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Note

Measurement of trazodone in biological fluids by high-performance liquid chromatography

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Trazodone, 2-[3-(4-*m*-chlorophenyl-1-piperazinyl)propyl]-1,2,4-triazolo[4,3-α]pyridin-3-(2H)-one, is structurally unrelated to the tricyclic antidepressants or other psychotropic drugs but nevertheless has antidepressant and anxiolytic activity [1]. Adverse reactions to trazodone include drowsiness and ataxia, and these were also the principal signs reported in twenty patients suspected of acute self-poisoning with this drug [2]. In order to facilitate further studies a simple method for the measurement of trazodone in biological specimens was required.

Published methods for plasma trazodone assay have used spectrophotofluorimetry [3], gas—liquid chromatography with flame-ionisation [4—6], nitrogen-selective [7, 8] or mass-fragmentographic [5] detection, or high-performance liquid chromatography (HPLC) using an organosilane-modified silica column together with UV [9—11] or electrochemical oxidation [12] detection. The method described here employs a silica column together with a non-aqueous ionic eluent [13] and fluorimetric detection, and has advantages of speed, simplicity, selectivity and small sample requirement.

EXPERIMENTAL

Materials and reagents

Trazodone hydrochloride was obtained from Roussel (Uxbridge, U.K.). The internal standard (dimethothiazine) was used as a solution in deionised water (20 mg/l) after dilution from a methanolic solution containing dimethothiazine mesylate (May & Baker, Dagenham, U.K.) (1.00 g/l free-base). The aqueous internal standard solution was stable for at least three months if stored at 4°C in the absence of visible light. Methanol and methyl *tert.*-butyl ether were

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HPLC grade (Rathburn, Walkerburn, U.K.) and perchloric acid (70%) and tris-(hydroxymethyl)methylamine (Tris) were analytical-reagent grade (BDH, Poole, U.K.). Tris solution (2 *M*, pH 11) was prepared in deionised water.

High-performance liquid chromatography

A constant-flow reciprocating pump (Applied Chromatography Systems 750/04) was used with a syringe-loading sample injection valve (Rheodyne 7125, 100-μl loop), and a stainless-steel column (125 × 5 mm I.D.) packed with Spherisorb S5W silica (5 μm average particle size) (Hichrom, Woodley, U.K.) used at ambient temperature. The column effluent was monitored by fluorescence (Kratos-Schoeffel FS970, excitation 200 nm, no emission filter, time constant 0.5 sec) and data capture was performed using a Hewlett-Packard 3392A recording integrator. The eluent was methanol containing 0.02% (v/v) perchloric acid (flow-rate 2.0 ml/min, back-pressure ca. 60 bar).

Sample preparation

Sample or standard (20 μl) was added to a glass test-tube (40 × 5 mm I.D., Dreyer tube) (A.A. Service, Croydon, U.K.). Internal standard solution (20 μl), Tris solution (50 μl) and methyl *tert.*-butyl ether (200 μl) were added using Hamilton repeating mechanisms fitted with Hamilton gas-tight luer-fitting glass syringes and stainless-steel needles. The contents of the tube were vortex-mixed (30 s) and centrifuged (9950 *g*, 2 min; Eppendorf 5412) and a portion of the extract (100 μl) was injected. Duplicate analyses were performed and the mean taken.

Instrument calibration

Solutions containing 0.25, 0.50, 1.0, 2.0, 3.0, 5.0 and 10.0 mg/l trazodone were prepared in equine serum (Gibco Europe, Paisley, U.K.) by dilution from a methanolic solution of trazodone hydrochloride (1.00 g/l free-base). Additional standards were prepared as appropriate for other specimens. These solutions were stable for at least six months if stored in small portions at −20°C in the absence of visible light. On analysis of these solutions the ratio of the peak area of trazodone to the peak area of dimethothiazine, when plotted against trazodone concentration, was linear and passed through the origin of the graph. Specimens containing trazodone at concentrations greater than 10 mg/l were reanalysed after dilution with drug-free equine serum.

RESULTS AND DISCUSSION

Sensitivity adequate for the measurement of the plasma trazodone concentrations attained following acute overdosage (ca. 2—20 mg/l) [14] may be obtained using HPLC with either UV absorption or electrochemical oxidation detection [9—12]. However, fluorescence detection provides good selectivity, sensitivity and reproducibility. No endogenous sources of interference have been found in plasma (Fig. 1) or in the other fluids studied. The retention times of trazodone and some other compounds which may be encountered are given in Table I. A number of additional compounds tested gave no response even at high concentrations. The design of the flow cell in the Schoeffel FS970

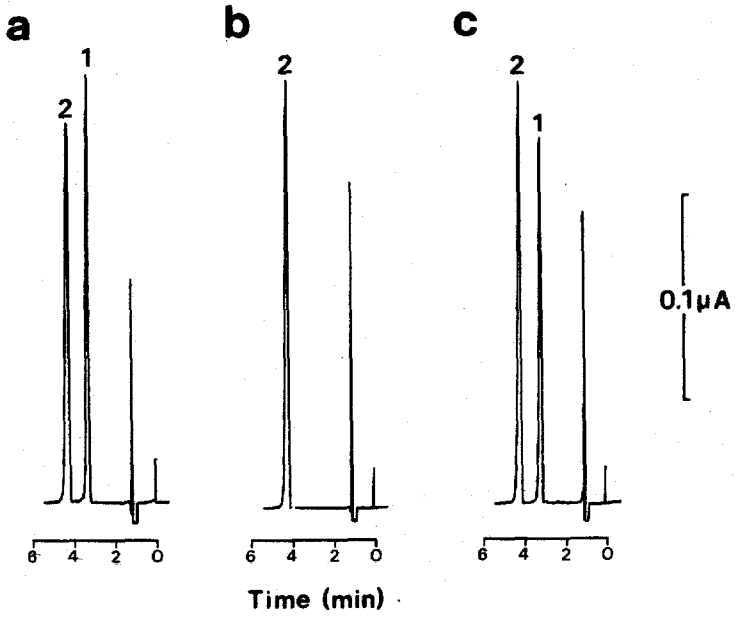

Fig. 1. Chromatograms obtained using the HPLC trazodone assay. The initial dimethothiazine concentration was 20 mg/l in each case; 100 μl of each extract were injected. Specimens: (a) Standard solution prepared in equine serum containing 3.0 mg/l trazodone. (b) Trazodone-free human plasma. (c) Plasma specimen from a trazodone overdose patient (trazodone concentration 2.5 mg/l). Peaks: 1 = trazodone; 2 = dimethothiazine.

may be a factor in ensuring that non-fluorescent species showed no response even though we did not use an emission filter.

Although trazodone gives rise to a number of metabolites in man [7, 15], no additional peaks have been observed on analysis of plasma specimens from patients who had ingested trazodone (Fig.1). However, two additional compounds were observed on analysis of urine specimens from trazodone overdose patients [retention times relative to dimethothiazine 0.60 (minor peak) and 0.72 (major peak)]. Of the synthetic trazodone metabolites available for study, 2-[3-carboxypropyl]-1,2,4-triazolo[4,3-*a*]pyridin-3(2H)-one (AF 2067; cf. ref. 9) was hardly retained (retention time relative to dimethothiazine 0.35), while 1-*m*-chlorophenylpiperazine (*m*-CPP, cf. ref. 7) was not detected on this system. TLC of urine extracts from trazodone overdose patients suggests that at least three metabolites are present in addition to the parent compound [14], *m*-CPP corresponding approximately to metabolite 3 while AF 2067 did not migrate from the origin.

The trazodone recovery from equine serum standards was assessed by comparison of the peak area obtained on analysis of methyl *tert.*-butyl ether extracts to those of a corresponding methanolic standard. The mean recovery varied between 95 and 102% in the range 2—10 mg/l. The intra- and inter-assay coefficients of variation obtained on analysis of standard trazodone solutions prepared in equine serum are given in Table II. Using a 20-μl specimen, the

TABLE I

RETENTION TIMES RELATIVE TO DIMETHOTHIAZINE OF TRAZODONE AND SOME OTHER COMPOUNDS

Compound	Relative retention time	Compound	Relative retention time
Nitrazepam*	0.57	Terazosin	0.69
Penbutolol	0.58	Nortrimipramine	0.70
Nadolol	0.59	Trazodone metabolite (see text)	0.72
Oxprenolol*	0.59	Desipramine	0.75
Acebutolol	0.60	Protriptyline	0.75
Ajmaline	0.60	Dextropropoxyphene*	0.76
Pyrimethamine*	0.60	Trazodone	0.80
Trazodone metabolite (see text)	0.60	Nortriptyline	0.82
Desalkylflurazepam	0.61	Verapamil	0.84
Flecainide	0.61	Butriptyline	0.85
Sotalol**	0.61	Normianserin	0.85
Mexiletine	0.62	Trimipramine	0.92
Pindolol	0.62	Amitriptyline	0.95
Triamterene	0.62	N-Acetylprocainamide	0.95
Atenolol**	0.63	Mianserin***	0.96
Doxazosin	0.63	Clomipramine	0.99
Prazosin	0.63	Orphenadrine***	0.99
Propranolol	0.63	Dimethothiazine	1.00
Timolol	0.63	Trimeprazine	1.01
Labetalol***	0.64	Chlorpromazine	1.04
Prajmalium	0.65	Methotrimeprazine	1.14
Dipyridamole	0.66	Imipramine	1.15
Nordextropropoxyphene*	0.66	Flurazepam***	1.50
Norverapamil	0.67	Procainamide***	2.5
Metoprolol	0.68	Quinine/quinidine***	6.9

*Poor fluorescence.
**Poor extraction efficiency.
***Tailing peak.

TABLE II

INTER- AND INTRA-ASSAY REPRODUCIBILITY ASSESSED USING STANDARD SOLUTIONS PREPARED IN EQUINE SERUM

n = 10 at each concentration

	Trazodone concentration (mg/l)	Coefficient of variation (%)
Intra-assay	2	3.4
	5	1.1
	10	1.6
Inter-Assay	2	4.4

limit of accurate measurement was 0.25 mg/l (intra-assay coefficient of variation 9.4%, n = 10), although should the need arise the sensitivity of the assay could be enhanced by increasing the sample size.

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REFERENCES

1 M.M. Al-Yassiri, S.I. Ankier and P.K. Bridges, Life Sci., 28 (1981) 2449.
2 J.A. Henry and C.J. Ali, Hum. Toxicol., 2 (1983) 353.
3 B. Catanese and R. Lisciani, Boll. Chim. Farm., 109 (1970) 369.
4 B. Munday, M.J. Kendall, M. Mitchard and T.A. Betts, Br. J. Clin. Pharmacol., 2 (1975) 19.
5 G. Belvedere, A. Frigerio and C. Pantarotto, J. Chromatogr., 112 (1975) 631.
6 W.H. Anderson and M.M. Archuleta, J. Anal. Toxicol., 8 (1984) 217.
7 S. Caccia, M. Ballabio, R. Fanelli, G. Guiso and M.G. Zanini, J. Chromatogr., 210 (1981) 311.
8 D.R. Abernathy, D.J. Greenblatt and R.I. Shader, Pharmacology, 28 (1984) 42.
9 S.I. Ankier, B.K. Martin, M.S. Rogers, P.K. Carpenter and C. Graham, Br. J. Clin. Pharmacol., 11 (1981) 505.
10 I. Root and G.B. Ohlson, J. Anal. Toxicol., 8 (1984) 91.
11 S.H.Y. Wong, S.W. Waugh, M. Draz and N. Jain, Clin. Chem., 30 (1984) 230.
12 R.F. Suckow, J. Liq. Chromatogr., 6 (1983) 2195.
13 R.J. Flanagan, G.C.A. Storey, R.K. Bhamra and I. Jane, J. Chromatogr., 247 (1982) 15.
14 J.A. Henry, C.J. Ali, R. Caldwell and R.J. Flanagan, Psychopathology, 17 (Suppl. 2) (1984) 77.
15 L. Baiocchi, A. Frigerio, M. Giannangeli and G. Palazzo, Arzneim.-Forsch., 24 (1974) 1699.