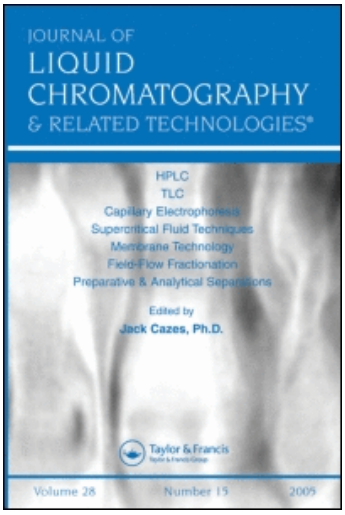

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A Rapid High Performance Liquid Chromatographic Method for the Simultaneous Measurement of Six Tricyclic Antidepressants

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A RAPID HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS MEASUREMENT OF SIX TRICYCLIC ANTIDEPRESSANTS

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ABSTRACT

A reversed-phase high pressure liquid chromatographic procedure has been developed for the quantitation of the concentration of six different tricyclic antidepressants in the plasma of patients undergoing routine drug therapy. Plasma samples were extracted into a 97:3 hexane:isoamyl alcohol solution and then extracted back into dilute acid. A Supelcosil C-8 column with 5 micron packing was employed in combination with an acetonitrile/phosphate buffer/diethylamine mobile phase. At an optimized mobile phase pH of 7.22, baseline separation of all six tricyclic antidepressants plus the internal standard was achieved within 8 minutes. UV detection at 254 nm resulted in limits of detection of 2.5 μg/L for each drug. The potential interferences from 13 different benzodiazepines and neuroleptics was investigated. Five of the 13 parent drugs and three metabolites were found to interfere with this tricyclic antidepressant assay.

INTRODUCTION

The growing practice of monitoring the concentration of tricyclic antidepressants (TCAs) in plasma from patients undergoing antidepressant therapy has required many clinical laboratories to introduce procedures for quantitation of the common antidepressant drugs (1,2). Therapeutic plasma levels of antidepressants are usually in the μg/L range and a number of concurrently administered medications may possibly interfere with the analysis (3,4). These considerations have resulted in many laboratories using high-pressure liquid chromatography (HPLC) for the plasma monitoring of the six common drugs: doxepin, desmethyldoxepin, imipramine, desipramine, amitriptyline and nortriptyline.

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Several authors have published normal-phase chromatographic methods for the separation of one or more TCAs (5-14); however, only Sutheimer has accomplished baseline separation of all six drugs listed above (15). Sutheimer's method is based on the earlier work of Vandemark et al. (6) in which hydrophilic endogenous components were found to cause background interference. Sutheimer did not apply the method to patient samples and, further, presented no information on interferences, precision, or the extraction procedure.

Reversed-phase HPLC techniques have less interference from plasma extracts and have achieved more rapid chromatography of a few TCAs (16-29). Kabra et al. (19) described a procedure providing baseline resolution of all six drugs listed above; however, the retention time for the last eluting component was about 13 min. Bannister et al (21) described a rapid system for the automatic extraction and quantitation of TCAs in plasma samples but routine use required two chromatographic runs with two different standards because all TCAs could not be simultaneously separated in a single run.

Before chromatographic analysis, TCAs must be extracted from the plasma samples. Several liquid-solid and liquid-liquid extraction techniques have been published (3). The liquid-solid techniques have required a second liquid-liquid extraction (28) or evaporation step to concentrate the samples (13). The liquid-liquid extraction methods have required long mechanical shaking times (19), freezing steps for the isolation of the organic phase (11), evaporation of solvent to concentrate the samples (7), or an elaborate autoextraction system not available to many clinical laboratories (21). All of these techniques are time consuming. Additionally, the use of an evaporation step results in increased variability and requires two internal standards (10).

In this paper we report the development of a reversed-phase HPLC method for the simultaneous quantitation of six TCAs in the plasma from patients undergoing routine antidepressant therapy.

EXPERIMENTAL

Apparatus

A Waters HPLC system (Waters Associates, Milford, Ma.) equipped with a Model 441 UV absorbance detector (operated at 254 nm), a Model 6000A solvent-delivery system, a Model 710 autoinjector, a Model 720 integrator and a Model 730 System Controller was employed. A Supelcosil C-8 reversed-phase column (4.6 x 250 mm with 5 μm packing), fitted with a C-8 guard column (4.6 x 50 mm with 40 μm packing) was used (Supelco, Inc., Bellefonte, Pa.).

The mobile phase used for routine analysis was prepared by mixing 53.3 volumes of acetonitrile, 45.1 volumes deionized water, 1 volume diethylamine, and 0.4 volumes 85% phosphoric acid. The pH was adjusted to 7.2 by dropwise addition of phosphoric acid or sodium hydroxide. Other mobile phase combinations were prepared as needed. All mobile phases were degassed by ultrasonic vibration prior to use. Column mobile phase flow rate was 2.0 mL/min.

Reagents

HPLC grade hexane, acetonitrile, and isopropanol, and reagent grade potassium carbonate, diethylamine, and isoamyl alcohol were obtained from Fisher Scientific Co. (Atlanta, Georgia). A 25% solution of potassium carbonate containing 0.1% diethylamine was prepared and stored at room temperature. A 97:3 mixture of hexane:isoamyl alcohol was stored in a glass container at room temperature.

Standards

A standard stock solution of tricyclic antidepressant drugs contained 1 g/L of each of the following pure drugs was prepared in isopropanol: doxepin (Pfizer Laboratories), desmethyldoxepin (Pfizer Laboratories), imipramine (USV Laboratories), desipramine (USV Laboratories), amitriptyline (Merck, Sharp & Dohme Laboratories), and nortriptyline (Merck, Sharp & Dohme Laboratories). This stock standard was stored at -15°C.

A stock internal standard containing 1 g/L of Loxapine (American Cyanamid Co.) was prepared in isopropanol. This solution was stored at -15°C.

A tricyclic working standard (100 μg/L) was made by a 10^4-fold dilution of the TCA stock standard. To prevent adsorption of the TCAs onto the glass container, diethylamine was added to a final concentration of 0.1%. The tricyclic working solution was stored at 4°C.

The working internal standard was prepared by a 10^3-fold dilution of the stock internal standard to a final concentration of 1 mg/L with diethylamine added (0.1%); this solution was stored at 4°C.

Samples

Patient blood samples were collected in Dark Blue stoppered Vacutainers (Bectin-Dickinson, Rutherford, N.J.) and centrifuged within 2 hours of sampling to obtain the plasma. The plasma was then stored at -15°C in a clean Dark Blue Vacutainer tube until analyzed.

Sample Preparation

Two mL of plasma was transferred to a 15 x 150 mm borosilicate glass disposable test tube. Into this tube was added 100 µL of working internal standard, 250 µL of the 25% potassium carbonate solution, and 5 mL of the 97:3 hexane:isoamyl alcohol solution. Each tube was vortexed rapidly for 30 s and then centrifuged for 3 min at 500 x g to break the emulsion. The aqueous layer was removed by aspiration and the organic layer was transferred to a 15 mL glass conical centrifuge tube. A 100 µL aliquot of 0.25 mol/L HCl was added and the tube vortexed rapidly for 30 s. The organic layer was discarded and 50 µL of the aqueous layer was injected onto the column.

Quantitation

The quantity of each drug in a sample was calculated by determining the ratio of the peak absorbance of that tricyclic drug to that of the working internal standard. The concentration was then calculated by comparison with a TCA standard curve generated with each run.

RESULTS

Chromatography

The pH of the mobile phase was found to be a crucial factor in obtaining baseline separation of the six TCAs plus the internal standard. Over the pH range of 3.9 to 7.8 the retention times of the solutes showed considerable variation (Figure 1). At the low pH extreme several of the drugs coelute. Doxepin and nortriptyline reversed in elution order at pH 7. Amitriptyline and loxapine (the internal standard) also reversed in elution order at high mobile phase pH (pH 7.8). The optimal regions of chromatographic separation were determined from the retention data of Figure 1 using the "window diagram" technique popularized by Laub and Purnell (30) and applied to reverse-phase HPLC by Deming and Turoff (31). Third-order models were fitted to the retention data for each of the seven individual peaks. These fitted equations were then employed to calculate the relative retention ratios for all pairs of peaks (21 different pairs in this case) over the pH range investigated. These results are plotted in Figure 2. Regions of best separation for the worst separated pair of peaks are darkened in as "windows" in Figure 2. The highest window occurs at pH 7.22 where the worst separated pairs show a relative retention of about 1.10. At this pH all seven components are completely resolved as shown in the chromatogram of Figure 3.

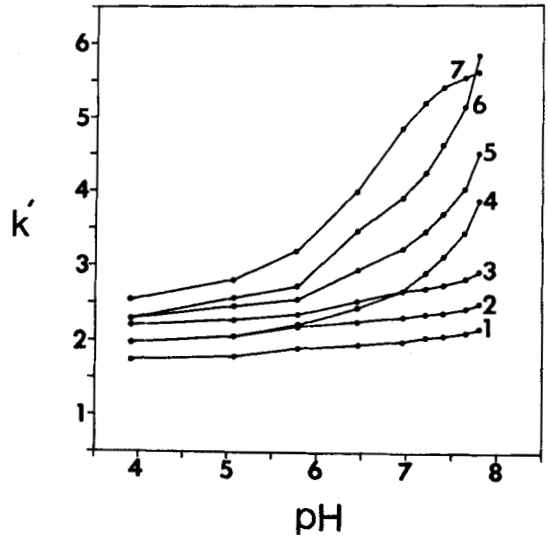

FIGURE 1. The relationship between k' and pH for the seven solutes (1 = desmethyldoxepin, 2 = desipramine, 3 = nortriptyline, 4 = doxepin, 5 = imipramine, 6 = amitriptyline, 7 = loxapine (internal standard)).

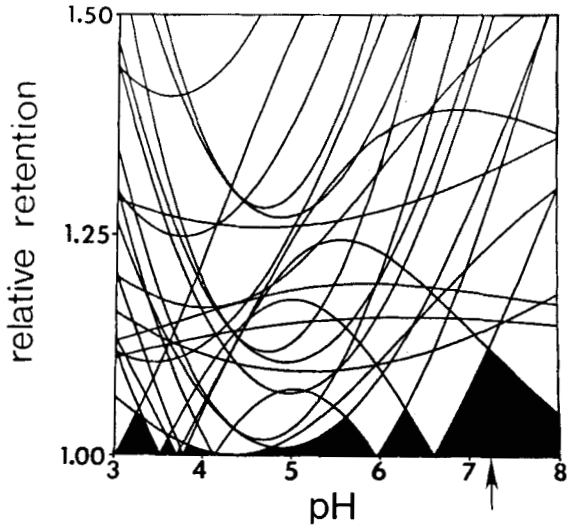

FIGURE 2. Window diagram of relative retention as a function of pH for all 21 pairs of tricyclic antidepressants. The location of the optimum pH is marked by an arrow.

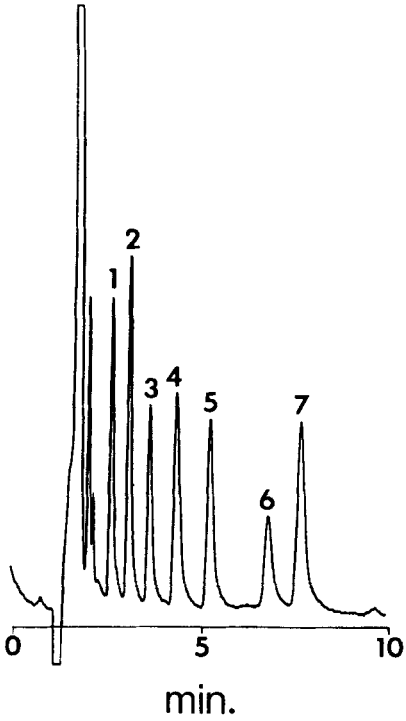

FIGURE 3. Chromatogram of a spiked plasma standard extract containing 100 µg/L of each of the tricyclic antidepressants. Peak identities: 1 = desmethyldoxepin, 2 = desipramine, 3 = nortriptyline, 4 = doxepin, 5 = imipramine, 6 = amitriptyline, 7 = loxapine (internal standard).

An amine modifier was employed to reduce peak tailing of the solutes. Diethylamine was chosen since it proved effective in improving peak symmetry at pH 7.2. The relatively high concentration of diethylamine (1%) not only improved peak shape, but also improved selectivity among solutes when compared with dibutylamine (Figure 4).

Precision, Accuracy and Recovery

The precision of analysis for a pooled serum sample spiked with 100 µg/L of each of the TCAs is shown in Table 1. The percent relative standard deviation ranged from 3.2 to 4.0%.

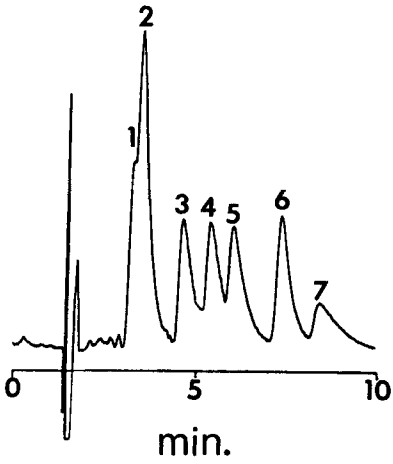

FIGURE 4. Chromatogram of tricyclic antidepressants with 1% dibutylamine as the amine modifier in the mobile phase. Peak identities as in Figure 3.

TABLE 1. Precision of the Method

	%RSD[a]	Mean[b]
Desmethyldoxepin	3.2	101.8
Desipramine	3.6	100.6
Nortriptyline	3.4	102.3
Doxepin	3.8	102.5
Imipramine	3.8	103.8
Amitriptyline	4.0	104.3

[a]n = 25 measurements; [b]µg/L

TABLE 2. Analytical Recovery of Tricycle Drugs from Pooled Serum

Drug	% Recovery[a]
Desmethyldoxepin	53.8
Desipramine	48.8
Nortriptyline	50.9
Doxepin	54.6
Imipramine	54.3
Amitriptyline	50.0

[a]concentration, 100µg/L

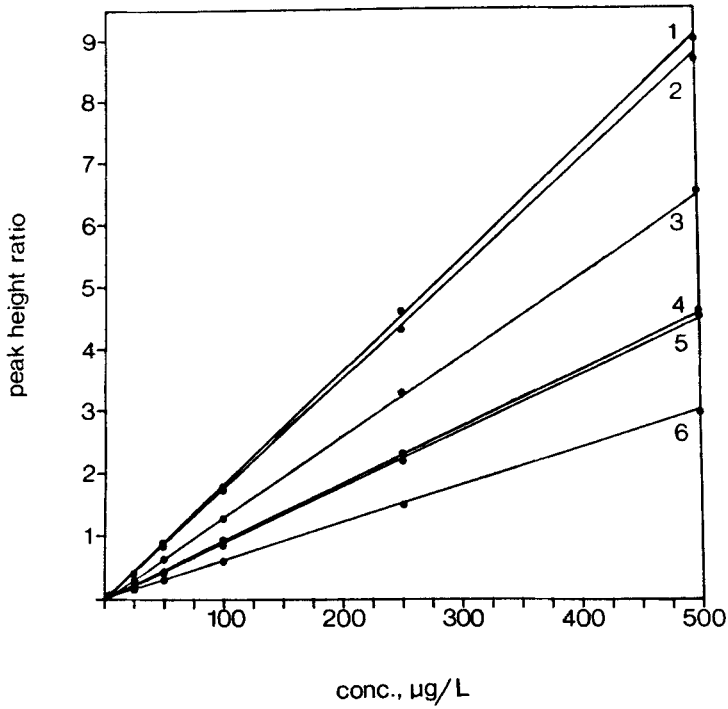

FIGURE 5. Calibration curves (1 = desmethyldoxepin, 2 = desipramine, 3 = nortriptyline, 4 = doxepin, 5 = imipramine, 6 = amitriptyline).

In Figure 5, a calibration curve is presented for each of the drugs over a concentration range of 2.5 to 500 µg/L. Using this technique, however, TCA concentrations up to 1000 µg/L can be measured without dilution.

The two stage liquid-liquid extraction can be completed within eight minutes. The percent recovery (Table 2) for these TCAs ranged from 48.8 to 54.6%.

Interference

Table 3 lists the k' of the TCAs and some additional psychotropic drugs which are often concurrently administered to patients receiving TCA therapy. To identify whether or not the antipsychotic drugs or metabolites interfered with the measurement of TCAs, plasma samples from patients receiving standard neuroleptic drug therapy were assayed. Each of the benzodiazepines was tested for interference using pooled human plasma

TABLE 3. k' for Tricyclic Drugs and Other Common Psychiatric Drugs and Metabolites

Tricyclic Antidepressants	k'
Desmethyldoxepin	2.50
Desipramine	2.95
Nortriptyline	3.45
Doxepin	4.18
Imipramine	5.00
Amitriptyline	6.41
Loxapine[a]	7.18

Benzoiazepines	k'
Desmethylchlordiazepoxide	2.06
Oxazepam	2.44
Chlordiazepoxide	2.53
Desmethyldiazepam	3.93
Diazepam	5.20

Neuroleptics	k'
Molindone	<2.00
Trifluoperazine	<2.00
Perphenazine	<2.00
Fluphenazine	2.67
Haloperidol	3.34
Thiothixene	3.39
Thioridazine metabolites	<2.00-4.14[b]
Chlorpromazine	8.23
Chlorpromazine metabolite (1)	3.02
Chlorpromazine metabolite (2)	3.65

[a]internal standard; [b]several unresolved peaks

spiked with drug standards since the metabolites of these compounds were available.

Among the benzodiazepines, three drugs were found to cause interference with this assay. Diazepam interferes with the measurement of imipramine, and the desmethyl metabolite of diazepam interferes with the measurement of doxepin. The third benzodiazepine, chlordiazepoxide, interferes with the analysis of desmethydoxepin.

Of the group of neuroleptic drugs tested, five were found to cause interference: Haloperidol, thiothixene, fluphenazine, chlorpromazine and thioridazine. Haloperidol and thiothixene elute with retention times which conflict with nortriptyline. Fluphenazine interferes with the measurement of desmethyldoxepin.

Although the parent compounds of chlorpromazine and thioridazine do not interfere with the measurement of TCAs, the metabolites do. Chlorpromazine metabolites interfere with the measurement of both desipramine and nortriptyline. The metabolites of thioridazine provide the greatest problems in this assay. In the plasma from a patient whose thioridazine treatment had been discontinued for seven days prior to the time the plasma sample was obtained, sufficient levels of thioridazine metabolites were still present to cause interference in the measurement of all six TCAs.

DISCUSSION

In this report, a rapid and sensitive HPLC technique is described for the simultaneous measurement of six tricyclic antidepressants (doxepin, desmethyldoxepin, imipramine, desipramine, amitriptyline, and nortriptyline) in human plasma. A simple extraction technique is used to prepare the samples for analysis by reversed-phase chromatography. The liquid-liquid extraction requires no evaporation step, small extraction volumes, and vortexing instead of mechanical shaking. The addition of the diethylamine to the potassium carbonate buffer prevents adsorption of the TCAs to the extraction glassware. These steps improve the reproducibility of the extraction procedure, with typical % RSDs for the complete assay ranging from 3.2 to 4.0% at a plasma concentration of 100 μg/L. Plasma samples can be prepared for chromatography within eight minutes.

Using this reversed-phase HPLC technique, the six tricyclic drugs and internal standard all elute within eight min. Thus, a total of only 16 minutes are required for both extraction and chromatography. Improvements in peak symmetry and selectivity were obtained by using the amine modifier diethylamine. The optimum mobile phase pH (7.22) was determined by conducting experiments over the pH range 3.9 to 7.8 and by plotting a window diagram of relative retention for each pair of peaks versus pH. Loxapine,

which provides acceptable extraction and chromatographic characteristics based on the structural similarities to the TCAs, was chosen as the internal standard.

It has been suggested that the use of a mobile phase pH above 7.0 in combination with an amine modifier may reduce the life of a silica based column by dissolving the silica (16). The method described in this report has been used for several years in both a routine clinical laboratory and in a research setting. Several thousand injections can be made on a single column before performance deteriorates. Degradation is primarily due to a buildup of lypophilic compounds on the column surface and is not due to the silica base of the column dissolving. The use of a guard column greatly extends the analytical column life by slowing lypophilic contamination.

Psychiatrists use TCAs as the drugs of choice for the treatment of affective disorders. TCA therapy is also sometimes combined with concurrent administration of other psychotropic agents. An evaluation, using the plasma from patients receiving some of these psychotropic drugs, showed limited interference from the parent compounds of three neuroleptics (thiothixene, haloperidol, and fluphenazine) and two benzodiazepines (diazepam and chlordiazepoxide). In addition, the metabolites of one of the benzodiazepines (diazepam) and two neuroleptics (chlorpromazine and thioridazine) caused interference in the assay.

This sensitive and rapid HPLC technique has been effectively applied for two years in a tricyclic drug monitoring program for both inpatients and outpatients at a major medical facility. Studies involving the pharmacokinetics and drug metabolism of TCAs are in progress.

ACKNOWLEDGMENT

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REFERENCES

(1) Risch, S. C., Huey, L. Y., and Janowsky, D. S., J. Clin. Psych., 40, 4-16 (1979).

(2) Risch, S. C., Huey, L. Y., and Janowsky, D. S., J. Clin. Psych., 40, 58-59 (1979).

(3) Scoggins, B. A., Maguire, K. P., Norman, T. R., and Burrow, G. D., Clin. Chem., 26, 5-17 (1980).

(4) Scoggins, B. A., and Maguire, K. P., Clin. Chem., 26, 805-815 (1980).

(5) Watson, I. D., and Steward, M. J., J. Chromatogr., 132, 155-159 (1977).

(6) Vandemark, F. L., Adams, R. F., and Schmidt, G. J., Clin. Chem., 24, 87-91 (1978).

(7) Sonsalla, P. K., Jennison, T. A., and Finkle, B. S., Clin. Chem., 28, 457-461 (1982).

(8) van den Berg, J. H. M., De Ruwe, H. J. J. M., and Deelder, R. S., J. Chromatogr., 138, 431-436 (1977).

(9) Dixon, R., and Martin, D., Res. Commun. Chem. Pathol. Pharmacol., 33, 537-545 (1981).

(10) Sonsalla, P. K., Jennison, T. A., and Findle, B. S., Clin. Chem., 28, 1401-1402 (1982).

(11) Godbillon, J., and Gauton, S., J. Chromatogr., 204, 303-311 (1981).

(12) Watson, I. D., and Steward, M. J., J. Chromatogr., 134, 182-186 (1977).

(13) Bidlingmeyer, B. A., Korpi, J., and Little, J. N., Chromatographia, 15, 83-85 (1982).

(14) De Ridder, J. J., Koppens, P. C. J. M., and Van Hal, H. J. M., J. Chromatogr., 143, 281-287 (1977).

(15) Sutheimer, C., Chromatogr. Newslett. (Perkin-Elmer), 7, 38-39 (1979).

(16) Proelss, H. F., Lohmann, H. J., and Miles, D. G., Clin. Chem., 24, 1948-1953 (1978).

(17) Kraak, J. C., and Bijster, P., J. Chromatogr., 143, 499-512 (1977).

(18) Jensen, K. M., J. Chromatography, 183, 321-329 (1980).

(19) Kabra, P. M., Mar, N. A., and Marton, L. J., Clin. Chem. Acta, 111, 123-132 (1981).

(20) Burke, D., and Sokoloff, H., J. Pharm. Sci., 69 (2), 138-140 (1980).

(21) Bannister, S. J., Van de Wal, S., Dolan, J. W., and Snyder, L. R., Clin. Chem., 27, 849-855 (1981).

(22) Bock, J. L., Giller, E., Gray, S., and Jatlow, P., Clin. Pharmacol. Ther., 609-616 (1982).

(23) Breutzmann, D. A., and Bowers, L. D., Clin. Chem., 27, 1907-1911 (1981).

(24) Wong, S. H. Y., McCauley, T., and Kramer, P. A., J. Chromatogr., 226, 147-154 (1981).

(25) Fekete, J., del Castilho, P., and Kraak, J. C., J. Chromatogr., 204, 319-327 (1981).

(26) Reece, P. A., and Zacest, R., J. Chromatogr., 163, 310-314 (1979).

(27) Suckow, R. F., and Cooper, T. B., J. Pharm. Sci., 70, 257-261 (1981).

(28) Thoma, J., and Bondo, P., Analytichem International, brochure no. 1 TCA 1001 (1979).

(29) Koteel, P., Mullins, R. E., and Gadsden, R. H., Clin. Chem., 28, 462-466 (1982).

(30) Laub, R. J., and Purnell, J. H., J. Chromatogr., 112, 71-79 (1975).

(31) Deming, S. N., and Turoff, M. L. H., Anal. Chem., 50, 546-548 (1978).