000 SW, 7.5 mm x 30 cm).

Samples (50 or 100 µl) were injected into the Waters column and 20 µl into the Altex column and monitored respectively at 215 and 254 nm.

In every case, HPLC columns were eluted at room temperature.

RESULTS

Influence of Different Chromatographic Parameters

We have first analysed by HPLC the crude extract from 2 fresh corpora cardiaca using different phases both as extraction and elution media :

EP_1 = distilled water ;

EP_2 = Tris 0.5 M ; NaCl 0.3 M ; pH = 7.4 ;

EP_3 = EP_2, with SDS 0.1 % ;

EP_4 = EP_2/MeOH, 2:1 vol/vol ;

EP_5 = H_2O/MeOH, 70:30 vol/vol.

EP_1 gave several peaks but this eluent phase was rapidly dismissed, as a high proportion of the biological material remains linked to the column. Increasing the ionic strength and buffering at pH = 7.4 (EP_2) dramatically improved the separation ; a further improvement was the addition of 0.1 % sodium dodecylsulfate (SDS), leading to the eluent phase EP_3 which gave a better resolution.

A typical chromatographic separation of the protein content of neurosecretory endings obtained with EP_3 is shown in fig. 1. Two high molecular weight fractions (Ia and Ib) were first eluted with a retention time (9-11 min) close to the retention time of serum albumin (MW : 67.000 ; t_r = 11 min). They were followed by a second apparently homogenous peak (II : t_r = 13 min) and by a heterogenous group III. The five constituent peaks (IIIa, IIIb, IIIc, IIId,

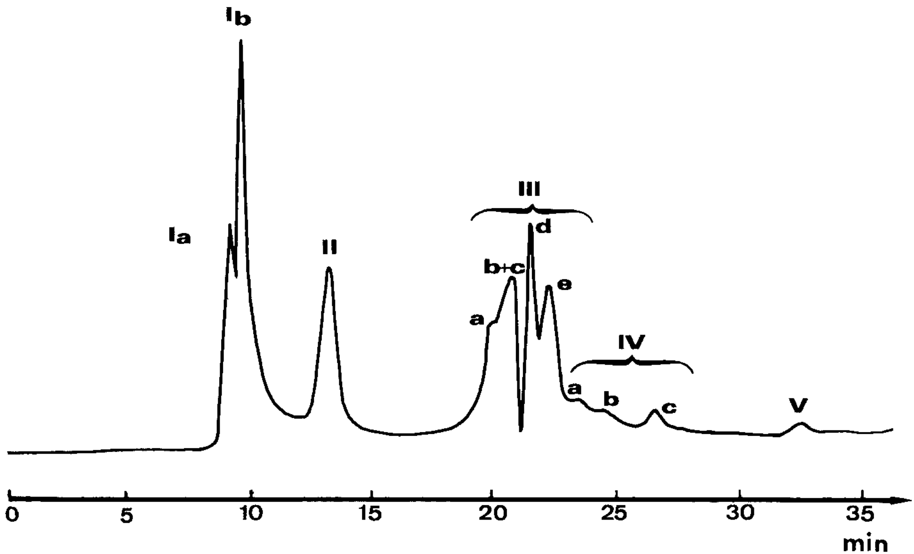


Figure 1.- HPLC separation of the proteins extracted from two fresh corpora cardiaca. The biological material was directly homogenized in the mobile phase EP_3 and centrifuged (10,000 g ; 20 min) ; the supernatant (20 µl) was loaded on an Altex column ; flow rate, 0.5 ml/min ; detection at 254 nm (0.01 AUFS) ; chart speed, 1 cm/min.

IIIe) with a retention time of 20-22 min correspond to fractions of molecular weight lower than ribonuclease (MW : 13.700 ; t_r = 17 min) and higher than insulin (MW : 6.000 ; t_r = 23 min). IIIa, IIIb and IIIc were poorly separated, especially IIIc which appeared more as a shoulder of IIIb than an individualized peak. The range of molecular weights of the III group peaks likely corresponds to the presence of neurosecretory products (1). The remaining elution peaks appeared on the diagram as a heterogenous group (IV : t_r = 23-27 min) with three minor peaks IVa, IVb and IVc. A last minor peak (V) was eluted with a retention time of 32-33 min (somatostatin, t_r = 32 min).

No major differences could be noted when modifying the concentration of Tris (0.01-0.5 M) or the final pH of the eluent

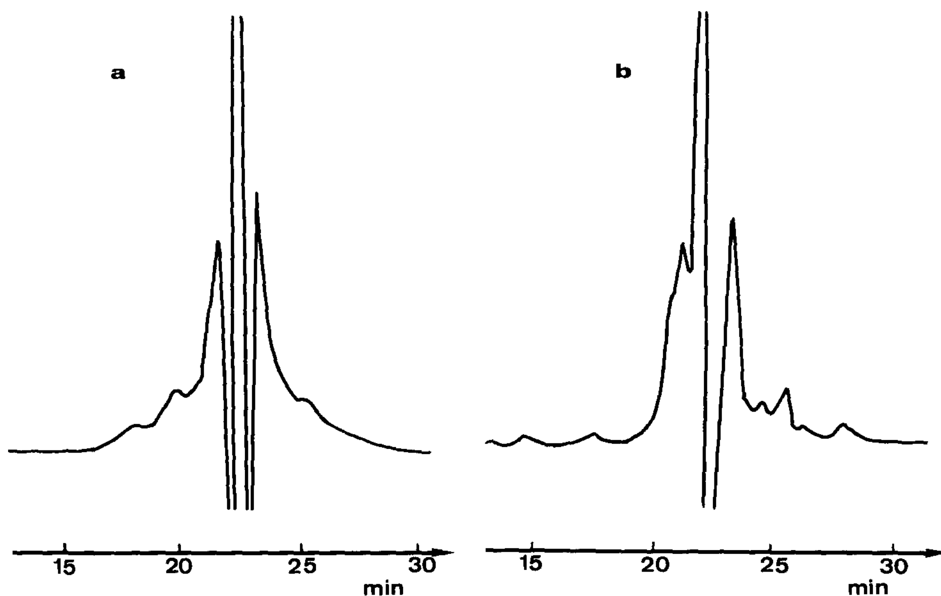


Figure 2.- Influence of methanol on the chromatographic profiles of group III peaks. The biological material (two fresh corpora cardiaca) was directly homogenized in either (a) EP_4 or (b) 30 % methanol ; in both cases, after a 20 min centrifugation at 10,000 g, the supernatant (20 µl) was loaded on an Altex column ; elution was performed with either EP_4 in (a) or EP_3 in (b) ; the chromatographic conditions were the same as in Fig. 1.

phase (pH = 3.0, 5.0 or 7.0) ; however, the higher salt concentration (0.5 M) and higher pH value (7.4) gave more reproducible results and were therefore finally selected. Addition of methanol (EP_4) considerably diminished the height of peak I ; peak II completely disappeared, and the different peaks of IV group were indistinct. The III group components were different, as only three peaks (instead of five) were noticeable ; the medium peak was the highest of the group, beginning and finishing in the negative part of the chart (fig. 2,a).

The effect of methanol was also demonstrated with solvent phases deprived of electrolytes. With EP_5, peak I was almost suppressed and appeared as at least four small separated peaks. The different components of peak III appeared as a unique small peak. As with EP_4, peak II and IV were absent.

The separation was usually performed with a flow rate of 0.5 ml/min ; similar results were obtained with a flow rate of 0.8 ml/min, but a further increase (1 or 1,5 ml/min) led to a lesser resolution of the peaks.

The chromatographic columns were loaded with crude extracts obtained from a maximum number of 6 corpora cardiaca (80 µg of proteins) ; a higher quantity of extracts led to the saturation of the column ; this was proved by the emergence of a series of new peaks which ressembled the usual elution diagram, after a further injection of pure eluent.

HPLC Analysis of Different Corpora Cardiaca Preparations

Crude methanolic extracts

Two fresh corpora cardiaca were extracted with 30 p. 100 methanol (sonification followed by centrifugation) and the chromatographic separation was performed with EP_3 as described. The modifications interested principally the two first peaks which almost disappear and group III, where two peaks were well resoluted (fig. 2,b). Increasing the methanol concentration to 70 p. 100 in the extraction medium with or without eliminating methanol under a stream of nitrogen, gave an extract very similar to the one obtained with 30 % methanol extraction.

Influence of storage

400 corpora cardiaca were sonificated in distilled water (4 ml) ; the homogenate was centrifuged (115.000 g ; 30 min) and the lyophilised supernatant stored at - 20°C. The lyophilised material was dissolved in EP_2 (4 ml) and analysed using EP_2. Peaks I and II almost disappeared ; the III group only showed four peaks. Contrary to the results obtained with fresh material, in which a very good reproductibility was always noticed, chromatograms from stored material showed a great variability in the four peaks

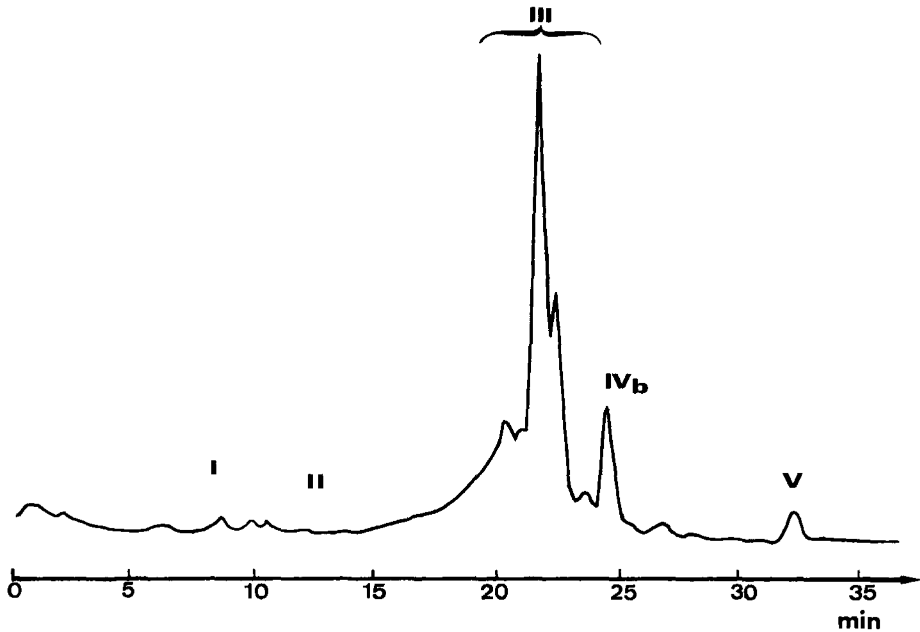


Figure 3.- Influence of storage on HPLC separation of the proteins. Corpora cardiaca (400 samples) were homogenized in distilled water (4 ml) : 30 min centrifugation at 115,000 g ; the supernatant was dissolved after lyophilisation in EP_2 (4 ml) ; 20 µl of the solution (corresponding to 2 corpora cardiaca) were loaded on an Altex column with the same chromatographic conditions as in Fig. 1.

of the III group, which differed in their heights from one batch to another and, in the same batch, from one injection to another. Storage of corpora cardiaca enhanced the importance of peak IVb and peak V (fig. 3).

Influence of a preliminary gel-filtration

Corpora cardiaca (2000 samples) were stored, extracted and centrifuged as previously described.

The extract was submitted to fractionation on Sephadex G-100. Three fractions a, b and c were collected at respectively 60, 67 and

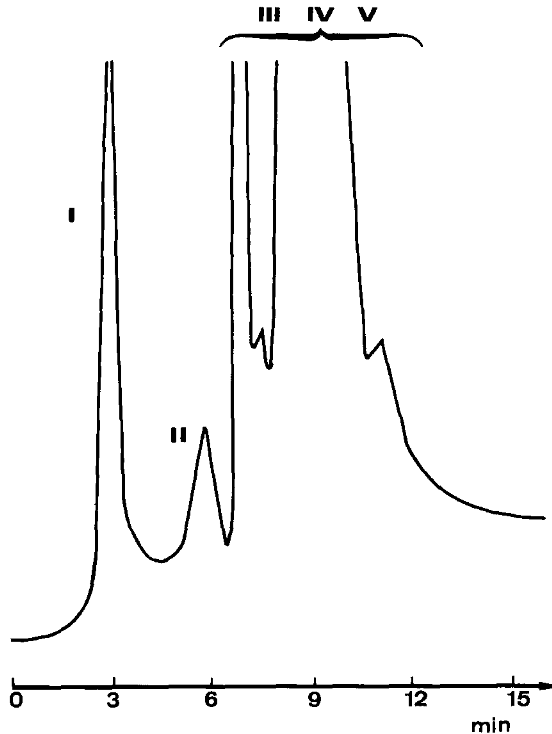


Figure 4.- HPLC separation of the proteins from fraction a obtained using Sephadex G-100 gel-filtration. Fraction a (50 µl collected at the top of the fraction) was loaded on a Waters column ; elution with EP_2 ; flow rate, 1.5 ml/min ; detection at 215 nm (0.01 AUFS) ; charter speed, 1 cm/min.

156 ml of elution volume. The a and b peaks were higher and sharper than c. Samples corresponding to the top of these peaks were submitted to HPLC with EP_2. The elution diagram of fraction a (fig. 4) is roughly similar to the one shown in fig. 1, with the exception of the different IV peaks which were all strongly enhanced. Peak I was absent in the elution diagrams of b and c fractions.

Neurosecretory vesicles extracts

The technique of Nordmann *et al.*, applied to dissected neurosecretory corpora cardiaca previously submitted to Potter treatment, gives rise to two bands (vol : 1 ml ; d = 1.3812 and 1.3848) which were carefully collected. The two bands appeared as a homogenous suspension of intact neurosecretory vesicles, with only a few contaminating mitochondria, as confirmed by electron microscopy.

The vesicles were collected by centrifugation (40.000 g ; 20 min), resuspended in distilled water containing peptidase inhibitors and sonificated. After a series of freezing and defreezing processes, a last centrifugation (40.000 g ; 20 min) gave a supernatant and a pellet which were both analysed by HPLC with EP_2 as eluent. In both cases, the elution diagram was perturbed by a very large plateau region (ranging approximatively from t_r = 7 min to t_r = 13 min) due to sucrose and metrizamide. The plateau corresponded to the retention time of the last peaks of the III group and of all group IV peaks, thus masking the occurrence of these fractions. Sucrose and metrizamide could not be eliminated neither by ultrafiltration of the samples on a Millipore membrane (cutting off point 1000 mw) nor by monitoring the detector at 280 nm. However, the occurrence of peak I could be proved in the pellet and not in the supernatant (fig. 5).

DISCUSSION

HPLC allowed a clear separation of the proteins of the neurosecretory endings of the locust. This could be achieved using a permeation column and a high osmotic strength elution medium, containing SDS. Our results agree with the conclusions of Jenik and Porter (2) and of Takagi et al. (6) : these two groups reported the beneficial influence of increasing the ionic strength and adding SDS in reducing the absorption of proteins and peptides to the matrix. The best separations were obtained with a low flow rate (0.5 ml/min). We have thus confirmed the previous results obtained by Meek and Rossetti (3), who emphazised the necessity of using a flow rate less than or equal to 1 ml/min.

The major advantage of HPLC was the possibility of exploring minute quantities of crude biological materials as the neurosecre-

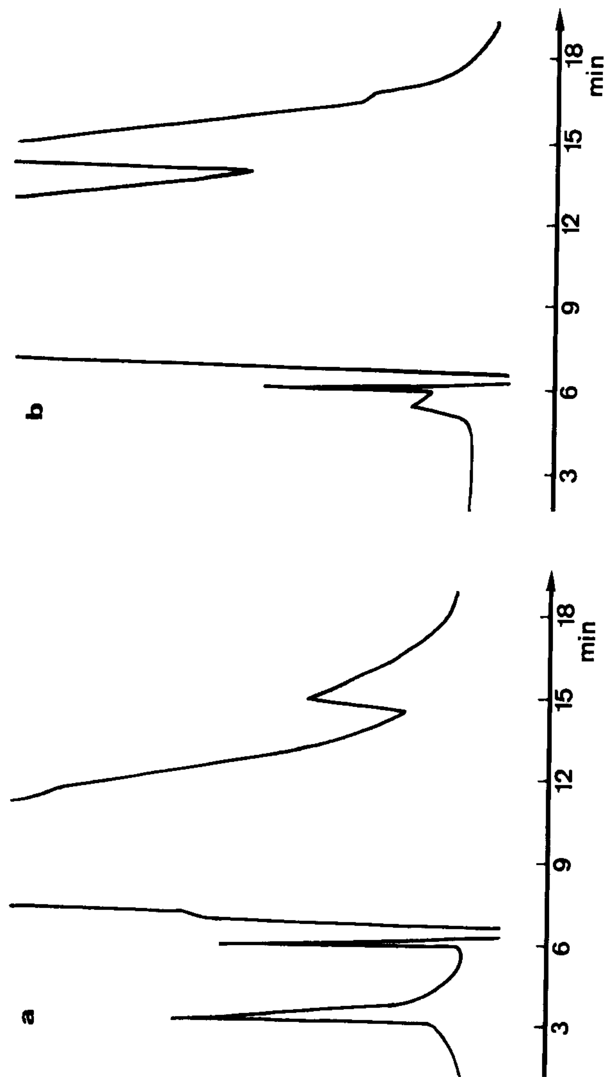


Figure 5.- HPLC analysis of the proteins from disrupted neurosecretory vesicles. (a) The pellet was homogenized in EP_2. (b) Supernatant. Chromatographic conditions as in Fig. 4.

tory endings of only two corpora cardiaca were needed, thus allowing analysis on fresh material in a wide variety of experimental conditions. A more heavier loading of the column diminished the quality of the separation, whereas the storage of the sample, an obligation when a larger weight of biological material is necessary, was followed by a low degree of reproductibility. Preliminary column gel-filtration did not improve the resolution.

Five groups of proteins of molecular weights ranging from 60,000 to 1,700 were distinctly separated. Peaks II and V always appeared homogenous ; Ia, Ib and II were present in all the samples analysed. Peaks belonging to groups III and IV showed the greatest variability in height. We generally observed that storage of the samples was associated with a decrease of group III peaks together with an enhancement of group IV peaks suggesting that the smaller compounds may be generated by an enzymatic attack of the larger protein molecules. Group III fractions include the range of molecular weights corresponding to the neurosecretory protein isolated from locust brain and corpora cardiaca by Freidel et al. (1). Using methanol in the extraction medium, an alcohol which allowed the extraction of two insect neuropeptides (5), was followed by a notable enhancement of peaks IIIb and IIIc.

Preparations of neurosecretory vesicles obtained by the technique of Nordmann et al. (4) appeared free from contaminating material and showed a good morphological quality. The constituants of the gradient unfortunately could not be eliminated and interfered with the subsequent spectrophotometric analysis. The exclusive occurence of the first compound in the pellet and its large molecular weight suggest that this compound can be a membrane protein, whereas group III compounds, entirely contained in the supernatant, appear to be neurosecretory products.

The results reported in this paper are currently applied to the isolation and biological characterization of the neurohormones of locust corpora cardiaca.

REFERENCES

1. Friedel, T., Loughton, B.G. and Andrew, R.D., Gen. comp. Endocrinol., 41, 487, 1980.

2. Jenik, R.A. and Porter, J.W., Anal. Biochem., 111, 184, 1981.

3. Meek, J.L. and Rossetti, Z.L., J. Chromatogr., 211, 15, 1981.

4. Nordmann, J.J., Louis, F. and Morris, S.J., Neuroscience, 4, 1367, 1979.

5. Stone, J.V. and Mordue, W., Insect Biochem., 10, 229, 1980.

6. Takagi, T., Takeda, K. and Okuno, T., J. Chromatogr., 208, 201, 1981.

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2513-2520 (1983)

HIGH-PRESSURE LIQUID CHROMATOGRAPHIC ASSAY OF AZLOCILLIN AND MEZLOCILLIN WITH AN ANION-EXCHANGE EXTRACTION TECHNIQUE

Claudine E. Fasching and Lance R. Peterson
Infectious Disease Section, Medical Service and
Microbiology Section, Laboratory Medicine Service,
Veterans Administration Medical Center
Minneapolis, Minnesota 55417

ABSTRACT

An anion-exchange column deproteination technique has been employed with the high-pressure liquid chromatographic (LC) assay for azlocillin and mezlocillin. The anion-exchange extraction gave excellent (97%-99%) drug recovery. Quantitation of antibiotics using the LC method compares favorably to the traditional biological assay technique with correlation coefficients for azlocillin = 0.998 and mezlocillin = 0.988. The anion-exchange extraction provides an interference-free chromatogram which aids in the LC assay of these drugs.

INTRODUCTION

Azlocillin and mezlocillin, ureidopenicillins, belong to a new class of semisynthetic penicillins possessing an extended spectrum of antimicrobial activity. Several methods for the LC assay of these agents have been described in the literature using organic extraction and Sep-Pak column (Waters Associates, Milford, MA.) protein separation techniques (1,2). We have developed an anion-exchange column procedure that works well for several cephalosporins (3,4) and have employed

Copyright © 1983 by Marcel Dekker, Inc.

0148-3919/83/0613-2513$3.50/0

this method for the LC assay of azlocillin and mezlocillin. The purpose of this report is to (i) apply anion-exchange deproteination techniques to the assay of azlocillin and mezlocillin, (ii) define drug recovery using this method, and (iii) compare quantitative results of LC method to the biological assay method.

EXPERIMENTAL

Antibiotic Extraction Technique

Anion-exchange columns were prepared by packing 6-ml syringes with DEAE A-25 Sephadex (Pharmacia Fine Chemical, Piscataway, N.J.) as described previously (3). Samples were processed by placing 0.5 ml serum on the column, followed by 0.5 ml phosphate buffered saline pH 7.2 (PBS) to rinse sample completely into column. Proteins were removed with a 4.0 ml PBS column wash, and antibiotics were then eluted from the column with 5.0 ml of 1.0 M sodium chloride. The final eluent is collected for LC analysis.

LC Assay

The chromatographic system used was a Varian (Walnut Creek, CA.) model LC 5020 liquid chromatograph with a Varichrom variable wavelength detector and a CDS 111L peak integrator with strip chart recorder. Sample eluates (100 µl) were chromatographed using an analytical octadecylsilane column (Waters Associates) of 30-cm length, 4 mm internal diameter, and 10 µm particle size. The mobile phase had a flow rate of 2.0 ml/min at isocratic conditions of 60% 0.01 M acetate buffer at pH 4.8 and 40% methanol. The UV detector was set at 220 nm and 0.1 A full scale. The calculations were based on an external standard of 20 µg/ml for both azlocillin and mezlocillin assays. Each sample was injected in duplicate and results averaged to obtain the concentration value.

Microbiological Assay

Antibiotic bioassays were performed using a standard well diffusion method with a 24-hour incubation at 37°C (5). The assay used *Micrococcus luteus* ATCC #9431 as the indicator organism in antibiotic medium no. 1 (Difco Laboratories, Detroit, MI.) at pH 6.6. Antibiotic concentrations were determined by reading zone diameters to the nearest 0.1 mm and comparing them to a standard line from 40 μg/ml to 1.25 μg/ml of either azlocillin or mezlocillin. All samples exceeding 40 μg/ml were diluted so as to fall within the standard line range. Bioassay of unknowns were run on three separate plates for each sample and then averaged.

Samples

Azlocillin and mezlocillin were added to pooled human serum *in vitro* to provide assay samples. LC assay linearity was determined for both drugs at concentrations of 100, 80, 60, 40, 20, 10, and 5 μg/ml. Peak area of antibiotic was plotted on a linear graph against drug concentration. Antibiotic recovery, using anion-exchange columns for sample preparation, was performed at 2 concentrations, 50 and 25 μg/ml. Recovery was calculated by comparing the antibiotic peak of a column eluate to one representing total drug. The described protein removal process dilutes original serum sample 10-fold, therefore saline samples containing 50 μg/ml and 25 μg/ml were diluted 1 to 10 in 1.0 M sodium chloride and used in drug recovery determination. Peak areas from serum samples were divided by the saline dilution peak areas and multiplied by 100 to give percent drug recovered. Each concentration's recovery was determined using two column extractions for both drugs. Antibiotic concentrations of approximately 100, 75, 50, 25, 10, and 5 μg/ml were prepared for each drug and si-

multaneous LC and biological ssays were performed to compare methodologies.

RESULTS

The retention times for azlocillin and mezlocillin were 4.0 and 4.4 minutes respectively, near the reported retention time of 5.25 minutes for piperacillin using the same mobile phase (6). The chromatograms from human serum samples are shown in Figure 1. The azlocillin and mezlocillin had linear detector response through range tested as shown in Figure 2. Quantitation of less than 5 µg/ml could be achieved by increasing the sensitivity of UV detector. The anion-exchange column extraction gave recoveries of 98% at 50 µg/ml for both azlocillin and mezlocillin, and at 25 µg/ml, 99% and 97% respectively. Comparison of bioassay and LC assay are shown in Figure 3 for azlocillin and Figure 4 for mezlocillin. Azlocillin comparison had a correlation coefficient of 0.998 and the mezlocillin comparison had a correlation coefficient of 0.988.

DISCUSSION

The anion-exchange column extraction provides excellent results for both quantitative drug recovery and serum interference removal for the azlocillin and mezlocillin assays, as previously found for cephalorporins in our reports using this extraction method (3,4). Since organic extractions have not given 100% antibiotic recovery (1) and involve several steps for sample preparation, a simpler and faster deproteination method is preferred. A Sep-Pak column preparation technique has also been described (2) and reports good antibiotic recovery, but at the present time Sep-Paks are costly and are used only once. The anion-exchange columns are made quickly and can be used at least ten times (3), which markedly reduces cost of sample preparation.

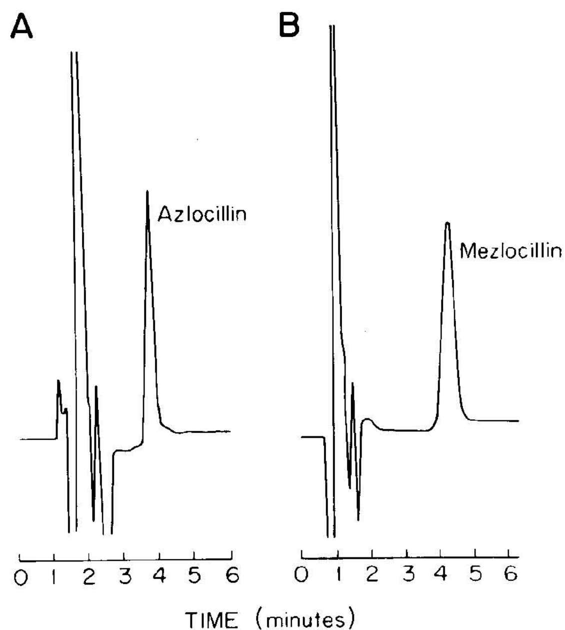


Figure 1. Sample chromatogram of azlocillin, 25 µg/ml (a) and mezlocillin, 40 µg/ml (b) from serum samples.

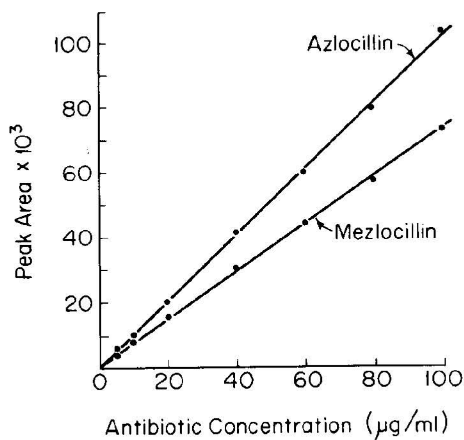


Figure 2. Comparison of detector responses (in peak area) to antibiotic concentration in sample for azlocillin and mezlocillin.

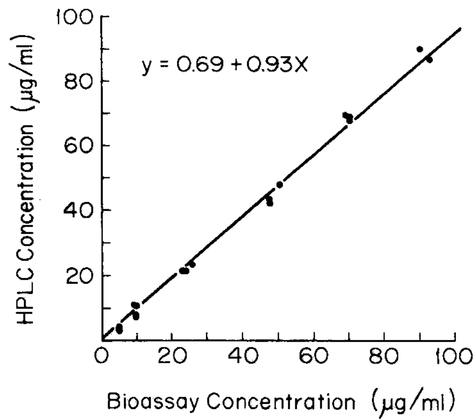


Figure 3. Linear regression of LC and bioassay for azlocillin.

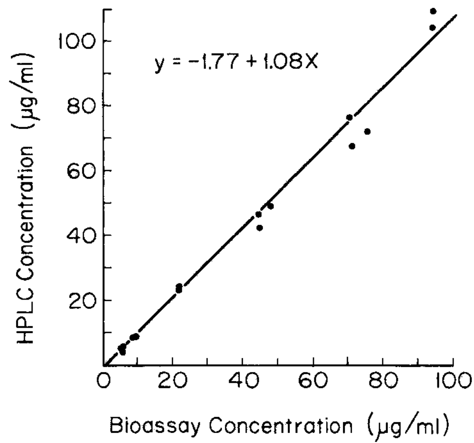


Figure 4. Linear regression of LC and bioassay for mezlocillin.

The results of linear regression analysis indicate that for both azlocillin and mezlocillin slope values obtained were near a perfect direct relationship of b=1.0. The correlation coefficient calculations represent the close agreement of the two groups of assay data obtained for each drug and reflects a favorable comparison for the two methods. Anion-exchange column extraction is a rapid, inexpensive technique for the deproteination of serum samples for use with the LC assay of azlocillin and mezlocillin. The LC method provides a sensitive, specific, and quantitative antibiotic assay.

ACKNOWLEDGMENTS

This study was supported by the Veterans Administration and by a grant from Miles Laboratories. The authors thank Evelyn C. Glatt and Alma Rollins for typing the manuscript.

REFERENCES

1. Gundert-Remy, V. and Devries, J.X., Determination of the ureidopenicillins azlocillin, mezlocillin, and Bay K 4999 in plasma by high-performance liquid chromatography. Brit. J. Clin. Pharmacol., 8:589, 1982.

2. Hildebrandt, R. and Gundert-Remy, V., Improved procedure for the determination of the ureidopenicillins, azlocillin and mezlocillin, in plasma by high-performance liquid chromatography. J. Chromatogr., 228:409, 1982.

3. Fasching, C.E. and Peterson, L.R., Anion-exchange extraction of cephapirin, cefotaxime, and cefoxitin from serum for liquid chromatography. Antimicrob. Agents Chemother., 21:628, 1982.

4. Fasching, C.E. and Peterson, L.R., High-pressure liquid chromatographic assay of ceftizoxime with an anion-exchange extraction technique. Antimicrob. Agents Chemother., 22:336, 1982.

5. Peterson, L.R., Gerding, D.N., Fasching, C.E. and Costas-Martinez, C., Assay of 27 antimicrobials using a microbiological method. Minnesota Medicine, 66:321, 1983.

6. Brisson, A.M. and Fourtillann, J.B., High-performance liquid chromatographic determination of piperacillin in plasma. Antimicrob. Agents Chemother., 21:664, 1982.

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2521-2541 (1983)

DEVELOPMENT OF A STANDARDIZED ANALYSIS STRATEGY FOR BASIC DRUGS, USING ION-PAIR EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

PART IV. Application to solid pharmaceutical dosage forms

G. Hoogewijs and D.L. Massart
Farmaceutisch Instituut, Vrije Universiteit Brussel
Laarbeeklaan 103, B-1090 Brussel
Belgium

ABSTRACT

The usefulness of two standardized HPLC-systems for the analysis of basic drugs in tablets and capsules is exemplified. The standardized HPLC systems both use a CN-column combined with either a polar or a non polar mobile phase. Also the sample preparation is standardized and simple; it involves suspension of the powder mixture in one of the mobile phase components, centrifugation and injection of the clear supernatant.

INTRODUCTION

In previous papers from this laboratory (1-3) the development of a standardized analysis strategy for basic drugs was reported. This strategy combines an ion-pair extraction technique with two preferred HPLC-systems using a CN-bonded phase and either acetonitrile-water-propylamine (90:10:0.01) or n-hexane-dichloromethane-acetonitrile-propylamine (50:50:25:0,1) as standard mobile phases. The strategy is applicable to the determination of

Copyright © 1983 by Marcel Dekker, Inc. 0148-3919/83/0613-2521$3.50/0

basic drugs in pharmaceutical dosage forms (syrups, ointments, emulsions...) (3), cosmetics (4), saliva and plasma (5). For solid pharmaceutical preparations such as tablets, the ion-pair extraction step can be omitted. The ground tablet can be suspended in the mobile phase component which is most appropriate for dissolving the analyte and following centrifugation the supernatant can directly be injected onto the nitrile bonded phase column. As reported earlier (3) the mobile phase composition can easily and rapidly be optimized for the particular analysis problem. The procedure is very convenient for the determination of basic drugs and is hence routinely applied in our laboratory in order to control label claims of tablets, capsules etc. The present paper describes some of these applications in order to exemplify the possibilities of the procedure. As in a previous paper (3) concerning the application of the strategy to syrups, ointments, etc. emphasis was laid on the extraction step and little or no account was given of how adaptation of the initial standardized mobile phase to the particular analysis problem was made, special attention will be paid in the present paper to the reasoning behind the alterations of the volume ratio of the mobile phase components.

EXPERIMENTAL

Apparatus

Chromatography was performed using either a Varian 5060 or a Varian 8500 liquid chromatograph, equipped with a Valco loop in-

jector (loop volume : 100 µl), a standard fixed wavelength (254 nm) UV detector, a Varian 9176 recorder and a Varian Vista CDS 401 data system. All analyses were performed using a LiChrosorb CN column, dp = 10 µm, 250 x 4 mm, except the caffeine analysis in which a Micro Pak CN-10 column, dp = 10 µm, 300 x 4 mm, was used. All analyses were performed at a flow rate of 2 ml/min and at maximum detector sensitivity in order to detect possible degradation products.

Chemicals and Reagents

n-hexane, dichloromethane and acetonitrile were HPLC grade and purchased from Fluka AG (Buchs, Switzerland) or E. Merck (Darmstadt, G.F.R.). Propylamine was obtained from Fluka AG (Buchs, Switzerland). Water was demineralized, double-distilled and further purified using a Water-I system (Gelman Sciences).

Composition of the Dosage Forms

Ponderal® Unicaps (Eutherapie Benelux, Belgium) : Fenfluramin. hydrochl. 60 mg - Hypromel. 50 - Cellul. - Saccharum. - Mononatrii phosphas. sic. - Talc - Magn. stear. - Eudragit S - Citroflex A_4 - Titan. oxyd. pro caps. gelat. una - Indigot. pro col. Titan. oxyd.

Vizocaf® coated tablets (Lab. Viselé, Belgium) : Diethylamid. Ac. Vanillic. 20 mg - Cafein. 20 mg - Amyl. - Sacch. lact. - Talc. - q.s. pro compressa - Accac. gum - Ti. oxy. - Talc. - Na. Indigotinodisulf. - Glycolpolyeth. - Sacch. pro obducta.

Rhinopront® capsules (Mack, G.F.R.) : Carbinoxamin. maleas 4 mg - Phenylephrin. hydrochlorid. 20 mg - Sacchar. - Maid. amyl. - Eudragit S mor. - Phtalic. acid. diaethylester - Talc. - Tartrazin. - Titan. dioxyd. - Natr. indigotinodisulfon. - Gelatin. pro capsula gelatinosa una.

Deanxit® coated tablets (Lundbeck, Denmark) : Flupentixol. dihydrochlorid. correspond. 0.5 mg basic. - Melitracen. hydrochlorid. correspond. 10 mg basic. - Amyl. - Sacchar. lact. - Gelatina - Talc. - Magnes. stearas pro tablet. compres. una - Indigotin. et Erythrosin. q.s. pro colore - Saccharo et Gelatina obducta.

Procedures

Determination of fenfluramine in Ponderal® Unicaps. The content of 20 capsules is pulverized in a mortar. An aliquot of the resulting powder, equivalent to 1/10 of the mean weight of a capsule is accurately weighed, brought into a 50 ml volume flask and suspended in about 40 ml 0.001 M HCl. After ultrasonification for 10-15 min. the flask is brought to volume. After centrifugation, 100 µl of clear supernatant are injected onto the HPLC-column. Quantitation is effected by intrapolation on a calibration curve (peak area versus concentration) of five aqueous standards in the 8 - 16 µg fenfluramine HCl/100 µl range. The calibration curve is linear in this concentration range.

Determination of caffeine in Vizocaf® coated tablets. 20 coated tablets are pulverized in a mortar. An aliquot of the resulting powder, equivalent to 1/20 of the mean weight of a coated tablet is accurately weighed, brought into a 50 ml volume flask and suspended in about 40 ml dichloromethane. After ultrasonication for 10 - 15 min the flask is brought to volume. After centrifugation, 100 µl of clear supernatant are injected onto the HPLC -column. Quantitation is effectuated by intrapolation on a calibration curve (peak area vs. concentration) of five standards in the 1 - 3 µg caffeine base/100 µl range, prepared in dichloromethane. The calibration curve is linear in this concentration range.

Determination of tetracaine in commercial tablets. 20 tablets are pulverized in a mortar. An aliquot of the resulting powder, equivalent to half the mean weight of a tablet is accurately weighed, brought into a 50 ml volume flask and suspended in about 40 ml dichloromethane. After ultrasonication for 30 min the flask is brought to volume. After centrifugation, 100 µl of the clear supernatant are injected onto the HPLC-column. Quantitation is effected by intrapolation on a calibration curve (peak area vs. concentration) of five standards in the 0.3 - 0.7 µg/100 µl concentration range, prepared in dichloromethane. The calibration curve is linear in this concentration range.

Determination of carbinoxamine and phenylephrine in Rhinopront® capsules. All manipulations are effected as good as

possible protected from light. The content of 10 capsules is ground in a mortar. An aliquot of the resulting powder, equivalent to the mean weight of 1 capsule is accurately weighed, brought into a 100 ml volume flask and suspended in about 80 ml 0.001 M HCl. After ultrasonication for 10 - 15 min the flask is brought to volume. After centrifugation, 50 µl of clear supernatant are injected onto the HPLC-column. Quantitation is effected by intrapolation on calibration curves (peak area vs. concentration) of five aqueous standards containing from 2 to 6 mg carbinoxamine maleate/100 ml and from 15 to 25 mg phenylephrine hydrochloride/100 ml. Both calibration curves are linear in this concentration range.

Determination of flupentixol and melitracen in Deanxit® coated tablets. 25 coated tablets are pulverized in a mortar. An aliquot of the resulting powder, equivalent to the mean weight of one tablet is accurately weighed, brought into a 50 ml volume flask and suspended in about 40 ml 0.001 M HCl. After ultrasonication for 10 - 15 min the flask is brought to volume. After centrifugation, 100 µl of clear supernatant is injected into the chromatograph. Quantitation is effected by intrapolation on calibration curves (peak area vs. concentration) of five aqueous standards containing from 0.5 to 1.5 µg flupentixol dihydrochloride/100 µl and from 15 to 25 µg melitracen hydrochloride/100 µl. Both calibration curves are linear in this concentration range.

RESULTS AND DISCUSSION

Fenfluramine in Ponderal®

The majority of the 100 basic drugs investigated previously (2) have capacity factors in the 1 - 10 range in both preferred eluents. Consequently both standardized eluents can be used for the chromatography of polar as well as non polar solutes. However, for fast optimization of the mobile phase composition for a drug not belonging to the original test set (2) we usually employ the polar mobile phase as initial investigation eluent for polar solutes and the non polar eluent for non polar molecules (3). Fenfluramine is a quite polar molecule and consequently the reversed phase system was used. In its original composition the acetonitrile-water-propylamine (90:10:0.01) eluent eluted fenfluramine with a retention time of 7.6 min at a flow rate of 2 ml/min. In order to shorten the analysis time the retention of the solute was reduced by increasing the propylamine content by a factor 3. A chromatogram of a capsule treated as described in the "Experimental section" is shown in Figure 1. As can be seen from Table 1, the recovery is excellent and the precision is acceptable.

Caffeine in Vizocaf®

The selection of an appropriate mobile phase for the determination of caffeine was very easy since its composition was identical to the eluent used for the determination of papaverine in blood (6). During method development for the latter assay the non

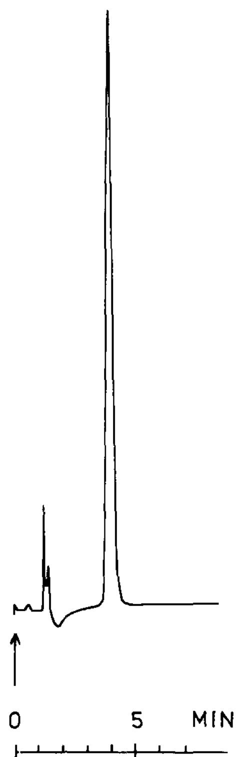


Figure 1 - Chromatogram of a Ponderal® capsule on a LiChrosorb-CN column (dp = 10 μm, 250 x 4 mm). Mobile phase : acetonitrile -water-propylamine (90:10:0.03). Flow rate : 2 ml/min. Detector sensitivity : 0.01 AUFS.

polar standardized eluent in its original composition n-hexane-dichloromethane-acetonitrile-propylamine (50:50:25:0.1) was altered by halving its dichloromethane content in order to resolve papaverine from caffeine and other matrix components. Since this (50:25:25:0.1) volume ratio allows caffeine to be chromatographed with good peak shape it was employed for the determination of caf-

TABLE 1

Chromatographic Conditions and Recoveries

Dosage Form	Column	Mobile Phase Composition		Analyte Retention time		Mean Recovery (% of Label Claim) ± S.D.		
Ponderal®	LiChrosorb-CN	Acetonitrile Water Propylamine	90 10 0.03	Fenfluramine	3.9	99.1	± 1.8%	(n=6)
Vizocaf®	MicroPak-CN	n-Hexane Dichloromethane Acetonitrile Propylamine	50 25 25 0.1	Caffeine	4.4	98.0	± 0.9%	(n=6)
Tetracaine tablets	LiChrosorb-CN	n-Hexane Dichloromethane Acetonitrile Propylamine	10 80 10 0.1	Tetracaine	3.0	64.9 (99.7	± 0.7% ± 0.9%)	(n=6) (n=3)
Rhino-pront®	LiChrosorb-CN	Acetonitrile Water Propylamine	80 20 0.05	Carbinoxamine Phenylephrine	2.7 5.2	97.9 100.2	± 0.7% ± 0.9%	(n=3)
Deanxit®	LiChrosorb-CN	Acetonitrile Water Propylamine	40 60 0.01	Flupentixol Melitracen	2.5 9.7	101.3 100.1	± 1.3% ± 1.8%	(n=6)

feine in Vizocaf® tablets. The column used was from the same manufacturer but from a different lot. It is striking to note that the retention time of caffeine (4.4 min) is much larger than on the column used for the papaverine determination from which caffeine eluted at 2.9 min. As stated previously (3) important differences between CN-columns of different batches and different brands indeed exist. This however does not jeopardize the standardized analysis strategy, since both preferred mobile phases are so versatile that the composition of the eluent can easily be finetuned in order to obtain retention times of a desired magnitude. Since in the case of the caffeine determination an analysis time of 5 min was considered sufficiently short, the mobile phase composition was not altered. A chromatogram of a tablet obtained as described in the "Experimental section" is shown in Figure 2. The recovery and standard deviation are excellent as can be seen from Table 1.

Tetracaine in Commercial Tablets

The determination was carried out because the tablets were suspected of being underdosed. The non polar standardized eluent in its original composition n-hexane-dichloromethane-acetonitrile-propylamine (50:50:25:0.1) resulted in strong retention of tetracaine. In order to shorten the time for analysing the tablet, the retention of tetracaine was reduced by drastically decreasing the hexane content of the mobile phase. The volume ratio of the mobile phase was then further finetuned to (10:80:10:0.1) in or-

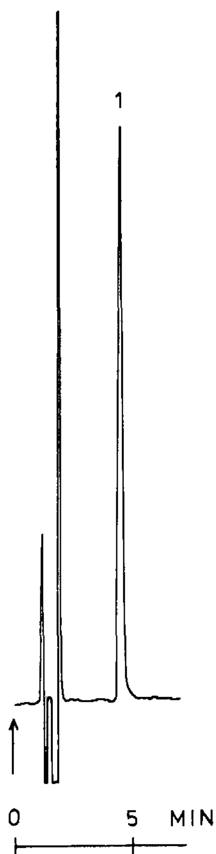


Figure 2 - Chromatogram of a Vizocaf® coated tablet on a Micro-Pak CN-10 column (dp = 10 µm, 300 x 4 mm). Mobile phase : n-hexane-dichloromethane-acetonitrile-propylamine (50:25:25:0.1). Flow rate : 2 ml/min. Detector sensitivity : 0.08 AUFS.

der to obtain a good peak shape. A chromatogram of a tablet treated as described in the "Experimental section" is given in Figure 3a. As can be seen from Table 1, the tablet contains only 64.9% of the tetracaine label claim which confirms the suspicion of underdosing. The precision of the method is excellent. In order to evaluate the accuracy of the method, a self made powder-mixture containing all ingredients of the commercially available tablet was analysed. The recovery of tetracaine was 99.7% $\pm$ 0.9% (n = 3) which confirms the accuracy of the method. The chromatograms of the commercial tablets showed only one peak (tetracaine) suggesting that no degradation of tetracaine had taken place. The main degradation product of tetracaine is p-n-butylaminobenzoic acid (7). Since we did not know whether this degradation product would be detectable with the chromatographic conditions used it was decided to proceed as follows. An aqueous tetracaine solution was subjected to hydrolysis at alkaline pH and at an elevated temperature ($\pm$ 90°C). An aliquot of this solution was extracted with dichloromethane after acidification. The extract was subjected to HPLC-analysis and in the chromatogram no peak at the retention time of tetracaine (t_R = 3.0 min) was observed but instead a peak with t_R = 1.7 min emerged. Consequently this peak was tentatively identified as p-n-butylaminobenzoic acid. The absence of such a peak in the chromatograms of the commercial tablets suggests that not hydrolysis but underdosing is responsible for the low tetracaine recovery. A chromatogram of both tetracaine and its degradation product, obtained by injecting a mixture

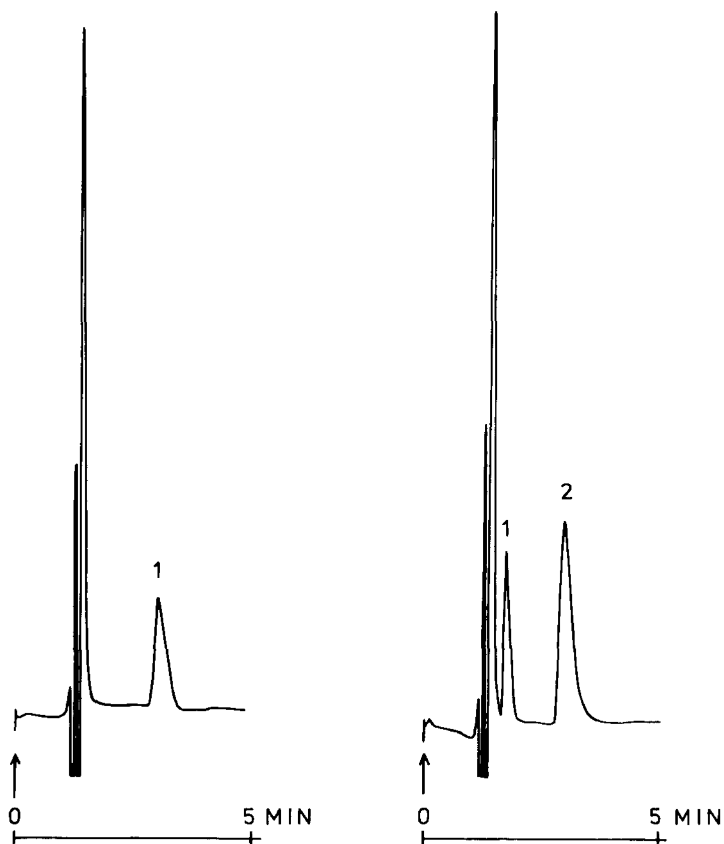


Figure 3 - a) Chromatogram of a commercial tetracaine tablet (Peak 1 = tetracaine) and; b) Chromatogram of a mixture of Tetracaine (= Peak 2) and its main degradation product (= Peak 1) on a LiChrosorb-CN column (dp = 10 µm, 250 x 4 mm). Mobile phase : n-hexane-dichloromethane-acetonitrile-propylamine (10:80:10:0.1). Flow rate : 2 ml/min. Detector sensitivity : 0.01 AUFS.

of the extract of the hydrolytic solution and a tetracaine standard is shown in Figure 3 b.

Other local anesthetics have also been chromatographed using eluents emanating from the nonpolar standardized mobile phase and the eluent has also been optimized for the separation of seven local anesthetics (8). A chromatogram of this separation using a (50:75:20:0.1) volume ratio is shown in Figure 4.

Carbinoxamine and Phenylephrine in Rhinopront®

The present application is reported in order to demonstrate the suitability of the CN-column for the simultaneous determination of a polar (phenylephrine) and a nonpolar (carbinoxamine) solute in a single chromatographic run with a fairly short analysis time. Usually this is difficult to achieve either on a C_{18}- or on a Si-column. On a Si-column it is indeed usually difficult to obtain sufficient retention of a nonpolar solute while avoiding excessive retention of the polar molecule. Similar difficulties occur with a C_{18}-column unless ion-pair chromatography would be used. The use of a CN-column which is of intermediate polarity offers however a more convenient solution. Optimization of the acetonitrile-water-propylamine (90:10:0.01) eluent was performed as follows. In its original composition this eluent resulted in a considerable retention time for phenylephrine and a rather unfavourable peak shape. Improvement could be obtained by increasing the water content of the eluent to 50-60% but this had a negative effect on the selectivity. It was therefore decided to increase

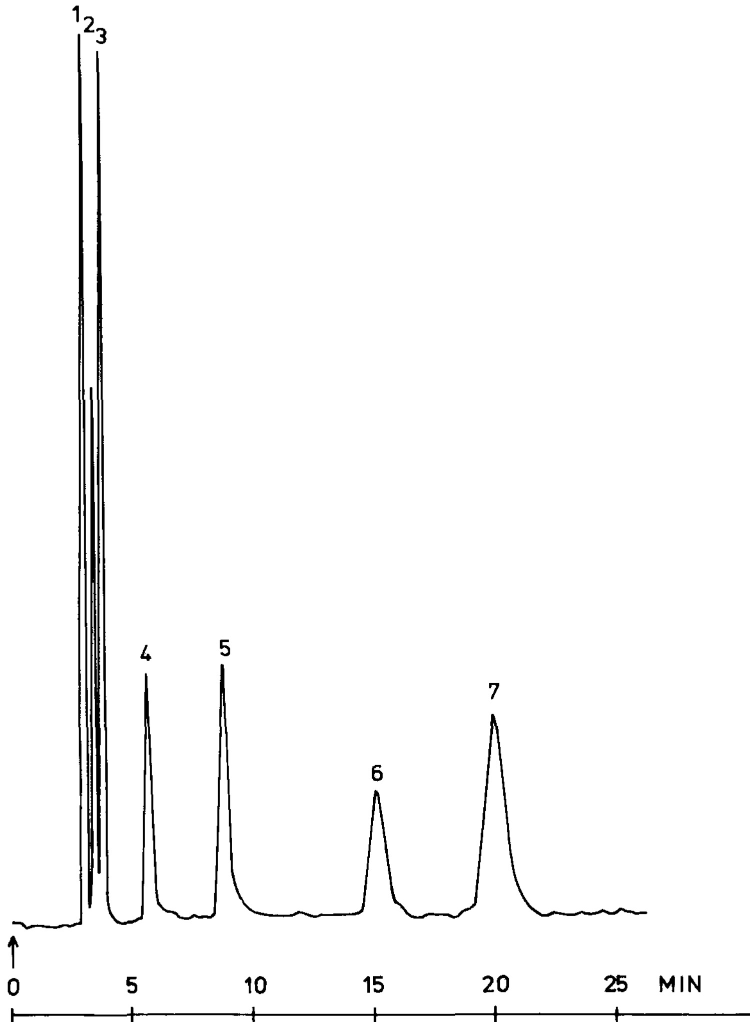


Figure 4 - Chromatogram of a mixture of local anesthetics on a MicroPak CN-10 column (dp = 10 µm; 300 x 4 mm). Peak identification : 1. Amylocaine, 2. Lidocaine, 3. Benzocaine, 4. Mepivacaine, 5. Piperocaine, 6. Procaine, 7. Tetracaine. Mobile phase : n-hexane-dichloromethane-acetonitrile-propylamine (50:75:20:0.1). Flow rate : 1 ml/min. Detector sensitivity : 0.01 AUFS.

the propylamine content by a factor 5 while increasing the water content to only 20%. The optimum volume ratio is hence : (80:20:0.05). A chromatogram (Figure 5) of a Rhinopront capsule treated as described in the "Experimental section" shows that both the polar and the nonpolar drug can be quantified simultaneously while limiting the analysis time to 7 min. The recoveries and standard deviations are also excellent as can be seen from Table 1.

Flupentixol and melitracen in Deanxit®

In the two foregoing examples in which the polar standardized mobile phase was used, the composition of the eluent had to be altered in order to reduce the retention of the polar analytes. If one wishes to chromatograph nonpolar solutes using the acetonitrile-water-propylamine eluent it sometimes is necessary to enhance retention. In the same way as for a classical reversed phase system using a C_{18}-column this usually can be done by increasing the water content of the mobile phase. The present application is reported to exemplify this. Flupentixol and melitracen are rather poorly retained when the original acetonitrile -water-propylamine (90:10:0.01) eluent is used. Drastically increasing the polarity of the mobile phase to a (50:50:0.01) volume ratio resulted in appropriate retention as can be seen in Figure 6. However, at this mobile phase composition flupentixol was not entirely separated from a tablet ingredient (probably one of the dyes). A baseline separation of flupentixol and the interfering sample ingredient could be achieved by further increasing the

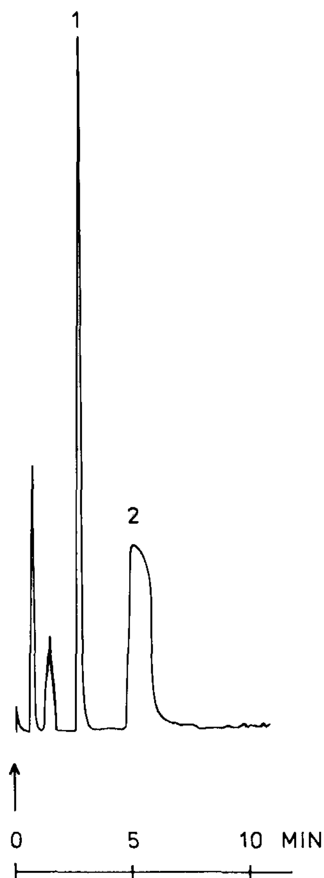


Figure 5 - Chromatogram of a Rhinopront® capsule on a LiChrosorb -CN column (dp = 10 µm, 250 x 4 mm). Peak identification : 1. carbinoxamine, 2. phenylephrine. Mobile phase : acetonitrile -water-propylamine (80:20:0.05). Flow rate : 2 ml/min. Detector sensitivity : 0.08 AUFS.

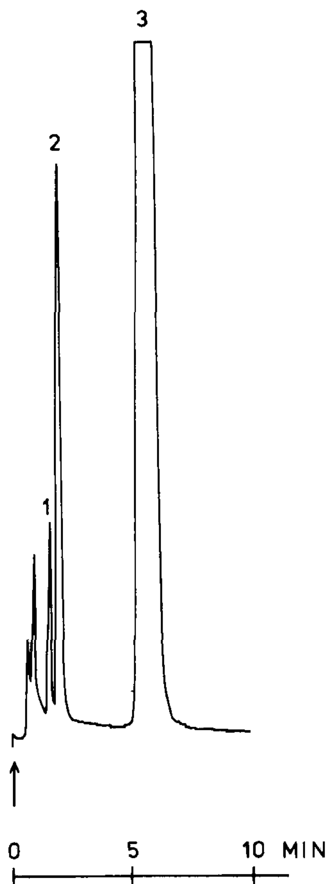


Figure 6 - Chromatogram of a Deanxit® coated tablet on a LiChrosorb-CN column (dp = 10 µm, 250 x 4 mm). Peak identification : 1. tablet ingredient, 2. flupentixol, 3. melitracen. Mobile phase : acetonitrile-water-propylamine (50:50:0.01). Flow rate : 2 ml/min. Detector sensitivity : 0.02 AUFS.

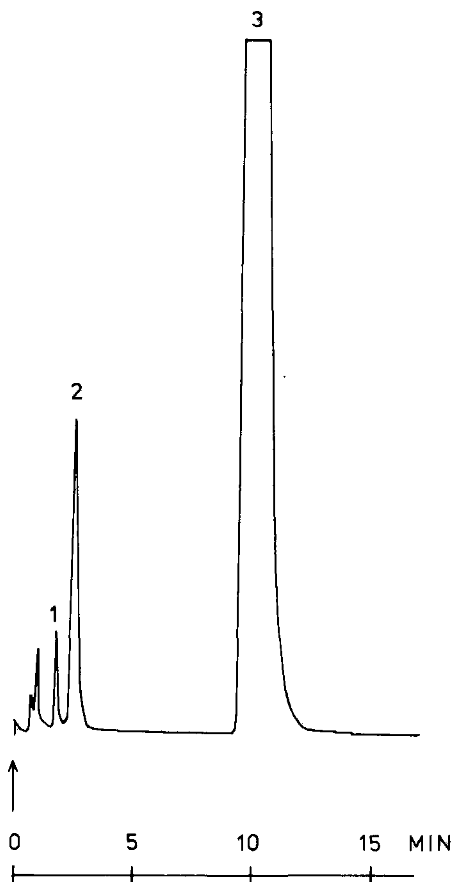


Figure 7 - Chromatogram of a Deanxit® coated tablet. Mobile phase : acetonitrile-water-propylamine (40:60: 0.01). All other parameters are the same as in Figure 6.

polarity of the eluent, at the cost however of also increasing the analysis time. A chromatogram of a Deanxit® coated tablet using a volume ratio of (40:60:0.01) is shown in Figure 7. The recoveries and standard deviations, presented in Table 1, are acceptable.

CONCLUSIONS

A few applications have been reported in order to exemplify the suitability of the standardized HPLC-systems for the determination of basic drugs in solid pharmaceutical dosage forms. The advantages of the approach are the following :

1) column selection can be omitted since all assays are performed using a CN-column
2) both polar and nonpolar solutes can be chromatographed on this column, and as was demonstrated, simultaneously if needed
3) both standardized mobile phases are very versatile and are easily and rapidly adaptable to the particular analysis problem
4) the excellent separation ability of the standardized HPLC-systems, even for stereoisomers (e.g. ref. 3,4) is profitable for stability indicating tests as was shown previously (3) and presently in the tetracaine case
5) the recovery and precision data show that the use of an internal standard is not necessary.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs. M. De Vreese and Mrs. L. Maes for skilful technical assistance and Mrs. S. Peeters for typing the manuscript.

REFERENCES

1. Hoogewijs, G. and Massart, D.L., Part I, submitted for publication, J. Pharm. Biom. Anal.

2. Detaevernier, M.R., Hoogewijs, G. and Massart, D.L., Part II, submitted for publication, J. Pharm. Biomed. Anal.

3. Hoogewijs, G. and Massart, D.L., Part III, submitted for publication, J. Pharm. Biomed. Anal.

4. Hoogewijs, G. and Massart, D.L., Part V, submitted for publication, J. Pharm. Belg.

5. Hoogewijs, G. and Massart, D.L., in preparation.

6. Hoogewijs, G., Michotte, Y., Lambrecht, J. and Massart, D.L., J. Chromatogr., 226, 423-430, 1981.

7. Menon, G.N. and Norris, B.J., J. Pharm. Sci., 70, 569-570, 1981.

8. Hoogewijs, G., Puttemans, M. and Massart, D.L., unpublished results.

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2543-2556 (1983)

LIGAND EXCHANGE CHROMATOGRAPHY OF CEPHALOSPORIN C ON POLYSTYRENE RESINS CONTAINING COPPER COMPLEX OF LYSINE DERIVATIVES

D.SACCO,E.DELLACHERIE
Laboratoire de Chimie Physique Macromoléculaire
C.N.R.S.ERA 23,ENSIC,1,rue Grandville
54042.NANCY Cedex - FRANCE.

ABSTRACT

A ligand-exchange chromatography procedure for the separation of cephalosporin C from an artificial mixture is described. Three new sorbents were synthesized by immobilizing the ligands ε-L-lysine,glycyl-ε-L-lysine and diglycyl-ε-L-lysine on a polystyrene matrix(SX 1 Bio-Rad).These resins were loaded with Cu(II)as a complexing agent.A good resolution of cephalosporin C was achieved only using the ε-L-lysine resin complexed with copper. Models of copper complexes involved are proposed, for the chromatographic separation of cephalosporin C.

INTRODUCTION

Cephalosporin C,the starting material for the synthesis of various antibiotics,is usually extracted from its biosynthesis medium by means of adsorption(1,2,3) or ion exchange(4) chromatography and this extraction is not always efficient because of the low selectivity of the sorbents usually employed.

In a previous study(5),we successfully purified cephalosporin C by using new selective sorbents.These sorbents were obtained by immobilizing three ligands(ε-L-lysine,glycyl-ε-L-lysine,and diglycyl-ε-L-lysine) on a polystyrene matrix(Bio-Beads S X_1).The best

Copyright © 1983 by Marcel Dekker, Inc.

0148-3919/83/0613-2543$3.50/0

results were achieved with the ε-L-lysine resin.However the selectivity of these sorbents depends greatly on the concentration of salt(NaCl)in the eluent solution. To overcome this drawback,the three resins were complexed with copper ions,when the binding of solutes to the copper-complexed ligands was not influenced by the presence of inorganic salts.

This paper describes a ligand-exchange chromatography procedure for the separation of cephalosporin C from by-products,which are usually found in its biosynthesis medium. The composition and the features of the eluent were varied in order to optimize the procedure.

EXPERIMENTAL

Reagents

The aminoacids,organic acids and sodium acetate were obtained from Fluka(Switzerland).The dipeptide glycylglycine was purchased from Bachem(Switzerland).The divinylbenzene-crosslinked polystyrene(Bio-Beads SX1,2% divinylbenzene,particle size 200-400 mesh) was obtained from Bio-Rad Labs(Richmond,California,USA).. Cephalosporin C and deacetylcephalosporin C were a gift from Roussel-Uclaf(Paris,France).The acetic acid was of reagent grade.

Synthesis

The sorbents were synthesized by a procedure described elsewhere(6).According to elemental analysis and potentiometric titration,the chemical capacities were 0.6-0.8 mmol(ε-L-lysine resin) and 0.4-0.6 mmol (glycyl-ε-L-lysine resin and diglycyl-ε-L-lysine resin)of fixed lysine per gram of dry resin. The copper complex was formed by treating the resin with aqueous acetate buffer solutions containing copper sulfate. Columns were packed by a conventional slurry method using a precolumn(7).

Instrumentation

The liquid chromatograph consisted of a Waters Model A.L.C.200 liquid chromatograph,equipped with a M 600 A pump,a M 440 UV detector with a 12.5 μl flow cell,a R 401 differential refractometer, and a U6K sample injector fitted with a 2 ml sample loop.

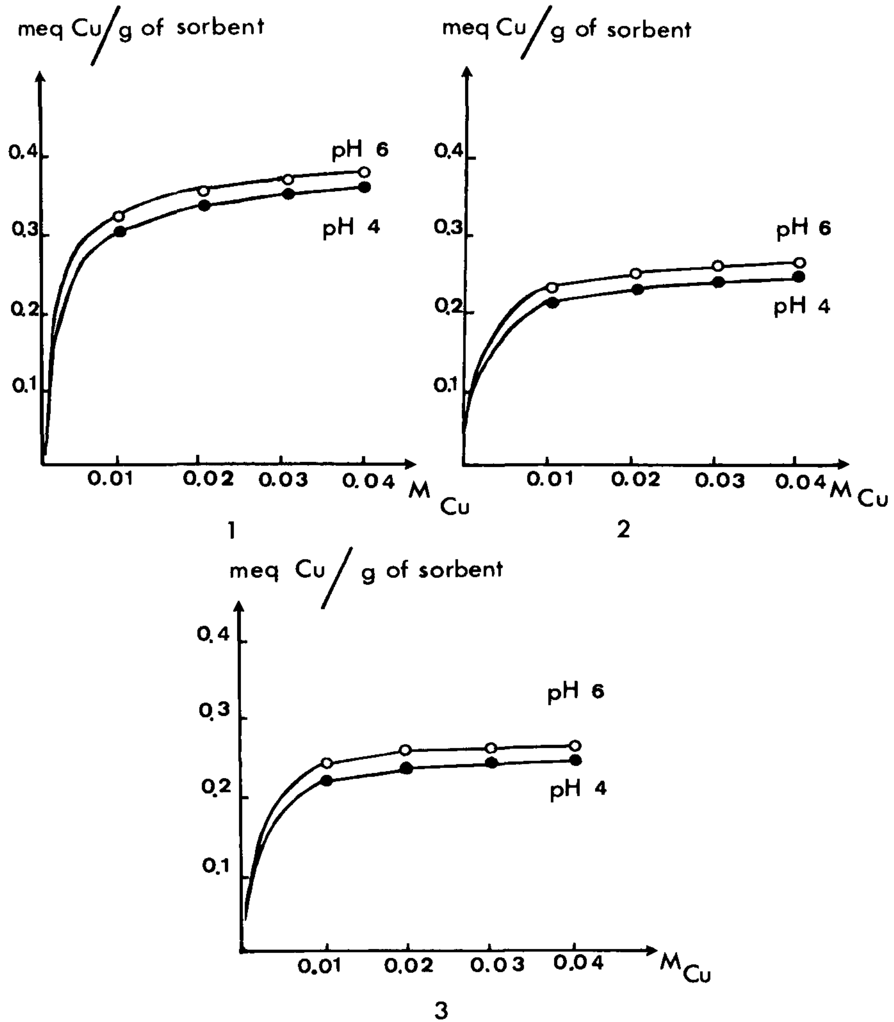
Stainless steel tubing (0.95 cm ID)30 cm long was purchased from Waters Associates. Volumes of 20 to 150 µl containing 6 mM solute were injected with a Hamilton syringe into the chromatographic system.

RESULTS AND DISCUSSION

Several buffers eluents,among those usually employed in ligand exchange chromatography(8,9,10)were tested,within the stability range of cephalosporin C towards pH(1 to 10).At pH 10,this compound undergoes a rapide inactivation,which leads to the loss of U.V. chromophore and the formation of two new titratable acid groups (11).On the other hand,at pH 1,cephalosporin C is transformed into a new substance,having antibacterial properties,namely deacetylcephalosporin C lactone. The antibiotic was irreversibly adsorbed on the three stationary phases,when it was injected in an isocratic flow with ammonium hydroxide solutions,or with ammonium phosphate buffers.On the other hand cephalosporin C was only retarded on the supports with acetate buffers as eluents.Therefore all the following experiments were performed in acetate buffers.

Isotherms of Cu(II)sorption on the three sorbents were determined by a batch process(Fig.1-3)(12,13).It should be noted that saturation with Cu(II)corresponded to 1 mole of Cu(II)per 2 moles of ligands,suggesting the formation of Resin-Cu-Resin bidentate complex. The features of resins complexed with copper are shown in Table 1. The swelling capacity of the sorbents fits the results obtained by Davankov and coworkers(12).On the other hand it appears that the sorption capacity was not greatly influenced by the pH of the acetate buffer in the range 4 to 6.

For the optimization of the conditions of separations,the effects of the pH and ionic strength of the eluent were thoroughly investigated. The various solutes were injected separately onto the three stationary phases. Capacity factors k' were calculated using the equation $k' = \frac{Ve - Vo}{Vo}$,where Ve is the elution volume for a chromatographic peak and Vo is the column void volume.



FIGURES 1-3: Adsorption isotherms of copper on the ε-L-lysine resin(1), glycyl-ε-L-lysine resin(2) and diglycyl-ε-L-lysine resin(3).
Copper adsorbed on the resin versus concentration of copper in the solution.

TABLE 1

Features of the resins in acetate buffers

Q_1 : quantity of sites per g of sorbent

Q_2 : quantity of copper per g of sorbent

S : swelling capacity is defined as the weight of water absorbed by unit weight of dry resin in 24 h.

Sorbents	Q_1	acetate concentration = 1 M			
		pH = 4		pH = 6	
	meq/g	S	Q_2 meq/g	S	Q_2 meq/g
ε-L-lysine resin	0.8	0.30	0.35	0.31	0.36
glycyl ε-L-lysine resin	0.6	0.29	0.25	0.29	0.25
diglycyl-ε-L-lysine resin	0.6	0.26	0.24	0.26	0.25

Diglycyl-ε-L-lysine resin complexed with copper

Figure 4 showsthe chromatographic peak corresponding to the elution of cephalosporin C. At all pH values and ionic strengths of the acetate buffer the antibiotic was eluted partly in the void volume,and in all cases the chromatographic peak obtained was very broad.This proves that copper-complexed diglycyl-ε-L-lysine resin is not very selective for cephalosporin C.

On the other hand, a comparison of selectivity factors of amino acids(Table 2)shows that their retention is due only to the participation of a carboxyl function in the side chain(Asp,Glu) in the copper complex or to weak hydrophobic interactions(Met,Phe). The structure of the copper complex on the stationary phase could explain these results.The diglycyl-ε-L-lysine ligand probably in-

TABLE 2

Capacity factors obtained on the diglycyl-ε-L-lysine-copper resin in acetate buffers of various concentration at pH 4.

* Solutes of both L and D configurations had identical k' values

** C=acetate concentrations.

*** Peaks too wide for the elution volume to be determined

Solutes*	k'		
	C = 0.25 M	C = 0.5 M	C = 1 M
Lysine	0	0	0
Glycine	0	0	0
Alanine	0	0	0
Methionine	0.09	0.09	0
Glutamic acid	0.95	0.45	0
γ-methyl glutamate	0	0	0
Aspartic acid	1.70	0.90	0.45
Phenylalanine	0.20	0.20	0.20
Cephalosporin C	***	***	***
Deacetylcephalosporin C	***	***	***

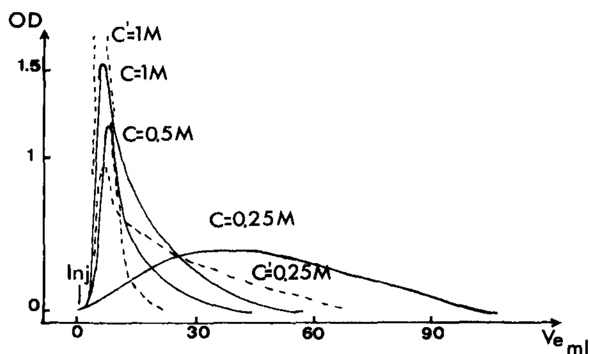


FIGURE 4 : Retardation of cephalosporin C on the diglycyl-ε-L-lysine-copper resin in acetate buffers of various concentrations at pH6 (C',---) and at pH 4 (C,——).

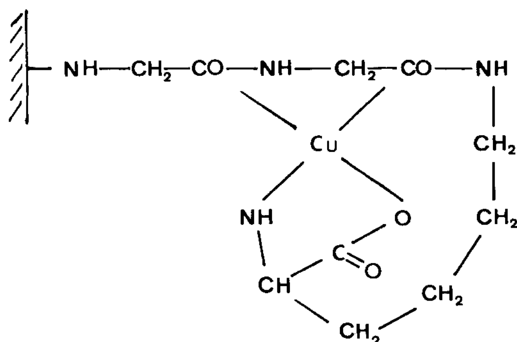


FIGURE 5 : Theoretical structure of the diglycyl-ε-L-lysine copper complex.

teracts in the copper coordination plane with the cation as shown in Figure 5.In such a case,solutes are only able to interact with the cation in an axial position. Thus,this structure does not favour the participation of the two functions of the solutes α-amino acid moiety in the mixed complex.As a matter of fact,the proximity of the carboxyl and amine functions does not allow the coordination of one of these,with the copper complex.In contrast,the carboxylic function of aspartic and glutamic acids,located on the lateral chain,could lie on the axis of the copper complex.Such a structure,which is in good agreement with molecular models,is consequently consistent with our results.

Glycyl-ε-L-lysine resin complexed with copper

As illustrated in Table 3,cephalosporin C was retarded more strongly than α-aminoacids on this stationary phase.The highest capacity factor for cephalosporin C was obtained at pH 4,and a decreased acetate concentration resulted in an increased retention. However the capacity factor of deacetylcephalosporin C varied similarly,and the resolution factor calculated between this compound and cephalosporin C was always less than 0.5 .Therefore the use of

TABLE 3

Capacity factors obtained on the glycyl-ε-L-lysine resin complexed with copper in sodium acetate buffers.

*C = acetate concentrations, ** Solutes of both L and D configurations had identical k' values.

Solutes**	k'				
	pH = 4			pH = 6	pH = 7
	C=0.25M	C=0.5M	C = 1M	C=0.5M	C=0.5M
Lysine	0	0	0	0	0.10
Glycine	0	0	0	0.10	0.10
Alanine	0	0	0	0.10	0.10
Methionine	0.20	0.20	0.20	0.25	0.50
Glutamic acid	0.70	0.60	0.30	0.30	0.30
γ-methyl glutamate	0	0	0	0.10	0.10
Aspartic acid	1.20	0.90	0.40	0.45	0.45
Phenylalanine	0.30	0.20	0.20	0.60	0.80
Cephalosporin C	3.90	2.65	1.20	1.65	1.90
Deacetyl-cephalosporin C	2.90	1.80	0.80	0.90	1.10

glycyl-ε-L-lysine resin does not make it possible to separate the antibiotic satisfactorily from its related contaminant.

On the other hand, neutral α-amino acids with hydrophobic features (Phe,Met) were the only ones to be retained on this support at pH 4. Furthermore, varying the concentration of the acetate buffer did not alter the capacity factors of these hydrophobic solutes. These facts indicate that the α-amino acid extremities of solutes do not participate in the copper complex and also that the retention of phenylalanine and methionine is due only to hydrophobic interactions. Moreover, the retention of dicarboxylic aminoacids can be attributed to an interaction between their β or γ

carboxyl function and the ligand complex.(See the retention of Asp,Glu and γ-methyl glutamate).

These results suggest that cephalosporin C interacts with the copper complex only by means of the carboxylfunction of its dihydrothiazine ring and that some additional hydrophobic interactions with the polymer matrix might increase the stability of this complex.

This fact could be explained by the structure of the copper ligand complex,if the amide function of the peptide bond can participate in the copper complex on the stationary phase.Thus the copper ion presumably coordinates both with the amide function and with the α-amino groups of lysine.In this case,one coordinate bond in the coordination plane and two in the axial position remain available. Obviously,the spatial disposition of these bonds prohibits the simultaneous interaction with copper,of the two functions of the solutes α-amino-acid moiety.As for the diglycyl-lysine resin the two functions of α-amino-acid ends are not able to interact in an axial position.Moreover the steric hindrance of the resin copper complex does not allow the coordination of one of these functions in the coordination plane.Therefore,this structure,which prohibits the participation of the solutes α-amino-acids ends in the mixed copper complex is in good agreement with our results.

ε-L-lysine resin complexed with copper

As shown in Table 4,above pH 4 cephalosporin C was irreversibly bound to the support,indicating that the ternary complex involving ε-L-lysine,a copper ion,and cephalosporin C is very stable.
At pH 4,the difference between the capacity factor of cephalosporin C and those of the other solutes illustrates the great affinity and the good specificity which this support has for the antibiotic.

It also appears that this compound is more strongly retained than deacetylcephalosporin C and consequently that this support may be used for a good resolution of cephalosporin C from its contaminants.

TABLE 4

Capacity factors obtained on the glycyl-ε-L-lysine resin in acetate buffers.

* C = acetate concentration

**Solutes of both L and D configurations had identical k'values

***Peaks too wide for the elution volume to be determined.

Solutes**	k'				
	pH = 4			pH=6	pH=7
	C=0.25M	C=0.5M	C = 1M	C=0.5M	C=0.5M
Lysine	0	0	0	0.55	1
Glycine	0.75	0.65	0.65	0.75	1.65
Alanine	0.75	0.65	0.65	0.75	1.65
Methionine	2.15	1.85	1.40	2.10	2.60
Glutamic acid	***	2.25	1.40	3	3.85
γ-methyl glutamate	0.75	0.65	0.65	0.75	1.65
Aspartic acid	***	5.25	2	6.25	7.30
Phenylalanine	6.25	3.55	2.75	4.30	9
Cephalosporin C	31	16.50	5.70	∞	∞
Deacetyl-cephalosporin C	13	8.20	2.20	***	∞

On the other hand,for the first time neutral α-amino-acids(Ala,Gly) show an affinity towards the sorbent which is greater,if the solutes are hydrophobic(Phe,Met).The retention of such compounds is due to the participation of the α-amino acid extremities in the mixed copper complex as well as to hydrophobic interactions with the polymer matrix.

By comparing the capacity factor of phenylalanine obtained using acetate buffers at pH 4 in the 0.5-0.2 M concentration range one can determine the importance of hydrophobic interactions.Moreover, the difference between the affinity of glutamic acid and that of

γ-methyl glutamate shows that the presence of another carboxyl function in the structure of solutes increases the stability of the ternary complex.May be such a function could lie on the axis position of the bound copper complex.

In the case of cephalosporin C and according to these results we can assume that the antibiotic coordinates the copper ion as a tridentate ligand.Its α-amino-acid extremity may interact in the copper coordination plane and the amide function in an axial position,with an additional ring copper interaction increasing the stability of the complex(14,15).For structural reasons it seems that the carboxyl function of the dihydrothiazine ring is not able to participate in the mixed copper complex.

On the other hand,it is well known that ε-L-lysine is able to interact with a copper cation in its coordination plane only by means of its α-amino-acid extremity(16).Because of structural reasons,the interaction of two bound lysines to form a bis-copper complex is forbidden(17).Thus,the available coordinate bonds located in the coordination plane and the two axial ones may be occupied by solutes functions.Such a resin complex structure is quite consistent with our above assumptions concerning the coordination bonds of cephalosporin C with the bound lysine complex.

Separations

On the basis of difference in k' values,separations were carried out on a chromatographic system containing ε-L-lysine resin.The best results were obtained using an isocratic flow of 1 M acetate buffer at pH 4.Figure 6 shows a typical separation of cephalosporin C from a model mixture containing several α-amino acids and deacetylcephalosporin C. In this case,the antibiotic was obtained 99% pure,but it was collected in a large volume (40 ml),and was therefore very diluted. To cope with this difficulty,another technique was developed in which the acetate concentration of the buffer increased during the elution process;cephalosporin C was then collected in half the previous volume.
An example of such a separation is shown in Figure 7.

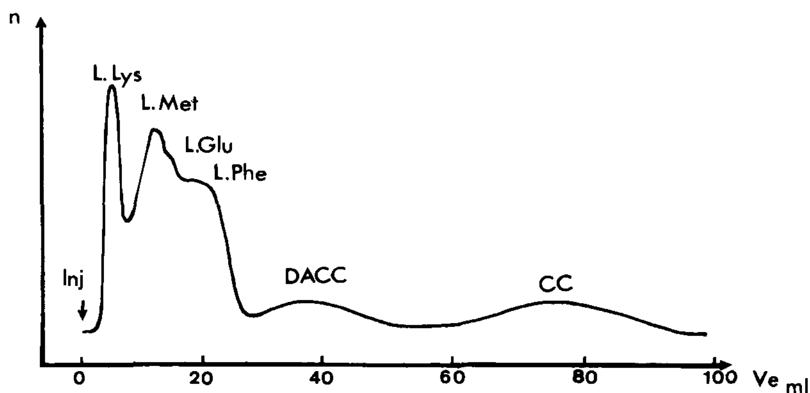


FIGURE 6 : Chromatogram of a mixture of cephalosporin C and various contaminants on the ε-L-lysine copper resin.
Refractometric detection(n)
Isocratic elution with a 1M acetate buffer ,pH 4.
Flow rate 1 ml per minute . Room temperature .
Cephalosporin C = CC, Deacetylcephalosporin C = DACC.

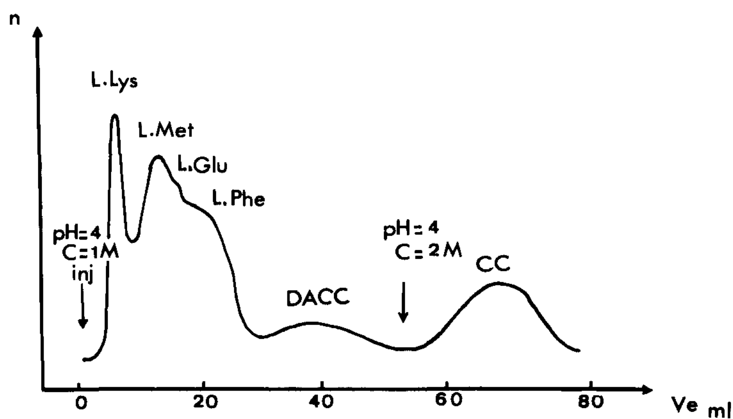


FIGURE 7 : Chromatogram of a mixture of cephalosporin C and various contaminants on the ε-L-lysine copper resin.
Elution with acetate buffers pH 4, 1M then 2M.
Refractometric detection(n) . Room temperature.
Flow rate 1 ml per minute.
CC = Cephalosporin C
DACC = Deacetylcephalosporin C.

CONCLUSION

Ligand exchange chromatography on a ε-L-lysine resin complexed with copper offers a new possibility to separate cephalosporin C from an artificial mixture of contaminants. This new sorbent has an affinity for the antibiotic which is not altered by the presence of inorganic salts(NaCl),in contrast to the results obtained using the uncomplexed ε-L-lysine resin. On the other hand,the copper glycyl-ε-lysine and copper diglycyl-ε-L-lysine ligands do not interact selectively with the antibiotic. These results can be explained by the structure of the copper complex on the stationary phases: the peptide bond present in the spacer arm can occupy some coordination bonds of the copper ion and prevent the solutes from interacting with the fixed complex. Application of the copper ε-L-lysine to a large scale separation of cephalosporin C is at present under investigation.

ACKNOWLEDGEMENTS

The authors thank Professor J.NEEL for many helpful discussions and Mrs.Th. GEOFFROY for the excellent technical assistance.

REFERENCES

1) Ciba S.A.,Brevet Fr.750,292,1970.
2) Voser W.,U.S.Pat.3,725,400,1973.
3) O'Connor S.C.,Methods Enzymol.,43,296,1975.
4) Mc.Cornick S.L.,Mack.H.,U.S.Pat.3,467,654,1969.
5) Sacco D.,Dellacherie E.,J.Chromatogr.,211,79,1981.
6) Sacco D.,Dellacherie E.,Makromol.Chem.,182,763,1981.
7) Kirkland J.J.,"Chromatographie en phase liquide" Ed.Gauthier-Villars,PARIS,1972.
8) Davankov V.A.,Semechkin A.V.,J.Chromatogr.,141,313,1977.
9) Gübitz G.,Jellenz W.,Santi V.,J.Liq.Chromatogr.,4,701,1981.
10) Kurganov A.A.,Zhuchkova L.Y.,Davankov V.A.,Makromol.Chem. 180,2101,1979.

11) Morin R.B.,Jackson B.G.,Forschritte Chem.Org. Naturst. 28,343,1970.
12) Boué J.,Audebert R.,Quivoron C.,J.Chromatogr.,204 ,185,1981.
13) Foucault A.,Caude M.,Oliveros L.,J.Chromatogr.,185,345,1979.
14) Muller D.,Jozefonvicz J.,Petit M.A.,J.Inorg.Nucl.Chem.,42, 1083,1980.
15) Brookes G.,Pettit L.D.,J.Chem.Soc.Dalton,1918,1977.
16) Greenstein J.P.,Winitz M.,"Chemistry of the amino acids"Vol.1 Ed.John Wiley and Sons,New York,1961.
17) Herring R.,Trenne K.,Neske P.,J.Prakt.Chem.32,291,1966.

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2557-2564 (1983)

A RAPID HPLC ANALYSIS OF DIAZEPAM IN ANIMAL FEED

A.P. Pakuts, R.H. Downie and T.I. Matula
Bureau of Drug Research
Health Protection Branch
Tunney's Pasture, Ottawa, Ontario
Canada, K1A 0L2

ABSTRACT

A rapid HPLC analysis is described for the methanolic extraction of diazepam from spiked animal feed. A short disposable reverse-phase extraction column is employed to remove interfering substances present in the animal feed. This is followed by direct injection of an aliquot into an analytical ODS column. The precision is better than 3.9% and the % recovery is 87.8 ± 0.8% resulting in a convenient, rapid method for the routine analysis of added diazepam in prepared animal diets.

INTRODUCTION

In a chronic diazepam spiked feed study, a method was needed to measure the concentration of diazepam fed to the experimental animals. An extensive literature search failed to produce a published method suitable for analysis of diazepam in feed. Excellent review articles by Tsuji (1) and Hailey (2) listed numerous spectrophotometric and GLC methods, developed to meet different analytical requirements. An attempt was made to modify the method of de Silva, Koechlin and Baker (3) but this classical organic extraction technique required considerable clean-up and had low recovery. HPLC methods using bonded phases (4,5) and UV detection have been used to measure diazepam and its metabolites in biological fluids. These methods employed an organic extraction, evaporation and reconstitution steps prior to injection into an analytical

Copyright © 1983 by Marcel Dekker, Inc. 0148-3919/83/0613-2557$3.50/0

reverse-phase column. The use of disposable, reverse-phase, extraction columns (6,7) has offered speed, higher recoveries and superior resolution in the clean-up of biological fluids than the classical organic extraction methods. The benzodiazepines were selectively adsorbed into Bond Elut, C_{18}, disposable columns, from serum at pH = 9.0. Methanol was used to elute the benzodiazepines from the column, followed by injection into the analytical column. In our method, the diazepam in the feed was extracted directly into methanol, then the methanolic extract was passed through a "Baker", disposable extraction column, which retained the interfering, UV absorbing peaks present in the feed. The methanol eluent was then injected into the chromatographic system. Methanol was compatible with the mobile phase, thus eliminating time consuming evaporation and reconstitution, resulting in a rapid, convenient, highly reproducible analysis of diazepam in animal feed.

MATERIALS

Chemicals

Diazepam and ^{14}C-diazepam, labelled in the 5-position with a specific activity of 197 µCi/mg were obtained from Hoffman-LaRoche Limited (Vaudreuil, Quebec, Canada). The ODS bonded (1.0 ml) "Baker" disposable extraction columns, HPLC grade acetonitrile and methanol were purchased from the J.T. Baker Chemical Co. (Phillipsburg, N.J., U.S.A.). Atomlight L.S.C. cocktail was bought from New England Nuclear (Boston, Mass., U.S.A.). The animal feed used for the chronic animal experiments was Master Laboratory Mash, purchased from Ritchie Feed and Seed Ltd. (Ottawa, Ont., Canada).

METHODS

HPLC Analysis

A Varian Aerograph Series 4100 liquid chromatography system (Walnut Creek, CA, U.S.A.) was used to deliver the mobile phase (70% acetonitrile/30% H_2O), isocratically at a flow rate of 60 ml/hr, at ambient temperature. A six-port Valco injection valve with a 40 µl fixed

loop was used to introduce all the samples. Guard columns (5 cm Brownlee Labs RP-8) from Technical Marketing Associates Limited (Ottawa, Ont., Canada) were used to protect the 25 cm x 4.5 mm Dupont Zorbax ODS column purchased from Fisher Scientific Company (Ottawa, Ont., Canada). The effluent was monitored at 242 nm with a variable wavelength UV spectrophotometer. A strip chart recorder (Linear Instruments Corp., Irvine, CA, U.S.A.) was used to record the chromatographs. Peak heights were used to quantify diazepam concentrations.

Standard Solutions and Feed Standards

All diazepam standard solutions were made by serial dilution of freshly prepared 125 mg diazepam/100 ml methanol. Forty µl aliquots of these standard solutions were introduced into the HPLC system to produce the standard curve shown in Table 1.

For the preparation of spiked feed samples, diazepam in 5.0, 3.75, 2.5, 1.25 mg amounts was dissolved per g of corn oil and mixed with 25 g ground animal feed in a Janke & Kunkel KG blender (Johns Scientific, Toronto, Ont., Canada). This procedure simulated the actual feed-diazepam preparation used for the animal feeding study. The final corn oil content of each spiked sample was 4% w/w (corn oil/feed). These feed standards generated the data in Table 2.

General Procedure

Triplicate 300 mg samples of animal feed standards or samples with unknown diazepam content were placed in vials with teflon-lined screw caps. Each sample was shaken with 6.0 ml of methanol (1:20 w/v) on a Burrel Wrist Action Shaker (Burrell Corporation, Pittsburgh, PA, U.S.A.) for 15 minutes in a single extraction. The feed mixture was then centrifuged for 10 minutes at 2000 g in a Clinical Centrifuge (Damon/IEC, Needham Hts., Mass., U.S.A.). One ml of the clear supernatant from each sample was placed on a "Baker" disposable extraction column, which was first pre-conditioned with methanol and then with water. The 1.0 ml sample was completely pushed through with a gentle pressure of nitrogen.

TABLE 1

Precision Data of Diazepam Standard Solutions (n=9)

Amount Injected ng	Amount Found $\bar{X}$ ng	± S.D.	% C.V.
25	25	1.4	5.5
50	49	0.9	1.9
100	100	1.2	1.2
125	127	0.6	0.5
250	253	2.0	0.8
400	394	1.8	0.5
500	504	2.4	0.5
		Mean =	1.6

TABLE 2

Precision Data of Diazepam Spiked Feed Standards
n = 9 except *(n = 6)

Amount Diazepam Added mg/25 g Feed	Amount Diazepam Found mg/25 g Feed $\bar{X}$ mg	± S.D.	% C.V.
1.25	1.27	0.50	3.9
2.50	2.47	0.06	2.4
*3.75	3.72	0.06	1.6
5.00	5.02	0.11	2.2
		Mean =	2.5

The column was then rinsed with 100 µl methanol and the combined effluent was analysed by injecting a 40 µl aliquot on the analytical ODS column.

^{14}C% Recovery

^{14}C-diazepam was diluted with cold diazepam in 1 g of corn oil to give a specific activity of 7.5 µCi/5.0 mg of diazepam. This solution was

then mixed in a blender with 25 g of ground feed. This concentration of drug corresponded to the highest spiked feed standard (5 mg/25 g). Ten 300 mg samples of radioactive feed were weighed out, extracted, cleaned-up and each total eluent was collected in a glass vial, to which 15 ml of Atomlight liquid scintillation fluid was then added. A 1.0 ml aliquot of the 6.0 ml methanolic feed extract prior to its passage through the "Baker" disposable extraction column was also sampled to establish the efficiency of only the methanol extraction step.

All samples were subsequently counted on a Beckman LS 8100 liquid scintillation counter equipped with automatic quench correction.

RESULTS

The data shown in Tables 1, 2 were analysed by linear regression. The standard errors of the means at each concentration ranged from ±0.02 to ±0.80. Table 1 represents the diazepam standards in methanol injected directly into the chromatographic system. Table 2 shows the diazepam spiked feed standards. Both plots were highly linear with a correlation coefficient of 0.999 and had respective slopes ±S.E. of 0.3929 ± .003 and 0.3637 ± .005 with negligible intercepts in both cases.

The precision data (% C.V.) for the diazepam standard solutions injected directly into the mobile phase averaged 1.6% (Table 1). The average % C.V. of the spiked feed standards was 2.5% with a very low C.V. of 3.9% at the lowest concentration tried (Table 2). These data were used to calculate the concentration of diazepam in the feed during the chronic feeding experiment. These results indicate that this analysis of diazepam in feed is highly reproducible.

Some of the actual determinations of diazepam in the feed used during the chronic animal feeding experiment are shown in Table 3.

The recovery of the diazepam during the extraction procedure was determined with radio-labelled diazepam. The recovery of the initial methanolic extraction step alone was 96.9% and the total recovery for 10 samples was 87.8 ± 0.8%. This recovery compares well with the 88% to 95% values for diazepam extracted from plasma in HPLC analysis (6,7).

TABLE 3

Determination of "Unknown" Diazepam Spiked Feed Samples

(A) Actual Diazepam Found mg/25 g Feed	(E) Expected Concentration mg/25 g Feed	% A/E
0.45	0.45	100%
0.46	0.40	115%
2.34	2.50	94%
4.36	4.32	101%
37.80	40.00	95%
44.30	45.80	97%
	Mean =	100%

The chromatograms A,B (Figure 1) show that the clean-up procedure removes all interfering peaks from the complex feed mixture and that diazepam travels as a sharp band with a retention time of 7.8 min. The capacity factor k' was calculated to be 2.8.

DISCUSSION

Although methanol is not the solvent of choice for a lipophilic compound such as diazepam it was tried because a) methanol could be injected directly into a reverse-phase clean-up system without evaporation and re-constitution and b) because the feed did not float in methanol thereby producing a clear supernatant on centrifugation. Ethyl ether was tried but it is incompatible with a reverse-phase solvent system, and it produced a cloudy supernatant. Other methods (8) have used hot chloroform in a forward phase system but we found many more interfering peaks were extracted. Also the buoyancy of the feed in chloroform made it difficult to sample. The high recovery (96.9%) with methanol and its compatibility with reverse-phase chromatography made it an ideal solvent for extraction of diazepam from ground animal feed. Although acetonitrile is similar to methanol in polarity it failed to extract diazepam from feed.

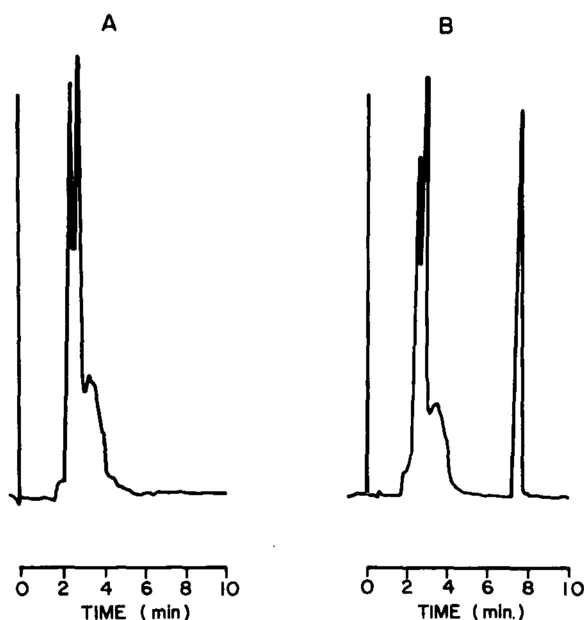


FIGURE 1. HPLC analysis of control feed (A), and diazepam-spiked feed (B), 3.75 mg/25 g, methanol extracts run in 70% acetonitrile/H_2O, at 60 ml/hr., attenuation 0.200 AUFS, at 242 nm.

The methanolic feed extract contained large peaks which masked the diazepam peak. Running the methanol extract through a short "Baker" disposable extraction column resulted in a clean, sharp, diazepam peak. The disposable clean up column retains most of the large, broad, interfering peaks, letting the diazepam through in the eluent. The methanolic extract when placed directly into a Dupont Zorbax column with a 70% acetonitrile/water mobile phase produced no chromatographic distortion. All our work reported was done on this column. Changing the guard column always restored the column to its original capabilities. The solvent system, 70% acetonitrile/30% H_2O, produced a very sharp peak which widened if we substituted 70% methanol.

This analytical procedure has proven to be a rapid, reliable and convenient method for monitoring diazepam in spiked animal feed. It is a method that could be readily adapted to the analysis of other drugs or contaminants in feed preparations.

REFERENCES

1. Tsuji, K., GLC and HPLC Determination of Therapeutic Agents, part 2, Marcel Dekker Inc., 1978.

2. Hailey, D.M., Chromatography of 1,4-Benzodiazepines, J. Chromatog., 98, 527, 1974.

3. de Silva, J.A., Koechlin, B.A., and Bader, G., Blood Level Distribution Patterns of Diazepam and its Major Metabolite in Man, J. Pharm. Sci., 55, 692, 1966.

4. Brodie, R.R., Chasseaud, L.F., and Taylor, T., High-Performance Liquid Chromatographic Determination of Benzodiazepines in Human Plasma, Journal of Chromatog., 150, 361, 1978.

5. Cotler, S., Puglisi, C.V., and Gustafson, J.H., Determination of Diazepam and its Major Metabolites in Man and in the Cat by High-Performance Liquid Chromatography, J. Chromatog., 222, 95, 1981.

6. Srininas N. Rao, Dhar, A.K., Kutt, H., and Okamoto, M., Determination of Diazepam and its Pharmacologically Active Metabolites in Blood by Bond Elut™ Column Extraction and Reversed-Phase High-Performance Chromatography, J. Chromatog., 231, 341, 1982.

7. Good, J.T., and Andrews, S.J., The Use of Bonded-Phase Extraction Columns for Rapid Sample Preparation of Benzodiazepines and Meta bolites from Serum for HPLC Analysis, J. Chromatog. Sci., 19, 562, 1981.

8. Hurst, W.J., and Martin, R.A., Jr., The HPLC Analysis of Caffeine and Theobromine in Animal Diets, J. Liquid Chromatog., 5, 585, 1982.

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2565-2570 (1983)

DEGRADATION OF HIGH PURITY WATER ON STORAGE

Raymond Gabler*, Ramesh Hegde#, and David Hughes#

*Biotechnology Division
#Analytical Services
Millipore Corporation
Ashby Road
Bedford, Massachusetts 01730

ABSTRACT

Experimental results are presented which indicate that the quality of high purity water degrades with storage time. HPLC trace enrichment techniques were used to monitor the amount of organics in water stored in various types of containers. Starting with initial, high purity water, it was shown that organics could be detected in as little as one hour after storage in plastic containers. Organics could also be detected if the water was stored in glass.

INTRODUCTION

The purpose of this note is to authenticate the fact that the quality of high purity water degrades with storage; that this degradation can be detected within hours; that the degradation generally increases with time, and that the storage container can influence the rate of degradation. This work illustrates the little realized fact that high quality water is a very powerful solvent, and that high quality water can leach materials of construction out of storage containers.

As techniques for analysis in general become more sophisticated and sensitive, the quality of the reagents used for diluting, for controls, for blanks, for standards, etc. becomes increasingly more critical, and in some cases may be the limiting factor in an analytical procedure (1). Impurities found in reagents are particularly important in those analytical procedures where trace concentrations are being measured.

Copyright © 1983 by Marcel Dekker, Inc.

0148-3919/83/0613-2565$3.50/0

Water has always been the most ubiquitous reagent in many types of analyses, and this is especially so in measurements performed on biological systems. Water quality is also a dominating factor in biological work because water is a universal solvent for biomolecules and cells where impurities can affect enzymatic activities, bacterial, mammalian and plant cell growth. The fact that water quality is important in general analytical and biochemical measurements is noted by the water quality standards that have been established by a number of organizations (ACS, ASTM, NCCLS, CAP). Systems are commercially available which can produce high purity water; however, storing this water for future use results in a degradation in the original quality that has not been readily documented.

EXPERIMENTAL

The design of our work was to simulate common laboratory practices that are routinely used to store water, and to monitor the general organic quality of this water as a function of time upon storage. No arduous or extraordinary measures were taken to preserve the quality of the initially pure water other than those which would normally be practiced in a typical biochemical or biological laboratory.

Water quality was measured qualitatively via HPLC using a trace enrichment technique (2). In this procedure, 40ml of test water is loaded into a reverse phase C18 column (Waters Associates) at 2ml/min. A linear gradient of 100% water to 100% acetonitrile over 20 minutes is then run to elute organics adsorbed to the column. The initial high purity water used was produced from a Milli-Q[R] system with an Organex-Q[R] cartridge (Millipore Corporation). Water from this system has been shown to be low in organics content and suitable for LC work(1).

The following materials were tested for storage containers: a 4 liter polyethylene bottle (Scientific Products, B-75-10-128); a 4 liter polypropylene bottle (B-75-13-128), and a 4 liter brown glass bottle (J. T. Baker) that is normally used for shipping LC grade water. In order to prevent inadvertent contamination due to handling, a Teflon[R] tube was inserted through the closed tops of the respective containers so the water could be introduced to the LC columns with little or

no extra handling or manipulation. Each container was initially rinsed thoroughly with water followed by rinsings with the Milli-Q^R water. Milli-Q^R with the Organex-Q^R cartridge water was also used as the mobile phase in all LC runs. Acetonitrile was LC grade (Burdick and Jackson). Detection of organics eluted by the gradient was performed using a UV detector at both 254nm and also 214nm (Waters Associates). Water was stored and measurements were taken at various time intervals for four weeks.

After the initial storage test was completed, the test water was removed, the container recleaned, and a new test cycle was restarted lasting one week. The purpose of this second trial was to determine if the first load of high purity water and storage for four weeks had exhausted all of the leachable material from the containers, or whether more material could be leached by a fresh batch of water. It is common in biochemical laboratories to "condition" storage containers by exhaustingly rinsing any leachates out of plastic containers for several weeks.

RESULTS

The results of the storage experiments as a function of time for polyethylene, polypropylene and glass containers are shown in Figures 1, 2, and 3 respectively. Chart speed was 0.60cm/minute and AUFS is 0.02. The lower most LC trace in each set is for the Milli-Q^R water that was initially placed in all these containers. A similar pattern was obtained for the Milli-Q^R water used as the mobile phase each time as a sample was tested. For both the polyethylene and the polypropylene containers, one can notice a degradation in water quality beginning in as short a time as one hour. Each peak represents presumably one organic species that is being leached from the plastic containers. No effort was made to determine the identification of what organics these various peaks represented. The LC traces for one day, one week and two weeks are similar to that of one hour except that certain peaks are intensified, and the general trend is seen that the amount of contaminants detected increases with time. The LC traces for the water stored in the glass containers show the same general character as for the water in the plastic containers. The peaks, however, are not as pronounced, nor do they appear as rapidly, compared to the corresponding plastic storage systems.

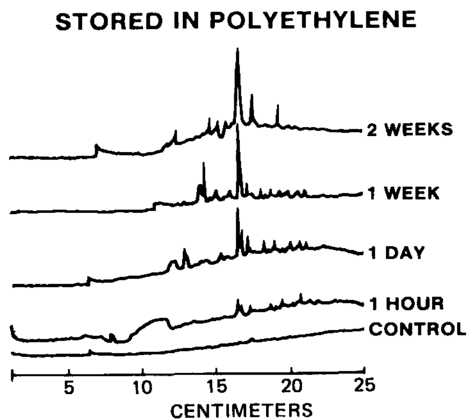


Figure 1: Trace enrichment chromatograms of high purity water stored in a polyethylene container.

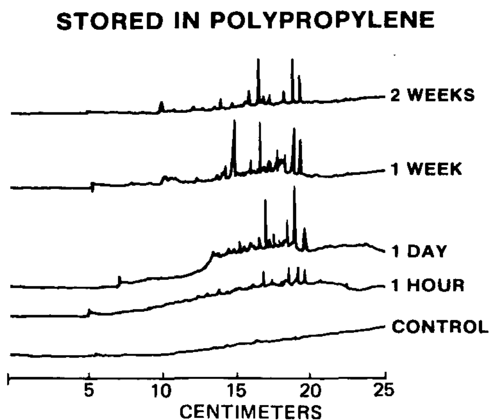


Figure 2: Trace enrichment chromatograms of high purity water stored in a polypropylene container.

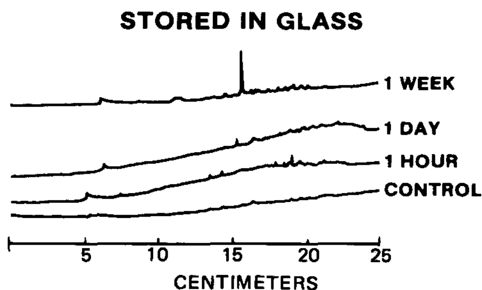


Figure 3: Trace enrichment chromatograms of high purity water stored in a glass container.

LC traces at 214nm showed similar trends with time as those at 254nm. Also, the results of the second trial with fresh, high purity water followed a pattern similar to the first trial.

DISCUSSION

Healy (3) showed that doubly distilled water could leach inorganic ions out of Pyrex[R] containers during a storage period of two weeks. Our study has illustrated a corresponding situation for organics in plastic containers. Upon storage, the general quality of initially pure water degrades as measured by the increase in organic constituents.

Although the relative contributions are unknown at this time, there are basically three sources for the organics detected in the LC traces.

1. Plasticizers, monomers, mold release agents and the oligomers used in the construction and manufacture of any container can be leached out by the water.
2. Bacteria can grow in the stored water and excrete waste products, thus contributing to the general organics (4).
3. Organics from other reagents, laboratory personnel's respiration, and shedding from clothing can all diffuse through the air and dissolve in the water.

It is quite possible that a succession of these mechanisms occur as a function of time.

Water quality can play an influential role in analytical LC measurements of biochemical components (5), and also in the growth of tissue culture systems. Even if high purity water is initially produced, that same water quality may not exist a short time later. For those analytical and biological systems that are extremely sensitive to water quality, care should be taken not to store the water, but to use the water as it is produced.

REFERENCES

1. Reust, J. B., Meyer, V. R., Organic Contaminants in Ultrapure Water: Determination by Reversed-phase HPLC and UV-detection, The Analyst, 107, 673, 1982.

2. Bristol, D. W., Detection of Trace Organic Impurities in Binary Solvent Systems: A Solvent Purity Test, J.of Chromatography, 188, 193, 1980.

3. Healy, G. M., Morgan, J. F., and Parker, R., Trace Metal Content of Some Natural and Synthetic Media, J. Biol. Chem., 198, 305, 1952.

4. Favero, M. S., Carson, L. A., Bond, W. W., and Petersen, H. J., Pseudomonas Aeruginosa: Growth in Distilled Water from Hospitals, Science, 173, 836, 1971.

5. Sampson, R. L., High Purity Water for Liquid Chromatography, American Laboratory, May, 1977.

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2571 (1983)

LETTER TO THE EDITOR

We feel that a correction is necessary regarding results attributed to us in a citation in the recent paper by Akintonwa et al., [J. Liquid Chromatogr. 6 (1983) 1513-1522]. The sentence on page 1521, lines 4-7 reads: "Further, during chronic daily administration of 300 mg. of chloroquine base, blood chloroquine concentrations ranged from approximately 150-500 ng/ml [3]."

In actuality, as clearly pointed out in our article (Staiger et al., 1981), administration was weekly, dosing was with chloroquine diphosphate (300 mg. expressed as base), and the values provided by this earlier, less selective method of ours represented not chloroquine alone, but a slight underestimation of the sum of chloroquine and desethylchloroquine in whole blood. Incidentally, we have subsequently published two methods which quantify chloroquine and desethylchloroquine separately (Churchill et al., 1983 and Patchen et al., 1983).

Frederick C. Churchill
Scientist Director
Control Technology Branch
Division of Parasitic Diseases
Center for Infectious Diseases

Staiger, M.A., Nguyen-Dinh, P., Churchill, F.C. "Sensitive HPLC Analysis for Chloroquine in Body Fluids: Application to Studies of Drug Resistance in Plasmodium Falciparum" (Analytical Division, 181st National Meeting of the American Chemical Society, Atlanta, Ga., April 2, 1981, Abstract No. 169) J. Chromatogr., 225 (1981) 139-149.

Churchill, F.C. II, Mount, D.L., Schwartz, I.K., "Determination of Chloroquine and Its Major Metabolite in Blood Using Perfluoroacylation Followed by Fused-Silica Capillary Gas Chromatography with Nitrogen-Sensitive Detection," J. Chromatogr., 274 (1983) 111-120.

Patchen, L.C., Mount, D.L., Schwartz, I.K., Churchill, F.C., "Analysis of Filter-Paper-Absorbed, Finger-Stick Blood Samples for Chloroquine and Its Major Metabolite Using HPLC with Fluorescence Detection" J. Chromatogr. (1983) in press.

Copyright © 1983 by Marcel Dekker, Inc.

0148-3919/83/0613-2571$3.50/0

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2573-2575 (1983)

LC CALENDAR

1983

NOVEMBER 3-4 ACS 18th Midwest Regional Meeting, Lawrence, Kansas. Contact: W. Grindstaff, SW Missouri State Univ., Springfield, MO, 65802, USA.

NOVEMBER 9-11: ACS 34th SE Regional Meeting, Charlotte, NC. Contact: J. M. Fredericksen, Chem. Dept., Davidson College, Davidson, NC, 28036, USA.

NOVEMBER 10-11: Electrofocusing and Electrophoresis Workshop, Birmingham, AL, USA. Contact: Workshop Registrar, LKB Instruments, Inc., 9319 Gaither Rd., Gaithersburg, MD, 20877, USA.

NOVEMBER 14-16: 3rd Int'l. Sympos. on HPLC of Proteins, Peptides and Polynucleotides, Monte Carlo, Monaco. Contact: S. E. Schlessinger, 400 East Randolph, Chicago, IL, 60601, USA.

NOVEMBER 16-18: Eastern Analytical Symposium, New York Statler Hotel, New York City. Contact: S. David Klein, Merck & Co., P. O. Box 2000, Rahway, NJ, 07065, USA.

NOVEMBER 22-23: Short Course: "Sample Handling in Liquid Chromatography," sponsored by the Int'l. Assoc. of Environmental and Biological Samples in Chromatography, Palais de Beaulieu, Lausanne, Switzerland. Contact: Dr. A. Donzel, Workshop Office, Case Postale 130, CH-1000 Lausanne 20, Switzerland.

NOVEMBER 24-25: Workshop: "Handling of Environmental and Biological Samples in Chromatography," sponsored by the Int'l. Assoc. of Environmental Anal. Chem., Palais de Beaulieu, Lausanne, Switzerland. Contact: Dr. A. Donzel, Workshop Office, Case Postale 130, CH-1000 Lausanne 20, Switzerland.

NOVEMBER 29-30: Electrofocusing and Electrophoresis Workshop, San Francisco, CA, USA. Contact: Workshop Registrar, LKB Instruments, Inc., 9319 Gaither Road, Gaithersburg, MD, 20877, USA.

DECEMBER 6-7 and 8-9: Electrofocusing and Electrophoresis Workshop, Los Angeles, CA, USA. Contact: Workshop Registrar, LKB Instruments, Inc., 9319 Gaither Road, Gaithersburg, MD, 20877, USA.

1984

FEBRUARY 12-16: 14th Australian Polymer Symposium, Old Ballarat Travel Inn, Ballarat, Australia, sponsored by the Polymer Div., Royal Australian Chemical Inst. Contact: Dr. G. B. Guise, RACI Polymer Div., P. O. Box 224, Belmont, Victoria 3216, Australia.

FEBRUARY 20-22: International Symposium on HPLC in the Biological Sciences, Regent Hotel, Melbourne, Australia. Contact: The Secretary, Int'l Symposium on HPLC in the Biological Sciences, St. Vincent's School of Medical Research, 41 Victoria Parade, Fitzroy 3065, Victoria, Australia.

APRIL 8-13: National ACS Meeting, St. Louis, MO. Contact: Meetings, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

MAY 20 - 26: 8th Intl. Symposium on Column Liquid Chromatography, New York Statler Hotel, New York City. Contact: Prof. Cs. Horvath, Yale University, Dept. of Chem. Eng., P. O. Box 2159, Yale Stn., New Haven, CT, 06520, USA.

JUNE 18-21: Symposium on Liquid Chromatography in the Biological Sciences, Ronneby, Sweden, sponsored by The Swedish Academy of Pharmaceutical Sciences. Contact: Swedish Academy of Pharmaceutical Sciences, P. O. Box 1136, S-111 81 Stockholm, Sweden.

AUGUST 26-31: National ACS Meeting, Philadelphia, PA. Contact: Meetings, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

OCTOBER 1-5: 15th Int'l. Sympos. on Chromatography, Nurenberg, West Germany. Contact: K. Begitt, Ges. Deutscher Chemiker, Postfach 90 04 40, D-6000 Frankfurt Main, West Germany.

DECEMBER 16-21: International Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, sponsored by the Chemical Inst. of Canada, Chemical Soc. of Japan, and the American Chem. Soc. Contact: PAC CHEM '84, International Activities Office, American Chem. Soc., 1155 Sixteenth St., NW, Washington, DC, 20036, USA.

1985

FEBRUARY 11-14: Polymer 85, Int'l Symposium on Characterization and Analysis of Polymers, Monash University, Melbourne, Australia,

sponsored by the Polymer Div., Royal Australian Chemical Inst. Contact: Polymer 85, RACI, 191 Royal Parade, Parkville Victoria 3052, Australia.

APRIL 28 - MAY 3: 189th National ACS Meeting, Miami Beach. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

SEPTEMBER 8-13: 190th National ACS Meeting, Chicago. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA

1986

APRIL 6-11: 191st National Am. Chem. Soc. Mtng., Atlantic City, NJ. Contact: A. T. Winstead, ACS, 1155 16th Streeet, NW, Washington, DC, 20036, USA.

SEPTEMBER 7-12: 192nd National Am. Chem. Soc. Mtng., Anaheim, Calif. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA

1987

APRIL 5-10: 193rd National Am. Chem. Soc. Mtng., Denver, Colo. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

AUGUST 30 - SEPTEMBER 4: 194th National Am. Chem. Soc. Mtng., New Orleans, LA. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

The Journal of Liquid Chromatography will publish announcements of interest to liquid chromatographers in every issue of the Journal. To be listed in the LC Calendar, we will need to know: Name of the meeting or symposium, sponsoring organization, when and where it will be held, and whom to contact for additional details. You are invited to send announcements to Dr. Jack Cazes, Editor, Journal of Liquid Chromatography, P. O. Box 1440-SMS, Fairfield, CT, 06430, USA.

LIQUID CHROMATOGRAPHY DIRECTORY

A Comprehensive Listing of Suppliers of LC Equipment

This is a special section of *Journal of Liquid Chromatography*, Volume 6, Number 14, 1983

MARCEL DEKKER, INC. New York and Basel

JOURNAL OF LIQUID CHROMATOGRAPHY, 6(13), 2579-2619 (1983)

LC DIRECTORY

The LC DIRECTORY has been assembled to help LC users and researchers in locating products used in the LC laboratory. Every effort has been made to list all manufacturers of LC equipment. The data contained in the LC DIRECTORY was provided directly by the manufacturers and is believed to be accurate. Where available, multiple addresses for a manufacturer are given. It is intended that the LC DIRECTORY will be truly an international listing. The Journal of Liquid Chromatography will update the LC DIRECTORY on an on-going basis and will publish an updated version annually.

Suppliers and manufacturers of LC equipment are listed alphabetically in the Manufacturers Section. For each company, a list of codes for its products is given. This section should therefore be used to determine which products a given company offers.

To locate all of the companies that supply a given product, use the Products Section. Products are listed alphabetically in this section.

Manufacturers who are not yet included in the LC DIRECTORY are invited to submit their product information for inclusion in the next edition. Comments and suggestions from users of the LC DIRECTORY are invited. Send all correspondence to The Editor, LC DIRECTORY, Journal of Liquid Chromatography, P.O.Box 1440-SMS, Fairfield, CT, 06430, USA.

NUMERICAL PRODUCT INDEX

A1 LC Instruments, Complete
A2 Injection Systems, Manual
A3 Refractometric Detectors
A4 Infrared Detectors
A5 UV/Visible Detectors
A6 Electrochemical Detectors
A7 Radiation Detectors
A8 Fluorescence Detectors
A9 Light-Scattering Detectors
B1 Chemicals, Standards, Reagents
B2 Solvents, Eluents
B3 Column Packing Devices
B4 Water Purifiers
B5 Solvent Del. Syst., Pumps
B6 Injection Systems, Automatic
B7 Sample Prep'n Equipment
B8 Fraction Collectors
B9 Programmers, Controllers
C1 Valves, Tubing, Fittings
C2 Packed Columns

Copyright © 1983 by Marcel Dekker, Inc.

0148-3919/83/0613-2579$3.50/0

C3 Column Packing Materials	C4 Data Systems, Computers
C5 Guard- & Pre-Columns	C6 Recorders, Plotters
C7 Column Ovens, Heaters	C8 LC/MS Interfaces
C9 Derivatization Devices	D1 TLC Plates, Anal. & HPTLC
D2 TLC Adsorbents, Media	D3 TLC Spotters, Streakers
D4 TLC Quantitation Devices	D5 TLC Plates, Preparative
D6 TLC Spreaders	D7 TLC Developing Chambers
D8 TLC Visualization Reagents	D9 TLC Visualization Devices
E1 Countercurrent Chrom. Equipt.	E2 Open-Column LC Equipment
E3 Paper Chromatography Equipt.	F1 Mass Detectors
F2 Conductivity Detectors	F3 Optical Filters
F4 Polarimetric Detectors	F5 Industrial-Scale LC Equipt.
F6 Dielectric Constant Detectors	Z1 Contract Laboratories

ALPHABETICAL PRODUCT INDEX

Chemicals - B1
Column Ovens - C7
Column Packing Materials - C3
Complete LC Instruments - A1
Contract Laboratories - Z1
Countercurrent Chrom. Equipt.- E1
Derivatization Devices - C9
Detectors, Dielectric Const.- F6
Detectors, Fluorescence - A8
Detectors, Light-Scattering - A9
Detectors, Polarimetric - F4
Detectors, Refractometric - A3
Eluents - B2
Fittings - C1
Guard Columns - C5
Injection Systems, Automatic - B6
LC/MS Interfaces - C8
Packings, Column - C3
Plotters - C6
Programmers - B9
Reagents - B1
Sample Prep'n Equipt.- B7
Solvents, Eluents - B2
TLC Adsorbents, Media - D2
TLC Plates, Anal. & HPTLC - D1
TLC Quantitation Devices - D4
TLC Spreaders - D6
TLC Visualization Reagents - D8
Valves - C1
Column Heaters - C7
Column Packing Devices - B3
Columns, Packed - C2
Computers - C4
Controllers - B9
Data Systems - C4
Detectors, Conductivity - F2
Detectors, Electrochemical - A6
Detectors, Infrared - A4
Detectors, Mass - F1
Detectors, Radiation - A7
Detectors, UV/Visible - A5
Filters, Optical - F3
Fraction Collectors - B8
Industrial-Scale LC Equipt.- F5
Injection Systems, Manual - A2
Open-Column LC Equipt.- E2
Paper Chromatography Equipt.- E3
Pre-Columns - C5
Pumps - B5
Recorders - C6
Solvent Delivery Systems - B5
Standards - B1
TLC Developing Chambers - D7
TLC Plates, Preparative - D5
TLC Spotters, Streakers - D3
TLC Visualization Devices - D9
Tubing - C1
Water Purifiers - B4

MANUFACTURERS SECTION

A. S. I. Suite B-24 1400 Coleman Ave.
Santa Clara, CA 95050 USA
408/779-1901
B3 C1 C2 C3 C5

ABC Laboratories P. O. Box 1097
Columbia, MO 65205 USA
314/474-8579
C2 C3

Accuspec Corp. 347 Fifth Avenue Suite 508
New York, NY 10016 USA
212/696-5550
A4 A5

Ace Glass, Inc. P. O. Box 688 1430 NW Blvd.
Vineland, NJ 08360 USA
609/692-3333
C2 E2

Ace Scientific Supply Co. 40A Cotters Lane
East Brunswick, NJ 08816 USA
201/254-0400
B1 B2 B3 B5 B6 B7 C1 C2 C3 C4 C5 C6 C7 C8 D1 D2 D5 D7 D8

Alltech Associates UK Ltd. 32, New Street Carnforth
Lancashire LA5 9BX United Kingdom
0524-734451
A2 A5 B1 B2 B3 B5 B7 B9 C1 C2 C3 C5 C6 C7 C9 D1 D2 D5 D7

Alltech Associates, Inc. Applied Science Labs 2051 Waukegan Road
Deerfield, IL 60015 USA
312/948-8600
A2 B1 B2 B3 B4 B5 B6 B9 C1 C2 C3 C4 C5 C6 C7 D1 D2 D3 D5 D6 D7 D8

Alpha Chem. & Biomed. Labs Park Plaza Profess. Center 245 Kentucky St.
Petaluma, CA 94952 USA
707/778-8607
Z1

American Scientific Products 1430 Waukegan Road
McGaw Park, IL 60085 USA
312/689-8410
A2 A3 A4 A5 A8 B1 B2 B3 B4 B5 B6 B7 B8 B9 C2 C3 C4 C5 C6 C7 C8 C9 D1 D2

American Type Culture Coll. 12301 Parklawn Drive
Rockville, MD 20852 USA
301/881-2600
B1

Amicon Corp. Scientific Systems Div. 17 Cherry Hill Drive
Danvers, MA 01923 USA
617/777-3622
B7 C2 C5

Analtech, Inc. 75 Blue Hen Drive P. O. Box 7558
Newark, DE 19711 USA
302/737-6960 or 800/441-7540
C3 D1 D2 D3 D5 D7

Analytichem Internat'l 24201 Frampton Ave.
Harbor City, CA 90710 USA
213/539-6490 or 800/421-2825
B6 B7 C2 C3 C5 C9 D3

Anspec Company P. O. Box 7730
Ann Arbor, MI 48107 USA
313/665-9666
A1 A2 A3 A4 A5 A6 A7 A8 B5 B6 B8 B9 C2 C3 C4 C8 D1 D2 D5 D6 E1 E2

Antibodies, Inc. P. O. Box 1560
Davis, CA 95617 USA
800-824-8540 or 916/758-4400
C3

Applied Analytical Ind./Camag TLC Route 6 Box 55, New Hanover Air Pk.
Wilmington, NC 28405 USA
800/334-8395
D1 D2 D3 D4 D5 D6 D7 D8 E3

Applied Automation, Inc. Pawhuska Road
Bartlesville, OK 77004 USA
918/661-9635
A1 A3 A5 B7 B9 C1 C4 C6 F5 F6

Applied Chromatography Syst., Ltd. Concorde House Concorde St., Luton
Bedfordshire LU2 OJE United Kingdom
(0582)-410505
A1 A2 A3 A5 A6 B3 B5 B6 B8 B9 C2 C4 C6 C7 C9 F1

Applied Chromatography Systems 409 South Push Street
State College, PA 16801 USA
814/237-1709
A1 A2 A3 A5 A9 B5 B6 B9 C1 C4 C5 C6 C7 F1

Barnstead Co. Sybron Corporation 225 Rivermoor St.
Boston, MA 02132 USA
617/327-1600
B4

BDH Chemicals, Inc. Broom Road
Poole, Dorset BH12 4NN United Kingdom
Parkstone 745520
B1 B2 C3 D2 D8

Beckman Instruments, Inc. Altex Scientific Operations, 1716 East Fourth Street
Berkeley, CA 94710 USA
415/527-5900
A1 A2 A3 A5 A8 B5 B6 B9 C1 C2 C3 C4 C5 C6 C7

Benson Company P. O. Box 12812
Reno, NV 89510 USA
702/825-1493
B1 C2 C3 C5

Berthold Instruments, Inc. Beta Analytical Div. 136 Bradford Avenue
Pittsburgh, PA 15205 USA
412/922-2635
A7 D9

Berthold, Lab. of Prof. Dr. Kambacherstr. 22
D-7547 Waldbad 1 West Germany

A7 D9

Bio-Rad Laboratories 2200 Wright Avenue
Richmond, CA 94804 USA
415/234-4130
A1 A2 A3 A4 A5 B1 B5 B9 C1 C2 C3 C5 C6 C7 E2

Bioanalytical Systems, Inc. 1205 Kent Avenue
West Lafayette, IN 47906 USA
317/463-2505
A1 A2 A5 A6 B1 B5 B7 B8 C2 C5 C7 C9

Brinkmann Instruments, Inc. Cantiague Road
Westbury, NY 11590 USA
516/334-7500
A5 A6 B7 B8 C6 D1 D2 D5 D7 E1

Brownlee Labs, Inc. 2045 Martin Avenue Suite 204
Santa Clara, CA 95050 USA
408/727-1346
B5 C2 C5

Buck Scientific, Inc. 58 Fort Point Street
East Norwalk, CT 06855 USA
203/853-9444
A3 A4 A5 C6

Burdick & Jackson, Inc. 1953 South Harvey Street
Muskegon, MI 49442 USA
616/726-3171
B1 B2

Cajon Co. 32550 Old S. Miles Rd.
Solon, OH 44139 USA
216/248-0200
C1

Camag, GmbH P. O. Box 410865
D-1000 Berlin 41 West Germany
(030)-795-10-91
D1 D2 D3 D4 D5 D6 D7 D8 E3

Camag, Inc. Sonnenmattstrasse 11
CH-4132 Muttenz Switzerland
(061)-61 34 34
D1 D2 D3 D4 D5 D6 D7 D8 E3

Carolina Biol. Supply Co. 2700 York Rd.
Burlington, NC 27215 USA
919/584-0381
B1 B2 B3 B4 C2 C3 D1 D2 D5 D8 E2 E3

Cecil Instruments Ltd. Milton Industrial Estate
Cambridge CB4 4AZ United Kingdom
0223-66821
A1 A5 B5 C1 C2 C6

Chem Service, Inc. 660 Tower Lane P. O. Box 194
West Chester, PA 19380 USA
215/692-3026
B1

Chromatix, Inc. LDC/Milton Roy Corp. 560 Oakmead Parkway
Sunnyvale, CA 94086 USA
408/736-0300
A1 A2 A3 A4 A5 A8 A9 B5 B6 B8 C1 C2 C4 C5 C6 C7

Chromatographic Specialties P. O. Bag 1150 300 Laurier Blvd.
Brockville, Ont., K6V 5W1 Canada
613/342-4678
A2 B4 B6 B7 B9 C1 C2 C3 C5 D1 D5 D7 D8

Chromatography Services, Ltd. Carr Lane Industrial Estate Hoylake,
Merseyside United Kingdon
(051)-632-5884
A2 B1 B2 B3 B5 B7 C1 C2 C3 C5 C6 C7 C8 C9 D1 D2 D3 D4 D5 D6 D7 D8 E2

Chrompack, Inc. P. O. Box 3 Kuipersweg 6
4330 AA Middelburg The Netherlands
01180-11251
A2 B1 B6 C1 C2 C3 C4 C5 C6 C7

Chrompack, Inc. P. O. Box 6795
Bridgewater, NJ 08807 USA
201/722-8930
A2 B1 B6 C1 C2 C3 C4 C5 C6 C7

Corion Corporation 73 Jeffrey Avenue
Holliston, MA 01746 USA
617/429-5065
F3

Crescent Chemical Co. 1324 Motor Pkway
Hauppauge, NY 11788 USA
516/348-0333
B1 B2 B3 C2 C3 D1 D2 D5

Crippen Labs, Inc. 4027 New Castle Avenue
New Castle, DE 19720 USA
302/571-8882
B1 C2 C5 Z1

Cyborg Corporation 55 Chapel Street
Newton, MA 02158 USA
617/964-9020
C4 C6

Desaga, GmbH Maassstrasse 26-28 P. O. Box 101969
D-6900 Heidelberg 1 West Germany
06221-81013
B5 D1 D3 D6 D7

Digilab Division Bio-Rad Laboratories, Inc. 237 Putnam Avenue
Cambridge, MA 02139 USA
617/868-4330
A4

Dionex (UK), Ltd. The Parade, Frimley Camberley
Surrey GU16 5HY United Kingdom
01-464-7632
A1 A5 A6 A8 B1 B5 B6 B9 C1 C2 C3 C4 C6 C7

Dionex Corp. 1228 Titan Way
Sunnyvale, CA 94086 USA
408/737-0700
A1 A5 A6 A8 B1 B5 B6 B9 C1 C2 C3 C4 C6 C7

Drummond Scientific Co. 500 Parkway
Broomall, PA 19008 USA
215/353-0200
D3

DuPont SA 50-52 Route des Acacias
CH-1211 Geneva Switzerland
022-27-81-11
A1 A2 A3 A4 A5 B5 B6 B9 C1 C2 C3 C4 C5 C6 C7

Dynatech Precision Sampling Corp. 8275 W. El Cajon Dr. P. O. Box 15886
Baton Rouge, LA 70895 USA
504/927-1128
A2 B6

E. I. duPont de Nemours & Co. Photoproducts Dept. Instrument Systems Div.
Wilmington, DE 19898 USA
302/772-5500
A1 A2 A3 A4 A5 B5 B6 B9 C1 C2 C3 C4 C5 C6 C7

E. Merck Frankfurterstr. 250 Postfach 4119
D-6100 Darmstadt 1 West Germany
06151-72-0
A1 A5 B1 B2 B4 C1 C2 C3 C5 D1 D2 D3 D5 D8

Eastman Kodak Co. Eastman Organic Chemicals 343 State Street
Rochester, NY 14650 USA
716/724-4977
B1 B2 D1 D7 D8

Eldex Laboratories 3551 Haven Avenue
Menlo Park, CA 94025 USA
415/364-8159
B5 B8 B9 C7

Elf Aquitaine Devel. Corp. Elf Technologies, Inc. 9 West 57th Street
New York, NY 10019 USA
212/750-1140
A1 F5

EM Science, Inc. 480 Democrat Road
Gibbstown, NJ 08027 USA
609/423-6300
A1 A2 A5 B1 B2 B5 C2 C3 C5 C6 D1 D2 D3 D5 D8 E2

Erma Optical Works, Ltd. Sci. Instrum. Div. 2-4-5 Kajicho, Chiyoda-ku
Tokyo 101 Japan
03-251-0350
A1 A3 A5 B3 B5 B7 C2 C5 C6 C7

ES Industries 8 South Maple Avenue
Marlton, NJ 08053 USA
609/983-3616
B6 B8 C1 C2 C3 C5

ESA, Inc. 45 Wiggins Avenue
Bedford, MA 01730 USA
617/275-0100
A6

Farrand Optical Co., Inc. Commercial Products Div. 117 Wall Street
Valhalla, NY 10595 USA
914/428-6800
A5 A8 C2 D4

Fisher Scientific Co. 711 Forbes Avenue
Pittsburg, PA 15219 USA
412/562-5749
B1 B2 B4 B8 C1 C2 C3 C5 C6 D1 D2 D3 D5 D7 D8

Fisons Scientific Equipt.
Loughborough, Leicester United Kingdom

B1 B2

Floridin Company 3 Penn Center
Pittsburgh, PA 15235 USA
412/243-7500
C3 D2

Fluid Metering, Inc. 29 Orchard Street
Oyster Bay, NY 11771 USA
516/922-6050
B5

Fluka Chemical Corp. 255 Oser Avenue
Hauppauge, NY 11788 USA
516/273-0110
B1 D1 D2 D5

Foxboro/Analabs 80 Republic Drive
North Haven, CT 06473 USA
203/288-8463
A1 A2 B1 B2 B3 B4 B5 C1 C2 C3 C5 D1 D3 D5 D7 D8

Gelman Sciences, Inc. 600 S. Wagner Road
Ann Arbor, MI 48106 USA
313/665-0651
B4 B7 D4 D7 E3

Gilson Medical Electronics, Inc. P. O. Box 27
Middleton, WI 53562 USA
608/836-1551
A1 A5 A8 B5 B6 B8 B9 C4

Gilson SA 72, rue Gambetta B. P. 45
95400 Villiers-le-Bel France
(3)-990-54-41
A1 A5 A8 B5 B6 B8 B9 C4

Glas-Col Apparatus Co. 711 Hulman Street
Terre Haute, IN 47802 USA
812/235-6167
B9

Gow-Mac Instrument Co. P. O. Box 32
Bound Brook, NJ 08805 USA
201/560-0600
A1 A2 A5 B5 C4 C6

Haake Buchler Instruments, Inc. 244 Saddle River Road
Saddle Brook, NJ 07662 USA
201/843-2320
B7 B8 E2

Hamilton Company P. O. Box 10030
Reno, NV 89510 USA
702/786-7077
A2 B7 C1 C2 C3 D3

Harrick Scientific Corp. 88 Broadway P. O. Box 351
Ossining,NY 10562 USA
914/762-0020
A4 A5 A8 B7

HETP Jarman Crest, Jarman Road Sutton
Macclesfield, Cheshire SK11 OHJ United Kingdom
Sutton (02605)-2515
B3 C1 C3 C5 C9

Hewlett-Packard GmbH Hewlett-Packard Str.
D-7517 Waldbronn 2 West Germany
07243-6021
A1 A3 A5 B8 C2 C4 C8

Hewlett-Packard SA 7, rue du Bois du Lan
CH-1217 Geneva Switzerland
41-22-82-70-00
A1 A2 A3 A5 B5 B6 B8 B9 C1 C2 C4 C6 C7 C8

Hewlett-Packard, Inc. Analytical Group 1601 California Avenue
Palo Alto, CA 94304 USA
415-857-1501
A1 A2 A3 A5 B5 B6 B8 B9 C1 C2 C4 C6 C7 C8

Hitachi Scientific Instruments 460 East Middlefield Rd.
Mountain View, CA 94043 USA
415/969-1100
A1 A2 A5 B5 B9 C6

HPLC Technology - Phenomenex 405 Via Corta
Palos Verdes, CA 90274 USA
213/373-0903
A1 A2 A3 A5 A6 A8 B1 B3 B5 B6 B8 B9 C1 C2 C3 C4 C5 C6 C7 C9

HPLC Technology, Ltd. Wellington House, Waterloo St. West Macclesfield
Cheshire SK11 6EF United Kingdom
(0625)-613848
A1 A2 A3 A5 A6 A8 B1 B3 B5 B6 B8 B9 C1 C2 C3 C4 C5 C6 C7 C9

IBM Instruments, Inc. Orchard Park P. O. Box 332
Danbury, CT 06810 USA
203/796-2500 or 800/243-7054
A1 A2 A3 A5 A6 A8 B5 B6 C1 C2 C3 C4 C5 C6 C7

Instruments SA Div. Jobin Yvon 16-18 rue du Canal
F-91160 Longjumeau France
(6)-909 34 93
A1 A3 B5 B8 B9 C6

Interaction Chemicals, Inc. 800 Jordan Avenue
Los Altos, CA 94022 USA
415/494-1055
C2 C3

ISCO, Inc 4700 Superior Street P. O. Box 5347
Lincoln, NE 68505 USA
402/464-0231
A1 A5 B5 B6 B8 B9 C2 C6 E2

Isolab, Inc. Drawer 4350
Akron, OH 44321 USA
216/825-4528
E2

J & S Scientific P. O. Box 396
Crystal Lake, IL 60014 USA
815/455-4255
B1 B4 C2 C3 C5 C6 D1

J & W Scientific, Inc. 3871 Security Park Drive
Rancho Cordova, CA 95670 USA
916/351-0387
A2 A5 B7 C2 C4 C5

J. T. Baker, Inc. Research Products Div. 222 Red School Lane
Phillipsburg, NJ 08865 USA
201/859-2151
B1 B7 C2 C3 C5 D1 D2 D3 D5 D8 E2

Jasco International Co., Ltd. 2967-5, Ishikawa-cho Hachioji-City
Tokyo 192, Japan
0426-46-4128
A1 A2 A3 A4 A5 A8 B3 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C8 C9

Jones Chromatography, Inc. 914A Boscastle Court
Columbus, OH 43212 USA
614/459-4263
A2 B1 B2 B3 B6 B7 B8 C1 C2 C3 C4 C5 C6 C7 C9

Jones Chromatography, Ltd. Colliery Road Llanbradach
Mi-Glamorgan CF8 3QQ United Kingdom
(0222)-861661
A2 B1 B2 B3 B6 B7 B8 C1 C2 C3 C4 C5 C6 C7 C9

Jordi Associates, Inc. 397 Village Street
Millis, MA 02054 USA
617/376-8883
C2 C5 Z1

Kontes Glass Co. Spruce Street P. O. Box 729
Vineland, NJ 08360 USA
609/692-8500
D3 D4 D7

Kontron AG Analytical Division Reruerstr. Sud 169
8048-Zurich Switzerland
01-4354111
A1 A2 A5 A7 A8 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 E2

Kontron Electronics, Inc. 630 Price Avenue
Redwood City, CA 94063 USA
415/361-1-12
A1 A2 A5 A7 A8 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 E2

Kontron Instruments Ltd. Kontron House Campfield Road
St Albans, Herts AL1 5JG United Kingdom

A1 A2 A5 A7 A8 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 E2

Kratos Anal. Instr. Co. 170 Williams Dr.
Ramsey, NJ 07446 USA
201/934-9000
A1 A2 A5 A8 B5 C2 C4 C5 C6 C7 C8 C9 D4

Kratos GmbH Karlsburgstr. 6
D-7500 Karlsruhe 41 West Germany
(0721)-40-40-91
A1 A2 A5 A8 B5 C2 C4 C5 C6 C7 C8 C9 D4

Kratos Ltd. Barton Dock Road Urmston
Manchester M31 2LD United Kingdom
(061)-865-4466
A1 A2 A5 A8 B5 C2 C4 C5 C6 C7 C8 C9 D4

Kratos SA 88, rue Phillippe
F-75018 Paris France
(010)-201-71-00
A1 A2 A5 A8 B5 C2 C4 C5 C6 C7 C8 C9 D4

Labindustries, Inc. 620 Hearst Avenue
Berkeley, CA 94710 USA
800/227-0128
B7

Lachat Chemicals, Inc. 10500 N. Port Washington Rd.
Mequon, WI 53092 USA
414/241-3872
A2 A3 B1 B2 B6 C6 D1 D4

LC Company, Inc. Chemical Research Supplies Div. P. O. Box 72125
Roselle, IL 60172 USA
312/529-1880
B1 B3 B5 B6 B7 C1 C2 C3 C5 C6 C7

LC Services Corp. 165 New Boston Street
Woburn, MA 01801 USA
617/938-1700
Z1

LDC/Milton Roy Co. 3661 Interstate Ind. Pk. Rd. P. O. Box 10235
Riviera Beach, FL 33461 USA
305/844-5241
A1 A2 A3 A5 A6 A8 A9 B5 B6 B9 C1 C2 C4 C5 C6 C7 C9 E2 F2

LeCroy Research Systems, Inc. 700 S. Main Street
Spring Valley, NY 10977 USA
914/425-2000
C4

LKB Instruments, Inc. 9319 Gaither Road
Gaithersburg, MD 20877 USA
301/963-3200
A1 A2 A5 B5 B8 B9 C1 C2 C3 C4 C5 C6 E2

LKB Produkter AB Fredsforsstigen 22-24
S-161 25 Bromma Sweden
(08)-980040
A1 A2 A3 A5 A6 B5 B6 B8 B9 C1 C2 C3 C4 C5 C6 C7 E2

Luft Instruments, Inc. Hillside Road
Lincoln, MA 01773 USA
617/259-9215
B9

Macherey-Nagel GmbH Werkstrasse 6-8
D-5160 Duren West Germany
(02421)-61071
B2 B3 C1 C2 C3 C5 D1 D2 D5

Mallinckrodt, Inc. Science Products Div. P. O. Box 5840
St. Louis, MO 63134 USA
314/895-2333
B1 B2

Manville International Corp. Filtration and Minerals Div. P. O. Box 5108
Denver, CO 80217 USA
303/978-2000
C3

Marco Scientific, Inc. 1055 Sunnyvale-Saratoga Rd #8
Sunnyvale, CA 94087 USA
408/739-9418
A6 D4

Micromeritics Instrument Corp. 5680 Goshen Springs Road
Norcross, GA 30093 USA
404/448-8282
A1 A2 A3 A5 B3 B5 B6 C2 C4 C6 C7 C9

Micromeritics, Ltd. 2 Orchard Way Eaton Bray
Bedfordshire LU6 2SS United Kingdom
0525-220639
A1 A2 A3 A5 B3 B5 B6 B9 C2 C3 C7 C9

Miles Scientific 30 W 475 North Aurora Rd
Naperville, IL 60566 USA
312/357-3720
C3

Munhall Company 5850 N. High Street
Worthington, OH 43085 USA
614/888-7700
A1 A2 A3 A5 A6 A7 A8 B1 B3 B4 B5 B6 B7 B8 B9 C1 C2 C3 C4 C5 C6 C7 C9 D1

N & R Scientific Co., Inc. 462 11th Street
Palisades Park, NJ 07650 USA
201/592-1864
A1 A3 A5 A8 B5 B6 B7 B8 C6 D4 D6 E3

Nelson Analytical, Inc. 10061 Bubb Road
Cupertino, CA 95014 USA
408/725-1107
C4

Nicolet Analytical Instruments Senefelderstrasse 162
D-6050 Offenbach Am Main West Germany
0611-837001
A4

Nicolet Instrument Corp. 5225 Verona Road
Madison, WI 53711 USA
608/271-3333
A4

Nihon Waters Ltd. Shuwa Kioicho Park Bldg. 3, Kioicho, Chiyoda-ku
Tokyo Japan
264-8005
A1 A2 A3 A5 A8 C1 C2 C3 C4 C5 C6 C7 C9 B1 B2 B3 B4 B5 B6 B7 B9 D2 E2

Nu-Chek-Prep, Inc. P. O. Box 295
Elysian, MN 56028 USA
507/267-4689
B1

Owens Polyscience Ltd 28 Water Street
Macclesfield, Cheshire SK11 6PH United Kingdom
(0625)-610 118
A2 A3 A5 A6 B1 B5 B6 B7 B8 C1 C2 C5 C6

PC, Inc. 11805 Kim Place
Potomac, MD 20854 USA
301/299-9386
E1

Perkin-Elmer Corp. Main Avenue
Norwalk, CT 06856 USA
203/762-1000
A1 A2 A3 A5 A6 A8 B5 B6 B8 B9 C1 C2 C4 C5 C6 C7 C9 F2 F4

Perkin-Elmer GmbH P. O. Box 1120
D-7770 Uberlingen West Germany
07551-811
A1 A2 A3 A5 A6 A8 B5 B6 B8 B9 C1 C2 C4 C5 C6 C7 C9 F2 F4

Perkin-Elmer Ltd. Post Office Lane Beaconsfield
Bucks HP9 1QA United Kingdom
(04-946)-6161
A1 A2 A3 A5 A6 A8 B5 B6 B8 B9 C1 C2 C4 C5 C6 C7 C9 F2 F4

Pharmacia Fine Chemicals AB Box 175
S-75104 Uppsala Sweden
(018)-163000
A1 A2 A5 B1 B5 B6 B8 B9 C1 C2 C3 C4 C6 E2

Pharmacia, Inc. Fine Chemicals Div. 800 Centennial Ave.
Piscataway, NJ 08854 USA
201/457-8000
A1 A2 A5 B1 B5 B8 B9 C1 C2 C3 C6

Pickering Labs, Inc. 1951 Colony Street Suite S
Mountain View, CA 94043 USA
415/968-9502
B1 B2 C2 C5 C7 C9

Pierce Chemical Co. P. O. Box 117
Rockford, IL 61105 USA
815/968-0747 or 800/435-2960
B1 B2 B4 B5 B7 C1 C2 C3 C5 D1 D2 D5 D7 D8

Polymer Laboratories, Inc. Box 1581
Stow, OH 44224 USA

A3 B1 B5 C2 C4 C5

Polymer Laboratories, Ltd. Essex Road Church Stretton
Shropshire SY6 6AX United Kingdom
(0694)-723581
A3 B1 B5 C4 C5

PolyScience Corp. 7800 Merrimac Ave.
Niles, IL 60648 USA
312/967-0611
B1

Polysciences, Inc. Paul Valley Industrial Pk. 400 Valley Rd.
Warrington, PA 18976 USA
215/343-6484
B1 C3

Pressure Chem. Co. 3419 Smallman Street
Pittsburgh, PA 15201 USA
412/682-5882
B1

Princeton Applied Research-E G & G 7 Roszel Rd. P. O. Box 2565
Princeton, NJ 08540 USA
609/452-2111
A6

Pye Unicam, Ltd. York Street
Cambridge CB1 2PX United Kingdom
(0223)-358866
A1 A2 A3 A5 A6 A8 B1 B5 B6 B8 B9 C1 C2 C3 C4 C5 C6 C7 C9

Radiomatic Instr. & Chem. Co. 5102 S. Westshore Blvd.
Tampa, FL 33611 USA
813/837-1090
A7 B1 B9

Rainin Instrument Co. Mack Road
Woburn, MA 01801 USA
617/935-3050 or 800/225-5392
A1 A2 A3 A5 A6 A8 B1 B2 B3 B4 B5 B6 B7 B9 C2 C3 C4 C5 D1 D2 D3 D5

Rainin Instrument Co. 1715 64th Street
Emeryville, CA 94608 USA
415/654-9142
A1 A2 A3 A5 A6 A8 B1 B2 B3 B4 B5 B6 B7 B9 C2 C3 C4 C5 D1 D2 D3 D5

Regis Chemical Co. 8210 Austin Avenue
Morton Grove, IL 60053 USA
312/967-6000
B1 C2 C3 C5 D7

Rheodyne, Inc. P. O. Box 966
Cotati, CA 94928 USA
707/664-9050
A2 B6 C1 C2 C5

Rocky Mountain Labs, Inc. 2107 Templeton Gap Road
Colorado Springs, CA 80907 USA
303/636-2883
C2 Z1

Schleicher & Schuell, Inc. 543 Washington Street
Keene, NH 03431 USA
603/352-3810
B7 D1

Scientific Systems, Inc. 1120 West College Avenue
State College, PA 16801 USA
814/234-7311
B3 B6 C1 C2 C5 C7

Serdary Res. Labs 1643 Kathryn Drive
London, Ontario N6G 2R7 Canada
519/434-4419
B1

Shandon Southern Instruments 515 Broad Street
Sewickley, PA 15143 USA
412/741-8400 or 800/245-6212
A2 B3 C1 C2 C3 C5 D3 D6 D7

Shandon Southern Products 93 Chadwick Rd. Runcorn
Cheshire WA7 1PR United Kingdom
09285-66611
A2 B3 C1 C2 C3 C5 D3 D6 D7 E1

Shimadzu Corp. P. O. Box 209 Shinjuku-Mitsui Bldg 1-1 Nishishinjuku 2-chome
Shinjuku, Tokyo Japan
(03)-346-5641
A1 A2 A3 A5 A8 B5 B6 B9 C2 C4 C6 C7

Shimadzu Europa GmbH Ackerstrasse 111
D-4000 Duesseldorf 1 West Germany
(0211)-66 63 71
A1 A2 A3 A5 A8 B5 B6 B9 C2 C4 C6 C7 D4

Shimadzu Scientific Instr., Inc. 9147 Red Branch Rd.
Columbia, MD 21045 USA
301/997-1227
A1 A2 A3 A5 B5 B6 B9 C2 C4 C6 C7 D4

Showa Denko America 280 Park Ave. W-Bldg. 27th Floor
New York, NY 10017 USA
212/687-0773
A3 A6 C2 C5

Showa Denko KK 13-9 Shiba Daimon 1-chome Minato-ku
Tokyo 105 Japan
(03)-432-5111
A1 A3 B1 C2 C5

Spark-Holland P. O. Box 388
7800 Ajemmen The Netherlands
5910-31700
A6 B6 B7 B9 C7 C9

Spectra Physics, Inc. Autolab Division 3333 North First Street
San Jose, CA 95134 USA
408/946-6080
A1 A2 A3 A5 A8 B5 B6 C1 C2 C3 C4 C5 C6 C7

Spectronics Corp. 956 Brush Hollow Road P. O. Box 483
Westbury, NY 11590 USA
516/333-4840
A5 A8 D9 E3

Springborn Laboratories
Enfield, CT 06082 USA
203/749-8371
Z1

Supelco, Inc. Supelco Park
Bellefonte, PA 16823 USA
814/359-2784
A2 B1 B2 B4 B6 C1 C2 C3 C5 C7 D1 D2 D7 D8

Supelco, SA Route de Celigny 3
CH-1299 Crans Switzerland
(022)-76-31-10
A2 B1 B2 B4 B6 C1 C2 C3 C5 C7 D1 D2 D7 D8

SynChrom, Inc. P. O. Box 110
Linden, IN 47955 USA
317/339-4668
C2 C3 C5 C9

Systec, Inc. 3816 Chandler Dr.
Minneapolis, MN 55421 USA
612/788-9701
B9 C7

System Instruments Corp. of America 106 Centre Street
Dover, MA 02030 USA
617/785-2048
C4

The Separations Group P. O. Box 867
Hesperia, CA 92345 USA
714/244-6107
C2 C3 C5 F2

Touzart & Matignon 8, rue Eugene Henaff
F-94403 Vitry sur Seine France
680-85-21
A1 A2 A3 A5 A6 A8 B1 B3 B5 B6 B7 B9 C2 C3 C4 C9 D1 D5 D6 D7 D8 E2 E3

Tracor Instruments, Inc. 6500 Tracor Lane
Austin, TX 78721 USA
512/929-2014
A1 A2 A3 A5 B5 B6 B8 B9 C1 C2 C4 C5 C6 C7

Unimetrics Corp. 1853 Raymond Avenue
Anaheim, CA 92801 USA
714/879-3777 or 800/854-6931
A2 B3 C1 C2 C3 C5

Universal Scientific, Inc. Suite 101 2070 Peachtree Industrial Ct.
Atlanta, GA 30341 USA
404/455-1140
A1 A2 A3 A4 A5 A6 A8 B5 B6 B8 B9 C2 C3 C4 C9 D1 D2 D3 D4 D5 E2

Upchurch Scientific, Inc. 2969 North Goldie Road
Oak Harbor; WA 98277 USA
206/679-2528 or 800/426-0191
B3 C1 C3 C5

US Biochem. Corp. P. O. Box 22400
Cleveland, OH 44122 USA
216/663-0330
B1 C8

Utopia Instrument Corp. Caton Farm Road P. O. Box 863
Joliet, IL 60434 USA
815/727-5431
A1 A3 A4 A5 B5 C2 C3 C5 C6

UVP, Inc. 5100 Walnut Grove Avenue
San Gabriel, CA 91778 USA
213/285-3123
A8 D4

Vacumetrics, Inc. 2261 Palma Drive
Ventura, CA 93003 USA
805/644-7461
B9 C1 C4 C6 C8

Valco Instruments, Inc. P. O. Box 55603
Houston, TX 77255 USA
713/688-9345
A2 B3 B6 B7 B8 B9 C1 C5 C9

Varex Corp. 12221 Parklawn Drive
Rockville, MD 20852 USA
301/984-7760
A2 A3 A5 A9 B5 B6 C1 C2 C4 C6

Varian Associates, Inc. Instrument Div. 611 Hansen Way
Palo Alto, CA 94303 USA
415/493-4000
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 C1 C2 C3 C4 C5 C6 C7 C9

VG Instruments, Inc. 300 Broad Street
Stamford, CT 06901 USA
203/322-4546
C8

Waters AB Box 17067
S-40261 Goteborg 17 Sweden
31-447340
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2

Waters Assoc (Inst.) Ltd 324 Chester Rd. Hartford
Northwich, Cheshire CW8 2AH United Kingdom
(606)-883846
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2

Waters Assoc. AS Sondre Ringvej 24
DK-4000 Roskilde Denmark
(3)-361080
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2

Waters Assoc. Pty. Ltd. 82-96 Myrtle Street Fourth Floor
Sydney, NSW 2008 Australia
(2)-699-1377
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2

Waters Assoc., NV Pontbeekstraat 33
B-1720 Bijgaarden Belgium
(2)-465-5088
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2

Waters Associates, Inc. 34 Maple Street
Milford, MA 01757 USA
617/478-2000
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2 F5

Waters BV Penningweg 33 Postbus 166
NL-4870 AD Etten-Leur The Netherlands
1608-22000
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2

Waters Espanola, S.A. La Coruna, 29
Barcelona 26 Spain
225-14-68 or 225-14-69
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2

Waters GmbH Herzog-Adolphstrasse 4
D-6240 Koenigstein-Taunus West Germany
6174-4021
A1 A2 A3 A5 A8 C1 C2 C3 C4 C5 C6 C7 C9 B1 B2 B3 B4 B5 B6 B7 B9 D2 E2

Waters SA 18, rue Goubet
F-75940 Paris Cedex 19 France
1-200-60 08
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C4 C5 C6 C7 C9 D2 E2

Waters Scientific Ltd. Unit #4 6480 Viscount Rd.
Mississauga, Ont. L4V 1H3 Canada
416/254-1111
A1 A2 A3 A5 A8 B1 B2 B3 B4 B5 B6 B7 B9 C1 C2 C3 C$ C5 C6 C7 C9 D2 E2

Wescan Instruments, Inc. 3018 Scott Blvd.
Santa Clara, CA 95050 USA
408/727-3519
A1 A5 A6 B5 B6 B9 C2 C5 C6 F2

Whatman, Inc. 9 Bridewell Place
Clifton, NJ 07014 USA
201/773-5800
A1 C2 C3 C5 D1 D2 D3 D5 D6 D7 D8 E2 E3 F5

Zymark Corporation Zymark Center
Hopkinton, MA 01748 USA
617/435-9041
B7 B8 B9 C9

PRODUCTS SECTION

CHEMICALS, REAGENTS, STANDARDS (B1)

Ace Scientific Supply Co.
American Scientific Products
BDH Chemicals, Inc.
Bio-Rad Laboratories
Burdick & Jackson, Inc.
Chem Service, Inc.
Chrompack, Inc.
Crescent Chemical Co.
Dionex (UK), Ltd.
E. Merck
EM Science, Inc.
Fisons Scientific Equipt.
Foxboro/Analabs
HPLC Technology, Ltd.
J. T. Baker, Inc.
Jones Chromatography, Ltd.
LC Company, Inc.
Munhall Company
Nu-Chek-Prep, Inc.
Pharmacia, Inc.
Pierce Chemical Co.
Polymer Laboratories, Ltd.
Polysciences, Inc.
Pye Unicam, Ltd.
Rainin Instrument Co.
Regis Chemical Co.
Showa Denko America
Supelco, Inc.
Touzart & Matignon
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Alltech Associates, Inc.
American Type Culture Coll.
Benson Company
Bioanalytical Systems, Inc.
Carolina Biol. Supply Co.
Chromatography Services, Ltd.
Chrompack, Inc.
Crippen Labs, Inc.
Dionex Corp.
Eastman Kodak Co.
Fisher Scientific Co.
Fluka Chemical Corp.
HPLC Technology - Phenomenex
J & S Scientific
Jones Chromatography, Inc.
Lachat Chemicals, Inc.
Mallinckrodt, Inc.
Nihon Waters Ltd.
Pharmacia Fine Chemicals AB
Pickering Labs, Inc.
Polymer Laboratories, Inc.
PolyScience Corp.
Pressure Chem. Co.
Radiomatic Instr. & Chem. Co.
Rainin Instrument Co.
Serdary Res. Labs
Showa Denko KK
Supelco, SA
US Biochem. Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

COLUMN OVENS, HEATERS (C7)

Ace Scientific Supply Co.
Alltech Associates, Inc.
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Bioanalytical Systems, Inc.
Chromatography Services, Ltd.
Chrompack, Inc.
Dionex Corp.
E. I. duPont de Nemours & Co.
Erma Optical Works, Ltd.
Alltech Associates UK Ltd.
American Scientific Products
Applied Chromatography Systems
Bio-Rad Laboratories
Chromatix, Inc.
Chrompack, Inc.
Dionex (UK), Ltd.
DuPont SA
Eldex Laboratories
Hewlett-Packard SA

Hewlett-Packard, Inc.
HPLC Technology, Ltd.
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LC Company, Inc.
LKB Produkter AB
Micromeritics, Ltd.
Nihon Waters Ltd.
Perkin-Elmer GmbH
Pickering Labs, Inc.
Scientific Systems, Inc.
Shimadzu Europa GmbH
Spark-Holland
Supelco, Inc.
Systec, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
HPLC Technology - Phenomenex
IBM Instruments, Inc.
Jones Chromatography, Inc.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LDC/Milton Roy Co.
Micromeritics Instrument Corp.
Munhall Company
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pye Unicam, Ltd.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Spectra Physics, Inc.
Supelco, SA
Tracor Instruments, Inc.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

COLUMN PACKING DEVICES (B3)

A. S. I.
Alltech Associates UK Ltd.
American Scientific Products
Carolina Biol. Supply Co.
Erma Optical Works, Ltd.
HETP
HPLC Technology, Ltd.
Jones Chromatography, Inc.
LC Company, Inc.
Micromeritics Instrument Corp.
Munhall Company
Rainin Instrument Co.
Scientific Systems, Inc.
Shandon Southern Products
Unimetrics Corp.
Valco Instruments, Inc.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Ace Scientific Supply Co.
Alltech Associates, Inc.
Applied Chromatography Syst., Ltd.
Crescent Chemical Co.
Foxboro/Analabs
HPLC Technology - Phenomenex
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Macherey-Nagel GmbH
Micromeritics, Ltd.
Nihon Waters Ltd.
Rainin Instrument Co.
Shandon Southern Instruments
Touzart & Matignon
Upchurch Scientific, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA

COLUMN PACKING MATERIALS (C3)

A. S. I.
Ace Scientific Supply Co.
Alltech Associates, Inc.
Analtech, Inc.
Anspec Company
ABC Laboratories
Alltech Associates UK Ltd.
American Scientific Products
Analytichem Internat'l
Antibodies, Inc.

BDH Chemicals, Inc.
Benson Company
Carolina Biol. Supply Co.
Chromatography Services, Ltd.
Chrompack, Inc.
Dionex (UK), Ltd.
DuPont SA
E. Merck
ES Industries
Floridin Company
Hamilton Company
HPLC Technology - Phenomenex
IBM Instruments, Inc.
J & S Scientific
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron Electronics, Inc.
LC Company, Inc.
LKB Produkter AB
Manville International Corp.
Micromeritics, Ltd.
Munhall Company
Pharmacia Fine Chemicals AB
Pierce Chemical Co.
Pye Unicam, Ltd.
Rainin Instrument Co.
Shandon Southern Instruments
Spectra Physics, Inc.
Supelco, SA
The Separations Group
Unimetrics Corp.
Upchurch Scientific, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Whatman, Inc.
Beckman Instruments, Inc.
Bio-Rad Laboratories
Chromatographic Specialties
Chrompack, Inc.
Crescent Chemical Co.
Dionex Corp.
E. I. duPont de Nemours & Co.
EM Science, Inc.
Fisher Scientific Co.
Foxboro/Analabs
HETP
HPLC Technology, Ltd.
Interaction Chemicals, Inc.
J. T. Baker, Inc.
Jones Chromatography, Inc.
Kontron AG
Kontron Instruments Ltd.
LKB Instruments, Inc.
Macherey-Nagel GmbH
Micromeritics Instrument Corp.
Miles Scientific
Nihon Waters Ltd.
Pharmacia, Inc.
Polysciences, Inc.
Rainin Instrument Co.
Regis Chemical Co.
Shandon Southern Products
Supelco, Inc.
SynChrom, Inc.
Touzart & Matignon
Universal Scientific, Inc.
Utopia Instrument Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

COLUMNS, PACKED (C2)

A. S. I.
Ace Glass, Inc.
Alltech Associates UK Ltd.
American Scientific Products
Analytichem Internat'l
Applied Chromatography Syst., Ltd.
Benson Company
Bioanalytical Systems, Inc.
Carolina Biol. Supply Co.
Chromatix, Inc.
Chromatography Services, Ltd.
Chrompack, Inc.
Crippen Labs, Inc.
Dionex Corp.
E. I. duPont de Nemours & Co.
EM Science, Inc.
ES Industries
ABC Laboratories
Ace Scientific Supply Co.
Alltech Associates, Inc.
Amicon Corp.
Anspec Company
Beckman Instruments, Inc.
Bio-Rad Laboratories
Brownlee Labs, Inc.
Cecil Instruments Ltd.
Chromatographic Specialties
Chrompack, Inc.
Crescent Chemical Co.
Dionex (UK), Ltd.
DuPont SA
E. Merck
Erma Optical Works, Ltd.
Farrand Optical Co., Inc.

Fisher Scientific Co.
Hamilton Company
Hewlett-Packard SA
HPLC Technology - Phenomenex
IBM Instruments, Inc.
ISCO, Inc
J & W Scientific, Inc.
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LDC/Milton Roy Co.
LKB Produkter AB
Micromeritics Instrument Corp.
Munhall Company
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pickering Labs, Inc.
Polymer Laboratories, Inc.
Rainin Instrument Co.
Regis Chemical Co.
Rocky Mountain Labs, Inc.
Shandon Southern Instruments
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Showa Denko KK
Supelco, Inc.
SynChrom, Inc.
Touzart & Matignon
Unimetrics Corp.
Utopia Instrument Corp.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Wescan Instruments, Inc.
Foxboro/Analabs
Hewlett-Packard GmbH
Hewlett-Packard, Inc.
HPLC Technology, Ltd.
Interaction Chemicals, Inc.
J & S Scientific
J. T. Baker, Inc.
Jones Chromatography, Inc.
Jordi Associates, Inc.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LC Company, Inc.
LKB Instruments, Inc.
Macherey-Nagel GmbH
Micromeritics, Ltd.
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Pierce Chemical Co.
Pye Unicam, Ltd.
Rainin Instrument Co.
Rheodyne, Inc.
Scientific Systems, Inc.
Shandon Southern Products
Shimadzu Europa GmbH
Showa Denko America
Spectra Physics, Inc.
Supelco, SA
The Separations Group
Tracor Instruments, Inc.
Universal Scientific, Inc.
Varex Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Whatman, Inc.

COMPLETE LC INSTRUMENTS (A1)

Anspec Company
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Bioanalytical Systems, Inc.
Chromatix, Inc.
Dionex Corp.
E. I. duPont de Nemours & Co.
Elf Aquitaine Devel. Corp.
Erma Optical Works, Ltd.
Gilson Medical Electronics, Inc.
Gow-Mac Instrument Co.
Hewlett-Packard SA
Hitachi Scientific Instruments
HPLC Technology, Ltd.
Instruments SA
Applied Automation, Inc.
Applied Chromatography Systems
Bio-Rad Laboratories
Cecil Instruments Ltd.
Dionex (UK), Ltd.
DuPont SA
E. Merck
EM Science, Inc.
Foxboro/Analabs
Gilson SA
Hewlett-Packard GmbH
Hewlett-Packard, Inc.
HPLC Technology - Phenomenex
IBM Instruments, Inc.
ISCO, Inc

Jasco International Co., Ltd.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LDC/Milton Roy Co.
LKB Produkter AB
Micromeritics, Ltd.
N & R Scientific Co., Inc.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Rainin Instrument Co.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Spectra Physics, Inc.
Tracor Instruments, Inc.
Utopia Instrument Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Whatman, Inc.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LKB Instruments, Inc.
Micromeritics Instrument Corp.
Munhall Company
Nihon Waters Ltd.
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pye Unicam, Ltd.
Rainin Instrument Co.
Shimadzu Europa GmbH
Showa Denko KK
Touzart & Matignon
Universal Scientific, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Wescan Instruments, Inc.

COMPUTERS, DATA SYSTEMS (C4)

Ace Scientific Supply Co.
American Scientific Products
Applied Automation, Inc.
Applied Chromatography Systems
Chromatix, Inc.
Chrompack, Inc.
Dionex (UK), Ltd.
DuPont SA
Gilson Medical Electronics, Inc.
Gow-Mac Instrument Co.
Hewlett-Packard SA
HPLC Technology - Phenomenex
IBM Instruments, Inc.
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LDC/Milton Roy Co.
LKB Instruments, Inc.
Munhall Company
Nihon Waters Ltd.
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Polymer Laboratories, Ltd.
Rainin Instrument Co.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
System Instruments Corp. of America
Tracor Instruments, Inc.
Vacumetrics, Inc.
Varian Associates, Inc.
Alltech Associates, Inc.
Anspec Company
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Chrompack, Inc.
Cyborg Corporation
Dionex Corp.
E. I. duPont de Nemours & Co.
Gilson SA
Hewlett-Packard GmbH
Hewlett-Packard, Inc.
HPLC Technology, Ltd.
J & W Scientific, Inc.
Jones Chromatography, Inc.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LeCroy Research Systems, Inc.
LKB Produkter AB
Nelson Analytical, Inc.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Polymer Laboratories, Inc.
Pye Unicam, Ltd.
Rainin Instrument Co.
Shimadzu Europa GmbH
Spectra Physics, Inc.
Touzart & Matignon
Universal Scientific, Inc.
Varex Corp.
Waters AB

Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

CONTRACT LABORATORIES (Z1)

Alpha Chem. & Biomed. Labs
Jordi Associates, Inc.
Rocky Mountain Labs, Inc.
Crippen Labs, Inc.
LC Services Corp.
Springborn Laboratories

COUNTERCURRENT CHROMATOGRAPHY EQUIPMENT (E1)

Anspec Company
PC, Inc.
Brinkmann Instruments, Inc.
Shandon Southern Products

DERIVATIZATION DEVICES (C9)

Alltech Associates UK Ltd.
Analytichem Internat'l
Bioanalytical Systems, Inc.
HETP
HPLC Technology, Ltd.
Jones Chromatography, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LDC/Milton Roy Co.
Micromeritics, Ltd.
Nihon Waters Ltd.
Perkin-Elmer GmbH
Pickering Labs, Inc.
Spark-Holland
Touzart & Matignon
Valco Instruments, Inc.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
American Scientific Products
Applied Chromatography Syst., Ltd.
Chromatography Services, Ltd.
HPLC Technology - Phenomenex
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kratos GmbH
Kratos SA
Micromeritics Instrument Corp.
Munhall Company
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pye Unicam, Ltd.
SynChrom, Inc.
Universal Scientific, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Zymark Corporation

DETECTORS, CONDUCTIVITY (F2)

LDC/Milton Roy Co.
Perkin-Elmer GmbH
The Separations Group
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Wescan Instruments, Inc.

DETECTORS, DIELECTRIC CONSTANT (F6)

Applied Automation, Inc.

DETECTORS, ELECTROCHEMICAL (A6)

Anspec Company
Bioanalytical Systems, Inc.
Dionex (UK), Ltd.
ESA, Inc.
HPLC Technology, Ltd.
LDC/Milton Roy Co.
Marco Scientific, Inc.
Owens Polyscience Ltd
Perkin-Elmer GmbH
Princeton Applied Research-E G & G
Rainin Instrument Co.
Spark-Holland
Universal Scientific, Inc.
Applied Chromatography Syst., Ltd.
Brinkmann Instruments, Inc.
Dionex Corp.
HPLC Technology - Phenomenex
IBM Instruments, Inc.
LKB Produkter AB
Munhall Company
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pye Unicam, Ltd.
Rainin Instrument Co.
Touzart & Matignon
Wescan Instruments, Inc.

DETECTORS, FLUORESCENCE (A8)

American Scientific Products
Beckman Instruments, Inc.
Dionex (UK), Ltd.
Farrand Optical Co., Inc.
Gilson SA
HPLC Technology - Phenomenex
IBM Instruments, Inc.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
Munhall Company
Nihon Waters Ltd.
Perkin-Elmer GmbH
Pye Unicam, Ltd.
Rainin Instrument Co.
Shimadzu Europa GmbH
Spectronics Corp.
Universal Scientific, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Anspec Company
Chromatix, Inc.
Dionex Corp.
Gilson Medical Electronics, Inc.
Harrick Scientific Corp.
HPLC Technology, Ltd.
Jasco International Co., Ltd.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LDC/Milton Roy Co.
N & R Scientific Co., Inc.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Rainin Instrument Co.
Shimadzu Corp.
Spectra Physics, Inc.
Touzart & Matignon
UVP, Inc.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

DETECTORS, INFRARED (A4)

Accuspec Corp.
Anspec Company
Buck Scientific, Inc.
Digilab Division
E. I. duPont de Nemours & Co.
Jasco International Co., Ltd.
Nicolet Instrument Corp.
Utopia Instrument Corp.
American Scientific Products
Bio-Rad Laboratories
Chromatix, Inc.
DuPont SA
Harrick Scientific Corp.
Nicolet Analytical Instruments
Universal Scientific, Inc.

DETECTORS, LIGHT-SCATTERING (A9)

Applied Chromatography Systems
LDC/Milton Roy Co.
Chromatix, Inc.
Varex Corp.

DETECTORS, MASS (F1)

Applied Chromatography Syst., Ltd.
Applied Chromatography Systems

DETECTORS, POLARIMETRIC (F4)

Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Perkin-Elmer GmbH

DETECTORS, REFRACTOMETRIC (A3)

American Scientific Products
Applied Automation, Inc.
Applied Chromatography Systems
Bio-Rad Laboratories
Chromatix, Inc.
E. I. duPont de Nemours & Co.
Hewlett-Packard GmbH
Hewlett-Packard, Inc.
HPLC Technology, Ltd.
Instruments SA
Lachat Chemicals, Inc.
LKB Produkter AB
Micromeritics, Ltd.
N & R Scientific Co., Inc.
Owens Polyscience Ltd
Perkin-Elmer GmbH
Polymer Laboratories, Inc.
Pye Unicam, Ltd.
Rainin Instrument Co.
Shimadzu Europa GmbH
Showa Denko America
Spectra Physics, Inc.
Tracor Instruments, Inc.
Utopia Instrument Corp.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Anspec Company
Applied Chromatography Syst., Ltd
Beckman Instruments, Inc.
Buck Scientific, Inc.
DuPont SA
Erma Optical Works, Ltd.
Hewlett-Packard SA
HPLC Technology - Phenomenex
IBM Instruments, Inc.
Jasco International Co., Ltd.
LDC/Milton Roy Co.
Micromeritics Instrument Corp.
Munhall Company
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Polymer Laboratories, Ltd.
Rainin Instrument Co.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Showa Denko KK
Touzart & Matignon
Universal Scientific, Inc.
Varex Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

DETECTORS, SCINTILLATION/RADIATION (A7)

Anspec Company
Berthold, Lab. of Prof. Dr.
Kontron Electronics, Inc.
Munhall Company
Berthold Instruments, Inc.
Kontron AG
Kontron Instruments Ltd.
Radiomatic Instr. & Chem. Co.

DETECTORS, UV/VISIBLE (A5)

Accuspec Corp.
American Scientific Products
Applied Automation, Inc.
Applied Chromatography Systems
Bio-Rad Laboratories
Brinkmann Instruments, Inc.
Cecil Instruments Ltd.
Dionex (UK), Ltd.
DuPont SA
E. Merck
Erma Optical Works, Ltd.
Gilson Medical Electronics, Inc.
Gow-Mac Instrument Co.
Hewlett-Packard GmbH
Hewlett-Packard, Inc.
HPLC Technology - Phenomenex
IBM Instruments, Inc.
J & W Scientific, Inc.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LKB Instruments, Inc.
Micromeritics Instrument Corp.
Munhall Company
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Rainin Instrument Co.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Spectronics Corp.
Tracor Instruments, Inc.
Utopia Instrument Corp.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Wescan Instruments, Inc.
Alltech Associates UK Ltd.
Anspec Company
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Bioanalytical Systems, Inc.
Buck Scientific, Inc.
Chromatix, Inc.
Dionex Corp.
E. I. duPont de Nemours & Co.
EM Science, Inc.
Farrand Optical Co., Inc.
Gilson SA
Harrick Scientific Corp.
Hewlett-Packard SA
Hitachi Scientific Instruments
HPLC Technology, Ltd.
ISCO, Inc
Jasco International Co., Ltd.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LDC/Milton Roy Co.
LKB Produkter AB
Micromeritics, Ltd.
N & R Scientific Co., Inc.
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pye Unicam, Ltd.
Rainin Instrument Co.
Shimadzu Europa GmbH
Spectra Physics, Inc.
Touzart & Matignon
Universal Scientific, Inc.
Varex Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

FRACTION COLLECTORS (B8)

American Scientific Products
Applied Chromatography Syst., Ltd.
Brinkmann Instruments, Inc.
Eldex Laboratories
Fisher Scientific Co.
Gilson SA
Hewlett-Packard GmbH
Hewlett-Packard, Inc.
HPLC Technology, Ltd.
ISCO, Inc
Jones Chromatography, Ltd.
LKB Produkter AB
Anspec Company
Bioanalytical Systems, Inc.
Chromatix, Inc.
ES Industries
Gilson Medical Electronics, Inc.
Haake Buchler Instruments, Inc.
Hewlett-Packard SA
HPLC Technology - Phenomenex
Instruments SA
Jones Chromatography, Inc.
LKB Instruments, Inc.
Munhall Company

N & R Scientific Co., Inc.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Tracor Instruments, Inc.
Valco Instruments, Inc.
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pye Unicam, Ltd.
Universal Scientific, Inc.
Zymark Corporation

GUARD COLUMNS, PRE-COLUMNS (C5)

A. S. I.
Alltech Associates UK Ltd.
American Scientific Products
Analytichem Internat'l
Beckman Instruments, Inc.
Bio-Rad Laboratories
Brownlee Labs, Inc.
Chromatographic Specialties
Chrompack, Inc.
Crippen Labs, Inc.
DuPont SA
E. Merck
Erma Optical Works, Ltd.
Fisher Scientific Co.
HETP
HPLC Technology, Ltd.
J & S Scientific
J. T. Baker, Inc.
Jones Chromatography, Inc.
Jordi Associates, Inc.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LC Company, Inc.
LKB Instruments, Inc.
Macherey-Nagel GmbH
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pierce Chemical Co.
Polymer Laboratories, Ltd.
Rainin Instrument Co.
Regis Chemical Co.
Scientific Systems, Inc.
Shandon Southern Products
Showa Denko KK
Supelco, Inc.
SynChrom, Inc.
Tracor Instruments, Inc.
Upchurch Scientific, Inc.
Valco Instruments, Inc.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Whatman, Inc.
Ace Scientific Supply Co.
Alltech Associates, Inc.
Amicon Corp.
Applied Chromatography Systems
Benson Company
Bioanalytical Systems, Inc.
Chromatix, Inc.
Chromatography Services, Ltd.
Chrompack, Inc.
Desaga, GmbH
E. I. duPont de Nemours & Co.
EM Science, Inc.
ES Industries
Foxboro/Analabs
HPLC Technology - Phenomenex
IBM Instruments, Inc.
J & W Scientific, Inc.
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LDC/Milton Roy Co.
LKB Produkter AB
Munhall Company
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pickering Labs, Inc.
Polymer Laboratories, Inc.
Pye Unicam, Ltd.
Rainin Instrument Co.
Rheodyne, Inc.
Shandon Southern Instruments
Showa Denko America
Spectra Physics, Inc.
Supelco, SA
The Separations Group
Unimetrics Corp.
Utopia Instrument Corp.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Wescan Instruments, Inc.

INDUSTRIAL SCALE LC EQUIPMENT (F5)

Applied Automation, Inc.
Elf Aquitaine Devel. Corp.
Waters Associates, Inc.
Whatman, Inc.

INJECTION SYSTEMS, AUTOMATIC (B6)

Ace Scientific Supply Co.
American Scientific Products
Anspec Company
Applied Chromatography Systems
Chromatix, Inc.
Chrompack, Inc.
Dionex (UK), Ltd.
DuPont SA
E. I. duPont de Nemours & Co.
Gilson Medical Electronics, Inc.
Hewlett-Packard SA
HPLC Technology - Phenomenex
IBM Instruments, Inc.
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron Electronics, Inc.
Lachat Chemicals, Inc.
LDC/Milton Roy Co.
Micromeritics Instrument Corp.
Munhall Company
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pye Unicam, Ltd.
Rainin Instrument Co.
Scientific Systems, Inc.
Shimadzu Europa GmbH
Spark-Holland
Supelco, Inc.
Touzart & Matignon
Universal Scientific, Inc.
Varex Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Alltech Associates, Inc.
Analytichem Internat'l
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Chromatographic Specialties
Chrompack, Inc.
Dionex Corp.
Dynatech Precision Sampling Corp.
ES Industries
Gilson SA
Hewlett-Packard, Inc.
HPLC Technology, Ltd.
ISCO, Inc
Jones Chromatography, Inc.
Kontron AG
Kontron Instruments Ltd.
LC Company, Inc.
LKB Produkter AB
Micromeritics, Ltd.
N & R Scientific Co., Inc.
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Rainin Instrument Co.
Rheodyne, Inc.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Spectra Physics, Inc.
Supelco, SA
Tracor Instruments, Inc.
Valco Instruments, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Wescan Instruments, Inc.

INJECTION SYSTEMS, MANUAL (A2)

Alltech Associates UK Ltd.
American Scientific Products
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Bioanalytical Systems, Inc.
Chromatographic Specialties
Chrompack, Inc.
DuPont SA
E. I. duPont de Nemours & Co.
Alltech Associates, Inc.
Anspec Company
Applied Chromatography Systems
Bio-Rad Laboratories
Chromatix, Inc.
Chromatography Services, Ltd.
Chrompack, Inc.
Dynatech Precision Sampling Corp.
EM Science, Inc.

Foxboro/Analabs
Hamilton Company
Hewlett-Packard, Inc.
HPLC Technology - Phenomenex
IBM Instruments, Inc.
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
Lachat Chemicals, Inc.
LKB Instruments, Inc.
Micromeritics Instrument Corp.
Munhall Company
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pye Unicam, Ltd.
Rainin Instrument Co.
Shandon Southern Instruments
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Supelco, Inc.
Touzart & Matignon
Unimetrics Corp.
Valco Instruments, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Gow-Mac Instrument Co.
Hewlett-Packard SA
Hitachi Scientific Instruments
HPLC Technology, Ltd.
J & W Scientific, Inc.
Jones Chromatography, Inc.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LDC/Milton Roy Co.
LKB Produkter AB
Micromeritics, Ltd.
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Rainin Instrument Co.
Rheodyne, Inc.
Shandon Southern Products
Shimadzu Europa GmbH
Spectra Physics, Inc.
Supelco, SA
Tracor Instruments, Inc.
Universal Scientific, Inc.
Varex Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

LC/MS INTERFACES (C8)

Ace Scientific Supply Co.
Anspec Company
Hewlett-Packard GmbH
Hewlett-Packard, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
US Biochem. Corp.
VG Instruments, Inc.
American Scientific Products
Chromatography Services, Ltd.
Hewlett-Packard SA
Jasco International Co., Ltd.
Kratos GmbH
Kratos SA
Vacumetrics, Inc.

OPEN COLUMN CHROMATOGRAPHIC EQUIPMENT (E2)

Ace Glass, Inc.
Bio-Rad Laboratories
Chromatography Services, Ltd.
Haake Buchler Instruments, Inc.
Isolab, Inc.
Kontron AG
Kontron Instruments Ltd.
LKB Instruments, Inc.
Nihon Waters Ltd.
Touzart & Matignon
Waters AB
Anspec Company
Carolina Biol. Supply Co.
EM Science, Inc.
ISCO, Inc
J. T. Baker, Inc.
Kontron Electronics, Inc.
LDC/Milton Roy Co.
LKB Produkter AB
Pharmacia Fine Chemicals AB
Universal Scientific, Inc.
Waters Assoc (Inst.) Ltd

Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Whatman, Inc.

OPTICAL FILTERS (F3)

Corion Corporation

PAPER CHROMATOGRAPHY EQUIPMENT (E3)

Applied Analytical Ind./Camag TLC
Camag, Inc.
Gelman Sciences, Inc.
Spectronics Corp.
Whatman, Inc.
Camag, GmbH
Carolina Biol. Supply Co.
N & R Scientific Co., Inc.
Touzart & Matignon

PROGRAMMERS/CONTROLLERS (B9)

ABC Laboratories
Alltech Associates, Inc.
Anspec Company
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Chromatographic Specialties
Dionex Corp.
E. I. duPont de Nemours & Co.
Gilson Medical Electronics, Inc.
Glas-Col Apparatus Co.
Hewlett-Packard, Inc.
HPLC Technology - Phenomenex
Instruments SA
Jasco International Co., Ltd.
Kontron Electronics, Inc.
LDC/Milton Roy Co.
LKB Produkter AB
Micromeritics Instrument Corp.
Munhall Company
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Radiomatic Instr. & Chem. Co.
Rainin Instrument Co.
Shimadzu Europa GmbH
Spark-Holland
Touzart & Matignon
Universal Scientific, Inc.
Valco Instruments, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Wescan Instruments, Inc.
Alltech Associates UK Ltd.
American Scientific Products
Applied Automation, Inc.
Applied Chromatography Systems
Bio-Rad Laboratories
Dionex (UK), Ltd.
DuPont SA
Eldex Laboratories
Gilson SA
Hewlett-Packard SA
Hitachi Scientific Instruments
HPLC Technology, Ltd.
ISCO, Inc
Kontron AG
Kontron Instruments Ltd.
LKB Instruments, Inc.
Luft Instruments, Inc.
Micromeritics, Ltd.
Nihon Waters Ltd.
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pye Unicam, Ltd.
Rainin Instrument Co.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Systec, Inc.
Tracor Instruments, Inc.
Vacumetrics, Inc.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Zymark Corporation

PUMPS/SOLVENT DELIVERY SYSTEMS (B5)

Ace Scientific Supply Co.
Alltech Associates, Inc.
Anspec Company
Applied Chromatography Systems
Bio-Rad Laboratories
Brownlee Labs, Inc.
Chromatix, Inc.
Dionex (UK), Ltd.
DuPont SA
Eldex Laboratories
Erma Optical Works, Ltd.
Foxboro/Analabs
Gilson SA
Hewlett-Packard SA
Hitachi Scientific Instruments
HPLC Technology, Ltd.
Instruments SA
Jasco International Co., Ltd.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
LC Company, Inc.
LKB Instruments, Inc.
Micromeritics Instrument Corp.
Munhall Company
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Polymer Laboratories, Inc.
Pye Unicam, Ltd.
Rainin Instrument Co.
Shimadzu Europa GmbH
Spectra Physics, Inc.
Tracor Instruments, Inc.
Varex Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Alltech Associates UK Ltd.
American Scientific Products
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Bioanalytical Systems, Inc.
Cecil Instruments Ltd.
Chromatography Services, Ltd.
Dionex Corp.
E. I. duPont de Nemours & Co.
EM Science, Inc.
Fluid Metering, Inc.
Gilson Medical Electronics, Inc.
Gow-Mac Instrument Co.
Hewlett-Packard, Inc.
HPLC Technology - Phenomenex
IBM Instruments, Inc.
ISCO, Inc
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LDC/Milton Roy Co.
LKB Produkter AB
Micromeritics, Ltd.
N & R Scientific Co., Inc.
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pierce Chemical Co.
Polymer Laboratories, Ltd.
Rainin Instrument Co.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Touzart & Matignon
Universal Scientific, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Wescan Instruments, Inc.

RECORDERS, PLOTTERS (C6)

Ace Scientific Supply Co.
Alltech Associates, Inc.
Applied Automation, Inc.
Applied Chromatography Systems
Bio-Rad Laboratories
Buck Scientific, Inc.
Chromatix, Inc.
Chrompack, Inc.
Cyborg Corporation
Dionex Corp.
E. I. duPont de Nemours & Co.
Alltech Associates UK Ltd.
American Scientific Products
Applied Chromatography Syst., Ltd.
Beckman Instruments, Inc.
Brinkmann Instruments, Inc.
Cecil Instruments Ltd.
Chromatography Services, Ltd.
Chrompack, Inc.
Dionex (UK), Ltd.
DuPont SA
EM Science, Inc.

Erma Optical Works, Ltd.
Gow-Mac Instrument Co.
Hewlett-Packard, Inc.
HPLC Technology - Phenomenex
IBM Instruments, Inc.
ISCO, Inc
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron Electronics, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
Lachat Chemicals, Inc.
LDC/Milton Roy Co.
LKB Produkter AB
N & R Scientific Co., Inc.
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pye Unicam, Ltd.
Shimadzu Europa GmbH
Spectra Physics, Inc.
Utopia Instrument Corp.
Varex Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Fisher Scientific Co.
Hewlett-Packard SA
Hitachi Scientific Instruments
HPLC Technology, Ltd.
Instruments SA
J & S Scientific
Jones Chromatography, Inc.
Kontron AG
Kontron Instruments Ltd.
Kratos GmbH
Kratos SA
LC Company, Inc.
LKB Instruments, Inc.
Munhall Company
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Shimadzu Corp.
Shimadzu Scientific Instr., Inc.
Tracor Instruments, Inc.
Vacumetrics, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Wescan Instruments, Inc.

SAMPLE PREPARATION EQUIPMENT (B7)

Ace Scientific Supply Co.
American Scientific Products
Analytichem Internat'l
Bioanalytical Systems, Inc.
Chromatographic Specialties
Erma Optical Works, Ltd.
Haake Buchler Instruments, Inc.
Harrick Scientific Corp.
J. T. Baker, Inc.
Jones Chromatography, Inc.
Kontron AG
Kontron Instruments Ltd.
LC Company, Inc.
N & R Scientific Co., Inc.
Owens Polyscience Ltd
Rainin Instrument Co.
Schleicher & Schuell, Inc.
Touzart & Matignon
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Alltech Associates UK Ltd.
Amicon Corp.
Applied Automation, Inc.
Brinkmann Instruments, Inc.
Chromatography Services, Ltd.
Gelman Sciences, Inc.
Hamilton Company
J & W Scientific, Inc.
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron Electronics, Inc.
Labindustries, Inc.
Munhall Company
Nihon Waters Ltd.
Pierce Chemical Co.
Rainin Instrument Co.
Spark-Holland
Valco Instruments, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Zymark Corporation

SOLVENTS, ELUENTS (B2)

Ace Scientific Supply Co.
Alltech Associates, Inc.
BDH Chemicals, Inc.
Carolina Biol. Supply Co.
Crescent Chemical Co.
Eastman Kodak Co.
Fisher Scientific Co.
Foxboro/Analabs
Jones Chromatography, Ltd.
Macherey-Nagel GmbH
Nihon Waters Ltd.
Pickering Labs, Inc.
Rainin Instrument Co.
Supelco, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Alltech Associates UK Ltd.
American Scientific Products
Burdick & Jackson, Inc.
Chromatography Services, Ltd.
E. Merck
EM Science, Inc.
Fisons Scientific Equipt.
Jones Chromatography, Inc.
Lachat Chemicals, Inc.
Mallinckrodt, Inc.
Owens Polyscience Ltd
Pierce Chemical Co.
Rainin Instrument Co.
Supelco, SA
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

TLC ADSORBENTS, MEDIA (D2)

Ace Scientific Supply Co.
American Scientific Products
Anspec Company
BDH Chemicals, Inc.
Camag, GmbH
Carolina Biol. Supply Co.
Crescent Chemical Co.
EM Science, Inc.
Floridin Company
J. T. Baker, Inc.
Nihon Waters Ltd.
Rainin Instrument Co.
Supelco, Inc.
Universal Scientific, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA
Whatman, Inc.
Alltech Associates, Inc.
Analtech, Inc.
Applied Analytical Ind./Camag TLC
Brinkmann Instruments, Inc.
Camag, Inc.
Chromatography Services, Ltd.
E. Merck
Fisher Scientific Co.
Fluka Chemical Corp.
Macherey-Nagel GmbH
Pierce Chemical Co.
Rainin Instrument Co.
Supelco, SA
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.

TLC DEVELOPING CHAMBERS (D7)

Ace Scientific Supply Co.
Alltech Associates, Inc.
Applied Analytical Ind./Camag TLC
Camag, GmbH
Chromatographic Specialties
Desaga, GmbH
Fisher Scientific Co.
Gelman Sciences, Inc.
Pierce Chemical Co.
Alltech Associates UK Ltd.
Analtech, Inc.
Brinkmann Instruments, Inc.
Camag, Inc.
Chromatography Services, Ltd.
Eastman Kodak Co.
Foxboro/Analabs
Kontes Glass Co.
Regis Chemical Co.

Shandon Southern Instruments
Supelco, Inc.
Touzart & Matignon
Shandon Southern Products
Supelco, SA
Whatman, Inc.

TLC PLATES, ANALYTICAL & HPTLC (D1)

Ace Scientific Supply Co.
Alltech Associates, Inc.
Analtech, Inc.
Applied Analytical Ind./Camag TLC
Camag, GmbH
Carolina Biol. Supply Co.
Chromatography Services, Ltd.
Desaga, GmbH
Eastman Kodak Co.
Fisher Scientific Co.
Foxboro/Analabs
J. T. Baker, Inc.
Macherey-Nagel GmbH
Pierce Chemical Co.
Rainin Instrument Co.
Supelco, Inc.
Touzart & Matignon
Whatman, Inc.
Alltech Associates UK Ltd.
American Scientific Products
Anspec Company
Brinkmann Instruments, Inc.
Camag, Inc.
Chromatographic Specialties
Crescent Chemical Co.
E. Merck
EM Science, Inc.
Fluka Chemical Corp.
J & S Scientific
Lachat Chemicals, Inc.
Munhall Company
Rainin Instrument Co.
Schleicher & Schuell, Inc.
Supelco, SA
Universal Scientific, Inc.

TLC PLATES, PREPARATIVE (D5)

Ace Scientific Supply Co.
Alltech Associates, Inc.
Anspec Company
Brinkmann Instruments, Inc.
Camag, Inc.
Chromatographic Specialties
Crescent Chemical Co.
EM Science, Inc.
Fluka Chemical Corp.
J. T. Baker, Inc.
Pierce Chemical Co.
Rainin Instrument Co.
Universal Scientific, Inc.
Alltech Associates UK Ltd.
Analtech, Inc.
Applied Analytical Ind./Camag TLC
Camag, GmbH
Carolina Biol. Supply Co.
Chromatography Services, Ltd.
E. Merck
Fisher Scientific Co.
Foxboro/Analabs
Macherey-Nagel GmbH
Rainin Instrument Co.
Touzart & Matignon
Whatman, Inc.

TLC QUANTITATION DEVICES (D4)

Applied Analytical Ind./Camag TLC
Camag, Inc.
Farrand Optical Co., Inc.
Kontes Glass Co.
Kratos GmbH
Kratos SA
Marco Scientific, Inc.
Shimadzu Europa GmbH
Universal Scientific, Inc.
Camag, GmbH
Chromatography Services, Ltd.
Gelman Sciences, Inc.
Kratos Anal. Instr. Co.
Kratos Ltd.
Lachat Chemicals, Inc.
N & R Scientific Co., Inc.
Shimadzu Scientific Instr., Inc.
UVP, Inc.

TLC SPOTTERS, STREAKERS (D3)

Alltech Associates, Inc.
Analytichem Internat'l
Camag, GmbH
Chromatography Services, Ltd.
Drummond Scientific Co.
EM Science, Inc.
Foxboro/Analabs
J. T. Baker, Inc.
Rainin Instrument Co.
Shandon Southern Instruments
Universal Scientific, Inc.
Analtech, Inc.
Applied Analytical Ind./Camag TLC
Camag, Inc.
Desaga, GmbH
E. Merck
Fisher Scientific Co.
Hamilton Company
Kontes Glass Co.
Rainin Instrument Co.
Shandon Southern Products
Whatman, Inc.

TLC SPREADERS (D6)

Alltech Associates, Inc.
Applied Analytical Ind./Camag TLC
Camag, Inc.
Desaga, GmbH
Shandon Southern Instruments
Touzart & Matignon
Anspec Company
Camag, GmbH
Chromatography Services, Ltd.
N & R Scientific Co., Inc.
Shandon Southern Products
Whatman, Inc.

TLC VISUALIZATION DEVICES (D9)

Berthold Instruments, Inc.
Spectronics Corp.
Berthold, Lab. of Prof. Dr.

TLC VISUALIZATION REAGENTS (D8)

Ace Scientific Supply Co.
Applied Analytical Ind./Camag TLC
Camag, GmbH
Carolina Biol. Supply Co.
Chromatography Services, Ltd.
Eastman Kodak Co.
Fisher Scientific Co.
J. T. Baker, Inc.
Supelco, Inc.
Touzart & Matignon
Alltech Associates, Inc.
BDH Chemicals, Inc.
Camag, Inc.
Chromatographic Specialties
E. Merck
EM Science, Inc.
Foxboro/Analabs
Pierce Chemical Co.
Supelco, SA
Whatman, Inc.

TUBING, FITTINGS, VALVES (C1)

A. S. I.
Ace Scientific Supply Co.
Alltech Associates, Inc.
Applied Chromatography Systems
Bio-Rad Laboratories
Cecil Instruments Ltd.
Chromatographic Specialties
Chrompack, Inc.
Dionex (UK), Ltd.
DuPont SA
E. Merck
ABC Laboratories
Alltech Associates UK Ltd.
Applied Automation, Inc.
Beckman Instruments, Inc.
Cajon Co.
Chromatix, Inc.
Chromatography Services, Ltd.
Chrompack, Inc.
Dionex Corp.
E. I. duPont de Nemours & Co.
ES Industries

Fisher Scientific Co.
Hamilton Company
Hewlett-Packard SA
HPLC Technology - Phenomenex
IBM Instruments, Inc.
Jones Chromatography, Inc.
Kontron AG
Kontron Instruments Ltd.
LDC/Milton Roy Co.
LKB Produkter AB
Munhall Company
Owens Polyscience Ltd
Perkin-Elmer GmbH
Pharmacia Fine Chemicals AB
Pierce Chemical Co.
Rheodyne, Inc.
Shandon Southern Instruments
Spectra Physics, Inc.
Supelco, SA
Unimetrics Corp.
Vacumetrics, Inc.
Varex Corp.
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
Foxboro/Analabs
HETP
Hewlett-Packard, Inc.
HPLC Technology, Ltd.
Jasco International Co., Ltd.
Jones Chromatography, Ltd.
Kontron Electronics, Inc.
LC Company, Inc.
LKB Instruments, Inc.
Macherey-Nagel GmbH
Nihon Waters Ltd.
Perkin-Elmer Corp.
Perkin-Elmer Ltd.
Pharmacia, Inc.
Pye Unicam, Ltd.
Scientific Systems, Inc.
Shandon Southern Products
Supelco, Inc.
Tracor Instruments, Inc.
Upchurch Scientific, Inc.
Valco Instruments, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA

WATER PURIFIERS (B4)

Alltech Associates, Inc.
Barnstead Co.
Chromatographic Specialties
Fisher Scientific Co.
Gelman Sciences, Inc.
Munhall Company
Pierce Chemical Co.
Rainin Instrument Co.
Supelco, SA
Waters AB
Waters Assoc. AS
Waters Assoc., NV
Waters BV
Waters GmbH
Waters Scientific Ltd.
American Scientific Products
Carolina Biol. Supply Co.
E. Merck
Foxboro/Analabs
J & S Scientific
Nihon Waters Ltd.
Rainin Instrument Co.
Supelco, Inc.
Varian Associates, Inc.
Waters Assoc (Inst.) Ltd
Waters Assoc. Pty. Ltd.
Waters Associates, Inc.
Waters Espanola, S.A.
Waters SA

INSTRUCTIONS FOR PREPARATION OF MANUSCRIPTS FOR DIRECT REPRODUCTION

Journal of Liquid Chromatography is a bimonthly publication in the English language for the rapid communication of liquid chromatographic research.

Directions for Submission

One typewritten manuscript suitable for direct reproduction, carefully inserted in a folder, and two (2) copies of the manuscript must be submitted. Since all contributions are reproduced by direct photography of the manuscripts, the typing and format instructions must be strictly adhered to. Noncompliance will result in return of the manuscript to the authors and delay its publication. To avoid creasing, manuscripts should be placed between heavy cardboards and securely bound before mailing.

Manuscripts should be mailed to the Editor:

Dr. Jack Cazes
Journal of Liquid Chromatography
P. O. Box 1440-SMS
Fairfield, Connecticut 06430

Reprints

Owing to the short production time for articles in this journal, it is essential to indicate the number of reprints required upon notification of acceptance of the manuscript. Reprints are available in quantities of 100 and multiples thereof. For orders of 100 or more reprints, twenty (20) free copies are provided. A reprint order form and price list will be sent to the author with the notification of acceptance of the manuscript.

Format of Manuscript

1. The general format of the manuscript should be as follows: title of article; names and addresses of authors; abstract; and text discussion.

2. Title and Authors: The entire title should be in capital letters and centered on the width of the typing area at least 2 inches (5.1 cm) from the top of the page. This should be followed by three lines of space and then by the names and addresses of the authors in the following way (also centered):

A SEMI-AUTOMATIC TECHNIQUE FOR THE SEPARATION AND DETERMINATION OF BARIUM AND STRONTIUM IN SURFACE WATERS BY ION EXCHANGE CHROMATOGRAPHY AND ATOMIC EMISSION SPECTROMETRY

F. D. Pierce and H. R. Brown
Utah Biomedical Test Laboratory
520 Wakra Way
Salt Lake City, Utah 84108

3. Abstract: Three lines below the addresses, the title ABSTRACT should be typed (capitalized and centered on the page). This should be followed by a single-spaced, concise, abstract comprising less than 10% of the length of the text of the article. Allow three lines of space below the abstract before beginning the article itself.

4. Text Discussion: Whenever possible, the text discussion should be divided into such major sections as INTRODUCTION, MATERIALS, METHODS, RESULTS, DISCUSSION, ACKNOWLEDGMENTS, and REFERENCES. These major headings should be separated from the text by two lines of space above and one line of space below. Each heading should be in capital letters, centered, and underlined. Secondary headings, if any, should be flush with the left margin, underscored, and have the first letter of all main words capitalized. Leave two lines of space above and one line of space below secondary headings.

5. Paragraphs should be indented five (5) typewriter spaces.

6. Acknowledgment of collaboration, sources of research funds, and address changes for an author should be listed in a separate section at the end of the paper.

7. References (including footnotes) in the text will be numbered consecutively by numbers in parentheses. All references (and footnotes) should then be aggregated in sequence at the end of the communication. No footnotes should be shown at the bottom of pages. The reference list follows immediately after the text. The word REFERENCES should be capitalized and centered above the reference list. It should be noted that all reference lists should contain initials and names of all authors; *et al.* will not be used in reference lists. Abbreviations of journal titles and styles of reference lists will follow the American Chemical Society's Chemical Abstracts List of Periodicals. References should be typed single-spaced with one line space between each reference.

8. Each page of manuscript should be numbered lightly at the bottom of the sheet with a light blue pencil.

9. Only standard symbols and nomenclature approved by the International Union of Pure and Applied Chemistry should be used.

10. Any material that cannot be typed, such as Greek letters, script letters, and structural formulae, should be drawn carefully in black India ink (do not use blue ink).

Typing Instructions

1. The manuscript must be typewritten on good quality white bond paper measuring approximately 8½ x 11 inches (21.6 cm x 27.9 cm). Do not use Corrasible bond or its equivalent. The typing area of the article opening page, including the title, should be 5½ inches wide by 7 inches deep (14 cm x 18 cm). The typing area of all other pages should be no more than 5½ inches wide by 8½ inches deep (14 cm x 21.6 cm).

2. In general, the chapter title and the abstract, as well as the tables and references, are typed single-spaced. All other text discussion should be typed 1½-line spaced, if available, or double-spaced. Prestige elite characters (12 per inch) are recommended, if available.

3. It is essential to use black typewriter ribbon (carbon film is preferred) in good condition so that a clean, clear impression of the letters is obtained. Erasure marks, smudges, creases, etc., may result in return of the manuscript to the authors for retyping.

4. Tables should be typed as part of the text but in such a way as to separate them from the text by a three-line space at both top and bottom of each table. Tables should be inserted in the text as close to the point of reference as possible, but authors must make sure that one table does not run over to the next page, that is, no table may exceed one page. The word TABLE (capitalized and followed by an Arabic number) should precede the table and be centered on the page. The table title should have the first letters of all main words in capitals. Titles should be typed single-spaced. Use the full width of the type page for the table title.

5. Drawings, graphs, and other numbered figures should be professionally drawn in black India ink (do not use blue ink) on separate sheets of white paper and placed at the end of text. Figures should not be placed within the body of the text. They should be sized to fit within the width and/or height of the type page, including any legend, label, or number associated with them. Photographs should be glossy prints. A typewriter or lettering set should be used for all labels on the figures or photographs; they may not be hand drawn. Captions for the pictures should be typed single-spaced on a separate sheet, along the full width of the type page, and preceded by the word FIGURE and a number in arabic numerals. All figures and lettering must be of a size to remain legible after a 20% reduction from original size. Figure numbers, name of senior author, and arrow indicating "top" should be written in light blue pencil on the back or typed on a gummed label, which should be attached to the back of the illustration. Indicate approximate placement of the illustrations in the text by a marginal note in light blue pencil.

6. The reference list should be typed single-spaced although separated from one another by an extra line of space. Use Chemical Abstract abbreviations for journal titles. References to journal articles should include (1) the last name of all author(s) to any one paper, followed by their initials, (2) article title, (3) journal, (4) volume number (underlined), (5) first page, and (6) year, in that order. Books should be cited similarly and include (1) author, surname, first and middle initials, (2) title of book, (3) editor of book (if applicable), (4) edition of book (if any), (5) publisher, (6) city of publication, (7) year of publication, and (8) page reference (if applicable). E.g., Journals: Craig, L. C. and Konigsber, W., Use of Catechol Oxygenase and Determination of Catechol, Chromatogr., 10, 421, 1963. Books: Albertsson, P. A., Partition of Cell Particles and Macromolecules, Wiley, New York, 1960. Article in a Book: Walter, H., Proceedings of the Protides of Biological Fluids, XVth Colloquim, Pteeters., H., eds.. Elsevier, Amsterdam, 1968, p. 367.

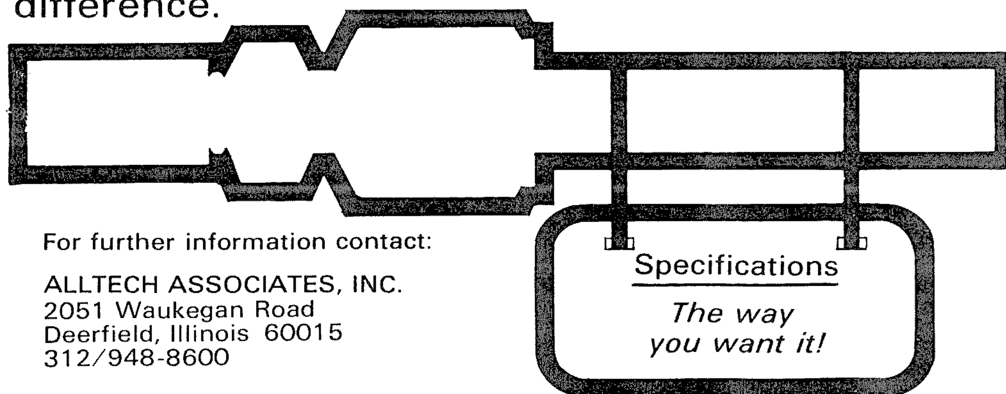
Each one is our special concern

CUSTOM PACKED HPLC COLUMNS

Custom packing HPLC columns has become our specialty. Any length, several ID's (including 3.2mm) and almost any commercially available packing material may be specified. We'll supply the column others won't.

With each column, you will receive the original test chromatogram plus a vial of the test mixture. Our advanced technology and computer testing is your assurance of a quality product.

When custom packing and testing is your special concern, we make the difference.



For further information contact:

ALLTECH ASSOCIATES, INC.
2051 Waukegan Road
Deerfield, Illinois 60015
312/948-8600

ALLTECH ASSOCIATES

Circle Reader Service Card No. 102