Br. J. clin. Pharmac. (1981), **11**, 505–509

TRAZODONE—A NEW ASSAY PROCEDURE AND SOME PHARMACOKINETIC PARAMETERS

S.I. ANKIER
Roussel Laboratories, Roussel House, Wembley Park, Middlesex HA9 0NF

B.K. MARTIN, M.S. ROGERS, P.K. CARPENTER & C. GRAHAM
Bios (Consultancy & Contract Research), College Ride, Bagshot, Surrey HU19 5ER

1 A simple and specific procedure is described for the determination of the new anti-depressant trazodone in human plasma utilising reverse-phase HPLC which is sensitive to 20 ng ml^{-1}.

2 Following oral administration of single 50 mg doses of two formulations of trazodone on separate occasions to healthy fasted volunteers, the peak plasma concentration, time to peak concentration, area under the curve, elimination rate constant and half-life were determined.

3 The two formulations are closely similar and they are considered to have comparable bioavailability.

Introduction

Trazodone (I, Figure 1) is the approved name for 2-[3-(4-*m*-chlorophenyl-1-piperazinyl) propyl]-1,2,4-triazolo [4,3-*a*] pyridin-3(2*H*)-one, and its hydrochloride (Molipaxin) is currently being marketed as a new anti-depressant agent. This compound, a triazolo-pyridine derivative, represents a novel structure unrelated to that of the tricyclics or other psychotropic drugs. Synthesised in 1966, its chemistry was described by Palazzo (1973). The pharmacology of trazodone has been reported by Silvestrini, Cioli, Burberi & Catanese (1968), and Silvestrini & Lisciani (1973), and its metabolism by Baiocchi, Frigerio, Giannangeli & Palazzo (1974). Udabe (1973), Ban & Lehman (1973), and Wheatley (1976) have described its clinical use. Recently, the profile of trazodone has been reviewed by Al-Yassiri, Ankier & Bridges (1981).

The estimation of trazodone in plasma using a fluorimetric procedure was described by Catanese & Lisciani (1970), and Jauch, Kopitar, Prox & Zimmer (1976) who made use of the ^{14}C-labelled compound. The gas chromatography of the compound, although difficult, was reported by Baiocchi, Chiari, Frigerio & Ridolfi (1973) and subsequently developed and applied by Munday, Kendall, Mitchard & Betts (1975) for the determination of trazodone in the plasma.

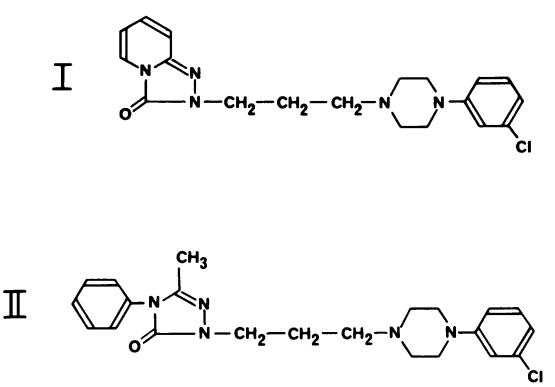

Figure 1 Structural formulae of trazodone (I) and the analogue used as internal standard (II).

The objective of the present study was to develop a method for the determination of trazodone in plasma, suitable for routine clinical application and for the assessment of some kinetic parameters of two capsule formulations of the drug.

0306–5251/81/050505–05 $01.00

Methods

Reagents

Diethyl ether:	Peroxide-free, stored over copper
Internal standard:	2-[3-(4-*m*-chlorophenyl-1-piperazinyl)-propyl]-5-methyl-4-phenyl-triazol-3-(2H)-one, (II, Figure 1)

HPLC apparatus consisted of a constant flow pump (Applied Chromatography Systems, model 750/03), an injection value provided with a 100 μl sample loop (Rheodyne, type 7120) and a u.v. photometric detector (Applied Chromatography Systems) provided with a 10 mm path length flow cell and with filters for measurement at 254 nm.

The column (25 cm × 4.6 mm i.d.) was packed with Spherisorb S5 ODS (Phase Separations), protected by a guard column (10 cm × 2 mm i.d.) packed with Co-Pell ODS (Whatman Laboratory Sales) and operated at ambient temperature.

Chromatograms were displayed at sensitivities 0.01 and 0.04 absorbance units for full scale deflection by use of a twin channel recorder to facilitte measurement of the wide range of concentrations encountered in the study.

Procedure

To plasma (1.0 ml) was added saturated tri-sodium orthophosphate solution (0.5 ml) and diethyl ether (5.0 ml). After extraction, an aliquot of the ether solution (2.5 ml) was transferred to a second vial and evaporated to dryness under a stream of nitrogen (oxygen-free) without heating. The residues were redissolved in a solution of internal standard (500 μl) in 0.05 M sulphuric acid (2 μg ml^{-1}) and analysed by HPLC using as mobile phase a mixture of acetonitrile and 0.05 M sulphuric acid solution (18:1) at a flow rate of 2 ml min^{-1}. Under these conditions, trazodone had a retention time of 6.8 min and the internal standard 6.0 min (Figure 2). For calibration, known quantities of trazodone were added to plasma at a concentration range of 0.1 to 2.5 μg ml^{-1}. The relationship of peak height ratio of drug to internal standard was linear with respect to drug concentration and the recovery from plasma exceeded 91%. No peaks which might cause interference were observed in plasma either before or after dosage. The metabolite of trazodone described by Baiocchi *et al.* (1974), 2-[3-carboxy-propyl]-1, 2, 4-triazolo[4,3-a] pyridin-3(2H)-one, elutes close to the solvent front in the described HPLC system and does not interfere in the quantitation of trazodone.

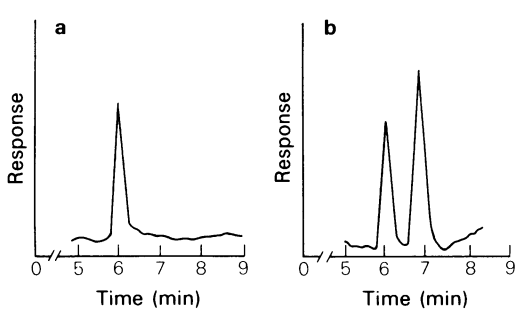

Figure 2 Typical HPLC chromatogram derived from (a) plasma blank plus internal standard, (b) plasma obtained after dosage with trazodone plus internal standard. The retention time of the internal standard was about 6 min while that for trazodone was about 7 min.

It is essential that the diethylether used as extractant is peroxide-free as the presence of peroxides leads to loss of trazodone. In this work the internal standard was added immediately prior to HPLC. It was not possible to obtain an internal standard that could be satisfactorily extracted from plasma under the conditions required for trazodone.

Methods for bioavailability study

The bioequivalence of two capsule formulations (A and B) of trazodone (50 mg) was compared in a crossover study using 13 healthy volunteers. Formulation A and B were administered to each volunteer on separate occasions according to a pre-arranged randomisation code with a 7 day gap between each treatment. The average weights of the 6 males and 7 females were 74.7 kg and 62.1 kg, respectively. Following an overnight fast, blood (10 ml) was collected from each volunteer into heparinised tubes just before and at 0.25, 0.5, 1, 1.5, 2, 2.5, 4, 6, 8, 25 and 30 h after dosage. No food or beverage was permitted for 3.5 h after dosage but water was allowed *ad lib.* The separated plasma was placed in a deep freeze within 1 h of venepuncture.

Plasma specimens were stored frozen below −15°C together with the series of spiked samples which were analysed alongside the clinical specimens in order to study the stability of trazodone in plasma under storage.

Table 1 Analysis of three spiked plasma samples stored at −15°C and assayed repeatedly over a period of 2 months

	Plasma concentration of trazodone (μg ml^{-1})		
Initial concentration	2.280	0.911	0.182
Mean determined values (n = 26)	2.390	0.981	0.199
s.d.	0.109	0.054	0.027
Mean recovery (%)	105	108	109

Table 2 Plasma concentration of trazodone ($\mu g\ ml^{-1}$) in 13 volunteers after a single oral 50 mg dose administered as formulation A or B.

Volunteer	*Formulation*	*Plasma concentration ($\mu g ml^{-1}$) after trazodone (h)*										
		0.25	*0.5*	*1*	*1.5*	*2*	*2.5*	*4*	*6*	*8*	*25*	*30*
1	A	bm	1.64	1.27	1.06	0.86	0.68	0.48	0.28	0.21	bm	bm
	B	bm	0.79	1.12	1.09	0.84	0.70	0.40	0.27	bm	bm	bm
2	A	bm	0.78	0.97	0.79	0.54	0.51	0.33	0.25	0.19	0.06	0.04
	B	bm	0.36	1.10	0.77	0.59	0.61	0.34	0.25	0.17	0.05	0.04
3	A	bm	0.40	0.72	0.83	0.84	0.72	0.48	0.33	0.28	0.06	bm
	B	bm	0.60	0.81	0.79	0.65	0.52	0.38	0.26	0.20	0.03	0.03
4	A	0.03	0.81	1.23	0.95	0.92	0.83	0.68	0.52	0.40	0.10	0.04
	B	0.05	0.04	0.62	0.59	0.77	0.87	0.71	0.52	0.41	0.03	0.06
5	A	0.11	0.96	0.80	0.72	0.60	0.51	0.35	0.22	0.13	bm	bm
	B	bm	1.22	0.98	0.79	0.65	0.58	0.36	0.26	0.16	bm	bm
6	A	bm	1.35	1.06	0.78	0.65	0.57	0.48	0.31	0.26	bm	bm
	B	bm	0.08	1.09	0.86	0.66	0.56	0.39	0.33	0.22	bm	bm
7	A	bm	0.55	0.93	0.73	0.60	0.59	0.40	0.32	0.26	0.06	0.03
	B	bm	0.68	0.93	0.79	0.57	0.52	0.38	0.30	0.23	0.07	bm
8	A	0.15	0.78	0.71	0.88	0.73	0.95	0.62	0.37	0.26	0.03	bm
	B	bm	0.24	0.59	0.89	0.93	0.90	0.60	0.37	0.27	0.07	bm
9	A	bm	0.19	0.92	0.75	0.66	ns	0.55	0.23	0.21	0.06	bm
	B	0.82	1.01	1.10	0.90	0.82	0.68	0.50	0.30	0.26	0.04	0.02
10	A	bm	1.67	1.17	1.24	0.96	0.84	0.58	0.42	0.32	0.06	bm
	B	0.19	2.34	1.06	0.79	0.64	0.55	0.41	0.33	0.25	0.05	bm
11	A	bm	0.08	0.06	0.07	0.16	0.21	0.49	0.28	0.20	0.02	bm
	B	bm	0.03	0.16	0.46	0.67	0.56	0.40	0.23	0.14	bm	bm
12	A	bm	0.04	0.18	0.85	0.82	0.67	0.46	0.28	0.21	bm	bm
	B	bm	0.15	0.76	0.81	0.63	0.55	0.38	0.29	0.22	0.04	0.04
13	A	bm	1.12	0.88	0.70	0.59	0.50	0.36	0.30	0.19	0.04	bm
	B	bm	0.67	0.64	0.67	0.66	0.53	0.37	0.27	0.16	0.04	bm
Mean	A	0.02	0.80	0.84	0.80	0.69	0.63	0.48	0.32	0.24	0.04	0.01
	B	0.08	0.63	0.84	0.79	0.70	0.63	0.43	0.31	0.21	0.03	0.02

bm = Below measurable concentration; ns = No sample

Results

The analysis of the spiked samples are summarised in Table 1, and the results show no significant loss of trazodone occurring during storage over two months. There is the indication that the recovery was slightly higher in this study than that initially determined.

The individual volunteer plasma concentrations of trazodone in the bioavailability study are shown in Table 2. The mean values are plotted in Figure 3.

The salient kinetic features are given in Table 3. The half-life of trazodone was calculated based on a first order elimination rate constant k which was estimated from the slope of the log plasma concentration versus time plot. The area under the plasma concentration versus time curve (AUC) from 0 to 30 h was calculated using the trapezoidal rule. All values for formulation A and B are closely similar and it was concluded that the bioavailability of the two formulations is comparable in terms of both the rate and extent of absorption.

Discussion

A sensitive and reproducible HPLC method has been developed for the routine assay of trazodone in human plasma and is sensitive in detecting concentrations of 20 ng ml^{-1}. Using the method, the plasma profile of two formulations of trazodone have been compared after the oral administration of a single dose of 50 mg.

The results obtained showed that the kinetic parameters of the two formulations were very similar. Thus the mean peak plasma concentrations for formulations A and B were 1.07 $\mu g\ ml^{-1}$ and 1.05 $\mu g\ ml^{-1}$ respectively. These are comparable to those reported in fasted subjects by Munday *et al.* (1975). However, Catanese & Lusciana (1970) and Allori, Catanese, Cioli & Interdonato (1978) reported lower peak concentrations which occurred at 2 h. This difference from the present results may be attributed to the fact that the latter authors studied non-fasted subjects.

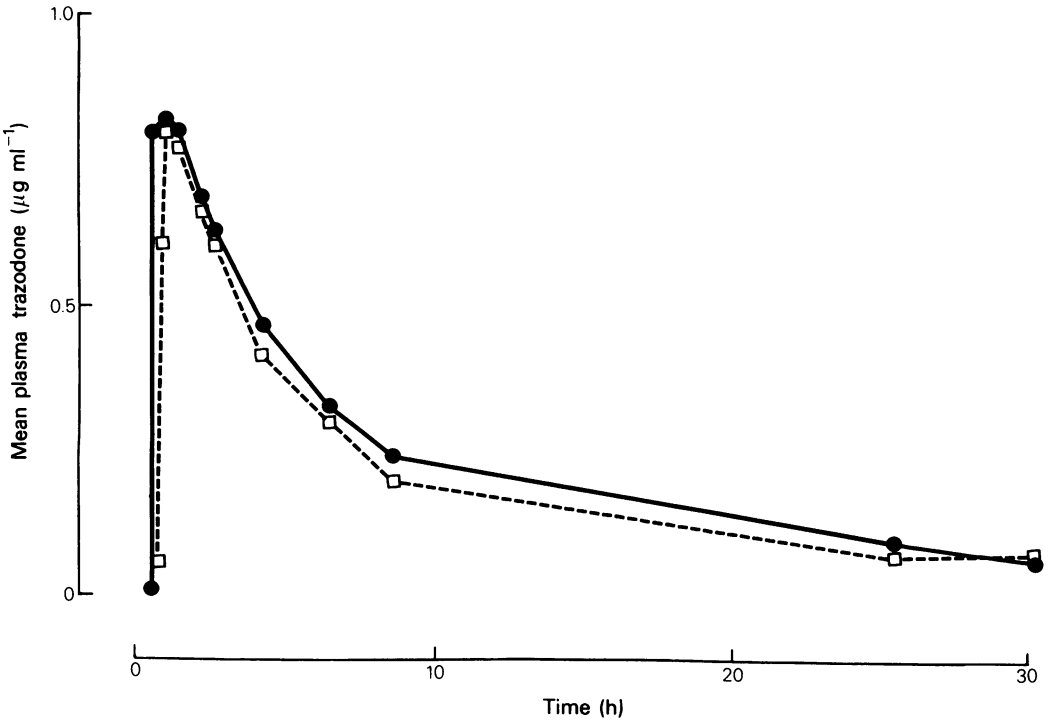

Figure 3 Mean plasma trazodone concentration in thirteen volunteers following oral administration of 50 mg as formulation A (●) and formulation B (□).

Table 3 Kinetic parameters of trazodone following administration of either formulation A or B

Volunteer	*Cp max* ($\mu g\ ml^{-1}$)		$t_{max}(h)$		*AUC* ($\mu g\ h\ ml^{-1}$)		$k(h^{-1})$		$T_{½}(h)$	
	A	*B*	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
1	1.64	1.12	0.5	1.0	6.29	3.76	0.21	0.20	3.4	3.5
2	0.97	1.10	1.0	1.0	5.60	5.34	0.14	0.17	5.0	4.0
3	0.84	0.81	2.0	1.0	6.88	5.36	0.14	0.16	5.1	4.3
4	1.23	0.87	1.0	2.5	9.92	8.54	0.13	0.14	5.2	5.1
5	0.96	1.22	0.5	0.5	1.69	4.92	0.25	0.20	2.8	3.4
6	1.35	1.09	0.5	1.0	6.25	5.33	0.15	0.14	4.5	4.9
7	0.93	0.93	1.0	1.0	6.14	6.47	0.13	0.11	5.5	6.4
8	0.95	0.93	2.5	2.0	7.07	7.32	0.22	0.20	3.2	3.5
9	0.92	1.10	1.0	1.0	7.25	7.11	0.22	0.21	3.1	3.3
10	1.67	2.34	0.5	0.5	8.71	7.02	0.15	0.12	4.7	5.6
11	0.49	0.67	4.0	2.0	4.92	3.71	0.22	0.26	3.1	2.7
12	0.85	0.81	1.5	1.5	4.97	5.58	0.23	0.18	3.0	3.8
13	1.12	0.67	0.5	1.0	5.48	4.91	0.16	0.21	4.3	3.3
Mean	1.07	1.05	1.3	1.2	6.32	5.80	0.18	0.18	4.1	4.1
s.e.mean	0.09	0.12	0.3	0.2	0.50	0.39	0.01	0.01	0.3	0.3

The mean plasma half-lives of trazodone from formulations A and B were found both to be 4.1 h. Jauch *et al.* (1976) quoted much higher values for the half-life of trazodone. However, this may be related to the fact that the values were based on the decline of total radio-activity in the plasma which may have included activity attributable to metabolite(s) as well as parent compound.

References

ALLORI, L., CATANESE, B., CIOLI, V. & INTERDONATO, N. (1978). A study on serum levels of trazodone produced in man following single or repeated oral administration. *Boll. Chim. Farm.*, **117**, 530–533.

AL-YASSIRI, M.M., ANKIER, S.I. & BRIDGES, P.K. (1981). Trazodone—a new antidepressant. *Life Sci.*, **22**, (in press).

BAIOCCHI, L., CHIARI, A., FRIGERIO, A. & RIDOLFI, P. (1973). Analytical profile of trazodone. *Arzneim. Forsch.*, **23**, 400–406.

BAIOCCHI, L., FRIGERIO, A., GIANNANGELI, M. & PALAZZO, G. (1974). Basic metabolites of trazodone in humans. *Arzneim. Forsch.*, **24**, 1699–1706.

BAN, T.A. & LEHMAN, H.E. (1973). Systematic clinical studies with trazodone. Methodological aspects. *Curr. Ther. Res.*, **15**, 764–768.

CATANESE, B. & LISCIANI, R. (1970). Investigations on the absorption and distribution of trazodone or AF 1161 in rats, dogs and humans. *Boll. Chim. Farm.*, **109**, 369–373.

JAUCH, R., KOPITAR, Z., PROX, A. & ZIMMER, A. (1976). Pharmakokinetik und Stoffwechsel von Trazodone beim Menschen. *Arzneim. Forsch.*, **26**, 2084–2089.

MUNDAY, B., KENDALL, M.J., MITCHARD, M. & BETTS, T.A. (1975). A single dose study of trazodone with an assessment of its effect on mood and arousal. *Br. J. clin. Pharmac.*, **2**, 19–24.

PALAZZO, G. (1973). Chemistry of trazodone. *Curr. Ther. Res.*, **15**, 745–748.

SILVESTRINI, B., CIOLI, V., BURBERI, S. & CATANESE, B. (1968). Pharmacological properties of AF 1161, a new psychotropic drug. *Int. J. Neuropharmac.*, **7**, 587–599.

SILVESTRINI, B. & LISCIANI, R. (1973). Pharmacology of trazodone. *Curr. Ther. Res.*, **15**, 749–754.

UDABE, U.R. (1973). Clinical experience with a new psychotropic drug—trazodone. *Curr. Ther. Res.*, **15**, 755–763.

WHEATLEY, D. (1976). Evaluation of trazodone in the treatment of anxiety. *Curr. Ther. Res.*, **20**, 74–83.

(Received May 19, 1980)