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## Note

# Determination of ketanserin in human plasma by high-performance liquid chromatography

A.T. KACPROWICZ, P.G. SHAW, R.F.W. MOULDS and R.W. BURY*

*Department of Medicine, University of Melbourne and Department of Clinical Pharmacology and Therapeutics, The Royal Melbourne Hospital, Parkville, Victoria 3050 (Australia)*

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Ketanserin, 3{2-[4-(fluorobenzoyl)-1-piperidinyl]ethyl}-2,4-(1H,2H)quinazolinedione (Fig. 1), is a new serotonin antagonist which is thought to selectively block serotonin-2 receptors [1—3]. There is evidence that this agent may be useful in the treatment of hypertension, congestive cardiac failure and other clinical conditions in which platelet activation may be occurring [4—6].

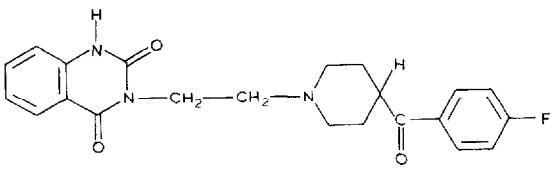

Fig. 1. Structural formula of ketanserin.

Methods for estimation of ketanserin in plasma using radioimmunoassay [7] and high-performance liquid chromatography (HPLC) [8] have been reported briefly in publications by Janssen Pharmaceutica, Beerse, Belgium, but the suitability of these for clinical studies has not been established. The present paper describes an improved alternative assay for ketanserin in human plasma using reversed-phase HPLC and the verification of its use in measuring plasma levels in man.

## MATERIALS AND METHODS

### *Sample preparation*

Plasma specimens (1 ml) were pipetted into conical glass tubes containing either 195 nmol/l or 585 nmol/l of the chloro derivative of ketanserin, the in-

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ternal standard (vide infra). After addition of 200 μl of 2 mol/l carbonate buffer (pH 12.0), the mixture was extracted with 3 ml 8% *tert.*-amyl alcohol in heptane for 3 min using a vortex mixer. The two phases were separated by centrifugation at about 1500 *g* for 5 min. The organic layer was then transferred to another conical glass tube, acidified with 500 μl of 1 mol/l hydrochloric acid and mixed thoroughly for 3 min using a vortex mixer. After centrifugation, the organic layer was discarded. The aqueous phase was rendered alkaline with 500 μl of saturated carbonate buffer and about 50 mg solid sodium chloride was added. This mixture was extracted with 8% *tert.*-amyl alcohol in heptane, as described above, and subjected to centrifugation. The organic phase was transferred to another conical tube and evaporated to dryness at 40° C under a gentle stream of nitrogen. The extract was reconstituted with 50 μl methanol and 20-μl aliquots were injected into the chromatograph.

*Chromatographic analysis*

A reciprocating dual-piston high-pressure pump (Constametric IIG; Laboratory Data Control, Riviera Beach, FL, U.S.A.), a sample injection valve containing a 20-μl loop (Rheodyne Model 7120; Rheodyne, Berkeley, CA, U.S.A.) and a variable-wavelength UV detector (Spectromonitor II; Laboratory Data Control) formed the basis of the chromatographic system. Analyses were performed on an octadecylsilane (ODS) reversed-phase column (Hibar LiChrosorb, 5 μm, RP-18, 125 mm × 4 mm, E. Merck, Darmstadt, G.F.R.), at ambient temperature, using methanol—acetonitrile—0.02 mol/l sodium acetate, pH 4.5 (1:4:6) as the mobile phase. The flow-rate was constant at 1.0 ml/min. Absorbance of the effluent from the column at 245 nm was recorded at a sensitivity of 0.005 or 0.01 a.u.f.s. using a strip chart recorder (Omniscribe, Houston Instruments, Austin, TX, U.S.A.).

To prolong the life of the analytical column, a pre-column (30 mm × 4 mm) containing 5-μm ODS reversed-phase packing was incorporated into the system. Reduction in chromatographic efficiency necessitated a change of pre-column after approximately 200 samples were assayed.

*Chemicals and solutions*

With the exception of methanol and acetonitrile which were HPLC grade (Ajax Chemicals, Melbourne, Australia), all chemicals used were of analytical quality. Water was distilled. Ketanserin was used as the tartrate monohydrate salt (R 49945; MW 563.5 daltons). The chloro derivative of ketanserin (R 46594) was supplied as the base (MW 411.9 daltons) and was used as the internal standard for the assay. These compounds were donated by Janssen Pharmaceutica, Belgium.

Stock solutions of ketanserin tartrate in methanol were prepared in concentrations ranging from 0.25 μg/ml (0.44 μmol/l) to 8.0 μg/ml (14.2 μmol/l). Two solutions of the internal standard in methanol were prepared in concentrations of 1.6 μg/ml (3.9 μmol/l) and 4.8 μg/ml (11.7 μmol/l). The solutions were stable for at least 6 months when stored at 4° C.

The following aqueous solutions were used: acetate buffer: 0.02 mol/l sodium acetate, pH adjusted to 4.5 with glacial acetic acid. Carbonate buffers: (a) 2 mol/l sodium carbonate, pH adjusted to 12.0 with saturated sodium bicar-

bonate solution, (b) saturated sodium carbonate, pH adjusted to 12.0 with saturated sodium bicarbonate solution.

*Assay calibration*

The assay was calibrated by addition of ketanserin and internal standard to 1-ml drug-free human plasma specimens. Calibration curves were established over a wide concentration range, and to ensure accuracy at the extremes, two concentrations of internal standard were used. The lower end of the range was calibrated with samples containing 0, 22, 44, 89 and 177 nmol/l ketanserin and 195 nmol/l internal standard. Samples containing higher concentrations, 355 and 710 nmol/l ketanserin, contained 585 nmol/l internal standard and to enable continuity of calibration, additional samples containing 0, 89 and 177 nmol/l ketanserin and the higher concentration of internal standard, were assayed.

Peak height ratios of ketanserin to the internal standard were used in quantitation of the assay.

## RESULTS

Typical chromatograms of extracted plasma specimens (Fig. 2) show that control samples are free from interfering peaks. Retention times for ketanserin and the internal standard were 5.2 min and 8.1 min, respectively. Any endogenous contaminants remaining in the extracts were eluted before ketanserin and injection of specimens could be repeated immediately after elution of the internal standard.

Calibration curves for ketanserin passed through the origin and were linear up to 710 nmol/l, the maximum concentration used. When the lower quantity of internal standard was used and absorbance was monitored at 0.005 a.u.f.s.,

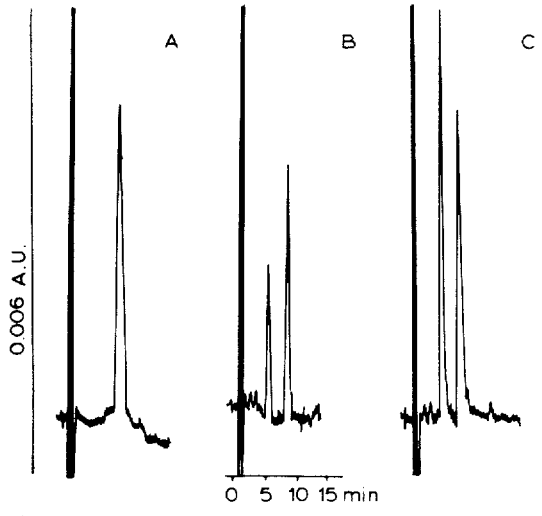


Fig. 2. Chromatograms of human plasma extracts: (A) blank plasma; (B) plasma containing 217 nmol/l ketanserin; (C) plasma containing 625 nmol/l ketanserin. All contain 585 nmol/l internal standard. The ordinate represents absorbance units (A.U.). Retention times were 5.2 min and 8.1 min for ketanserin and internal standard, respectively.

less than 20 nmol/l ketanserin was easily detectable. UV absorbance of ketanserin was monitored at 245 nm, the secondary absorbance peak. Maximum absorbance of ketanserin in the mobile phase occurred at 220 nm, but at this wavelength, the signal-to-noise ratio was lower than at 245 nm and background interference from contaminants remaining in the plasma extracts appeared in several samples.

Recovery of ketanserin from plasma following extraction was assessed by comparison of the peak heights from the plasma extracts with those from standard solutions of the drug in methanol. For ketanserin at concentrations of 44, 177 and 710 nmol/l, recovery ranged from 76 to 86%. At a concentration of 177 nmol/l, the intra-assay coefficient of variation was 4.2% ($n$ = 7). The day-to-day precision of the assay was determined over a period of eight weeks and the interassay coefficient of variation was 3.0% ($n$ = 7) at a concentration of 177 nmol/l, and was 8.5% ($n$ = 7) at a concentration of 22 nmol/l.

To demonstrate the effectiveness of the assay in a clinical situation, blood samples from a hypertensive patient who had received 30 mg ketanserin by intravenous infusion, were assayed. Plasma ketanserin concentrations at 10 min, 2 h and 8 h after cessation of the infusion were 708 nmol/l, 217 nmol/l and 58 nmol/l, respectively. In the same patient, after repeated oral therapy with ketanserin (40 mg twice daily) over a priod of four weeks, plasma levels of the drug in specimens taken 2—4 h after a dose, ranged from 144 to 265 nmol/l. No interference with the chromatographic measurement of ketanserin was observed despite concurrent medication with hydralazine, methyldopa, cyclopenthiazide, prazosin, metoprolol and labetalol.

## DISCUSSION

The HPLC assay method presented here is sufficiently sensitive and precise to determine plasma ketanserin concentrations in the clinically relevant concentration range. In addition, the procedure can be performed rapidly and over 30 plasma specimens per day can be assayed. This method should, therefore, be suitable for pharmacokinetic studies of ketanserin in man.

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