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High-performance Liquid Chromatographic Determination of Trazodone and 1-*m*-Chlorophenylpiperazine with Ultraviolet and Electrochemical Detector

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Abstract

A high-performance liquid chromatographic (HPLC) assay was developed for the determination of trazodone and its metabolite, 1-*m*-chlorophenylpiperazine (m-CPP), in plasma.

The high level of trazodone in plasma was detected by ultraviolet absorbance at 254 nm and the low level of m-CPP in plasma was detected by coulometric electrochemical detection at 840 mV on the series arrangement of two detectors. Pilsicainide as an internal standard for both compounds was monitored by both detectors. Trazodone and m-CPP in plasma were extracted by a rapid and simple procedure based on CN bonded-phase extraction, and C_8 reversed-phase HPLC separation.

Determination was possible for trazodone in the concentration range 100–2000 ng mL^{-1} and for m-CPP in the concentration range 5–100 ng mL^{-1}. The recoveries of trazodone and m-CPP added to plasma were 81·0–84·2 and 68·0–73·2%, respectively, with coefficients of variation of less than 7·3 and 8·2%, respectively. The method is applicable to high level monitoring of trazodone and low level monitoring of m-CPP in plasma of healthy volunteers and patients treated with trazodone.

Trazodone, 2-[3-(4-*m*-chlorophenyl-1-piperazinyl)propyl]-1, 2,4-triazolo[4,3-a]pyridin-3-(2H)-one (Fig. 1), is structurally unrelated to the tricyclic antidepressants and other psychotropic drugs but has antidepressant and anxiolytic activity (Agonston et al 1978). Although its mechanism of action is unknown, trazodone possesses 5-HT-antagonist activity and its major active metabolite, 1-*m*-chlorophenylpiperazine (m-CPP), possesses 5-HT-agonist activity (Fuller et al 1980). The optimal therapeutic range for trazodone and the contribution of m-CPP against the therapeutic effect of trazodone have not yet been established (Spar 1987; Monteleone et al 1989). Determination of the plasma concentrations of trazodone and m-CPP are required to establish the relationship between the plasma level of both drugs and the therapeutic effect.

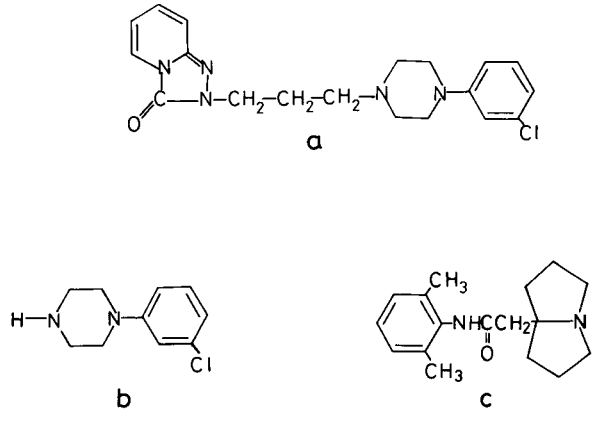

FIG. 1. Chemical structure of a. trazodone, b. m-CPP and c. pilsicainide.

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Several methods for the determination of trazodone by HPLC using ultraviolet detection at 214 nm (Wong et al 1984; Miller & Devane 1986), or 242 nm (Root & Ohlson 1984), with fluorescence detection (Caldwell & Flanagan 1985; Gupta & Lew 1985) and electrochemical detection (Suckow 1983) have been described. Also, m-CPP has been determined by HPLC using ultra violet detection at 214 nm (Miller & Devane 1986) and electrochemical detection (Suckow 1983). There are large differences in concentrations between trazodone and m-CPP in plasma. Suckow (1983) determined plasma levels of trazodone and m-CPP using a double internal standard, in which trazodone and m-CPP were detected by an electrochemical detector coupled with dual recorder set at different attenuations. This method is suitable for the determination of the two or more components in which the difference of concentrations is markedly large. In a previous paper, we reported the determination of two components which have a large difference of concentration by a double internal standard method (Ohkubo et al 1992).

Several extraction methods with liquid–liquid extraction have been described (Suckow 1983; Root & Ohlson 1984; Wong et al 1984; Caldwell & Flanagan 1985; Miller & Devane 1986), but such methods have tedious procedures. Gupta & Lew (1985) reported a simple extraction method for trazodone using a C_{18}-bonded solid phase. In the present paper, we describe a solid-phase extraction method for trazodone and m-CPP in plasma and HPLC determination.

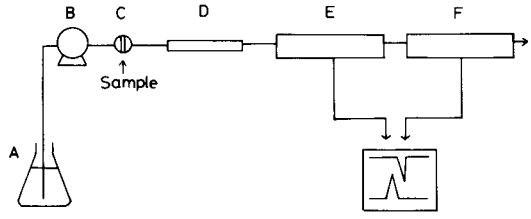

FIG. 2. Schematic diagram of the HPLC system. A = carrier solution; B = HPLC pump; C = injector; D = analytical column; E = UV detector; F = electrochemical detector.

Materials and Methods

Materials

Trazodone and m-CPP were kindly donated by Kanebou Pharmaceutical Co. Ltd (Tokyo, Japan) (Fig. 1), and pilsicainide by Daiichi Pharmaceutical Co. Ltd, (Tokyo, Japan) (Fig. 1). The Sep-Pak CN cartridge was purchased from Millipore Co. (Bedford, MA, USA). All other solvents used were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All other reagents and chemicals were purchased from Wako Pure Chemical Industries or Nakarai Tesque (Kyoto, Japan).

Apparatus

The apparatus used for HPLC was a Jasco Model PU-880 chromatography pump (Jasco, Tokyo, Japan) equipped with a Jasco Uvidec 100-III ultraviolet detector (Jasco) and with a Coulochem Model 5100A electrochemical detector (Environmental Sciences Assoc. Inc., Bedford, MA, USA) in series (Fig. 2). The wavelength and potential of detectors were set at 250 nm for trazodone and at 840 mV vs a reference electrode for m-CPP. Test samples were introduced using a Rheodyne Model 7120 injector (Rheodyne Inc., Cotati, CA, USA) with an effective volume of 100 μL. The HPLC column contained Develosil C_8-5 stationary phase (5 μm) (Nomura Chemical, Seto, Japan). A stainless-steel analytical column (150 × 4·6 mm i.d.) was packed in these laboratories by a conventional high-pressure slurry-packing procedure. The mobile phase consisted of 0·5% KH_2PO_4 (pH 2·5)–acetonitrile (77:23, v/v). Before mixing, the pH of the mobile phase was adjusted with 50% phosphoric acid and was degassed ultrasonically.

Extraction

Pilsicainide (4 μg) in methanol (8 μL) was added to the plasma sample (1 mL) as an internal standard and the plasma sample was diluted with 5 mL 1 M NaCl in 0·5% KH_2PO_4 (pH 10); the solution was briefly mixed. The mixture was applied to a Sep-Pak CN cartridge that had previously been activated with 5·0 mL acetonitrile and water. The cartridge was then washed with 5 mL water and the desired fraction was eluted with 5 mL acetonitrile and 5 mL ethanol. The elute was evaporated to dryness in vacuum at 60°C. The residue was dissolved in 160 μL mobile phase and filtered through a membrane filter (0·45 μm pore size). The filtrate was injected directly onto the HPLC apparatus.

Calibration

Known amounts of trazodone and m-CPP in the range 100–2000 and 5–100 ng mL^{-1}, respectively were added to blank plasma samples. These plasma samples were treated according to the described procedure. The peak-height ratios (trazodone:pilsicainide, UV detection; m-CPP:pilsicainide, electrochemical detector) were measured and plotted against the concentration of analyte as appropriate.

Recovery of trazodone and m-CPP

Samples were prepared by adding 100, 500 and 1000 ng mL^{-1} trazodone, and 10, 25 and 50 ng mL^{-1} m-CPP to blank plasma. Sample extraction and the subsequent HPLC was carried out as described above.

Drug administration and sampling

A trazodone tablet (50 mg) was orally administered to three healthy volunteers. Blood samples (2·5 mL) were collected by venepuncture at 1, 2, 3, 4, 5, 6 and 8 h after administration. Trazodone tablets (100–300 mg day^{-1}, 50 mg tablets) were orally administered to eight patients for seven days. Plasma samples were collected 12 h after each trazodone administration. Plasma was separated by centrifugation at 1900 *g* for 15 min, and stored at −40°C until analysis.

Results and Discussion

Initially, our efforts were directed towards developing an efficient chromatographic method for the determination of the large difference in concentration between trazodone and m-CPP in plasma of subjects dosed with trazodone.

In a previous paper, Suckow (1983) described the simultaneous determination of large differences in concentration between plasma trazodone and m-CPP using a double internal standard. However, this method has problems because of the difficulty in finding suitable internal standards which separate from each other and the endogenous components. Therefore, we aimed for the simultaneous determination of trazodone and m-CPP using a single

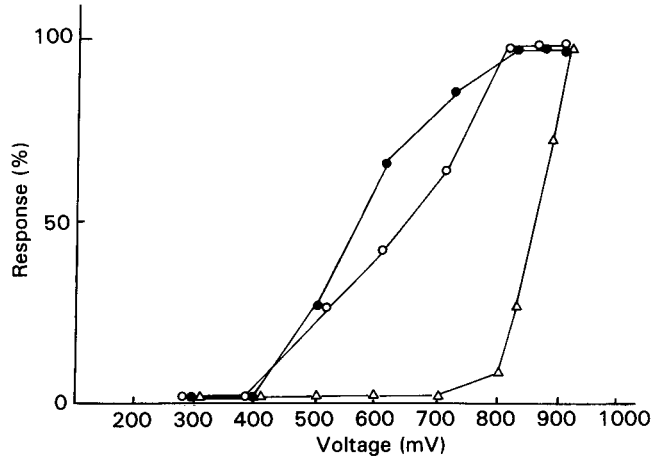

FIG. 3. The hydrodynamic voltammograms of ○, trazodone; ●, m-CPP; △, pilsicainide.

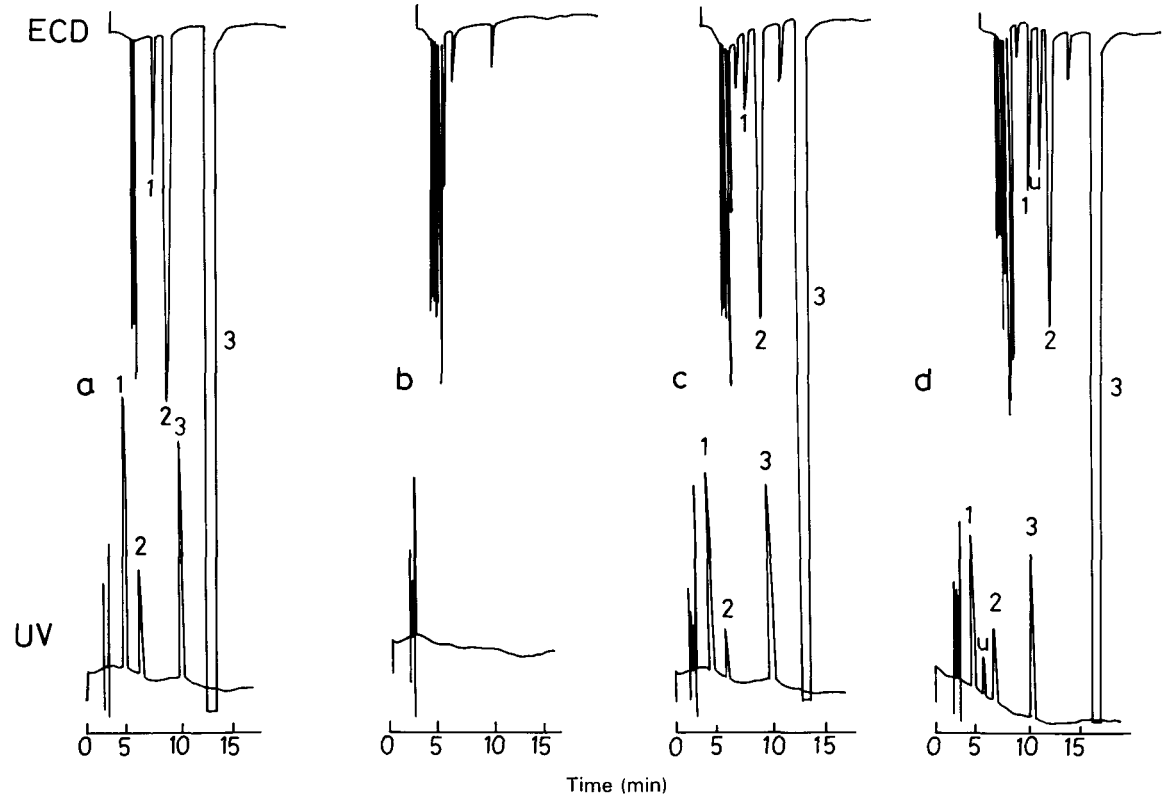

FIG. 4. Typical chromatogram of (a) standard sample, (b) plasma blank, (c) added standards in plasma, (d) plasma sample from healthy volunteer. Peaks: 1 = internal standard, 2 = m-CPP, 3 = trazodone, u = unknown peak.

internal standard, which would have different responses to ultraviolet and electrochemical detectors.

The hydrodynamic voltammograms of trazodone, m-CPP and pilsicainide are illustrated in Fig. 3. Trazodone and m-CPP showed a constant response above +850 mV, whereas the electrochemical response of pilsicainide was saturated at +950 mV. The amount of pilsicainide (4 μg) as an internal standard in this study was suitable for the determination of trazodone in the range 100–2000 ng mL^{-1} using the UV detector, whereas m-CPP in the range 5–100 ng mL^{-1} was not detected. m-CPP in the range 5–100 ng mL^{-1} could be detected by the electrochemical detector with pilsicainide as an internal standard at +850 mV. The electrochemical response of pilsicainide at +850 mV was about 27%, so that pilsicainide could be used as the internal standard using a sensitive electrochemical detector, with a suitable peak height in the chromatogram (Fig. 3). We then directed our project towards establishing a simple extraction method for trazodone, m-CPP and pilsicainide in plasma. In our preliminary experiments, higher

Table 1. Accuracy and precision of determination of trazodone and m-CPP in human plasma.

Trazadone added (ng mL^{-1})	Found (mean ± s.d.)	Recovery (%)	CV (%)	
			Between-day	Within-day
100	81·8 ± 3·9	81·8	4·7	3·4
500	405·8 ± 13·7	81·0	3·3	2·4
1000	842·5 ± 61·8	84·2	7·3	5·1
m-CPP				
10	7·3 ± 0·6	73·0	8·2	6·4
25	16·9 ± 0·7	68·0	4·1	4·0
50	36·6 ± 2·1	73·2	5·7	3·7

n = 6.

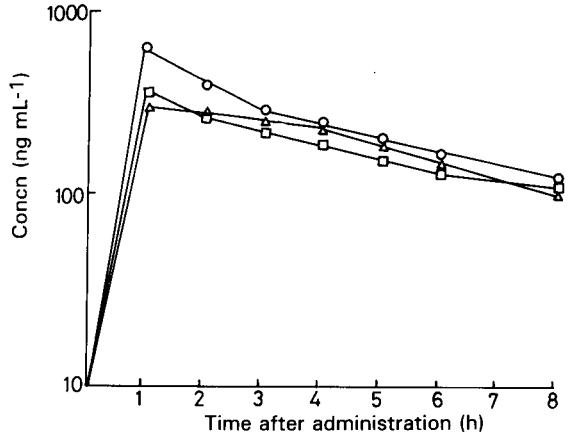

FIG. 5. Plasma concentration–time profile of trazodone after a single 50 mg oral dose of trazodone to three healthy volunteers.

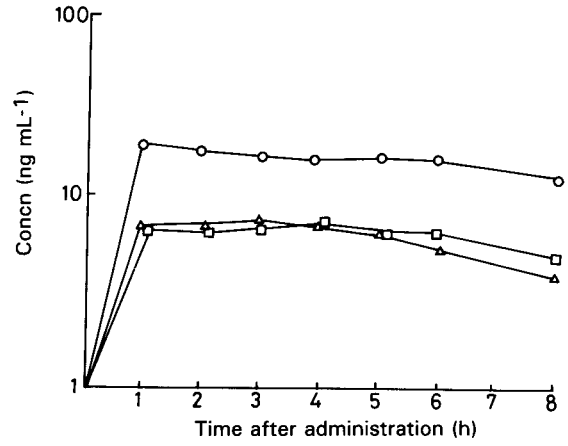

FIG. 6. Plasma concentration–time profile of m-CPP after a single 50 mg oral dose of trazodone to three healthy volunteers.

using Sep-Pak C_{18} or Sep-Pak CN cartridges were obtained with applied alkaline solution than with acidic or neutral solutions, and also with high ionic strength solvents than with solvents of low ionic strength; high ionic and alkaline solutions (1 M NaCl in 0·5% KH_2PO_4, pH 10·0) were, therefore, used for the adsorptions of plasma trazodone, m-CPP and pilsicainide on Sep-Pak stationary phases. Lower interference from endogenous components of plasma was obtained using Sep-Pak CN than for Sep-Pak C_{18}.

Fig. 4 shows the chromatogram of standard sample, blank plasma, plasma with added analyte, and plasma samples obtained from healthy volunteers treated with trazodone. The separation on the chromatogram of trazodone, m-CPP or pilsicainide from endogenous components derived from plasma was satisfactory. An unknown component before m-CPP on the chromatogram, is probably an unidentified metabolite of trazodone. Suckow (1983) described the presence of a similar unknown peak. Calibration graphs for trazodone and m-CPP in human plasma were linear in the range 100–2000 and 5–100 ng mL^{-1}, respectively. The limits of detection for trazodone and m-CPP were 50 and 2·5 ng mL^{-1}, respectively. The sensitivity and the calibration range of the present method were appropriate for therapeutic drug monitoring of trazodone and m-CPP in patients. The results of recovery studies are shown in Table 1. These results show that the proposed method is satisfactory with respect to accuracy and precision.

The time course of concentrations of trazodone and m-CPP in plasma samples from three healthy volunteers receiving 50 mg trazodone orally were determined using the proposed method (Figs 5, 6). The time required to reach the maximum concentration of trazodone was 1 h, and the concentration was 286–650 ng mL^{-1}. The half-life values were 3·3–4·4 h, and the AUC (0–18 h) values for three volunteers were 1471·3–2083 ng mL^{-1} h. The time required to reach the maximum concentration of m-CPP (3–20 ng mL^{-1}) was 2 h, the mean half-life was 13·4 h, and the mean AUC (0–8 h) of three volunteers was 68·7 ng mL^{-1} h.

The present method was applied to the monitoring of trazodone and m-CPP in patients (Table 2) diagnosed as suffering from major depression. The patients received the therapeutic treatment of trazodone over seven days. A good correlation between doses of trazodone and plasma concentration of trazodone or m-CPP was not obtained, although the ratio of trazodone to m-CPP was consistent with values reported by Miller & Devane (1986) and Suckow (1983). The proposed method for the determination of trazodone and m-CPP in clinical samples is satisfactory.

Acknowledgements

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Table 2. Plasma concentration of trazodone and m-CPP in trazodone-treated patients.

Case no.	Age (years)	Sex	BW (kg)	Diagnosis	Daily dose (mg)	Duration (Weeks)	Trazodone (ng mL^{-1})	m-CPP (ng mL^{-1})
1	56	F	60	MD	100	2	510	35
2	34	F	43	MD	100	2	529	49
3	63	F	62	MD	150	1	297	37
4	29	M	57	MD	150	1	859	57
5	27	M	73	MD	150	1	511	60
6	24	M	58	MD	150	2	468	37
7	39	F	58	MD	300	5	1189	200
8	27	M	60	MD	300	7	522	81

MD: major depression.

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