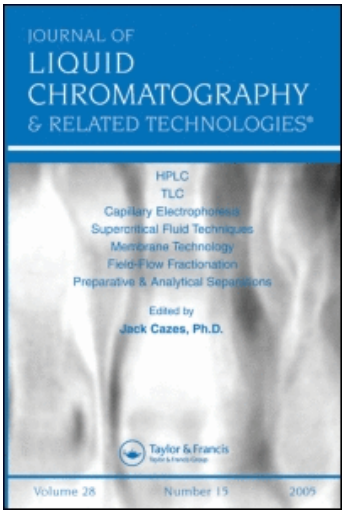

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An Improved Solid Phase Extraction Procedure for the Determination of Antidepressants in Serum by Column Liquid Chromatography

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AN IMPROVED SOLID PHASE EXTRACTION PROCEDURE FOR THE DETERMINATION OF ANTIDEPRESSANTS IN SERUM BY COLUMN LIQUID CHROMATOGRAPHY

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ABSTRACT

A convenient and economical procedure for the isolation of antidepressant drugs from serum is described. A 0.7 mL aliquot of the sample after the addition of trimipramine or protriptyline as the internal standard is vortex mixed with 0.7 mL of acetonitrile and centrifuged. The supernatant is applied to 1-mL BondElut C-18 silica extraction column which has been conditioned by subsequent washing with 1 M HCl, methanol, water, and 1% potassium bicarbonate solution. After passing the sample at a slow rate, the column is washed twice with water and once with acetonitrile. The desired compounds are then eluted with a 0.25 mL aliquot of a mixture of 35% perchloric acid and methanol (1:20). A 50-μL aliquot of the eluate is injected onto a 15 cm x 4.6 mm (i.d.) column packed with 5-μm C-8 silica particles, which is eluted at ambient temperature with a mobile phase containing tetramethylammoniun perchlorate and acetonitrile. The peaks are detected with an absorbance detector at 215 nm or 226 nm for fluoxetines. The resulting chromatogram shows only a few extraneous peaks. The extraction recovery of the commonly prescribed antidepressants is in the range of 90 % allowing their quantitation at a level of 5 ng/mL.

INTRODUCTION

The determination of antidepressant drugs which act by blocking neuronal uptake of biogenic amines, because of their widespread use and high toxicity, remains one of the most commonly requested drug assays in clinical laboratories (1). At present, column liquid chromatography coupled with solid phase extraction (SPE) is the most viable technique for a general procedure for the determination of these drugs (2-4). In all of these procedures, protein based sample is directly applied to the extraction column. Excellent results were obtained when we applied our fluoxetine SPE (5) to buffer or albumin based standards of amitriptyline or imipramine. However, when the procedure was applied to clinical and quality control samples, the concentration of drugs in a number of cases were quite different from the values obtained by a gas chromatographic procedure where the drugs were isolated from serum by liquid/liquid extraction (6). It was the object of this investigation to select conditions for SPE so as to minimize matrix effect.

EXPERIMENTAL

Materials

All reagents were of analytical grade. De-ionized water was distilled in an all glass still.

Stock solutions (1 mg/mL) of drugs and metabolites (Table 1) were prepared by dissolving appropriate amounts of drug salts corresponding to 10 mg of each compound as free base. These solutions were stored in tightly capped tubes at -20°C. Serum standards, 1000 ng/mL were prepared by diluting 25 μL of a stock solution with 25 mL of pooled drug free serum or blood bank plasma in a volumetric flask. Serum standards of other concentrations were prepared by appropriate dilution of the 1000 ng/mL standard. The standards were stored at -20°C in 1-mL portions. Working internal standard (IS) solutions were prepared by diluting 50 μL of TRI or 25 μL of PT to 10 mL with 5% potassium bicarbonate solution.

Procedures

Extraction. Wash 1-mL BondElut C-18 columns (Varian, Harbor City, CA 90710) once with 1 M HCl, once with methanol, once with water and once with 1% potassium carbonate, each time aspirating the liquid with a VacElut device. Place 50 μL of the appropriate IS solution, 0.7 mL of the sample and 0.7 mL of acetonitrile in 12 x 75 mm disposable glass tubes. Vortex mix the contents and centrifuge the tubes at 1500 x g for 5 min. Apply the supernatant to the conditioned columns. Let the liquid pass through the columns at a rate of approximately 1 mL/min. Then wash the columns twice with water and once with acetonitrile.

Place 1.5 mL plastic centrifuge tubes without lids in 12 x 75 mm glass tubes. Transfer the extraction columns in these tubes making sure that the tip of the column lies within the centrifuge tube. Apply 0.25 mL of a mixture

TABLE 1

RETENTION TIMES OF DRUGS SUITABLE FOR SOLID PHASE EXTRACTION

DRUG/METABOLITE	ABBREVIATION	RETENTION TIME, MIN.
Amitriptyline	AT	8.3
Clomipramine	CMI	12.6
Desmethylclomipramine	DCMI	11.1
Desmethyldoxepin	DDOX	4.4
Desmethylmaprotiline	DMAP	6.7
Desipramine	DMI	6.4
Doxepin	DOX	5.0
Desmethyltrimpramine	DTRI	8.4
Fluoxetine	FLU	10.9
Fluvoxamine	FLUV	6.8
Imipramine	IMI	7.1
Maprotiline	MAP	8.0
Norfluoxetine	NFLU	9.0
Nortriptyline	NT	7.4
Protriptyline	PT	6.6
Trimipramine	TRI	9.6

of 35% perchloric acid and methanol (1:20). Let the liquid pass through the column bed by gravity (10 min). Finally, drain the columns completely by centrifugation for 20 s. Save the extraction columns in a box. Cover the tubes containing the eluates with aluminium foil and load them in the autosampler with a modified rack so as to accept the centrifuge tubes in which eluates have been collected.

Chromatography. A modular chromatographic system comprising of a model LC-6A pump, a model SPD-10A absorbance detector, a model Sil-9A autosampler and a model C-R6A integrator plotter (all from Shimadzu Scientific Instrument Co., Columbia, Md 21406) was used.

A 15 cm x 4.6 mm (i.d.) Ultrasphere Octyl reversed phase column packed with 5-μm silica particles (Beckman Instruments, San Ramon, CA 94583) protected by a RP-8 1.5 cm guard cartridge packed with 7 μm silica particles (Brownlee Labs, Santa Clara, CA 95050) was used as the analytical column. The mobile phase consisting of acetonitrile (375 mL) + water (625 mL) + tetramethylammonium perchlorate (0.5 g) (Sigma) + 7% perchloric acid (0.5 mL) was pumped at a flow rate of 1.5 mL/min with an operating pressure of 9.2 MPa. The chromatography was performed at ambient temperature.

RESULTS AND DISCUSSION

The described SPE procedure was evaluated for the drugs and metabolites listed in Table 1. Trimipramine has been used as the IS as it is

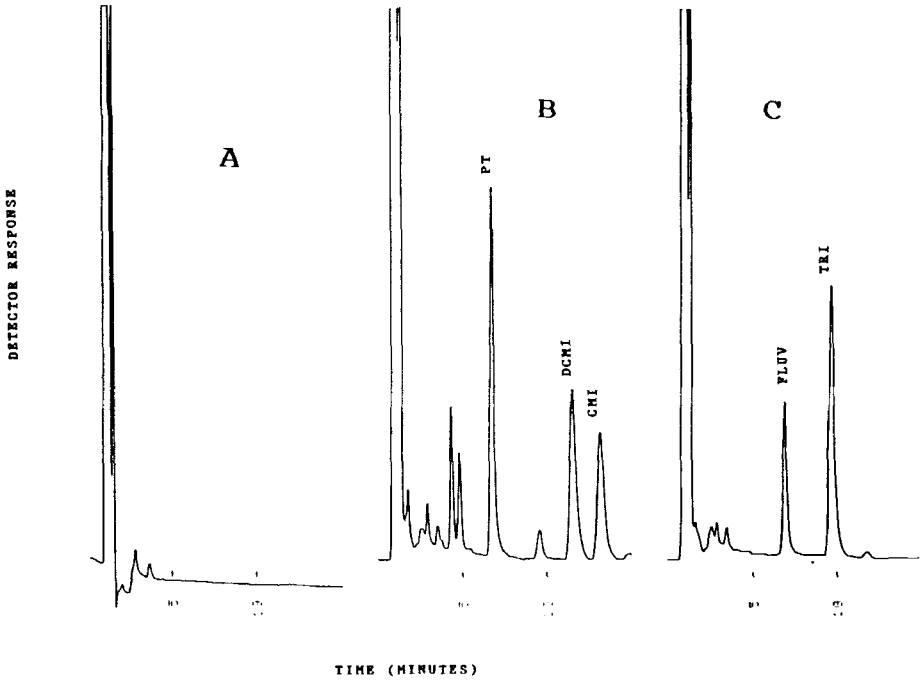

Figure 1. Chromatograms of extracts of (A) drug free serum; (B) serum of a patient receiving clomipramine (desmethylclomipramine = 210 ng/mL, clomipramine = 154 ng/ml; (C) serum of a patient receiving fluvoxamine (= 180 ng/mL).
Detector output = 1V, Range = 0.01 AUFS, Plotter attenuation = 3, Chart speed = 4mm

a commercially available compound (Sigma) and not a commonly prescribed drug. However, TRI cannot be used as the IS for FLU as it elutes close to FLU. It was also observed that TRI elutes close to an extraneous peak (Fig 1C) when clinical samples are processed for the determination of CMI. This extraneous peak is probably due to hydroxy

metabolites of CMI or DCMI. For the determination of FLU, CMI and TRI, PT was used as the internal standard.

Figure 1A shows that the baseline at 215 nm is quite stable and the extract of a drug free serum sample shows few extraneous peaks. There is a significant loss of sensitivity and an insignificant improvement in baseline stability or specificity when the chromatogram is monitored at 240 or 250 nm. Fluvoxamine is monitored at 215 nm though there is about 17% improvement in its detection sensitivity if it is monitored at 210 nm. Figure 1B is the chromatogram monitored at 215 nm of an extract of a patient's serum sample receiving fluvoxamine. The height of the fluvoxamine peak at a concentration of 180 ng/mL indicates adequate sensitivity. However, there is a significant loss in the sensitivity of detection of FLU at 215 nm. Fluoxetines are best detected at 226 nm.

The recovery of all the compounds (Table 1) determined at 4 different concentrations ranging from 25 to 600 ng/mL was in the range of 90% at all concentrations. There is no change in the ratio of peak areas of a drug and its internal standard after extraction by the described procedure. The recovery of a drug and the linearity of recovery are similar whether the standards are prepared in 0.1 M pH 7.4 phosphate buffer, 7% bovine albumin or human serum. The use of reversed phase extraction columns for the isolation of basic drugs is a complex process involving several retention mechanisms in addition to obvious hydrophobic interaction (7 - 9). Musch et al. have used 2 volumes of acetonitrile for the isolation of ß-

blockers, a class of basic drugs and a number of these drugs were recovered in high yield (10). Acetonitrile, which is an efficient eluent for reversed phase analytical chromatography, is only a poor solvent to adversely affect the adsorption of basic drugs on C-18 reversed phase extraction columns or to elute basic drugs adsorbed on reversed phase extraction columns. However, the efficiency of extraction has to be evaluated for every drug individually. Thus, salbutamol, nadolol, atenolol, trazodone, procainamide and thioridazine were recovered in poor yield by the described SPE procedure.

It is believed that binding of drugs with serum proteins is primarily responsible for the matrix effect observed in SPE of drugs (9,10). Precipitation with one volume of acetonitrile prior to the application of the sample to the conditioned extraction column is a simple step to eliminate the matrix effect to a large extent. Precipitation of proteins with acetonitrile simplifies the extraction procedure in another way as well. It is very difficult to control the flow rate of the sample through the extraction column when neat plasma or plasma diluted 1:1 with water or aqueous buffer is applied to the extraction column. However, the sample obtained after protein precipitation of plasma with acetonitrile, flows through the extraction column smoothly and the flow rate through all the columns placed on the vacuum manifold can be controlled collectively with the main vacuum control. The volume of 0.7 mL of serum has been selected so that the entire sample obtained after protein precipitation could be applied to the extraction

column without the use of an adapter and still have enough sample for adequate sensitivity. To obtain clean extracts and good, reproducible recoveries , control of pH in a narrow range of 7 - 9 is necessary. Basic drugs can be eluted from C-18 extraction columns with acetonitrile in the presence of acids and bases. In the present SPE procedure, there is a loss of reproducibility of extraction recoveries if bicarbonate is replaced by carbonate.

Basic drugs, including antidepressants have been isolated in high yield with minimal interfering peaks with the use of cation exchange (11) or mixed phase extraction columns (12). In this mode of extraction, elution of adsorbed drugs is carried out with an alkaline elution reagent and the eluate has to be evaporated prior to chromatography. Lin and Frade (3) have also evaporated the eluates although they have used C-18 extraction columns. In the present procedure, the eluate has been injected directly without any concentration or dilution as suggested by Lensmeyer and Evensen (2). There is no effect on the peak shape when 50 μL of the eluate are injected. Peaks are distorted when higher volumes of eluate are injected. Mazhar and Binder (4) also avoid evaporation but dilute their eluate 2:1 with water to avoid peak distortion. Avoiding the evaporation step is convenient. However, the sensitivity of detection is reduced as only 1/5th of the extract is injected, resulting in a relatively high limit of detection of 5 ng/mL.

In most cases, the extraction column has been used only once as suggested by the manufacturers. Some serum components can be irreversibly bound to the extraction adsorbent adversely affecting its extraction efficiency. We analyzed plasma spiked with IMI + DMI @ 500 ng/mL each with 6 new columns. Then we repeated the extraction of plasma control using the same columns five times. The conditioning, washing and elution steps with the used columns are carried out in the same manner as with the new columns. The absolute recovery of drugs and within batch CV of the ratios of IMI/IS or DMI/IS were similar in all the five sets of extraction. It appears that some of the unwanted impurities are coprecipitated with proteins and others are not adsorbed on the extraction column because of the presence of acetonitrile. The decision to use extraction columns 5 times only, and not more, is arbitrary. Kwong et al. have used the extraction columns 3 times where diluted plasma samples were applied to the columns without protein precipitation (13). For economy and to reduce harmful waste for the environment, we have recycled the mobile phase as proposed by Lensmeyer and Evenson (2). The detector effluent line was put in the 1 L mobile phase bottle for the first 200 injections and then the effluent line was put in the waste bottle. No significant effect on the baseline or on the retention times of drugs was observed. Only 10 mL of methanol is added to 1L of mobile phase after 200 injections.

The present extraction procedure is fairly specific in that only strongly basic drugs are recovered in the final eluate. Acidic, neutral and weakly

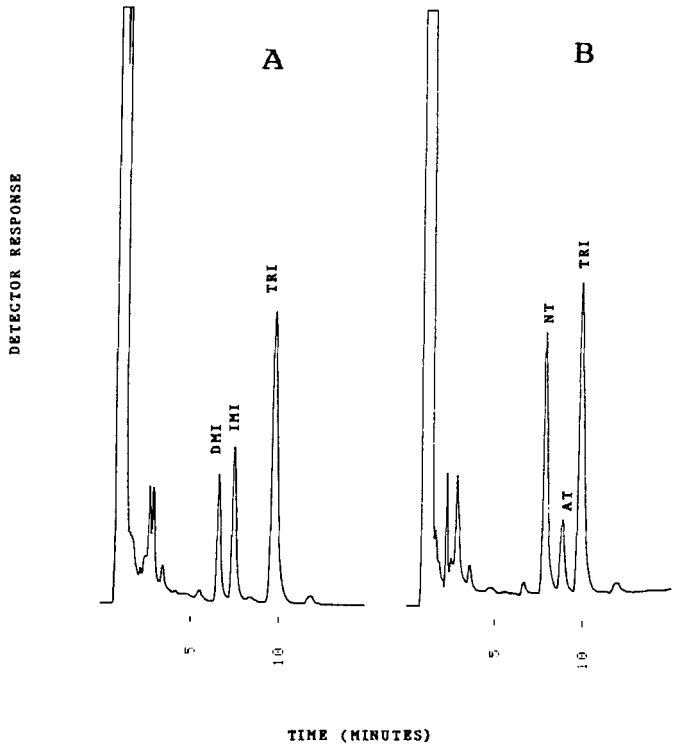

Figure 2. Chromatograms of extracts of (A) serum of a patient receiving imipramine (desipramine = 87 ng/mL, imipramine = 100 ng/mL); (B) serum of a patient receiving amitriptyline (nortriptyline = 110 ng/mL, amitriptyline = 40 ng/mL).

basic drugs are either not adsorbed in the presence of acetonitrile and unfavourable pH or are removed during acetonitrile wash. Thus, commonly prescribed drugs like salicylate, acetaminophen, benzodiazepines and antiepileptic drugs are completely eliminated. However, antihistamines, phenothiazines and antiarrhythmic drugs are co-extracted. In most cases, these drugs are separated from the desired peaks during chromatography.

TABLE 2

PRECISION OF THE METHOD

	AMITRIPTYLINE			NORTRIPTYLINE			IMIPRAMINE			DESIPRAMINE		
	Mean[a]	N	CV,%	Mean	N	CV,%	Mean	N	CV,%	Mean	N	CV,%
Antidep I	104 (83-125)[b]			90 (72-108)			160 (128-192)			159 (127-191)		
Within batch	112	10	4.2	110	10	4.7	157	10	5.5	157	10	5.0
Between batch	102	5	5.5	100	5	4.0	152	5	4.8	154	5	3.6
Antidep II	475 (380-570)			455 (364-546)			547 (438-656)			463 (370-556)		
Within batch	525	10	5.4	521	10	5.9	543	10	4.7	541	10	4.8
Between batch	504	5	4.3	516	5	4.0	514	5	4.2	524	5	4.3

[a] ng/ml

[b] Mean values (and range) obtained by GC/HPLC as reported in Ciba-Corning's Product insert

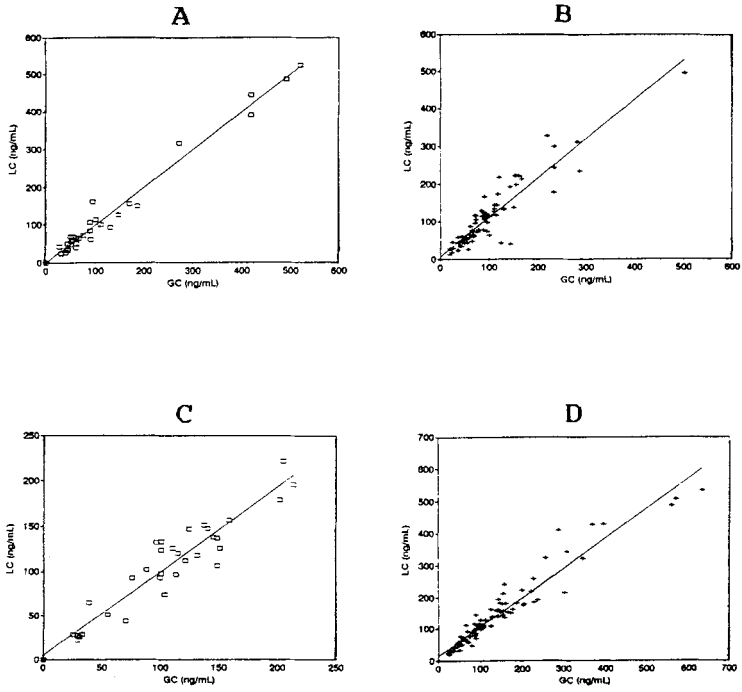

Figure 3. Correlations between drug concentrations of patient samples determined by liquid chromatography with solid phase extraction and gas chromatography with liquid/liquid extraction.
A. Amitriptyline (N = 34, R = 0.99); B. Nortriptyline (N = 80, R = 0.92; C. Imipramine (N = 34, R = 0.95); D. Desipramine (N = 88, R = 0.96)

We have not investigated the recovery of hydroxy metabolites of antidepressants. However, additional peaks, some of which may be due to hydroxy metabolites, are present when the samples obtained from patients receiving these drugs are processed by the described procedure (Figs.1C, 2A & 2B).

We have evaluated this procedure extensively for imipramine and amitriptyline and their corresponding metabolites. To check the accuracy and reproducibility, we used commercially available bi-level tricyclic controls for these drugs (Ciba Corning Diagnostics, Corp. Irvine,CA 92714) and the results are summarized in Table 2. Patient samples receiving AT, NT, IMI or DMI which were analyzed by gas chromatography with liquid/liquid extraction were analyzed by the present SPE procedure and column liquid chromatography. As shown in figure 3 a good correlation has been observed between the 2 sets of results.

In conclusion, a simple protein precipitation step with acetonitrile eliminates matrix effect in the SPE of antidepressants and also makes the extraction procedure convenient as the samples flow through different extraction columns at a similar flow rate, smoothly.

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REFERENCES

1. R.N. Gupta, J. Chromatogr. 576: 183-211(1992)
2. G.L. Lensmeyer, M.A. Evenson, Clin.Chem. 30: 1774-1779(1984)
3. W. Lin, P.D. Frade, Ther. Drug Monit. 9: 448-455(1987)

4. M. Mazhar, S.R. Binder, J.Chromatogr. 497: 201-212(1989)

5. R.N. Gupta, M. Steiner, J.Liq.Chromatogr. 13: 3785-3797(1990)

6. R.N. Gupta, M. Stefanec, F. Eng, Clin.Biochem. 16: 94-97(1983)

7. B. Law, S. Weir, N.A. Ward, J.Pharm.Biomed.Anal. 10: 167-179(1992)

8. B. Law, S. Weir, J.Pharm.Biomed.Anal. 10: 181-186(1992)

9. B. Law, S. Weir, J.Pharm.Biomed.Anal. 10: 487-493(1992)

10. G. Musch, Y. Buelens, D.L. Massart, J.Pharm. Biomed.Anal. 7: 483-497(1989)

11. B.K. Logan, D.T. Stafford, I.R. Tebbett, C.M. Moore, J.Anal.Toxicol. 14: 154-159(1990)

12. X.-H. Chen, J.-P. Franke, J. Wijsbeek, R.A. de Zeeuw, J.Anal.Toxicol. 16: 351-355(1992)

13. T.C. Kwong, R. Martinez, J.M. Keller, Clin.Chim. Acta, 126: 203-208(1982)

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