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Short Communication

Solid-phase extraction of fluoxetine and norfluoxetine from serum with gas chromatography–electron-capture detection

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ABSTRACT

A rapid, selective, and sensitive method is described for the purification and analysis of fluoxetine and norfluoxetine using a solid-phase extraction column and gas chromatography–electron-capture detection. Linear quantitative response curves for fluoxetine and norfluoxetine are generated over a concentration range of 20–200 ng/ml. Overall extraction efficiency of the extraction procedure is found to be >90% and >75% with correlation coefficients of 0.997 and 0.993 for fluoxetine and norfluoxetine, respectively.

INTRODUCTION

The use of tri- and tetracyclic antidepressant (TCA) drugs is becoming increasingly prevalent in the treatment of depression [1]. The potential clinical significance of active drug metabolites indicates the need for sensitive and specific assays capable of measuring several antidepressants and their active metabolites. Common analytical techniques have been reviewed by Scoggins *et al.* [2]. Traditionally these techniques involve liquid–liquid extraction of the drugs from biological fluids followed by high-performance liquid chromatography (HPLC) [3–5], and gas chromatographic (GC) analysis [2]. Monitoring the concentrations of these drugs in plasma for clinical chemistry laboratories is a challenging task. More recently solid-phase extraction techniques have also been used [4,6,7] for the extraction of these TCAs from serum.

Fluoxetine, an antidepressent [8] and chemically unrelated to these tricyclic and tetracyclic antidepressants, is a potent drug being used in the treatment of depressed outpatients whose diagnoses correspond most closely to the DSM-III catagory of major depressive disorders. The action of the drug is presumed to be linked to its inhibition of the central nervous system's neuronal uptake of serotonin. The therapeutic dosage for fluoxetine is 20 mg per day which is metabolized in the liver to norfluoxetine and other unidentified metabolites. Overdoses of

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fluoxetine have been reported to cause death. The plasma concentrations of the drug in these fatalities are 1.93–4.57 μg/ml [9].

This article describes a sensitive, specific, qualitative, and quantitative extraction procedure for fluoxetine and its metabolite norfluoxetine using Bond Elut Certify™, a solid-phase extraction column. Bond Elut Certify is a chemically modified silica gel material bearing three different types of interactions: hydrophobic, polar, and ion exchange which provide extremely clean extracts. This cleanliness is due to the fact that most of the interferences are removed during the column rinse process, while some of the impurities present in the serum are irreversibly retained on the column.

EXPERIMENTAL

Materials

Bond Elut Certify extraction columns and a Vac Elut® vacuum manifold (AI 6000) were provided by Analytichem International (Harbor City, CA, U.S.A.). A vortex mixer was obtained from Scientific Industries (Bohemia, NY, U.S.A.). A Reacti Therm™ heating module and a Reacti Vap™ evaporator were purchased from Pierce (Rockford, IL, U.S.A.).

Equipment

GC–electron-capture detection (ECD) chromatograms were obtained on a Varian Model 3500 instrument (Walnut Creek, CA, U.S.A.). The GC instrument was equipped with a split–splitless injector and a 30 m × 0.25 mm I.D., 0.25 μm film thickness DB-1 capillary column. The oven temperature was programmed at 110°C, increased at a rate of 10°C/min and held at 200°C for 2 min. The injector and detector temperatures were set at 300°C.

Reagents

Fluoxetine, norfluoxetine, and tomoxetine (internal standard, I.S.) were obtained from Lilly (Indianapolis, IN, U.S.A.) and pentafluoropropionic anhydride (PFPA) was obtained from Pierce. Methanol, acetonitrile, hexane, and ethyl acetate were purchased from EM Science (Cherry Hill, NJ, U.S.A.). Serum and all other chemicals were purchased from Fisher Scientific (Tustin, CA, U.S.A.).

Extraction procedure

Serum (1 ml) spiked with fluoxetine and norfluoxetine was added to a large test tube followed by 2 ml of 100 mmol/l KH_2PO_4 (pH 6.0). The Bond Elut Certify columns were connected to a Vac Elut and conditioned with 2 ml of methanol. Excess methanol was removed by washing with 2 ml of 100 mmol/l KH_2PO_4 (pH 6.0) buffer. Serum samples containing fluoxetine, norfluoxetine, and the I.S. were applied to each column. The sample was passed through the bed at a low flow-rate by applying vacuum at approximately 51–76 mmHg. The column was

washed with 2 ml of methanol, 2 ml of acetonitrile, and 2 ml of hexane–ethyl acetate (1:1), respectively. The sorbent was dried for 3 min under full vacuum (380 mmHg).

The tips of the Vac Elut delivery needles were wiped and a rack with labeled collection tubes was placed in the Vac Elut. The drugs were eluted with 2 ml of dichloromethane–isopropanol (8:2) containing 2% ammonium hydroxide. The Vac Elut was disassembled and the test tubes were removed and placed in the Reacti Therm evaporator. The solvent was evaporated to half volume, one drop of 0.3 *M* hydrochloric acid–methanol was added, and the mixture was vortex-mixed and evaporated to dryness at room temperature. The samples were derivatized prior to analysis.

Derivatization

The PFPA derivative of fluoxetine, norfluoxetine and the I.S. were prepared for GC–ECD analysis. A 100-μl volume of 1% triethylamine in toluene was added to the dried extracted sample. The sample was vortexed and 50 μl of PFPA were added to the solution. The reaction mixture was vortex-mixed and heated at 90°C for 30 min. The derivatized sample was cooled and evaporated to dryness under a slow stream of nitrogen at room temperature. Hexane (100 μl) was added to this mixture and vortex-mixed. A 1-μl volume of the sample was injected into the GC system equipped with an electron-capture detector.

RESULTS AND DISCUSSION

The solid-phase extraction procedure described here provides a rapid, reliable, and reproducible isolation of fluoxetine and its metabolite from a spiked serum sample. The bonded phase selectively retains and elutes the drugs by a mixed-mode interaction mechanism. Fig. 1 shows the GC–ECD profiles of the blank specimen extracted on a Bond Elut Certify column. As demonstrated by the chromatogram, the extracts are clean and no interfering peaks are found at the retention times of I.S. and the drugs.

Fig. 2 illustrates the GC–ECD profile of the PFPA derivatives of the drugs and I.S. at a concentration of 75 ng/ml. The recoveries and precision data for the drugs are listed in Table I. The data show overall absolute recoveries calculated from spiked serum samples at concentrations of 25, 50, and 75 ng/ml. The average absolute recoveries for fluoxetine and norfluoxetine were found to be greater than 90 and 75%, respectively, over the concentration range with standard deviations of 3.06 and 2.52. Relative standard deviations (coefficients of variation, C.V.) were calculated to be 3.3% for both drugs.

The linearity was verified by adding known amounts of fluoxetine and norfluoxetine to serum (20–200 ng/ml) and subjecting these to the extraction procedure and chromatography. The plots of the peak-area ratios of fluoxetine and norfluoxetine against I.S. *versus* concentrations were found to be linear (for fluoxetine:

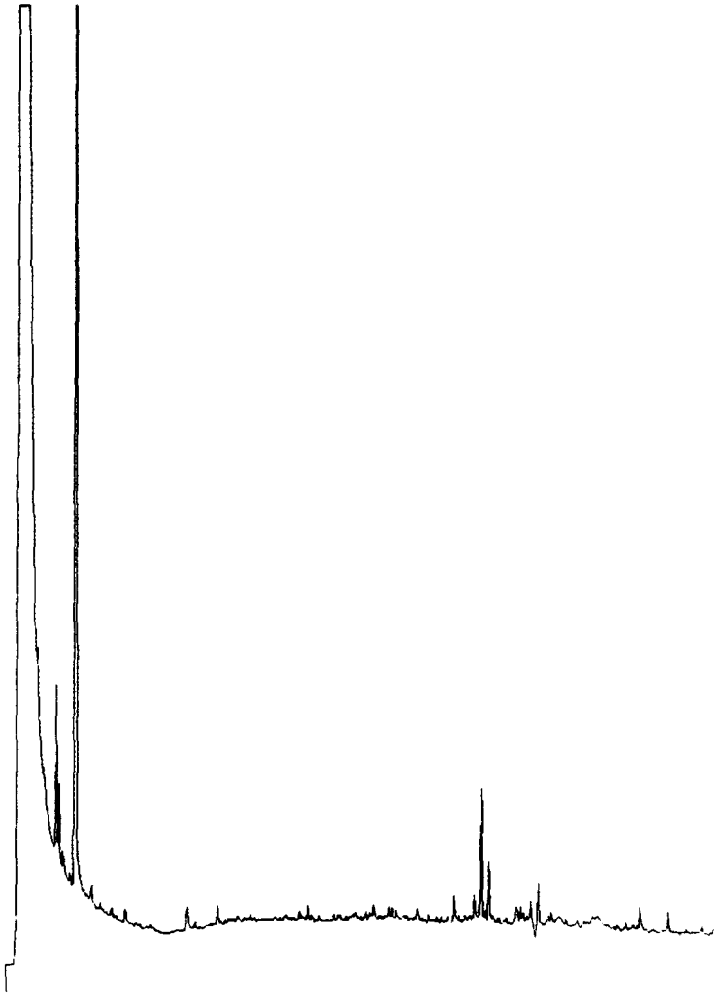

Fig. 1. GC–ECD profile of blank serum.

Fig. 2. GC–ECD profile of PFPA derivatives of fluoxetine, norfluoxetine, and tomoxetine (I.S.) spiked at 75 ng/ml. Peaks: FLU = fluoxetine (t_R = 12.08 min); NOR = norfluoxetine (t_R = 12.77 min); IS = internal standard (t_R = 13.38 min).

TABLE I

RECOVERIES AND PRECISION DATA OF FLUOXETINE AND NORFLUOXETINE

Numbers given represent the mean values for the drug and the metabolite in triplicate determinations at each concentration.

Concentration (ng/ml)	Fluoxetine		Norfluoxetine	
	Recovery (mean ± S.D.) (%)	C.V. (%)	Recovery (mean ± S.D.) (%)	C.V. (%)
25	91 ± 3.06	3.4	75 ± 1.53	2.2
50	93 ± 3.22	3.5	77 ± 2.65	3.4
75	97 ± 2.31	2.4	80 ± 4.16	5.2
Average	94 ± 3.06	3.3	77 ± 2.52	3.3

TABLE II

COMPARISON OF LIQUID–LIQUID EXTRACTION *VERSUS* SOLID-PHASE EXTRACTION PROCEDURE OF FLUOXETINE AND NORFLUOXETINE

Numbers given represent the concentrations obtained from positive serum samples.

Patient sample	Concentration (ng/ml)			
	Liquid–liquid extraction		Solid-phase extraction	
	Fluoxetine	Norfluoxetine	Fluoxetine	Norfluoxetine
1	120	122	335	322
2	68	107	149	122
3	128	189	256	263
4	457	338	1096	445

$y = 0.0950x - 0.637$, $r^2 = 0.997$; for norfluoxetine: $y = 0.272x - 0.299$, $r^2 = 0.993$).

Four positive serum samples at steady-state concentrations, obtained from patients receiving 20–60 mg fluoxetine per day [10], were extracted using the solid-phase extraction procedure and compared with a standard liquid–liquid extraction procedure. The recoveries were found to be two to three times better than the liquid–liquid extraction procedure as shown in Table II.

CONCLUSIONS

A solid-phase extraction procedure has been developed for fluoxetine and its metabolite norfluoxetine from serum using Bond Elut Certify columns. The method is fast and clean, and allows multiple samples to be processed at the same time.

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