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Antidepressants in serum determined by isolation with two on-line sorbent cartridges and liquid chromatography

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ABSTRACT

A selective method for measuring tricyclic antidepressants in serum is reported. A single assay can be done within *ca.* 30 min and eight samples can be assayed in less than 150 min. A 1-ml serum sample was diluted and the drugs were extracted from it by passage through a graphitized carbon black (Carbopack B) cartridge. After one washing, this cartridge was connected on line to another one containing a silica-based strong acid exchanger. The tricyclics were removed from the Carbopack surface and selectively readsorbed onto the cation-exchange surface by passing 4 ml of methylene chloride–methanol (60:40, v/v) through the two cartridges. After another wash, the drugs were desorbed from the cation-exchange surface with 0.8 ml of acetonitrile–methanol–water (72:18:10, v/v) saturated with potassium chloride. An aliquot of this solution was chromatographed on a cyano column, and the absorbance of the effluent was measured at 215 nm. The mean analytical recoveries of tricyclic antidepressants added to serum within the range 10–200 μg/l exceeded 90%, except for 8-hydroxyamoxapine (mean recovery 85.3%) and amoxapine (mean recovery 83.8%) at the lowest serum concentration considered.

INTRODUCTION

Routine determination of tricyclic antidepressants (TCAs) in serum is generally performed by either enzyme immunoassay [1–4] or liquid chromatographic (LC) techniques [5]. Enzyme-multiplied immunoassay technique (EMIT) with monoclonal antibodies is simple and easily adaptable to different automated analysers. However, at present, this technique is usable only for assays of first-generation TCAs. Moreover, as recently shown by Dorey *et al.* [6], the EMIT assay is inappropriate for assaying low concentrations of TCAs in plasma and cannot be modified to eliminate interferences from chlorpromazine [1] and thioridazine. LC methods in the literature vary widely with regard to sample preparation techniques. Increasing use of solid-phase extraction (SPE) in disposable cartridges, with high recovery, is evident [7–10], this being more suitable than the traditional solvent-extraction technique for assaying large numbers of specimens. Recently, in a novel approach, an SPE procedure involving the use of a silica-based weak cation-exchange cartridge, was proposed to achieve selectivity without excessive sample manipulation [11].

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Many proposed analytical schemes involve a solvent evaporation step before chromatographic quantitation of TCA. Many authors point to this step as the major source of wide variations in analytical recovery of TCA drugs [7,12,13].

Recently, there have been many efforts to devise analytical procedures for TCAs in serum that do not involve any concentration step [9,14,15]. This paper describes a selective, sensitive and reliable method for determining 7-hydroxyamoxapine (7-OH-AMOX), 8-hydroxyamoxapine (8-OH-AMOX), amoxapine (AMOX), doxepin (DOX), desmethyldoxepin (DESD), amitriptyline (AMI), nortriptyline (NOR), imipramine (IMI), desipramine (DESI), maprotiline (MAP) and desmethylmaprotiline (DESM) in serum samples. For combined extraction and isolation of TCAs we used SPE with two traps on line. One cartridge was filled with graphitized carbon black, Carbopack B, which has already proved valuable for extracting estrogens and their conjugates [16–18] from body fluids; the other contained a strong acid exchanger. Subsequent resolution on an isocratic LC system involving recycled solvent was accomplished by direct injection of the final extract. The method minimizes variability and lends itself to routine use as it requires minimum sample manipulation. Non-basic drugs, benzodiazepines and commonly prescribed phenothiazines do not interfere.

EXPERIMENTAL

Reagents

Acetonitrile and methanol (LC grade) were obtained from Carlo Erba (Milan, Italy). All other solvents and reagents were ACS reagent grade and were used as supplied (Carlo Erba).

AMI, IMI, NOR, DESI and DOX hydrochloride salt solutions (1.0 g/l, in terms of their free bases) were from Supelco (Bellefonte, PA, U.S.A.). Other pure drugs used as standards were gifts from pharmaceutical companies: DESD (Pfizer, New York, NY, U.S.A.); AMOX, 7-OH-AMOX and 8-OH-AMOX (Lederle Labs., American Cyanamid, Pearl River, NY, U.S.A.); MAP (Ciba Geigy, Basel, Switzerland); DESM methane sulphonate (Ciba Geigy, Saronno, Italy).

Individual stock solutions (1.0 g/l, free base) for all the pure drugs in methanol were prepared. The working composite standard (5.0 mg/l) was prepared by combining an aliquot of each of the eleven stock solutions and diluting the mixture with water. All the solutions were stored at 4°C. Serum-based working standards were prepared by adding the working composite standard to drug-free serum to give final concentrations of 10, 25, 50 and 200 μg/l. We distributed aliquots of these standards among polypropylene tubes and stored them in the freezer until use.

Carbopack B (120–400 mesh), a silica-based sulphonic acid-type cation exchanger (SCX), 1- and 3-ml polypropylene tubes, polyethylene frits (20 μm pore size) and adapters for connecting cartridges in series were kindly supplied by Supelco.

Procedure

The Carbopack B trap was prepared by pouring 80 mg of this material into a 1-ml polypropylene tube with one polyethylene frit in the bottom. The tube was tapped gently and a second frit was placed on the top of the Carbopack bed. Similarly, the SCX cartridge was prepared by pouring 200 mg of it into a 3-ml tube.

Both the Carbopack B and SCX cartridges were fitted into side-arm filtering flasks where pressure could be reduced by running-water pumps. The Carbopack cartridge was primed by passing 1 ml of methylene chloride–methanol (60:40, v/v) at a flow-rate of *ca.* 2 ml/min, followed by 1 ml of methanol and 1 ml of water. The cation-exchange material was converted from the Na form into the H form by passing through the cartridge 8 ml of 0.12 *M* methanolic HCl at a flow-rate of *ca.* 1 ml/min, followed by 2 ml of methanol.

A 1-ml volume of serum was diluted with 1 ml of distilled water. A 3-ml empty tube was attached to the primed Carbopack B cartridge by an adapter, and the sample mixture was poured in. The pressure in the filtering flask was reduced to get a flow-rate of *ca.* 0.5 ml/min. After the sample was passed through, the reservoir was removed and the cartridge washed with 2 ml of distilled water. Water was removed from the Carbopack bed by drawing room air through it for *ca.* 30 s.

Thereafter, the Carbopack B cartridge was connected to that containing the primed exchanger. This cartridge tandem assembly was fitted to the vacuum apparatus, a 3-ml plastic tube was attached to the top of the Carbopack cartridge, and 1 ml of methanol was poured in. The pressure in the flask was adjusted to obtain a flow-rate of 1 ml/min. After the methanol had been passed through, TCAs were moved from the Carbopack surface to the SCX one by passing 4 ml of methylene chloride–methanol (60:40, v/v). The Carbopack cartridge was discarded and the SCX bed washed with 2 ml of 0.1 *M* KCl in methanol–water (15:85, v/v). After this washing, a glass vial was introduced into the flask and placed under the cartridge. TCAs were eluted by applying acetonitrile–methanol–water (72:18:10, v/v) saturated with KCl to the top of the SCX bed, and passing this eluent system at a flow-rate not greater than 1 ml/min. We prepared this solution by adding to 1 ml of 1 *M* KCl in water 9 ml of acetonitrile–methanol (80:20, v/v). Separate addition of the two organic solvents to water provoked the formation of a floccular precipitate of KCl. The first 1.2 ml of the eluate were collected and diluted with the same volume of water containing 50 μg/l procainamide (internal standard); 200 μl of the final solution were injected.

LC apparatus

A Model 5000 liquid chromatograph (Varian, Walnut Creek, CA, U.S.A.) equipped with a Rheodyne Model 7125 injector with 200-μl loop and with a Model 2050 UV detector (Varian) was used. The output of the detector was connected to an electronic integrator SP 4270 (Spectra-Physics). The 25 cm × 4.6

mm I.D. column filled with 5-μm (average particle size) cyanopropyl reversed-phase packing (LC-PCN) was from Supelco. A 7.5 cm × 4.6 mm I.D. column filled with 18-μm silica packing (Supelco) was inserted between the pump and the injector to minimize dissolution of packing. Acetonitrile–methanol–phosphate buffer (10 m*M*; pH 7.8) (52:13:35, v/v) was used as mobile phase. The flow-rate was 2.0 ml/min. TCAs were monitored with the detector set at 215 nm.

RESULTS AND DISCUSSION

LC column variables

Initially, according to a previous report [8] dealing with the separation of first-generation TCAs, the cyano reversed-phase column was used with the mobile phase buffered at pH 7. Under these conditions, however, AMOX and 7-OH-AMOX co-eluted. Moreover, AMOX and its two metabolites had retention times very similar to an endogenous compound in serum, which produced a very broad peak. Further, procainamide interfered with the analysis of DOX. Increasing the water content in the mobile phase had the sole effect of increasing the total analysis time. The goal of quantifying all the TCAs considered in a single, isocratic chromatographic run was reached by increasing the pH to 7.8. At this pH, the silica-based LC column slowly deteriorated, but this effect was minimized by using a mobile phase silica saturating column. By this expedient, no deterioration of the column was observed after six months of daily use. Finally, 1 l of mobile

TABLE I

ANALYTICAL RECOVERY OF TCAs ADDED TO POOLED SERUM

Results of six replicate analyses each.

Analyte	Recovery (%)					
	Added: 10 μg/l		Added: 50 μg/l		Added: 200 μg/l	
	Mean	Range	Mean	Range	Mean	Range
8-OH-AMOX	85	79–93	92	87–95	93	90–95
7-OH-AMOX	90	84–94	95	90–97	97	94–98
AMOX	84	79–92	90	86–94	92	90–94
DOX	95	91–101	98	95–101	99	97–99
AMI	99	94–102	99	98–102	99	98–100
IMI	96	90–101	97	95–100	96	95–99
DESM	100	94–102	99	96–101	100	96–99
DESD	97	92–102	97	95–100	98	96–99
NOR	96	92–98	97	94–100	97	95–99
DESI	97	93–103	97	95–101	98	95–100
MAP	95	91–99	96	94–100	97	95–99

phase could be recycled at least three times with no observable negative effect in terms of sensivity and resolution of the TCAs. More prolonged use of the same mobile phase had the effect of producing a large negative peak that disturbed correct quantification of DESM.

Recovery

The analytical recovery at subtherapeutic, medium and high therapeutic concentrations of TCAs in serum was assessed by assaying serum-based working standards containing the analytes at individual concentrations of 10, 50 and 200 μg/l. The absolute analytical recovery was calculated by comparing peak areas from extracted samples with peak areas of standards that were not extracted (Table I). Some decrease of the extraction efficiency for AMOX and its metabolites occurred at the lowest drug concentrations considered. The same effect was observed when these three compounds were dissolved in water at 5 μg/l, and 2 ml of this solution were carried through the procedure. It is probable that the partial

TABLE II

WITHIN-RUN PRECISION FOR TCAs IN POOLED SERUM

Results of nine replicate analyses each.

Analyte	Concentration (mean ± S.D.) (μg/l)	C.V. (%)
8-OH-AMOX	22.1 ± 1.46	6.6
	190.8 ± 3.62	1.9
7-OH-AMOX	23.1 ± 1.25	5.4
	196.4 ± 3.34	1.7
AMOX	21.7 ± 1.52	7.0
	188.6 ± 3.96	2.1
DOX	24.1 ± 1.27	5.3
	197.4 ± 3.55	1.8
AMI	24.7 ± 0.79	3.2
	198.6 ± 2.78	1.4
IMI	23.6 ± 1.11	4.7
	193.6 ± 3.87	2.0
DESM	24.9 ± 0.77	3.1
	199.4 ± 3.39	1.7
DESD	24.3 ± 1.09	4.5
	195.8 ± 3.92	2.0
NOR	24.2 ± 0.60	2.5
	193.4 ± 2.71	1.4
DESI	24.4 ± 0.95	3.9
	196.7 ± 3.34	1.7
MAP	24.0 ± 0.79	3.3
	194.6 ± 3.11	1.6

loss of AMOX and its derivatives was due to strong adsorption of these compounds on few, unknown chemical heterogeneities contaminating the Carbopack B surface.

Precision

The within-run precision of the method was evaluated by repeated analyses ($n=9$) of serum-based working standards at two different concentrations, corresponding roughly to the lower and upper limits of therapeutic range reported for most TCAs (Table II). Moreover, by assaying each sample five times during one month, it was observed that the between-run precision was not significantly different from the within-run precision.

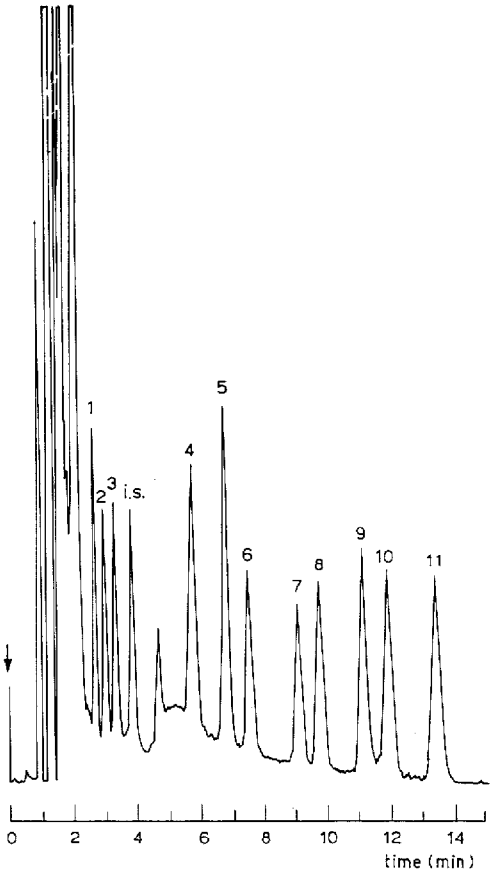

Fig. 1. Typical chromatogram of a serum sample supplemented with each drug at 50 μg/l. Peaks: i.s. = internal standard; 1 = 8-OH-AMOX; 2 = 7-OH-AMOX; 3 = AMOX; 4 = DOX; 5 = AMI; 6 = IMI; 7 = DESM; 8 = DESD; 9 = NOR; 10 = DESI; 11 = MAP.

TABLE III

RETENTION TIMES OF COMMON DRUGS

Drug	Retention time (min)	Drug	Retention time (min)
Flunarizine	2.2	Chlorpromazine	6.5
Verapamil	2.5	AMI	6.9
8-OH-AMOX	2.6	IMI	7.8
7-OH-AMOX	3.0	Thioridazine	8.7
AMOX	3.4	DESM	9.2
Procainamide	3.9	DESD	10.0
Acebutolol	4.1	NOR	11.4
Propranolol	5.2	DESI	12.1
Trifluoperazine	5.9	MAP	13.5
DOX	5.9		

Limit of detection

The limit of detection (three times the signal-to-noise ratio) ranged from 3 to 4.5 μg/l for all compounds considered. Fig. 1 shows a typical chromatogram obtained by this procedure.

Selectivity

The extent of background interference eluting at the same times as the drugs considered was evaluated by analysing fifteen individual, drug-free serum samples. The background absorbances were less than 1.0 μg/l of serum, and this does not interfere. The method was tested for possible interferences by drugs co-administered with TCAs by dissolving drugs in water and extracting. The use of a dual precolumn effectively eliminates any non-basic drug, such as barbiturates, salicylate, caffeine, theophylline and 8-chlorotheophylline. Among basic drugs, benzodiazepines, which are weakly basic in nature, were extracted by Carbopack but they passed almost unretained through the second cartridge. Moreover, under the LC conditions selected, they were eluted well before TCAs. Drugs with a basicity similar to that of TCAs, *e.g.* phenothiazines, β-adrenergic blocking drugs and antiarrhythimics, might be co-eluted with TCAs from the two-cartridge extraction assembly. Table III lists the absolute chromatographic retention times of TCAs and some of these basic drugs. Trifluoperazine, which has virtually the same retention time as DOX, does not interfere with the analysis of the latter compound: when an aqueous solution containing phenothiazines at individual concentrations of 1 mg/l was carried through the extraction procedure, complete loss of trifluoperazine was unaccounted for in the final extract.

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