Effect of Amitriptyline on Polarography of Chlordiazepoxide

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Abstract □ With increasing amounts of electroinactive amitriptyline, each of the three chlordiazepoxide reduction waves shifted to more cathodic half-wave potentials and decreased in limiting current. The shift was most pronounced up to the 1:1 mole ratio but continued up to ratios of 200:1. This behavior was observed in several supporting electrolytes and was not due to change in pH since this factor was maintained constant as the amitriptyline concentration was increased. Shifts in $E_{1/2}$ and reductions in limiting current may arise in several ways, such as complex formation between the two drugs or adsorption of the amitriptyline onto the surface of the dropping mercury electrode hindering chlordiazepoxide reduction. Most data point to adsorption as the cause.

Keyphrases □ Amitriptyline—effect on polarographic analysis of chlordiazepoxide, commercial products and synthetic mixtures □ Chlordiazepoxide—polarographic analysis, effect of amitriptyline, commercial products and synthetic mixtures □ Polarography—analysis, chlordiazepoxide, effect of amitriptyline on commercial products and synthetic mixtures □ Tranquilizers—chlordiazepoxide, polarographic analysis, effect of amitriptyline, commercial products and synthetic mixtures □ Antidepressants—amitriptyline, effect on polarographic analysis of chlordiazepoxide, commercial products and synthetic mixtures

Chlordiazepoxide and amitriptyline, in the ratio of 1:2.5, are marketed as a combination product[1]. Since it is well documented that chlordiazepoxide is electrochemically active (1–4) while amitriptyline is not, a study to determine chlordiazepoxide polarographically in this combination was initiated. In synthetic mixtures of the two drugs, the half-wave potentials shifted to more cathodic potentials and the limiting currents decreased for all three chlordiazepoxide waves with increasing concentrations of amitriptyline. Oelschlaeger *et al.* (5), in a paper describing chlordiazepoxide analysis in combinations including several that contained amitriptyline and clidinium bromide, reported a similar effect but attributed it to excipients present in the formulations, apparently being unaware that the change occurs even in their absence.

Shifts in $E_{1/2}$ and decreases in the limiting current of a polarographic wave may be attributed to several factors. One is complex formation between the two drugs. Recently, similar electrochemical evidence was used to show that an interaction exists between chlorpromazine hydrochloride and trifluoperazine hydrochloride and flavin coenzymes (6). The polarography of several charge transfer complexes was studied, and their respective stabilities were determined by measuring the shift in the half-wave potential caused by the electroinactive component (7).

A second possibility is adsorption of amitriptyline onto the dropping mercury electrode surface hindering chlordiazepoxide reduction. Weber *et al.* (8) demonstrated theoretically that the adsorption of an electroinactive substance shifts the half-wave potential and decreases the diffusion current for an irreversible electrode reaction. Subsequently, a theoretical treatment of reversible reactions predicted a decrease in current but no shift in the half-wave potential if the reaction is completely reversible (9). Experimental examples of these predictions also were presented (10, 11).

A more unusual case of a surface-active electroinactive species shifting the half-wave potential and decreasing current but increasing the reversibility of the reaction was reported by McCue and Kennedy (12). They demonstrated that titanium(IV), the polarographically active substance, complexed with procarbazine on the electrode surface, enhancing the reversibility.

The purpose of this study was to distinguish between these possibilities.

EXPERIMENTAL

Apparatus and Equipment—Experiments were performed with a commercial polarograph[2] and x–y recorder[3]. The polarographic cell contained a dropping mercury electrode as the working electrode, a silver–silver chloride reference with a potassium chloride–agar salt bridge, and a platinum wire counterelectrode. In later work, both carbon paste and glassy carbon served as working electrodes. The dropping mercury electrode had an open circuit drop time of 7.13 sec in 0.1 N HCl at a column height of 60 cm and a mercury flow rate of 0.95 mg/sec. All experiments were performed at 25 ± 0.1°. Dissolved air was removed from the solutions by bubbling prepurified nitrogen through the cell for 5 min and passing it over the solution during polarography.

Cyclic voltammetry was performed with the same instrumentation, employing a hanging mercury drop electrode[4] as the working electrode.

Chemicals and Reagents—All chemicals used as supporting electrolytes and for the preparation of buffer solutions were reagent grade. The mercury used in the dropping and hanging mercury electrodes was triple distilled[5]. Chlordiazepoxide hydrochloride[6], amitriptyline hydrochloride[6], clidinium bromide[6], and diazepam[6] were high purity (minimum 99%).

Procedure—The five supporting electrolytes were prepared using double-distilled water: 0.1 N HCl, 0.1 N HCl containing 20% methanol, citrate–phosphate buffer (pH 3.1), phosphate buffer containing 40% methanol (apparent pH 5.1), and citrate–phosphate buffer containing 45% methanol (apparent pH 7.0). Methanol was added to solutions of low pH to prevent polarographic maxima (2). At higher pH values, methanol was added to help maintain chlordiazepoxide and amitriptyline in solution.

Stock solutions (3 mM) of both drugs were prepared in the appropriate supporting electrolyte. These stock solutions were then diluted to obtain the working solutions of 0.6 mM chlordiazepoxide with the various amitriptyline concentrations. In a separate experiment to obtain higher mole ratios of amitriptyline, a 20 mM stock solution of this drug in 0.1 N HCl was made and subsequently diluted with 0.1 mM chlordiazepoxide to produce the appropriate amitriptyline concentration.

For the polarography of chlordiazepoxide alone, another 20 mM stock solution was prepared in 0.1 N HCl. The appropriate aliquots were measured and diluted with additional 0.1 N HCl. In the polarography of the clidinium bromide–chlordiazepoxide and amitriptyline–diazepam combinations, 3 and 3.5 mM stock solutions of the polarographically

[1] Limbitrol, Hoffmann-La Roche Inc., Nutley, NJ 07110.

[2] Princeton Applied Research model 174 polarographic analyzer.
[3] Hewlett-Packard model 7001AM.
[4] Princeton Applied Research model 932.
[5] Bethlehem Apparatus Co., Hellertown, Pa.
[6] Roche Laboratories, Nutley, N.J.

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Table I—Effect of Amitriptyline on Chlordiazepoxide at Various pH Values [a]

Mole Ratio of Amitriptyline to Chlordiazepoxide (0.6 mM)	$-E_{1/2}$, v *versus* Silver–Silver Chloride										Limiting Current, μamp									
	First Wave				Second Wave						First Wave [b]				Second Wave [b]					
	a	pH 1	pH 3	pH 5	pH 7	a	pH 1	pH 3	pH 5	pH 7	a	pH 1	pH 3	pH 5	a	pH 1	pH 3	pH 5
0:1	0.24	0.23	0.42	0.63	0.76	0.59	0.58	0.72	0.88	0.97	2.74	2.42	1.25	1.66	2.92	2.68	1.38	2.04
0.2:1	0.27	0.28	0.45	0.64	0.77	0.62	0.62	0.73	0.89	0.98	2.71	2.30	1.14	1.66	2.74	2.53	1.23	2.02
0.5:1	0.32	0.34	0.50	0.65	0.79	0.66	0.68	0.76	0.89	0.98	2.45	2.22	1.13	1.60	2.54	2.46	1.24	2.06
1:1	0.35	0.39	0.54	0.66	0.86	0.69	0.71	0.78	0.90	1.00	2.42	2.28	1.00	1.48	2.42	2.42	1.14	2.04
1.5:1	0.37	0.41	0.55	0.67	0.88	0.70	0.72	0.79	0.91	1.01	2.40	2.21	0.91	1.41	2.32	2.40	1.09	1.98
2:1	0.38	0.43	0.57	0.68	0.89	0.71	0.73	0.80	0.92	1.01	2.40	2.15	0.87	1.36	2.32	2.37	1.08	1.94
3:1	0.41	0.45	0.59	0.69	0.91	0.72	0.74	0.81	0.92	1.02	2.40	2.10	0.84	1.28	2.40	2.36	1.10	1.86
3.5:1	0.42	0.45	0.59	0.69	0.91	0.72	0.74	0.81	0.92	1.02	2.40	2.08	0.84	1.20	2.42	2.36	1.12	1.80
4:1	0.42	0.45	0.60	0.70	0.92	0.72	0.74	0.81	0.93	1.02	2.40	2.07	0.82	1.19	2.39	2.36	1.12	1.80

[a] Electrolytes used were: a, 0.1 *N* HCl containing 20% methanol; pH 1, 0.1 *N* HCl; pH 3, phosphate–citrate buffer; pH 5, 40% methanolic phosphate buffer; and pH 7, 45% methanolic phosphate–citrate buffer. [b] Overlap precluded accurate current measurements at pH 7.

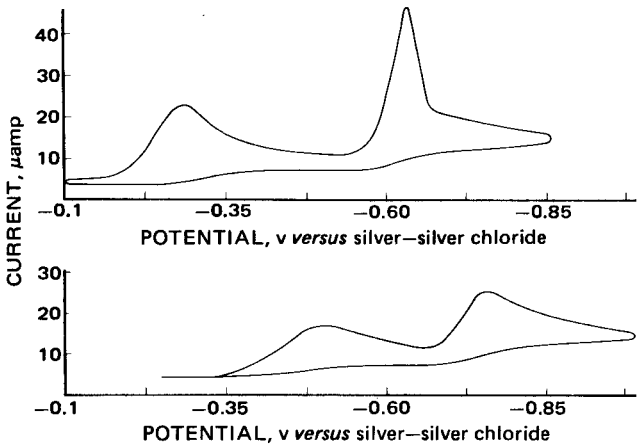

Figure 1—*Cyclic voltammogram of 0.6 m*M *chlordiazepoxide (top scan) and 0.6 m*M *chlordiazepoxide plus 1.2 m*M *amitriptyline (bottom scan) in 0.1* N *HCl. Scan rate was 0.2 v/sec.*

active component were prepared, respectively, in 0.1 *N* HCl. Stock solutions of the inactive component of the same concentrations were also made in 0.1 *N* HCl. Working solutions of 0.6 and 0.7 m*M* chlordiazepoxide and diazepam, respectively, then were prepared with the various concentrations of inactive component.

RESULTS

Chlordiazepoxide Polarography—The polarographic reduction of chlordiazepoxide at the dropping mercury electrode closely resembled the previously reported work (1–4). The compound displayed two well-defined polarographic waves in solutions buffered at apparent pH values from 1 to 7. A third wave appeared at lower pH values but was lost in the electrolyte wave near pH 7. The reduction was pH dependent, as evident from changes in the $E_{1/2}$ of the first wave from −0.23 to −0.76 v and of the second wave from −0.58 to −0.97 v as the pH was raised from 1 to 7.

Plots of $h^{1/2}$ (where h is mercury column height) *versus* the limiting currents of the first two waves were linear, indicating a diffusion-controlled process. Control by diffusion was confirmed by the fact that a 12° rise in temperature increased the limiting current by 20%. Generally, in diffusion-controlled processes, the limiting current increases 1.5%/degree (13).

Cyclic voltammetry studies in 0.1 *N* HCl showed the first two waves to be irreversible (Fig. 1). An electrocapillary curve of a solution containing the drug revealed a large decrease in drop time over a considerable potential range (in comparison to a solution containing only supporting electrolyte), signifying strong adsorption at the electrode. As a result of its surface activity and irreversibility, it would be expected that the half-wave potential would vary with concentration. This view is borne

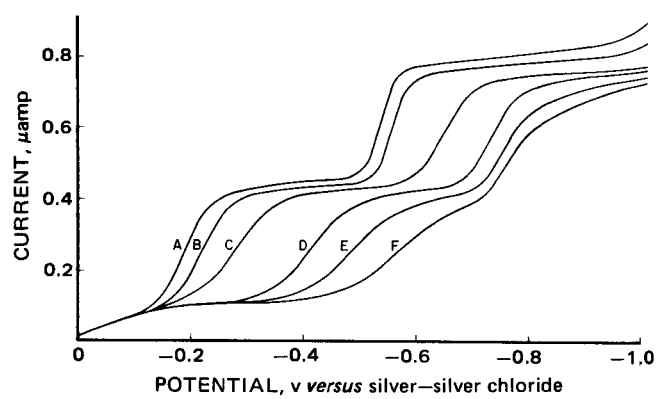

Figure 2—*Effect of amitriptyline on polarography of 0.1 m*M *chlordiazepoxide in 0.1* N *HCl. Key (mole ratio of amitriptyline to chlordiazepoxide): A, no amitriptyline; B, 1:1; C, 2:1; D, 10:1; E, 50:1; and F, 200:1.*

Table II—Effect of Amitriptyline on Chlordiazepoxide at High Mole Ratios [a]

Mole Ratio of Amitriptyline to Chlordiazepoxide (0.1 m*M*)	First Wave			Second Wave		
	$-E_{1/2}$, v	$E_{3/4} - E_{1/4}$, mv	i_{lim}, μamp	$-E_{1/2}$, v	$E_{3/4} - E_{1/4}$, mv	i_{lim}, μamp
0:1	0.17	52	0.312	0.52	28	0.318
1:1	0.21	56	0.312	0.55	31	0.328
5:1	0.37	68	0.240	0.70	42	0.256
10:1	0.39	73	0.240	0.72	45	0.248
25:1	0.44	77	0.240	0.74	50	0.248
50:1	0.47	85	0.240	0.75	52	0.256
100:1	0.50	86	—[b]	0.75	53	0.248
200:1	0.54	87	—[b]	0.75	55	0.240

[a] Supporting electrolyte was 0.1 *N* HCl. [b] It was difficult to measure i_{lim} because wave was merging into second wave.

out experimentally. Up to 0.1 m*M* chlordiazepoxide, the half-wave potentials of the two waves were constant at −0.17 and −0.52 v, respectively. At concentrations greater than 0.1 m*M*, the $E_{1/2}$ values shifted to more cathodic values, becoming −0.32 and −0.69 v, respectively, at 10.0 m*M*.

Effect of Amitriptyline on Chlordiazepoxide Polarography—Amitriptyline was electrochemically inactive in the potential region scanned. However, when solutions of chlordiazepoxide containing amitriptyline were examined in 0.1 *N* HCl containing 20% methanol, the $E_{1/2}$ values of the first two waves shifted to more cathodic potentials and the limiting current decreased as the amitriptyline concentration increased (Table I). The pH of these solutions was confirmed by measurement to be constant, independent of amitriptyline concentration. Therefore, a secondary effect where the base amitriptyline raises the pH, which could cause a shift, can be ruled out.

Similar results (Table I) were obtained in the four other supporting electrolytes used: 0.1 *N* HCl (pH 1), phosphate–citrate buffer (pH 3), phosphate buffer containing 40% methanol (pH 5), and phosphate–citrate buffer containing 45% methanol (pH 7). In each case, as the amitriptyline mole ratio increased, the shift increased and the limiting current decreased. This shift was most pronounced up to the 1:1 mole ratio, slowed in the rate of increase beyond this region, but still occurred up to 200:1 ratios (Fig. 2). The $E_{3/4} - E_{1/4}$ values also increased, indicating that the electrode reaction was becoming more irreversible (Table II).

At a 2:1 mole ratio, the reduction was still diffusion controlled; but in the cyclic scan (Fig. 1), the two peaks were broader and moved to more negative potentials with peak currents lower than those of the scan of chlordiazepoxide alone, indicative of increased irreversibility (14).

Electrocapillary curves of amitriptyline solutions showed a larger decrease in drop time over a similar potential range than did chlordiazepoxide solutions (Fig. 3). Attempts to eliminate the strong adsorption between amitriptyline and the dropping mercury electrode by replacing it with solid electrodes and to study the possibility of interaction between the two drugs without this interfering factor were successful.

A single wave was observed for chlordiazepoxide solutions in the −0.7–−0.8-v region, close to the electrolyte current cutoff, on both glassy carbon and carbon paste electrodes. The wave was reproducible and better defined on the glassy carbon electrode. Within the reproducibility of the measurement, the presence of amitriptyline did not affect the $E_{1/2}$ of the chlordiazepoxide wave.

Other Polarographic Systems—Diazepam, another common benzodiazepine, underwent a similar $E_{1/2}$ shift and decrease in the limiting current of its lone polarographic wave in the presence of increasing amitriptyline concentrations (Table III). Electroinactive clidinium bromide exhibited the same effect as amitriptyline on the polarography of chlordiazepoxide, although it was somewhat reduced in extent (Table IV). Clidinium bromide also was adsorbed onto the dropping mercury electrode, as evident from its electrocapillary curve (Fig. 3).

Table III—Effect of Amitriptyline on Polarography of Diazepam [a]

Mole Ratio of Amitriptyline to Diazepam (0.7 m*M*)	$-E_{1/2}$, v	i_{lim}, μamp
0:1	0.62	2.42
0.1:1	0.62	2.40
0.5:1	0.64	2.35
1:1	0.65	2.34
1.5:1	0.66	2.32
2:1	0.67	2.29
3:1	0.68	2.26

[a] Supporting electrolyte was 0.1 *N* HCl containing 20% methanol.

DISCUSSION

The electrochemical properties of chlordiazepoxide, *i.e.*, the shift of the half-wave potential to more negative values with increasing pH and chlordiazepoxide concentration, the limiting currents of the waves being diffusion controlled, drug adsorption onto the dropping mercury electrode, and the irreversibility of the two waves, were reported previously (4). With ac polarography, a strong adsorption between the drug and mercury was established, and the $E_{1/2}$ shift was attributed to the electrode being completely covered with a monomolecular layer of adsorbed material when the chlordiazepoxide concentration was above 0.1 m*M* (4). A further increase in concentration produced the shift; below 0.1 m*M*, however, the $E_{1/2}$ remained constant.

The presence of amitriptyline increased the irreversibility of the chlordiazepoxide reduction waves. This effect by amitriptyline could be explained by either its surface activity or interaction with chlordiazepoxide. However, enhancement of reversibility by complexation at the electrode surface, as described by McCue and Kennedy (12), probably was not occurring in this case.

Amitriptyline is very strongly adsorbed onto the dropping mercury electrode. Since adsorption of electroinactive substances can influence the half-wave potential of irreversible systems (8, 10–12) and reduce the limiting current of irreversible systems (12), this adsorption probably is the basis for the action of amitriptyline. If chlordiazepoxide concentrations greater than 0.1 m*M* exhibit shifts in half-wave potential, then it is reasonable to expect that higher concentrations of a more surface-active compound will show a similar, or greater, effect. Comparison of several examples of adsorption *versus* complexation gives even greater evidence that an electrode surface phenomenon occurred.

In the proposed interaction between chlorpromazine hydrochloride and flavin mononucleotide, the $E_{1/2}$ of the flavin coenzyme changed only 40 mv in a 1000:1 mole ratio of the two when the coenzyme concentration was 10^{-4} *M* (6). Peover (7) measured a $E_{1/2}$ shift of 20 mv when a 500-fold excess of electroinactive hexamethylbenzene complexed 0.7 m*M* chloranil and an 80-mv shift when a 750-fold excess of hexamethylbenzene complexed 0.6 m*M* dichlorodicyanoquinone. On the other hand, only 0.2 m*M* of surface-active procarbazine displaced the half-wave potential of 0.6 m*M* titanium(IV) by 130 mv (12). The $E_{1/2}$ of 1 m*M* lead(II) was transposed 360 mv when the concentration of surface-active polyoxyethylene lauryl ether was increased from 2×10^{-6} to 10^{-3} *M* (11). The present experimental values are certainly more in line with an adsorption phenomenon: 160-mv shift for a 1:1 mole ratio of 0.6 m*M* amitriptyline and chlordiazepoxide in 0.1 *N* HCl.

Table IV—Effect of Clidinium Bromide on Polarography of Chlordiazepoxide [a]

Mole Ratio of Clidinium Bromide to Chlordiazepoxide (0.6 m*M*)	First Wave		Second Wave	
	$-E_{1/2}$, v	i_{lim}, μamp	$-E_{1/2}$, v	i_{lim}, μamp
0:1	0.23	2.54	0.58	2.70
0.2:1	0.24	2.53	0.59	2.60
1:1	0.26	2.45	0.63	2.52
2:1	0.28	2.44	0.65	2.50
3:1	0.29	2.46	0.67	2.54

[a] Supporting electrolyte was 0.1 *N* HCl.

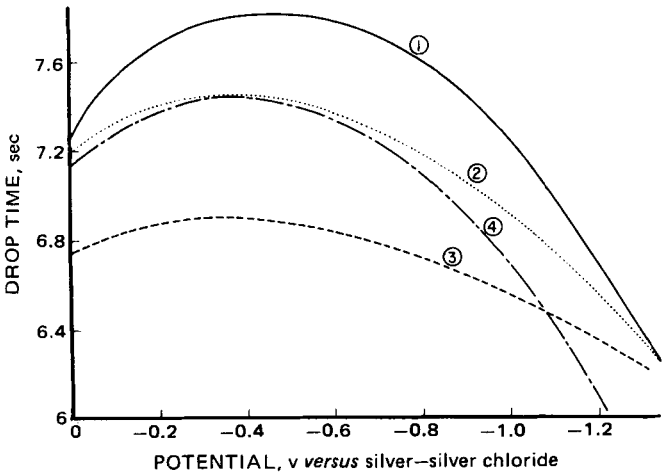

Figure 3—*Electrocapillary maximum curves of 0.1* N *HCl (curve 1), 0.8 mM chlordiazepoxide in 0.1* N *HCl (curve 2), 0.8 mM amitriptyline in 0.1* N *HCl (curve 3), and 0.8 mM clidinium bromide in 0.1* N *HCl (curve 4).*

Other polarographic systems, *i.e.*, amitriptyline with diazepam and clidinium bromide with chlordiazepoxide, underwent similar $E_{1/2}$ shifts and reductions in limiting current. The action of surface-active excipients on the polarography of chlordiazepoxide was studied by Oelschlaeger *et al.* (5) who showed that they all produced, to various degrees, results similar to the action of amitriptyline. It is highly unlikely that all of these dissimilar chemicals would form complexes with chlordiazepoxide.

Solid electrode evidence also points to adsorption as the underlying cause. The $E_{1/2}$ of the single chlordiazepoxide wave observed on carbon electrodes, where no adsorption is expected, was not significantly affected by the presence of amitriptyline in ratios as high as 2:1 of amitriptyline to chlordiazepoxide. On the dropping mercury electrode, the $E_{1/2}$ shift with amitriptyline concentration was most pronounced with ratios below 2:1. Thus, a solution interaction between amitriptyline and chlordiazepoxide appears improbable. If such an interaction exists, it does not significantly contribute to the observed polarographic phenomena.

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High-Pressure Liquid Chromatographic Separation and Determination of Anomeric Forms of Streptozocin in a Powder Formulation

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Abstract □ A high-pressure liquid chromatographic assay for streptozocin in a sterile powder formulation (1.0 g/vial) is described. The method effectively separates the α- and β-anomeric forms of streptozocin. Quantitative results are presented for the drug based on the use of an internal standard and peak height measurements.

Keyphrases □ Streptozocin—high-pressure liquid chromatographic analysis and separation of anomers in a commercial dosage form □ High-pressure liquid chromatography—analysis and separation of anomers of streptozocin in a commercial dosage form □ Antineoplastic agents—streptozocin, high-pressure liquid chromatographic analysis and separation of anomers in a commercial dosage form

Streptozocin is used for the treatment of malignant insulinoma. An assay was sought for this drug that would satisfy quality control requirements with respect to accuracy, precision, and specificity. The described high-pressure liquid chromatographic (HPLC) technique separates the two anomeric forms of streptozocin, 2-deoxy-2-(3-methyl-3-nitrosoureido)-α(and β)-D-glucopyranose. The mutarotation of streptozocin was studied by using HPLC and optical rotation. With these data, the optimum experimental conditions could be chosen for routine assay of both bulk drug and formulated product.

EXPERIMENTAL

Apparatus—A commercial liquid chromatograph[1] was used at an ambient temperature with UV detection at 254 nm. The column was stainless steel (type 316), 4 × 300 mm, prepacked with 10-μm microparticulate C_{18} bonded to silica gel[2]. Chromatographic recordings were made with a standard 1-mv, commercially available recorder[3].

Reagents and Solutions—The mobile phase was 0.1 *M* acetic acid in water–methanol (97:3). The pH was adjusted to 4.0 with 50% NaOH. The internal standard was a 2-mg/ml solution of potassium acid phthalate. A 1-mg/ml reference standard solution of streptozocin was prepared in 0.1 *M* acetate buffer (pH 4.0). Exactly 5.0 ml of this reference standard solution and 5.0 ml of the internal standard solution were mixed

[1] Model ALC202, Waters Associates, Milford, Mass.
[2] μBondapak C_{18}, Waters Associates, Milford, Mass.
[3] Model HP7123A, Hewlett-Packard, Palo Alto, Calif.

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