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Note

Determination of ketanserin and its major metabolite (reduced ketanserin) in human plasma by high-performance liquid chromatography

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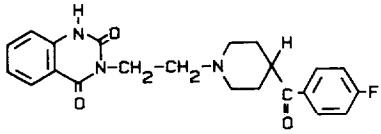
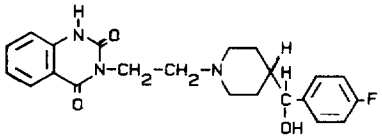
Ketanserin (R-41,468), a quinazoline derivative (Fig. 1), is a potent inhibitor of the contractile responses to serotonin (5-HT) of isolated rat caudal, canine basilar, carotid, coronary and splenic arteries. Besides its strong affinity to 5-HT_2 receptors and its inaffinity to 5-HT_1 receptors, it has a five-fold lower affinity for histamine H_1 receptors and adrenergic α_1 receptors [1].

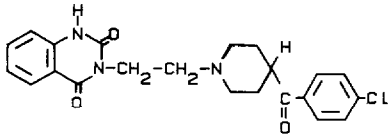
Clinically, ketanserin in a dose of 10 mg given intravenously causes a distinct fall in supine systemic arterial, right-atrial and pulmonary capillary wedge



Ketanserin



Reduced Ketanserin



R-46,594

Fig. 1. Chemical structures of ketanserin, reduced ketanserin and the internal standard, R-46,594.

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pressures, whereas cardiac output, renal blood flow, and glomerular filtration rate show no persistent changes [2]. Ketanserin further improved cardiac output in patients with severe cardiac failure, who had already been treated with digitalis and diuretics, it reduced both pre- and after-load and caused a significant fall in blood pressure while cardiac output and stroke work indices both increased [3], and in patients with thrombophlebitis it reduced pain and swelling [4]. Ketanserin may also be useful in the treatment of Raynaud's phenomenon [5] and in the treatment of postoperative hypertension following coronary artery bypass surgery [6].

Previously a rapid method for the determination of ketanserin by high-performance liquid chromatography (HPLC) was described [7]. One of the metabolites of ketanserin is reduced ketanserin (R-46,742) (Fig. 1). It is not yet understood whether this metabolite is pharmacologically active or not [8]. In this present article a method is described for the determination of both ketanserin and reduced ketanserin using HPLC and ultraviolet (UV) absorbance detection at a fixed wavelength of 214 nm.

EXPERIMENTAL

Chemicals and reagents

Ketanserin, {3-[2-[4-(4-fluorobenzoyl)-1-piperidinyl] ethyl] -2,4(1H,3H)-quinazoline dione}, reduced ketanserin {3-[2[4-[(4-fluorophenyl)hydroxy-methyl] -1-piperidinyl] ethyl] -2,4(1H,3H)-quinazoline dione} and the internal standard {3-[2-[4-(4-chlorobenzoyl)-1-piperidinyl] ethyl] -2,4(1H,3H)-quinazoline dione} (R-46,594) (Fig. 1) were provided by Janssen Pharmaceutica (Beerse, Belgium). R-46,594 was chosen as internal standard because of its structural similarity to ketanserin. HPLC-grade acetonitrile and HPLC-grade water were obtained from J.T. Baker Chemicals (Deventer, The Netherlands). *n*-Heptane spectroscopic grade was obtained from E. Merck (Darmstadt, F.R.G.). All other chemicals were reagent grade.

Instrumentation

The HPLC system was manufactured by Waters Assoc. (Milford, MA, U.S.A.) and consisted of an M-45 solvent delivery system, a Waters intelligent sample processor Model 710 B, and a Model 441 UV absorbance detector with the wavelength fixed at 214 nm. The recorder was a Philips PM 8220 pen recorder with a 10-mV output. For the extractions a Vortex mixer (Wilton & Co, Etten-Leur, The Netherlands) was used.

Chromatographic conditions

The column was a Chrompack CP^{tm} Spher C8 standard column, 25 cm × 4.6 mm I.D., with particle size of 8 μm. The flow-rate was set at 1 ml/min which resulted in a precolumn pressure of 68.9 bar. Detection was at 214 nm with sensitivity at 0.02. The chart speed was 0.5 cm/min.

Mobile phase preparation

To prepare the mobile phase, 550 ml of acetonitrile were filtered through a type FH 0.5-μm Millipore filter and 450 ml of water were filtered through

a type HA 0.45-μm Millipore filter. These liquids were combined and then were added 2 ml of a buffer solution, pH 5.8, consisting of 30% acetic acid and 100% diethylamine (10:4.25). The mobile phase was placed in an ultrasonic bath for 15 min to remove dissolved air.

Extraction procedure

Plasma (2 ml) was pipetted into 15-ml glass centrifuge tubes and spiked with 0.2 μg of the internal standard. Then 0.5 *M* sodium hydroxide (100 μl) was added and the plasma extracted twice with *n*-heptane—isoamyl alcohol (95:5, 2 ml) by vortexing for 1 min, and centrifuged at 4500 *g* for 5 min. The organic layers were combined and extracted with 0.05 *M* sulfuric acid (4 ml) by vortexing for 1 min, and centrifuged at 4500 *g* for 5 min. The aqueous layer was removed and, after adjusting the pH to 9 by adding 25% ammonium hydroxide (200 μl), extracted with *n*-heptane—isoamyl alcohol (95:5, 4 ml) by vortexing for 1 min, and centrifuged at 4500 *g* for 5 min. The organic layer was transferred into a conical tube and evaporated to dryness under a nitrogen stream in a waterbath at a temperature of 50°C. The residue was dissolved in the mobile phase (100 μl) and a 25-μl aliquot was injected into the HPLC apparatus.

Preparation of calibration standards

Ketanserin, reduced ketanserin and the internal standard were dissolved in methanol to produce stock solutions containing 1 mg/ml of these compounds. These stock solutions were further diluted with methanol to produce standard solutions containing 1 μg/ml. The stock solutions were stored in the dark at 4°C for one month; the standard solutions were prepared freshly when needed.

RESULTS AND DISCUSSION

The retention times under the conditions described were 4.9 min for reduced ketanserin, 6.4 min for ketanserin and 8.1 min for the internal standard. In Fig. 2 chromatograms are shown of control plasma (Fig. 2a), control plasma spiked with ketanserin, reduced ketanserin and the internal standard (Fig. 2b), and of a plasma sample obtained from a patient receiving three tablets of ketanserin 40 mg daily (Fig. 2c). The sample was drawn about 4 h after ingestion of the last tablet. (The patient was a 44-year-old white female who suffered from Raynaud's phenomenon and was on this medication for eight days; concurrently she was receiving nitrazepam.) The calculated concentration of ketanserin was found to be 0.169 mg/l and the concentration of reduced ketanserin was 0.103 mg/l.

The standard curves for ketanserin and reduced ketanserin were obtained in a series of experiments in which varying amounts of ketanserin and reduced ketanserin ranging from 0.0125 to 0.200 μg/ml and a fixed concentration of 0.1 μg/ml of the internal standard were added to plasma obtained from healthy human volunteers. After the extraction procedure described above, the samples were injected into the HPLC apparatus and the ratio of the peak height of ketanserin and reduced ketanserin to internal standard was plotted against the known concentrations of each substance. Both plots were linear with $r = 0.999$ for ketanserin and $r = 0.991$ for reduced ketanserin. A concentration of 0.01 μg/ml can easily be measured.

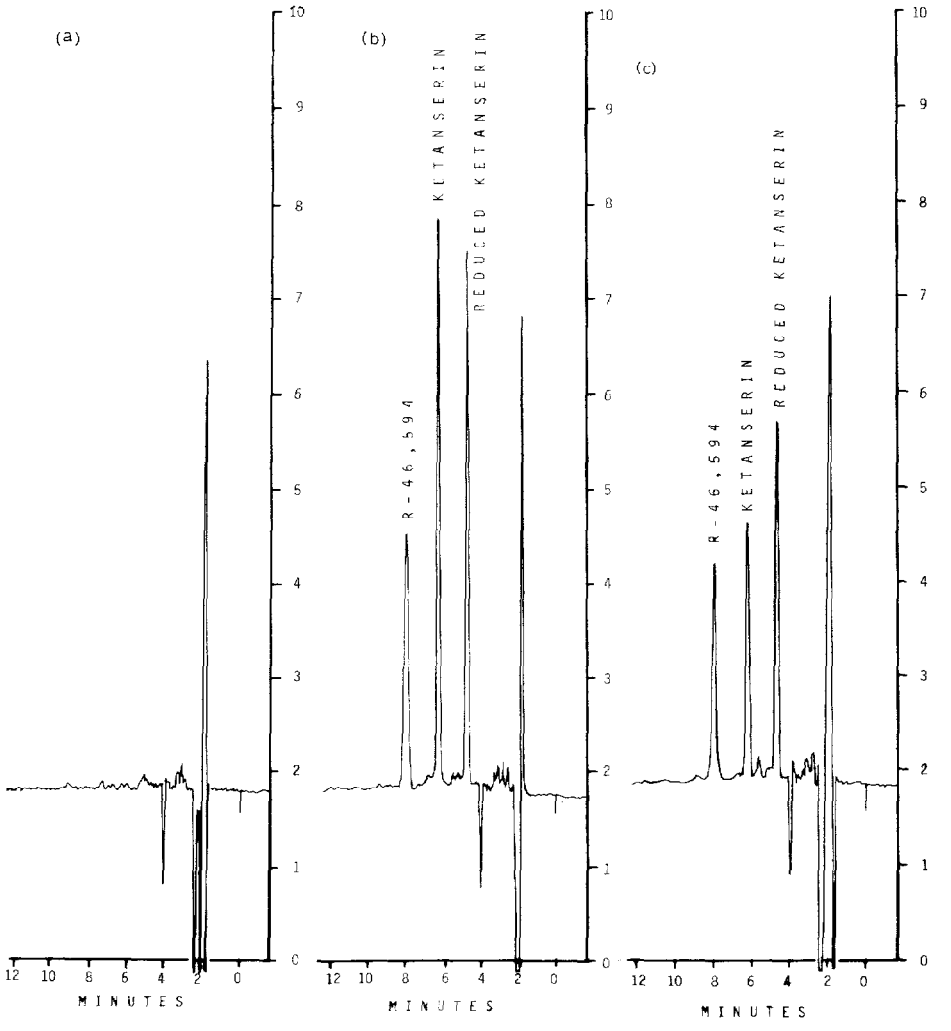

Fig. 2. High-performance liquid chromatograms of (a) control plasma, (b) control plasma spiked with ketanserin, reduced ketanserin (both 0.200 μg/ml) and internal standard (0.100 μg/ml), and (c) a patient's plasma sample.

The extraction efficiency was determined by comparing the peak height of ketanserin and reduced ketanserin extracted from plasma with the peak height of a solution of ketanserin and reduced ketanserin in the mobile phase (the internal standard was added after extraction). The recovery of ketanserin was 82.7 ± 7% and of reduced ketanserin 81.0 ± 8% (n = 10).

The reproducibility of the method was tested by determining the intraassay coefficient of variation; at a concentration of 0.100 μg/ml this was 3.2% for ketanserin and 3.6% for reduced ketanserin (n = 10). Also the interassay coefficient of variation was determined over a period of two months; at a concentration of 0.100 μg this was 4.2% for ketanserin and 4.9% for reduced ketanserin (n = 10).

The method reported here is useful for determination of the clinical phar-

macokinetics of ketanserin, as both the concentrations of ketanserin and reduced ketanserin can be measured in one run.

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