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## Note

# Determination of trazodone in human plasma by liquid chromatography with fluorescence detection

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Trazodone is one of the newly introduced antidepressant drugs. It has been claimed that unlike tricyclic antidepressants, trazodone does not have anticholinergic side-effects and is relatively less cardiotoxic [1, 2]. Optimal therapeutic range for this drug has not yet been established [3]. However, knowledge of plasma concentration of trazodone is required to check compliance and to decide treatment failures. Trazodone has been determined by gas chromatography in therapeutic [3, 4] and in toxic concentrations [5]. This drug has also been determined by liquid chromatography (LC) using UV detection at 254 nm [6], at 242 nm [7] or at 214 nm [8] and with electrochemical detection with an oxidation potential of 1.15 V [9]. Trazodone has been determined fluorometrically in the eluates of spots corresponding to the $R_F$ value of trazodone obtained by the separation of plasma extracts by thin-layer chromatography [10]. We describe an LC procedure for sensitive determination of trazodone using its native fluorescence for its detection.

## EXPERIMENTAL

### *Reagents*

All reagents were of analytical-reagent grade. Solvents had been distilled in glass by the supplier (Caledon Labs., Georgetown, Canada). Deionized water was distilled in an all-glass still.

### *Standards*

A 1 g/l stock solution of trazodone was prepared by dissolving 54.9 mg of

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trazodone hydrochloride (Bristol Meyer Canda) in 50 ml of methanol. The solution was stored at 4°C. A 100 mg/l solution was prepared by diluting 1 ml of stock trazodone to 10 ml with water. Plasma standard of 4 mg/l was prepared by adding 2 ml of diluted trazodone solution to 50 ml of drug-free pooled plasma: additional plasma standards of 2.0, 1.0, 0.5, 0.25, 0.125 and 0.62 mg/l were prepared by serial dilutions with drug-free plasma. In some cases bovine serum albumin solution (60 g/l) was used when drug-free plasma was not available. The standards were stored frozen at −15°C in 1-ml portions. The stock internal standard solution (100 mg/l) was prepared by dissolving 20 mg of harmine hydrochloride (Aldrich) in 200 ml of methanol and stored at 4°C. Working internal standard solution was prepared by diluting 10 μl of stock to 20 ml with 0.1 *M* sodium acetate solution.

*Sample preparation*

Bond-Elut $C_{18}$ 1-ml disposable extraction columns (Analytichem International) were washed under suction twice with methanol, once with water and once with 0.1 *M* sodium acetate. Working internal standard (200 μl) and plasma sample (0.5 ml) were applied to the washed columns. Suction was adjusted so that the liquid passed through the columns in 30–50 sec. The columns were washed twice with water and twice with methanol–water (10:90). The columns were transferred to labelled 100 × 16 mm disposable glass tubes. Methanol (0.5 ml) was added to each column and the tubes were centrifuged to elute the columns. An aliquot (25 μl) of the eluate was injected into the chromatograph.

*Chromatography*

The chromatographic separation is performed isocratically at room temperature with a single-piston reciprocating pump (Model 110A; Beckman) Injections are made with a syringe-loading injector with a 20-μl loop (Model 7125, Rheodyne). The peaks are detected with a fluorescence detector (Model RF-530; Shimadzu) at an excitation wavelength of 320 nm and an emission wavelength of 440 nm. A 15 cm × 4.6 mm Ultrasphere octyl column packed with particles of average diameter of 5 μm (Beckman) is used. The column is protected with a guard column (70 × 4.6 mm) packed with Co:Pell ODS of particle size 30–40 μm (Whatman). The mobile phase is prepared by mixing 500 ml acetonitrile, 500 ml water, 0.5 ml tetramethylammonium hydroxide (20 g/l) (Sigma) and 0.5 ml of 70% perchloric acid. It is pumped at a flow-rate of 1 ml/min with 6.9 MPa as back-pressure. The peaks are recorded with a recording integrator (Model CR 3A, Shimadzu). The integrator is connected to 1V full scale output of the detector.

## RESULTS AND DISCUSSION

Trazodone has been extracted from plasma with a variety of organic solvents at alkaline pH (6–8). Solid-phase extraction as described in the present report is rapid and simple. Trazodone is extracted in yields of 90–100%. There is no change in the ratio of drug to internal standard after extraction by the present procedure.

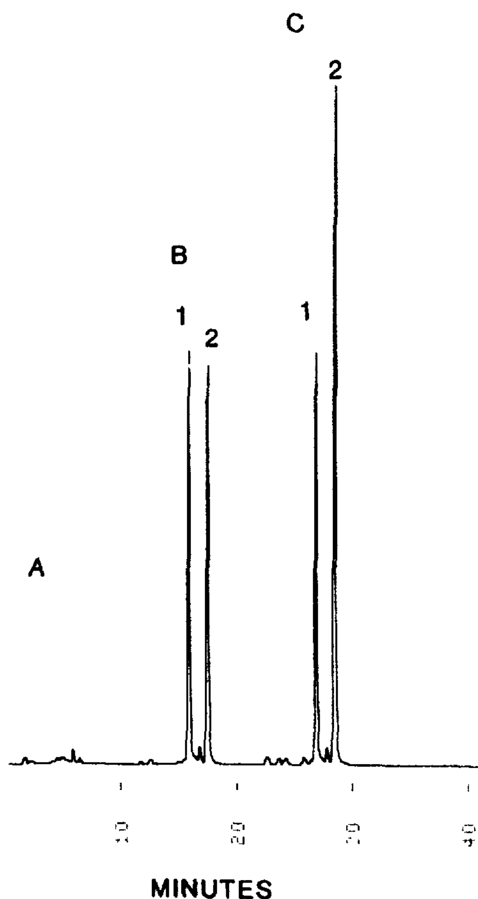


Fig 1. Liquid chromatograms of (A) drug-free plasma, (B) plasma with added trazodone (0.3 mg/l), (C) plasma of a patient receiving a daily trazodone dose of 150 mg. The trazodone peak corresponds to 0.52 mg/l. Detector: sensitivity high, integrator attenuation 3, chart speed 5 min/cm. Peaks: 1 = harmine; 2 = trazodone.

Fig. 1A shows a chromatogram of the extract of drug-free plasma. There are virtually no peaks due to endogenous components of plasma. Fig. 1B shows a chromatogram of an extract of plasma standard. The peaks of drug and of internal standard are sharp and well separated. The standard curve is linear for the range tested (0.05—4 mg/l) and passes through the origin.

Detection of native fluorescence provides high sensitivity and selectivity. As low as 1 ng of trazodone injected on-column can be quantitated. Because of high sensitivity of detection and relatively high therapeutic concentrations of this drug, evaporation of the plasma extract is not required. Harmine used as internal standard has a structure different from that of trazodone. However, it behaves similar to trazodone during extraction and is fluorescent at the excitation and emission wavelength selected for trazodone. However, fluorescence response of harmine under the described conditions is fifteen times that of trazodone. Etoperidone, an analogue of trazodone, which has been used as internal standard for the determination of trazodone [9] lacks

TABLE I

INTERFERENCE STUDIES

| Compound | Concentration in plasma (mg/l) | Retention time (min) |
|---|---|---|
| Harmine | | 5.6 |
| Trazodone | | 7.7 |
| Acetaminophen | 100 | —* |
| N-Acetylprocainamide | 20 | — |
| Amitriptyline | 1.0 | — |
| Clomipramine | 1.0 | — |
| Diazepam | 1.0 | — |
| Disopyramide | 10 | — |
| Doxepin | 1.0 | — |
| Imipramine | 1.0 | — |
| Lidocaine | 10 | — |
| Maprotiline | 1.0 | — |
| Procainamide | 20 | — |
| Propranolol | 0.5 | — |
| Protriptyline | 1.0 | 9.1 |
| Quinidine | 5.0 | 9.3 |
| Quinine | 5.0 | 8.8 |
| Salicylate | 50 | — |
| Trimipramine | 1.0 | — |

*Dash signifies no peak between 2 and 20 min.

TABLE II

ESTIMATION OF PRECISION

| *n* | Plasma trazodone level (mean ± S.D., mg/l) | Coefficient of variation (%) |
|---|---|---|
| *Within-batch* | | |
| 10 | 0.050 ± 0.003 | 5 1 |
| 10 | 0.501 ± 0.015 | 3.0 |
| *Between-batch* | | |
| 12 | 0.050 ± 0.003 | 6.5 |
| 12 | 0.500 ± 0.030 | 6.1 |

fluorescence at the excitation and emission wavelengths used for the detection of trazodone. Plasma standards spiked with a number of drugs (Table I) were analyzed by the present procedure. No interference has been observed. Only quinines and protriptyline show any response. Analysis of plasma of a patient receiving trazodone does not show any additional peaks (Fig. 1C). The procedure is satisfactorily reproducible (Table II).

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REFERENCES

1 M.M. Al-Yassiri, S.I Ankier and P.K. Bridges, Life Sci., 28 (1981) 2449.
2 R.N. Brogden, R.C. Heel, T.M. Speight and G.S. Avery, Drugs, 21 (1981) 401.
3 D.R. Abernethy, D.J. Greenblatt and R.I. Shader, Pharmacology, 28 (1984) 42.
4 S. Caccia, M. Ballabio, R Fanelli, G. Guiso and M.G. Zanini, J. Chromatogr., 210 (1981) 311.
5 W.H. Anderson and M.M. Archuleta, J. Anal. Toxicol., 8 (1984) 217.
6 S.I. Ankier, B.K. Martin, M.S Rogers, P.K. Carpenter and C. Graham, Brit. J. Clin. Pharmacol., 11 (1981) 505
7 I. Root and G.B. Ohlson, J. Anal. Toxicol., 8 (1984) 91.
8 S.H.Y. Wong, S.W. Waugh, M. Draz and N. Jain, Clin. Chem., 30 (1984) 230.
9 R.F. Suckow, J. Liq. Chromatogr., 6 (1983) 2195.
10 S. Putzolu, J.C. Pecknold, L. Baiocchi et al., Psychopharmacol. Bull., 12 (1) (1976) 40.