Determination of Trimipramine and Metabolites in Plasma by Liquid Chromatography with Electrochemical Detection

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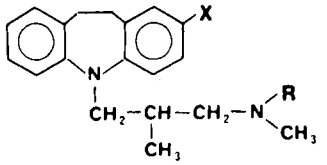
Abstract □ A procedure for the determination of trimipramine, the demethyl, 2-hydroxy, and 2-hydroxy demethyl metabolites in plasma by liquid chromatography with electrochemical detection is described. A 1-mL plasma sample is made alkaline with a carbonate buffer (pH 9.8) and extracted with 20% ethyl acetate in *n*-heptane. After back-extraction into an acid phosphate buffer, an aliquot is injected onto a reverse-phase trimethylsilyl-packed column and eluted with a phosphate buffer–acetonitrile mobile phase (65:35) containing *n*-butylamine. The peaks were detected at +1.1 V *versus* the silver–silver chloride reference electrode. The method provides absolute recoveries of 60–91% and a day-to-day precision of <9% for all compounds. The minimum quantifiable level for all compounds was 3 ng/mL. Steady-state plasma concentration data for 29 depressed patients receiving either 75 mg or 150 mg/d is reported.

Keyphrases □ Trimipramine—metabolite detection, HPLC, electrochemical detection, human plasma □ HPLC—electrochemical detection, trimipramine and its metabolites

Trimipramine (I) is a relatively recent addition to the structurally related dibenzazepine class of tricyclic antidepressants. It differs chemically from the prototype imipramine by a replacement of the *n*-propyl side chain with a 2-methyl propyl group. A review of its clinical use has been presented (1).

Trimipramine has been included in many tricyclic antidepressant methodologies either as an internal standard or for the purpose of demonstrating its chromatographic separation from other tricyclic antidepressants. Presently, there are two reports of its quantitation in plasma with respect to its therapeutic use. One method does not include its metabolites nor present any clinical data derived from its measurement (2). Another report (3) describes a GC procedure and its application to the pharmacokinetics of trimipramine only.

Since trimipramine is structurally very similar to imipramine one might expect its metabolic profile to be similar. We therefore developed an HPLC assay that will separate and quantitate I and its known major metabolites—the demethyl metabolite (II), the 2-hydroxy metabolite (III) and the 2-hydroxy demethyl metabolite (IV). Fixed-dose steady-state plasma concentration data for 29 patients are reported.



I: X = H, R = CH_3
II: X = H, R = H
III: X = OH, R = CH_3
IV: X = OH, R = H

EXPERIMENTAL SECTION

Apparatus and Reagents—Chromatography was performed using a dual piston solvent delivery pump[1] and an automatic sampler[2]. The column was 25 cm × 4.6 mm i.d. packed with 5-μm particle size trimethyl silyl material[3]. The detector system consisted of a thin-layer flow through electrochemical transducer[4]. The potential and current response were monitored by an amperometric controller[5] and recorder[6] and interfaced with a laboratory data acquisition system[7]. An aluminum column temperature control block was devised to fit the column and the temperature controlled by a circulating water bath[8]. Voltammograms were performed by a cyclic voltammetry instrument[9] and recorded on an X-Y recorder[10]. The potential was scanned from 0.0 to +1.3 V *versus* silver–silver chloride reference electrode at 180 mV/s. Glassy carbon was used as the working electrode.

Phosphoric acid[11], monobasic potassium phosphate[11], sodium carbonate[11], and potassium bicarbonate[11] were all analytical reagent grade. Acetonitrile-UV[12], *n*-heptane[11], *n*-butylamine[11], and β-glucuronidase[13] were used as received. Ethyl acetate[11] was purified through a silica-packed column prior to use. Distilled water was passed through a water purification system[14] before use.

Standards—One milligram (free base) per milliliter of stock solutions of trimipramine maleate (I)[15], the demethyl metabolite (maleate salt) (II)[15], the 2-hydroxy metabolite (fumarate salt) (III)[15], the 2-hydroxy demethyl metabolite (fumarate salt) (IV)[15], the didemethyl metabolite (maleate salt) (V)[15], and mianserin hydrochloride (VI)[16] were prepared in 0.01 M HCl. All stock solutions were further diluted with 0.01 M HCl to give working solutions of 1 μg/mL. Working standards of III and IV were prepared as 10 μg/mL for conjugate studies.

Standard curves were prepared containing five levels of spiked samples: 25, 50, 100, 200, and 400 ng/mL of I and II, and 12.5, 25, 50, 100, and 200 ng/mL of III and IV. For total III and IV, 100, 250, 500, 1000, and 2000 ng/mL spiked samples were prepared.

Extraction—The internal standard, mianserin (75 μL = 75 ng), and 0.5 mL of 0.6 M potassium bicarbonate–sodium carbonate buffer (pH 9.8) were added to 1.0 mL of plasma sample. Seven milliliters of 20% v/v ethyl acetate in *n*-heptane was added, the contents were mixed for 15 min and then centrifuged at 1500×*g* for 10 min. The organic layer was transferred to a 15-mL tapered centrifuge tube containing 0.25 mL of acidic phosphate buffer (0.025 M KH_2PO_4 adjusted to pH 2.5 with 85% H_3PO_4). After mixing for 10 min and centrifuging at 1500×*g* for 10 min, the organic layer was discarded and the aqueous portion was transferred to a small glass vial[17] and capped. Suitable aliquots were injected for chromatographic separation.

For the analysis of the 2-hydroxy and 2-hydroxy demethyl metabolites III and IV (total, free and conjugated), the pH of 0.5 mL of plasma was adjusted to 4.7 with the addition of 0.5 mL of 0.2 M KH_2PO_4. Then, 25 μL of β-glucuronidase solution was added to each tube and the mixture was incubated for 24 h at room temperature. Fifty microliters of 2 M NaOH, 50 μL (500 ng) of internal standard, mianserin, 0.5 mL of carbonate buffer, and 7 mL of 20% v/v ethyl acetate in *n*-heptane were added and the extraction was carried out as previously described. Standards containing III and IV were treated in the manner described above (including the incubation procedure).

[1] Model 6000A; Waters Associates, Milford, Mass.
[2] WISP 710B; Waters Associates.
[3] LC-1, Supelco; Bellefonte, Pa.
[4] TL-5A; Bioanalytical Systems, West Lafayette, Ind.
[5] Metrohm Model E-611 V/A Detector; Brinkmann Instruments Inc., Westbury, N.Y.
[6] Omniscribe Model B-5217B-2; Houston Instruments, Austin, Tex.
[7] PDP 11/34; Digital Equipment Corp., Maynard, Mass.
[8] Model FE; Haake Co., Saddlebrook, N.J.
[9] Model CV-1B; Bioanalytical Systems.
[10] Model 7034A; Hewlett Packard, Palo Alto, Calif.
[11] Fisher Scientific Co., Fair Lawn, N.J.
[12] Burdick-Jackson Laboratories, Muskegon, Mich.
[13] Glusulase; Endo Laboratories, Garden City, N.Y.
[14] Milli-Q; Millipore Corp., Bedford, Mass.
[15] Ives Laboratories Inc., New York, N.Y.
[16] Organon Laboratories, West Orange, N.J.
[17] Low Volume Inserts; Waters Associates.

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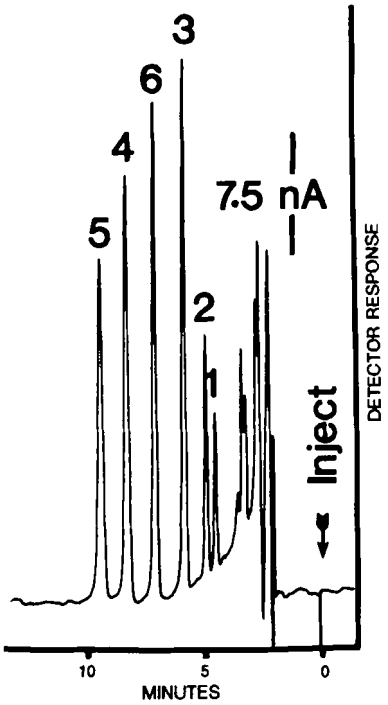

Figure 1—*A chromatogram of a spiked 1-mL plasma extract containing 25 ng each of III and IV (2 and 1) and 50 ng each of I, II, and V (5, 4, and 6). Actual patient samples showed little or no V (6). Mianserin (3) is the internal standard.*

Chromatographic Conditions—The mobile phase consisted of 0.05 M monobasic potassium phosphate-acetonitrile (65:35) with the addition of 1.2 mL/L of *n*-butylamine and 1.0 mL/L of phosphoric acid. The mixture was filtered and deaerated prior to use. The flow rate was 1.5 mL/min and the column temperature was set at 32°C. Detector potential was set at +1.10 V *versus* silver-silver chloride reference electrode.

Quantitation—All determinations were performed by calculating the peak height ratios of each compound to the internal standard. A linear regression analysis for each of the standard curves was performed by a computer program resulting in the calculations of slope, *x*-intercept, correlation coefficient, and standard error.

RESULTS AND DISCUSSION

Trimipramine (I) and its major metabolites (II, III, and IV) were separated in a single chromatogram in <10 min. Figure 1 illustrates a typical chromatogram of a spiked plasma sample. A blank plasma extract showed no endogenous interfering peaks.

The optimum oxidation potential of I–IV was determined by cyclic voltammetry. The presence of the 2-hydroxy groups on III and IV lowers the oxidation potential from that of the parent compound I. This profile, not surprisingly, resembles that of imipramine and its major analogous metabolites (4). Proposed mechanisms for the electrochemical oxidation of these compounds have been presented (5–7). The electrochemical oxidation profile for mianserin has been reported with the optimum being ~ +1.1 V *versus* the silver-silver chloride reference electrode (8).

The precision of this method was determined by spiking eight 1.0-mL aliquots of drug-free plasma with various levels of drug and metabolites. After the addition of 75 ng of internal standard, the samples were processed as described. The percent relative standard deviation for various levels are reported in Table I. Day-to-day accuracy was checked after runs over 7 consecutive days. The linear regression slopes as well as the quality control samples were assessed and are presented in Tables II and III.

Table I—Within-Run Variability of Trimipramine and Its Metabolites [a]

Concentration, ng/mL	*RSD*, %			
	I	II	III	IV
400	3.7	7.0		
200			2.1	4.4
100	1.6	3.7		
50			3.0	3.8
25	4.9	12.2		
20			3.7	5.2
5	5.1	9.6	10.4	12.1

[a] $n = 8$.

Table II—Linear Regression Data for the Standard Curve [a]

Compound	Slope, ng/mL	*x*-intercept, ng/mL	*r*
I	75.4 ± 2.58	1.63 ± 1.08	0.9999 ± 0.0001
II	63.1 ± 5.28	1.92 ± 4.22	0.9997 ± 0.0002
III	51.2 ± 3.28	1.72 ± 1.88	0.9995 ± 0.0005
IV	85.5 ± 7.47	1.21 ± 2.11	0.9992 ± 0.0015

[a] Data (mean ± *SD*) computed from seven consecutive standard curves.

Table III—Quality Control Data [a]

Compound	Amount added, ng/mL	Amount found, ng/mL	*RSD*, %
I	100	101	4.4
II	100	98	9.3
III	50	52	8.0
IV	50	51	6.5

[a] Data computed from quality control samples analyzed on seven consecutive days.

Table IV—Recovery of Trimipramine and Its Metabolites [a]

Compound	200 ng		25 ng	
	Percent	*RSD*, %	Percent	*RSD*, %
I	74	6.0	71	7.5
II	79	6.5	72	9.9
III	91	7.0	83	6.6
IV	60	6.4	59	5.7

[a] From 1 mL of plasma; $n = 8$.

The absolute recovery was determined by preparing a solution of known concentrations of all compounds, I–IV. The internal standard was added to each solution and the sample injected into the chromatograph. The same standards were added to 1 mL of plasma and processed routinely except for the internal standard. To the final acid extract, 75 ng of internal standard was added, mixed, and injected. The difference between the ratios of the standards to internal standard in processed sample *versus* the direct injection samples gave a measure of overall recovery (Table IV).

The absolute sensitivity ($S/n = 3$) of this method was 0.5-ng injected for all compounds (I–IV). In practical terms, however, the lowest quantifiable levels were ~3 ng/mL of plasma. Linearity was checked up to 500 ng.

Interfering peaks occurred in samples from patients receiving imipramine, desipramine, chlorimipramine, chlorpromazine, amoxapine, loxapine, trazodone, and haloperidol. Other tricyclic and tetracyclic antidepressants such as amitriptyline, nortriptyline, protriptyline, doxepin, and maprotiline do not interfere since they are not electroactive under these conditions. Some commonly used benzodiazepines (flurazepam, diazepam, and chlordiazepoxide) also do not interfere because they do not respond electrochemically at these conditions. This selectivity is one advantage of the electrochemical detector over a UV detector at the frequently used wavelength of 254 or 214 nm.

The extraction procedure was developed specifically for the simultaneous recovery of I–IV as well as VI. The pH of the plasma must be between 9–10 for optimum recovery of III and IV. The use of 20% v/v ethyl acetate in *n*-heptane was sufficiently polar enough to extract III and IV and at the same time did not cause variability in recoveries of I, II, and VI as was frequently encountered when using a 5–10% v/v isoamyl alcohol in *n*-heptane mixture or when using methyl *tert*-butyl ether.

When hydrolyzing plasma for the preparation of samples for the measurement of the total (conjugated and unconjugated) III and IV, an attempt was made to prevent decomposition of these hydroxy metabolites by the addition of an antioxidant. A previous report (9) indicated that the addition of ascorbic acid to the plasma hydrolysis buffer prevented a rapid decomposition of 2-hydroxydesipramine. We found this to be true not only for 2-hydroxydesipramine but for 2-hydroxyimipramine. However, the two hydroxy metabolites of trimipramine did not appear to be susceptible to this decomposition. Thus, no ascorbic acid was added to the hydrolysis buffer.

To check the validity of this assay, 100 samples were randomly selected and analyzed for I by GC with a nitrogen detector using a modified procedure developed initially for imipramine (10). The correlation coefficient between the two methods was 0.980, with HPLC = 0.942 (GC) −1.6.

Plasma samples from 29 different patients receiving either 75 or 150 mg/d

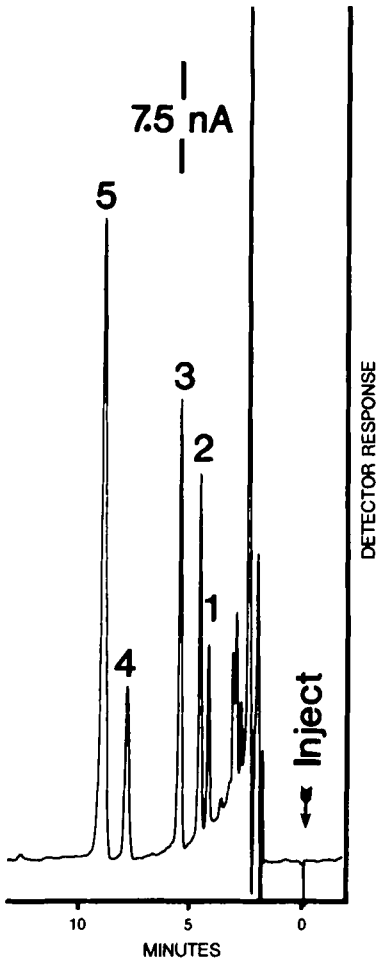

Figure 2—*A chromatogram of an extract of 1 mL of plasma from a patient receiving 150 mg/d of trimipramine. Concentrations were: I (5), 111 ng/mL; II (4), 25 ng/mL; III (2), 40 ng/mL; IV (1), 32 ng/mL.*

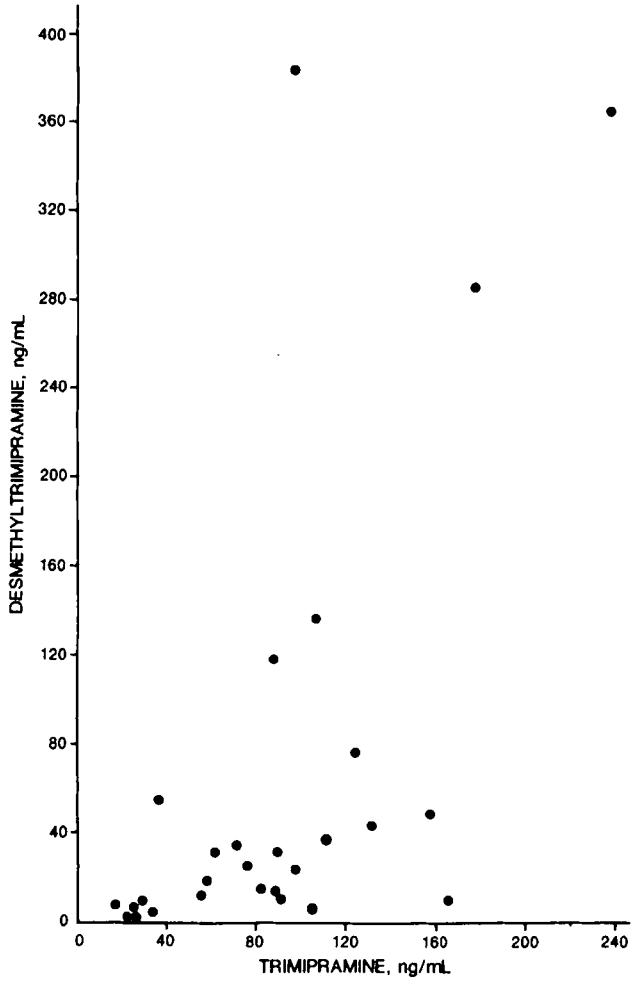

Figure 3—*Plasma concentrations of trimipramine I and the demethyl metabolite II in 28 patients. All patients were at steady state receiving either 75 or 150 mg of trimipramine (r = 0.62).*

were analyzed for trimipramine and its major metabolites (II, III, and IV). A typical chromatogram from one of these patients is shown in Fig. 2. In several patients who exhibited a relatively high level of II (>200 ng), another peak appeared at a retention time of ~7.0 min. This peak was identified as the didemethyl metabolite (V) by comparison with the retention time of a standard solution of V. A chromatogram of a spiked sample containing V as well as the other standards I–IV and VI is depicted in Fig. 1. Although never accurately quantitated, V was never found in any significant (>10 ng/mL) levels in over 120 samples tested.

To date, no clinical plasma concentrations for I and its major metabolites (II–IV) have been reported. From our preliminary data, we found that I undergoes the same metabolic pathway as imipramine: namely I is demethylated as well as hydroxylated in the 2-position, conjugated as the glucuronide, and excreted. Other minor pathways may exist for I such as *N*-oxidation, side-chain dealkylation, and others that again resemble the imipramine pathway. Metabolite II is also hydroxylated and, in a few instances, further demethylated to the primary amine (V).

Table V—Mean Data From 29 Patients Receiving 75 or 150 mg/d of Trimipramine [a]

Variables in Plasma	Mean, ng/mL	Range, ng/mL
I	86	11–241
II	65[b]	3–382
III (u)[d]	16	3–40
IV (u)	17[b]	3–49
III (T)[d]	379[b]	58–1126
IV (T)	317[b]	63–800
Ratios		
II/I	0.61[b]	0.07–3.71
III(u)/III(T)	0.04[b]	0.02–0.08
IV(u)/IV(T)	0.05[c]	0.01–0.10
III(u)/I	0.26	0.02–0.75
IV(u)/II	0.86	0.02–1.67

[a] n = 29; patient ages, 18–65. [b] n = 28. [c] n = 27. [d] Key: (u) unconjugated; (T) total.

Plasma concentrations of I and its metabolites (II–IV) varied widely among the 29 depressed patients (14 receiving 75 mg; 15 receiving 150 mg/d of I). This variability has been demonstrated for other tricyclic antidepressants such as imipramine, amitriptyline, *etc.* Table V lists the means, ranges, and various ratios for these 29 depressed patients. Demethylation of I does not appear to occur as readily as with imipramine or amitriptyline since it was reported that the ratios of the demethylated metabolites to the parent drug of both these compounds were 1.0–1.6 and 1.0 (11–13), respectively. The difference in the side chain probably accounts for this retardation in demethylation of I. A correlation between I and II is illustrated in Fig. 3.

The mean ratio of unconjugated III to I was 0.26, which is similar to the reported ratio of 2-hydroxyimipramine to imipramine (0.18–0.25) (12, 13). However, the ratio of unconjugated IV to II was 0.86 which is greater than that found for 2-hydroxydesipramine to desipramine (0.50) (12, 13). This increase in ratio of IV/II may occur because of the existence of an additional demethylation pathway from III to IV. Of the 29 patients, 3 were found to have a ratio of unconjugated III + IV/II + I ≤0.03 which could indicate the portion of the population that is considered deficient in the ability to hydroxylate (14). There was no correlation between either unconjugated III and I or unconjugated IV and II. This finding differs from the weak to moderate correlation between 2-hydroxyimipramine and imipramine and 2-hydroxydesipramine and desipramine reported by DeVane and Jusko (13) and Suckow and Cooper (4). Four to five percent of the total plasma hydroxy metabolites are unconjugated. Bock *et al.* (9) has reported 8% of the total plasma 2-hydroxydesipramine metabolite as unconjugated. This analytical procedure has sufficient sensitivity for single dose pharmacokinetic studies of trimipramine and its metabolites in plasma.

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Gas Chromatographic Analysis of Cetiedil, a Candidate Antisickling Agent, in Human Plasma With Nitrogen-Sensitive Detection

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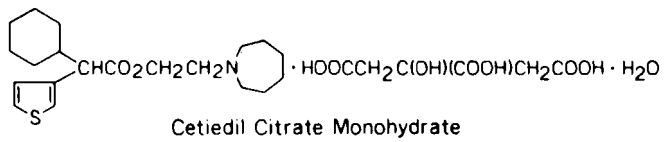
Received May 24, 1982, from the *Departments of Pharmacology and Pediatrics, University of South Alabama, College of Medicine, Mobile, AL 36688.* Accepted for publication August 24, 1983.

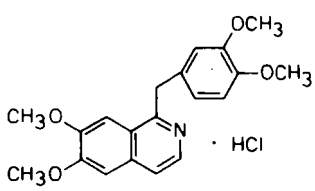
Abstract □ In this report a sensitive gas chromatographic assay for cetiedil, a candidate antisickling agent, in human plasma is described. After a triple extraction procedure, cetiedil was analyzed without derivatization with a nitrogen-phosphorus detector (with papaverine used as the internal standard.) Cetiedil was measured in plasma samples taken from human volunteers administered the drug intravenously.

Keyphrases □ Gas chromatography—cetiedil, human plasma, nitrogen-phosphorus detector □ Antisickling agents—cetiedil, GC analysis in human plasma, nitrogen-phosphorus detector □ Cetiedil—antisickling agent, GC analysis in human plasma, nitrogen-phosphorus detector

At present there is no pharmacological agent available to prevent or attenuate the sequelae of sickle cell crisis. For acute crisis, analgesics, hydration, and symptomatic care remain the standard therapy. In 1977, Cabannes reported on the clinical use of cetiedil citrate monohydrate for management of the painful thromboembolic crises characteristic of sickle cell disease (1).



Cetiedil Citrate Monohydrate



Papaverine Hydrochloride

Cetiedil[1] was first introduced for the treatment of peripheral and cerebral vascular disease (2). Subsequent studies have demonstrated that this vasoactive agent was also active in erythrocytes, in that it could prevent or reverse sickling by altering the permeability characteristics of the erythrocyte membrane (3–5). Several of its pharmacological properties (vasodilation, anticoagulation, analgesia, and inhibition of platelet aggregation) would be favorable for management of the thromboembolic episodes associated with sickle cell disease (6–8).

In this report, a reliable and sensitive gas chromatographic (GC) assay for cetiedil in human plasma, employing a triple extraction procedure and a nitrogen-phosphorus detector is described. The assay is similar to a previously reported method for analysis of papaverine in blood samples (9).

EXPERIMENTAL SECTION

Chemicals—Cetiedil citrate monohydrate[2] (white powder) and papaverine hydrochloride[3] were used without further purification. All solvents[4] were either nanograde or organic residue-analysis grade. All other reagents were certified ACS reagent grade.

Chromatographic Conditions—The gas chromatograph[5] was equipped with a nitrogen-phosphorus detector. A glass column (1.5 m × 2 mm i.d.) packed with 2% OV-101 on Chromosorb W HP 100–120-mesh support[6] was conditioned overnight (300°C). High-temperature septa[5] and graphite ferrules[6] were used. Air, helium, and hydrogen of the highest purity available[7]

[1] Stratene; Laboratoire Innothera, Arcueil, France.
[2] Johnson and Johnson Baby Products Co., Skillman, N.J.
[3] Sigma Chemical Co., St. Louis, Mo.
[4] American Scientific Products, Hanrahan, La.
[5] Model Sigma 3B; Perkin-Elmer, Norwalk, Conn.
[6] Supelco, Bellefonte, Pa.
[7] Air Products, Mobile, Ala.

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