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## Note

# Simultaneous determination of ketanserin and ketanserinol in biological fluids using ion-pair liquid chromatography and fluorometric detection

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Among novel 5-hydroxytryptamine antagonists (5-$HT_2$) ketanserin, a quinazoline derivative, is presently under clinical investigation as an antihypertensive [1—4]. Following oral doses of 40 mg of intravenous injection of 10 mg of ketanserin a decrease in the supine systemic arterial pressure can be achieved [4]. Furthermore, ketanserin increases cardiac output even in patients already under digitalis treatment [5]. Pharmacokinetic studies have been performed recently with young healthy volunteers [6] as well as with elderly subjects [7]. Ketanserin is converted to its major metabolite ketanserinol in humans, which accumulates considerably under chronic ketanserin treatment [7]. It has not yet been clarified whether this metabolite contributes pharmacological effects or may be reconverted to its parent compound.

Various methods for the determination of ketanserin have been described. Except for a radioimmunoassay, which allows ketanserin only to be quantitated [8], reversed-phase liquid chromatography was preferentially employed. In order to detect the slow terminal elimination phase recently described [6], it is necessary to measure concentrations of less than 1 ng/ml ketanserin. Using ultraviolet (UV) absorbance [9—12] the required detection limit could not be achieved. A sensitive method recently published [7] includes reversed-phase chromatography of ketanserin as the undissociated base at pH 12, and fluorometric detection. However, this procedure rapidly degrades reversed-phase column packing material and is not compatible with common high-performance liquid chromatography (HPLC) equipment.

We report here a selective and sensitive HPLC method, which involves ion-pairing and fluorometric detection at pH 5.2. Biological specimens from clinical

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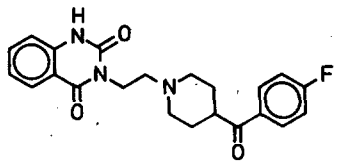
trials were analysed for ketanserin and ketanserinol simultaneously. Pharmacokinetic parameters derived from analytical data lead to improvement of ketanserin preparations, adequate dosage regimens for various patient populations and estimation of therapeutic risks dependent on drug accumulation.

## EXPERIMENTAL

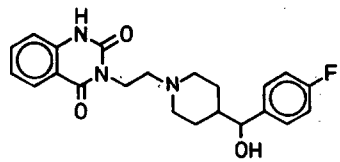
### *Chemicals and reagents*

Ketanserin (R-41,468, 3-{2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl}-2,4(1H,3H)-quinazoline dione), ketanserinol (reduced ketanserin, R-46,742, 3-{2-[4-(4-fluorobenzoyl)hydroxymethyl-1-piperidinyl]ethyl}-2,4(1H,3H)-quinazoline dione) and the internal standard (R-46,594, 3-{2-[4-(4-chlorobenzoyl)-1-piperidinyl]ethyl}-2,4(1H,3H)-quinazoline dione) were provided as reference compounds by Janssen Pharmaceutica, Beerse, Belgium (see Fig. 1 for structures).

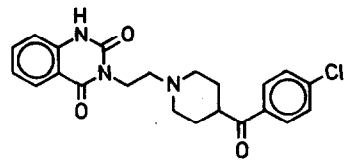
Isoamyl alcohol and pentane in nanograde quality, acetonitrile and methanol in ChromAr quality were purchased from Promochem (Wesel, F.R.G.). Ammonium acetate, acetic acid and all other reagents were obtained from Merck (Darmstadt, F.R.G.). Buffer solutions were prepared in doubly distilled water.




KETANSERIN




KETANSERINOL




INTERNAL STANDARD

**Fig. 1. Structures of ketanserin, ketanserinol and internal standard.**

### *Apparatus*

UV and fluorescence spectra were recorded on a Varian SuperScan 3 and a Perkin-Elmer 3000 fluorescence spectrometer, respectively. Liquid chromatography was performed on a Spectra-Physics solvent delivery system equipped with a Perkin-Elmer 3000 fluorescence detector and a Spectra-Physics SP 4200

integrator. At pH 5.2 the absorption and emission wavelengths were set at 310 and 370 nm, respectively, at excitation and emission slit widths of 10 μm. The computing integrator linked to the fluorescence detector was operated at a chart speed of 0.25 cm/min. Chromatography columns (25 cm × 4 mm I.D.) packed with Nucleosil 5-μm particles (Macherey, Nagel & Co., Düren, F.R.G.) were kept in an oven compartment at 32°C. A precolumn, 5 cm long, containing the same packing material was purchased from Knauer (Berlin, F.R.G.). The mobile phase consisted of 2% acetic acid—0.17 *M*/l ammonium acetate—acetonitrile (50:10:40). The solvent pump was operated at a flow-rate of 1.2 ml/min and a pressure of 250 atm.

*Sample preparation*

Standard solutions of ketanserin, ketanserinol and the internal standard in methanol were stored at 6°C. The stability under these conditions was demonstrated for a period of three months.

Patient control plasma was obtained prior to clinical trials. Following disproof of concomitant medication, blank samples were spiked with 1, 2, 5, 10 or 20 ng/ml ketanserin and ketanserinol. A stock solution of 100 μg/l internal standard was prepared, and 300 μl of this solution were added to 1 ml of each plasma sample. Following centrifugation and alkalinization with 1 ml of 0.05 *M* sodium hydroxide solution (pH 12), 4 ml of the extraction mixture (pentane—isoamyl alcohol, 95:5) were added, rotated for 10 min and centrifuged for 5 min. After removal of the organic layer the extraction procedure was repeated. The combined organic layers were evaporated to dryness in conical tubes under pre-cleaned nitrogen in a water bath at 50°C. The residue was dissolved in 100 μl of acetonitrile and injected into the column.

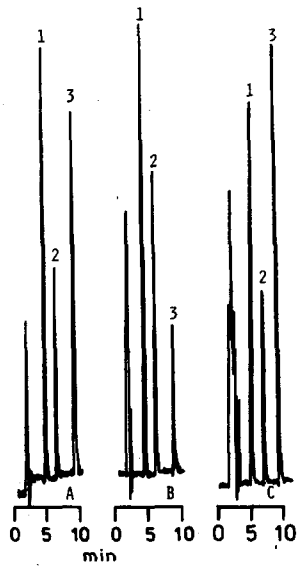
Patient plasma samples containing more than 40 ng/ml ketanserin or ketanserinol were diluted 1:10 with blank plasma. Urine samples of 0.2 ml were treated with a five-fold amount of 0.05 *M* sodium hydroxide solution. The alkalinized urine specimens (pH 12) were extracted twice with 4 ml of pentane—isoamyl alcohol (95:5) and subsequently treated in the same manner as described above.

*Peak detection and assignment*

Qualitative and quantitative detection of ketanserin, ketanserinol and the internal standard was established by spiking blank plasma with known amounts of these compounds and subsequent extraction using the described procedure. Recovery rates were 92% for ketanserin and 70% for ketanserinol and proved to be concentration-independent. Retention times and retention volumes under these conditions were 4.3 min (5.16 ml) for ketanserinol, 5.8 min (6.96 ml) for ketanserin and 8.9 min (10.68 ml) for the internal standard (Fig. 2).

The detection limits were 200 pg/ml for ketanserin and 100 pg/ml for ketanserinol.

Calibration curves were constructed using spiked plasma containing 1, 2, 5, 10 and 20 ng/ml of each compound. Detector responses calculated from integrated peak areas correlated in a linear fashion with the added amounts up to 40 ng/ml.



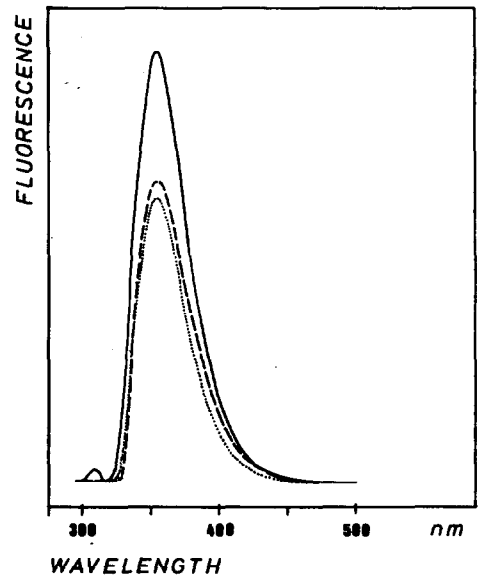




Fig. 2. Representative chromatograms of ketanserin, ketanserinol and internal standard. (A) Standard mixture of 3 ng of ketanserinol, 3 ng of ketanserin and 20 ng of internal standard; (B) human blank plasma sample spiked with 5 ng of ketanserinol, 5 ng of ketanserin and 5 ng of internal standard; (C) plasma sample from subject 10 h after ingestion of 40 mg of ketanserin (internal standard added). Peaks: 1 = ketanserinol; 2 = ketanserin; 3 = internal standard.

Fig. 3. Emission spectra of ketanserin (——), ketanserinol (- - -) and internal standard (· · · ·) recorded in mobile phase (absorption wavelength: 310 nm).

## RESULTS AND DISCUSSION

Fluorescence spectra were recorded under the described conditions. Fig. 3 shows emission spectra of ketanserin, ketanserinol and the internal standard with maximum intensity at 370 and 371 nm, respectively. Before employing acetic acid, various ion-pairing reagents with different lipophilicity were tested. However, retention times using these reagents ranged up to 40 min.

At basic pH values of the mobile phase, e.g. according to a method previously described [7], ketanserin and related compounds can be resolved on reversed-phase columns at pH values above 10 as undissociated bases. Applying this method RP-18 chromatography columns survive less than 40 h of operation. Furthermore, the valves and fittings of regular chromatographs are not suitable for continuous alkaline media.

UV absorbance between 210 and 254 nm yields minimum detection limits of 2 ng/ml [8—11]. Following single oral administration of ketanserin a slow terminal elimination phase could be demonstrated requiring measurement of concentrations between 5 and 0.2 ng of the parent compound [12]. The accurate measurement of the low drug concentrations during elimination periods is a prerequisite for estimation of drug accumulation under chronic treatment. In order to detect these levels fluorescence spectroscopy is the most suitable technique for ketanserin and its metabolite. Amounts and detector responses correlated over the range 0.2—40 ng in a linear fasion. The described technique proved to be twice as

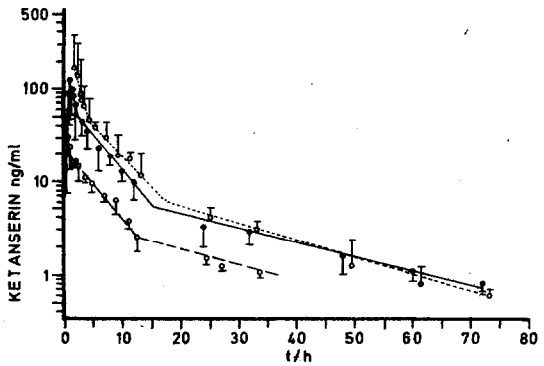



**Fig. 4. Ketanserin plasma levels obtained from an elderly subject, who received 40 mg of ketanserin as an oral solution (upper curve), 40 mg of ketanserin as tablets (middle curve) and 10 mg of ketanserin as an intravenous bolus (lower curve).**

sensitive for ketanserinol compared to its parent compound. Similar detector responses were found with equal molar concentrations of the internal standard and ketanserin. The following linear equations served as calibration curves: $y = 0.0495x + 0.00424$ (ketanserin) and $y = 0.0748x + 0.0554$ (ketanserinol), where $x$ = amount per ml and $y$ = ratio of detector responses of ketanserin(ol) to internal standard. The regression coefficient of both calibration lines was over 0.999. Reproducibility of the method tested by the intra-assay coefficient of variation at concentrations of 5 ng each amounted to 3.3% for ketanserin and 4.2% for ketanserinol.

The method was applied to plasma and urine specimens obtained from a pharmacokinetic study in elderly subjects (Fig. 4) [5]. All parameters (including materials, column and apparatus, except buffer solutions) remained stable for more than three months (equivalent to over 4000 determinations).

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