GLC–Mass Spectrometric Determination of Maprotiline and Its Major Metabolite Using Stable Isotope-Labeled Analog as Internal Standard

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Abstract □ A quantitative GLC–mass spectrometric assay was developed for the determination of maprotiline and its major metabolite, desmethylmaprotiline, in animal and human plasma. The assay utilizes selective-ion focusing to monitor, in a GLC effluent, the fragment ions and the base peaks of maprotiline and desmethylmaprotiline trifluoroacetamides generated by electron-impact ionization. Maprotiline-d_3 was the internal standard. The assay can measure 2 ng of maprotiline (and the metabolite)/ml of plasma with ~5% precision. The curves relating the amounts of maprotiline and the metabolite added *versus* the amounts experimentally found over a large concentration range were linear with nearly zero intercepts and slopes of 0.99 ± 0.01 and 0.98 ± 0.02, respectively. The method was used to study the pharmacokinetic pattern of the drug in rabbits as well as to analyze intact maprotiline and the metabolite in patients maintained on therapeutic doses of maprotiline. Assay specificity was confirmed by complete consistency of the mass spectra of maprotiline and desmethylmaprotiline with those of the authentic materials.

Keyphrases □ Maprotiline—GLC–mass spectrometric analysis, human and animal plasma □ Desmethylmaprotiline—GLC–mass spectrometric analysis, human and animal plasma □ GLC–mass spectrometry—stable isotope-labeled maprotiline, selected-ion monitoring, pharmacokinetics □ Antidepressants—maprotiline, GLC–mass spectrometric analysis, human and animal plasma

Maprotiline, 1-(3-methylaminopropyl)dibenzo[*b,e*]-bicyclo[2,2,2]octadiene[1,2], is a tetracyclic drug with sedative, antiaggressive, and antidepressant effects. In contrast to the tricyclic antidepressants, it has no influence on the central metabolism of 5-hydroxytryptamine and has weak anticholinergic actions (1). As an antidepressant, it has shown equal therapeutic efficacy but fewer side effects compared to tricyclic antidepressants (2–4); it also is less toxic (5, 6) and has less of an effect on the cardiovascular system (7, 8). Maprotiline has been used in Europe for the treatment of patients who had severe side effects with other antidepressants and for depressed patients with cardiovascular symptomatology. It is under evaluation for clinical use in the United States.

A precise and specific assay for maprotiline and its major metabolites in biological fluids and tissues is needed. Assays based on the double radioisotope derivative technique (9), electron-capture detection (10), and specific nitrogen detection (11) have been reported. This report describes a GLC–mass spectrometric assay for the intact drug and its major metabolite, desmethylmaprotiline, in the plasma of rabbits and humans.

GLC–mass spectrometry in the selected-ion monitoring (12–14) mode was used to quantitate plasma levels of the drug and its major metabolite, *N*-desmethylmaprotiline, in rabbits after maprotiline administration. The methodology also was applied to measure steady-state levels of the free drug and the metabolite in the plasma of patients on therapeutic doses. Stable isotope-labeled maprotiline, maprotiline-d_3 ([*N*-*methyl*-^{3}H]maprotiline), was synthesized and used as an internal standard for the estimation of both maprotiline and desmethylmaprotiline.

EXPERIMENTAL

Materials—Analytical grade maprotiline hydrochloride[1], desmethylmaprotiline methanesulfonate[2], trifluoroacetic anhydride[3], ethyl chloroformate[3], and lithium aluminum deuteride[4] were used without further purification. All solvents were analytical grade[5]. Silanized tubes[6] (10 ml) with screw caps[7] were used for extraction; final solvent evaporation was performed in 5-ml glass-stoppered centrifuge tubes[8]. Pasteur pipets with hand-drawn constricted tips were utilized for all solution transfers. Plasma samples were stored frozen at −10° until they were analyzed.

Maprotiline-d_3—Desmethylmaprotiline, upon treatment with ethyl chlorocarbonate, gave the corresponding carbonate (15) in a 78% yield. The carbonate was reduced with lithium aluminum deuteride in tetrahydrofuran to maprotiline-d_3 (16). The material showed satisfactory mass spectral (electron-impact ionization) characteristics. A selective-ion detection analysis of maprotiline-d_3 showed the presence of an ion equivalent to 99 ± 0.2% (n = 5) maprotiline-d_3.

Extraction from Plasma—To 1 ml of plasma was added an appropriate amount of maprotiline-d_3 (typically 70 ng/ml) as an internal standard. The plasma was diluted to 5 ml with borate buffer and sonicated for 0.5 min while keeping the temperature at ~6°. The homogenized material was adjusted to pH 9.5 with 0.1 *N* NH_4OH and extracted twice with 5 ml of benzene. The organic fractions were combined, 1 ml of 0.1 *N* HCl was added, and the solution was shaken for 15 min and centrifuged.

The organic layer was discarded, and the aqueous phase was adjusted to pH 9.5 with 1 *N* NH_4OH and extracted twice with 3 ml of benzene. The organic fractions were combined and dried, and the solvent was evaporated at 50° under a gentle nitrogen stream. Recovery of maprotiline and desmethylmaprotiline added to control plasma was studied at the 10-ng/ml level.

Formation of Derivatives—The dried extract was taken up in 100 μl of ethyl acetate, and 50 μl of trifluoroacetic anhydride was added. The material then was heated in an oil bath at 80° for 45 min. The solution was cooled to room temperature, and the solvents were evaporated at 40° under a gentle nitrogen stream. The dried extract was reconstituted in 50 μl of chloroform, and ~1 μl was injected into the gas–liquid chromograph–mass spectrometer for mass fragmentographic assay.

Instrumentation—The magnetic sector, single-focusing mass spectrometer[9] was interfaced with a gas chromatograph and equipped with a multiple-ion detector–peak matcher accessory (14, 17). GLC was performed on a 1.8-m × 2-mm i.d. glass column silanized with 5% dimethyldichlorosilane in toluene and packed with 1% OV-17 on 100–200-mesh Gas Chrom Q. The column was conditioned for 24 hr at 280° with a flow rate of 20 ml of helium/min. The column temperature was 225°, the flash heater was at 240°, the separator was at 250°, and the ion source was at 275°.

The accelerating voltage was 3.5 kv in the scan mode and 3.0 kv in the multiple-ion detection mode, the ionization potential was 70 ev, and the trap current was set at 60 μamp. The magnetic field was kept constant

[1] Ciba 34,276-Ba, Ludiomil, Ciba-Geigy AG, Basel, Switzerland.
[2] Kindly provided by Dr. W. Riess of Ciba-Geigy, Basel, Switzerland.
[3] Aldrich Chemical Co., Milwaukee, Wis.
[4] Isotopic purity of 99 atom % deuterium, Merck Sharp & Dohme, Canada Ltd., Montreal, Quebec, Canada.
[5] Fisher Scientific Co., Pittsburg, Pa.
[6] Kimble Owens, Illinois, Toledo, Ohio.
[7] Lined with Teflon, DuPont.
[8] Pyrex 8084.
[9] LKB 9000, LKB, Stockholm, Sweden.

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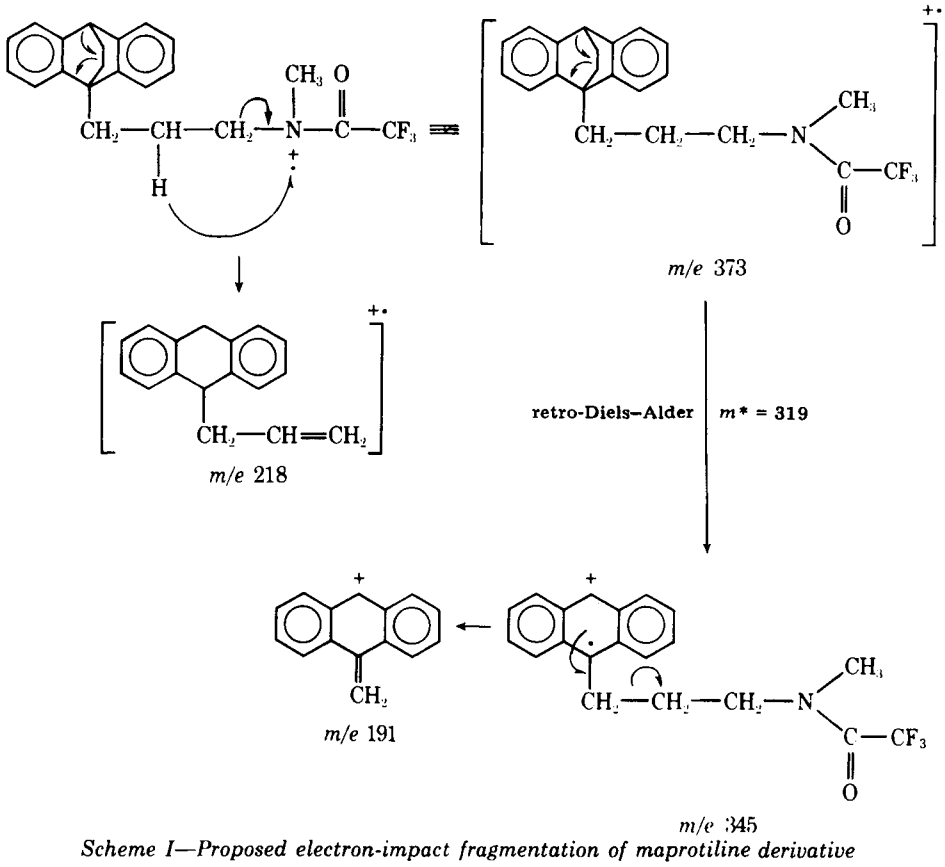

Scheme I—Proposed electron-impact fragmentation of maprotiline derivative

by focusing the background ion (column bleed) at *m/e* 355, and the additional voltages were 752, 600, and 569 v for measuring ion intensities at *m/e* 331, 345, and 348, respectively. The retention times of desmethylmaprotiline and maprotiline trifluoroacetamides were 2.0 and 3.0 min, respectively.

Experiments with Animals—Experiments were performed on rabbits to determine the time course of maprotiline and desmethylmaprotiline in plasma after the intravenous administration of varying drug doses. In a typical experiment, a 6-kg rabbit was given a single intravenous dose of 2.7 mg of maprotiline hydrochloride in water.

Blood samples were drawn and collected in heparinized tubes. The plasma was prepared as usual. An appropriate amount of the internal standard (typically 70 ng) was added to the plasma samples (1.0 ml), and the samples were processed as described. The amounts of endogenous maprotiline and desmethylmaprotiline were calculated from the ratio of ion intensities at *m/e* 345, 331, and 348, respectively.

Human Studies—Plasma of several patients on a normal therapeutic dose of maprotiline was analyzed for maprotiline and the metabolite. An appropriate amount of the internal standard was added to the plasma samples (0.25 ml), and the samples were processed as described. The amounts of endogenous maprotiline and desmethylmaprotiline were calculated from the ratio of ion intensities at *m/e* 345, 331, and 348, respectively.

RESULTS AND DISCUSSION

The mass spectrum of maprotiline trifluoroacetamide (Table I) shows a molecular ion at *m/e* 373, a base peak at *m/e* 345, an intense peak at *m/e* 191, and a peak of modest intensity at *m/e* 218. A reasonable mechanism for the fragmentation is presented in Scheme I. The molecular ion underwent a typical retro-Diels–Alder process (18, 19) to give a radical ion at *m/e* 345, the base peak. The driving force for this reaction must be the expulsion of a stable neutral ethylene molecule and the formation of an unusually stable radical ion at *m/e* 345. Since both the radical site and the cationic site are benzylic in this ion, obviously this very stable ion results in a base peak at *m/e* 345.

This interpretation is substantiated further by the presence of a metastable ion at *m/e* 319. The mass spectrum of maprotiline-d_3 trifluoroacetamide (Table I) confirms this interpretation, since in this case the molecular ion and the base peak, as expected, are shifted to a higher mass by 3 amu. The mass spectrum of desmethylmaprotiline trifluoroacetamide (Table I) shows a molecular ion at *m/e* 359, a base peak at *m/e* 331 as a result of a retro-Diels–Alder process, and a metastable ion at *m/e* 305. The ions at *m/e* 218 and 191 are the common fragment ions appearing in the spectra of maprotiline, maprotiline-d_3, and desmethylmaprotiline trifluoroacetamides.

GLC–Mass Spectrometric Quantitation—The ions at *m/e* 345 and 331 are specific for maprotiline and desmethylmaprotiline (*m/e* 348 for maprotiline-d_3) trifluoroacetamides and are not observed in the electron-impact ionization spectra of other tricyclic antidepressants (20, 21) and known metabolites of maprotiline (22). Consequently, the biological extract along with maprotiline-d_3 was treated with trifluoroacetic anhydride at 80°, the excess reagents were evaporated, and an aliquot of the reconstituted solution was injected into the gas–liquid chromatograph–mass spectrometer; maprotiline was quantitated by measuring the ion intensities at *m/e* 345 and 348 while desmethylmaprotiline was assayed by measuring the ion intensities at *m/e* 331 and 348.

Control plasma samples subjected to the described procedure showed no significant background ions at *m/e* 345, 348, and 331. Known amounts of maprotiline and desmethylmaprotiline and a fixed amount of the isotopic analog (maprotiline-d_3) were added to control plasma and processed as described. Maprotiline and desmethylmaprotiline were quantitated from the ratios of ion intensities at *m/e* 345/348 and 331/348, respectively. Analysis of the data for maprotiline (Fig. 1) gave a slope of 0.99 ± 0.01 and an intercept of 0.15 ± 0.1 ng. Similarly, the data for

Table I—Mass Spectral Analysis [a] of Trifluoroacetamides of Maprotiline, Maprotiline-d_3, and Desmethylmaprotiline

Presumed Fragmentation	*m/e* [b] Maprotiline	Maprotiline-d_3	Desmethylmaprotiline
M	373 (6)	376 (6)	359 (4)
M − 28	345 (100)	348 (100)	331 (100)
M − 28 part side chain	218 (11)	218 (11)	218 (6)
M − 28 side chain	191 (82)	191 (82)	191 (82)

[a] Only common major fragments are presented. [b] The values in parentheses represent the percent relative intensity.

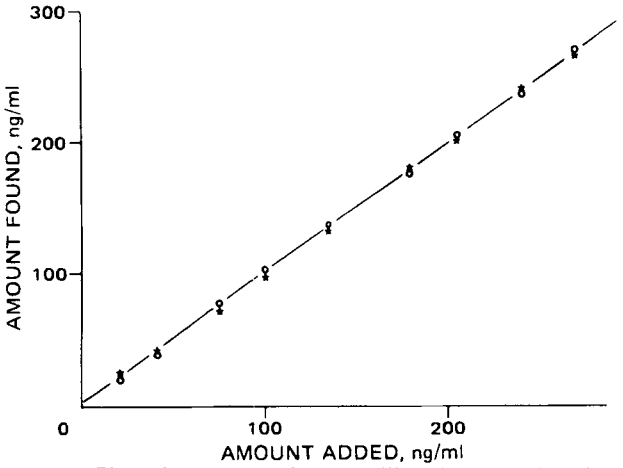

Figure 1—*Plot of amounts of maprotiline (0–285 ng) (O) and desmethylmaprotiline (0–285 ng) (×) added to control plasma versus the amounts found using the described method. Maprotiline-d_3 (70 ng/ml) was added as the internal standard.*

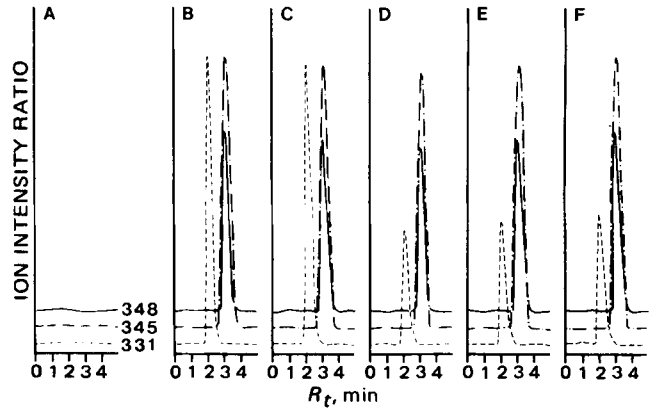

Figure 2—*Selective-ion chromatograms for maprotiline (m/e 345), desmethylmaprotiline (m/e 331), and maprotiline-d_3 (m/e 348). Key: A, control human plasma; B, control human plasma (1 ml) spiked with 10 ng each of maprotiline and desmethylmaprotiline and 7 ng of maprotiline-d_3; C, control rabbit plasma (1 ml) spiked as in B; and D, E, and F, typical rabbit and human plasma.*

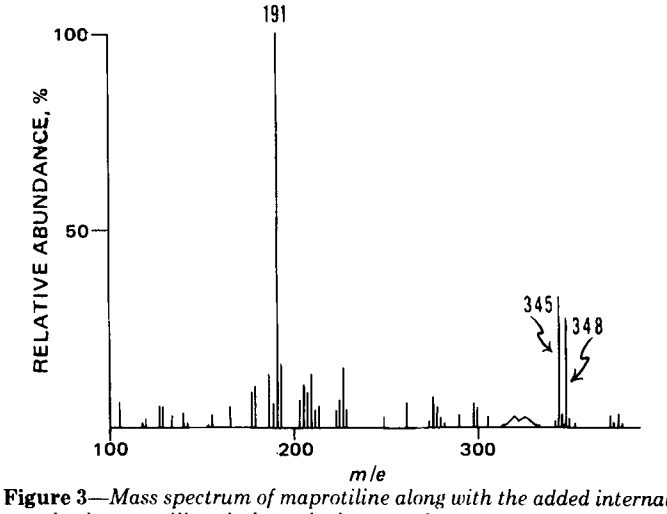

Figure 3—*Mass spectrum of maprotiline along with the added internal standard maprotiline-d_3 from the human plasma extract.*

desmethylmaprotiline (Fig. 1) gave a slope of 0.98 ± 0.01 and an intercept of 0.2 ± 0.11 ng. These data affirm a simple linear relationship between the ion intensity ratios and concentrations of maprotiline and desmethylmaprotiline and exclude any isotopic fractionation in the fragmentation process as well as in the physicochemical steps in the assay.

Six samples containing 10 ng each of maprotiline and desmethylmaprotiline/ml were analyzed using 7.0 ng of maprotiline-d_3/ml as the internal standard. The results were 9.91 ± 0.15 ng/ml for maprotiline and 9.87 ± 0.16 ng/ml for desmethylmaprotiline. These samples were assayed in duplicate; in this set, exactly the same amounts were taken as before, but the internal standard was added after the extraction. The recoveries for these samples, based on comparison of the ion intensity ratios of the two sets, were 82 ± 7% for maprotiline (*m/e* 345/348) and 85 ± 6% for desmethylmaprotiline (*m/e* 331/348). The variability in recoveries is to be expected in trace analysis and is attributed to variable glassware and GLC column adsorption.

Table II shows the concentration of intact drug and its major metabolite in rabbit plasma after a single intravenous dose. The decline of maprotiline concentration in plasma exhibited a typical fast absorption phase (α) followed by a slow disposition phase (β), while desmethylmaprotiline peaked out at ~50 hr after maprotiline administration.

The plasma levels of maprotiline and the metabolite varied considerably in different patients maintained on a normal therapeutic dose. More extensive data are needed for assessment of steady-state concentrations of these materials in human plasma.

Sensitivity and Specificity—The assay for maprotiline and its major metabolite presented here is sensitive, specific, and applicable to other body fluids and tissues. The assay sensitivity is a function of extraction efficiencies, GLC column conditions, and the ion source and cannot be quoted in absolute terms. With good mass spectrometer performance, a clean ion source, a freshly silanized GLC column and glassware, and >50% recoveries, an assay sensitivity of ~2 ng each of maprotiline and desmethylmaprotiline/ml of plasma is possible.

The results from the control plasma and plasma of patients show good specificity of the assay. The mass chromatograms obtained from biological extracts (Fig. 2) show clean and symmetrical peaks. Furthermore, these biological extracts were pure enough so that the entire mass spectra of maprotiline and desmethylmaprotiline trifluoroacetamides could be recorded. The mass spectrum of maprotiline trifluoroacetamide with added maprotiline-d_3 (Fig. 3) shows expected doublets at *m/e* 345–348. Also significant was a singlet of increased relative ion intensity at *m/e* 191, a common fragment ion from the two isotopic species. The mass spectrum of desmethylmaprotiline trifluoroacetamide in the biological extract was identical to that of the authentic material.

Table II—Analyses [a] of Maprotiline and Desmethylmaprotiline in the Plasma of Rabbits and Patients on Maprotiline Therapy

Sample	Hours	Desmethylmaprotiline	Maprotiline
		Rabbit Plasma[b], ng/ml	
1	1.25	6.3 ± 0.34	2320.0 ± 22
2	2.25	27.4 ± 1.1	1096.0 ± 13
3	5	46.0 ± 1.3	95.2 ± 2.8
4	18	120.0 ± 2.2	38.6 ± 2.6
5	48	280.0 ± 4.5	30.2 ± 2.1
6	68	35.4 ± 1.7	17.6 ± 0.9
7	97	14.0 ± 0.61	9.8 ± 0.28
		Human Plasma[c], ng/ml	
Patient 1		179.6 ± 3.1	1558.3 ± 14
Patient 2		88.1 ± 2.8	1160.6 ± 12
Patient 3		179.2 ± 3.4	677.6 ± 7.2
Patient 4		140.2 ± 3.3	353.4 ± 4.9

[a] Mean of duplicate determinations. [b] Analysis of maprotiline and desmethylmaprotiline in rabbits as a function of time after the administration of a single dose. [c] Steady-state plasma levels of maprotiline and desmethylmaprotiline in patients.

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Identification and Analysis of Persistent Contaminants Associated with Gemcadiol

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Abstract □ Application of several analytical probes indicated that certain batches of the investigational new drug gemcadiol (2,2,9,9-tetramethyl-1,10-decanediol) were contaminated with impurities whose nature and source were unknown. Subsequent study showed that these impurities consisted of polymeric material formed by self-condensation of the dialdehyde precursor. Gel permeation chromatographic techniques were found to be useful in the analysis of polymers present in both the aldehyde precursor and the finished drug.

Keyphrases □ Gemcadiol—identification and analysis of persistent contaminants □ Drug impurities—gemcadiol, identification and analysis of persistent contaminants □ Cholesterol-lowering agents, potential—gemcadiol, identification and analysis of persistent contaminants

Gemcadiol (2,2,9,9-tetramethyl-1,10-decanediol, I) is an investigational new drug that is being evaluated as a triglyceride- and cholesterol-lowering agent (1). This drug may be synthesized by a process whose last step involves sodium borohydride reduction of 2,2,9,9-tetramethyl-1,10-decanedial (II) (2).

When various batches of I were analyzed, it became apparent that significant interbatch variation existed for which the cause was not known. This study was conducted to determine the reason for the observed inconsistencies and to provide a more definitive method of analysis.

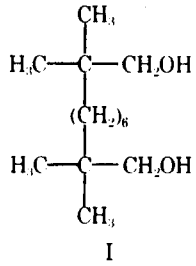
EXPERIMENTAL

Reagents—The solvents were analytical reagent grade. The sodium borohydride[1] was used as received.

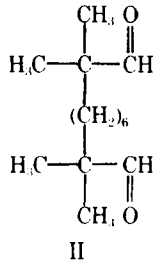
Apparatus—IR spectra were obtained using a grating IR spectrophotometer[2]. GLC was done on a programmable research chromatograph[3] equipped with a flame-ionization detector and an electronic integrator[4]. NMR spectra were obtained on a 90-MHz instrument[5] with deuterochloroform as the solvent and tetramethylsilane as the internal standard.

GLC Studies—GLC studies were performed using 183 × 0.64-cm stainless steel columns packed with 5% silicone gum rubber[6] on silanized diatomaceous earth[7]. Chromatograms were obtained under isothermal conditions (225°) after injection of 2 μl of a 10% solution of the sample in methanol. In certain cases, the solution injected was acidified by adding 1 drop of 3 *N* HCl to 1 ml of the methanolic sample solution.

Liquid Chromatography of I—Liquid chromatographic separation of the components present in impure samples of I was accomplished using modified dextran beads[8] packed in a 40 × 3-cm glass chromatographic column. Methanol (25% v/v) in benzene was the mobile phase. The column was charged with a sample of ~500 mg dissolved in 5 ml of the mobile



I



II

[1] Alfa Inorganics, Ventron Corp.

[2] Model 457, Perkin-Elmer Corp.
[3] Model 5750, F & M Scientific Corp.
[4] Model 3370A, Hewlett-Packard Corp.
[5] Bruker WH 90.
[6] OV-17, Hewlett-Packard.
[7] Chromasorb G, Hewlett-Packard.
[8] Sephadex LH-20, Pharmacia Fine Chemicals.

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