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Short Communication

Rapid method for the determination of ketanserin in rat serum by high-performance liquid chromatography with fluorimetric detection

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ABSTRACT

A simplified high-performance liquid chromatographic (HPLC) assay for the determination of ketanserin in rat serum is described. The chromatographic method allowed complete resolution of ketanserin from two of its metabolites. A protein precipitation extraction procedure was employed which allowed rapid sample preparation for injection into the HPLC system. Both intra- and inter-assay coefficients of variation at serum ketanserin concentration of 200 and 800 ng/ml were less than 6% and the accuracy was excellent. The assay has been applied for determining the elimination kinetics of ketanserin in the rat.

INTRODUCTION

Ketanserin, 3-{2-[4-(4-fluorobenzoyl)-1-piperidinyl]ethyl}-2,4(1*H*,3*H*)-quinazolinedione, is a serotonin S_2-receptor antagonist effective in the treatment of hypertension [1–3]. This agent has also been used extensively as a labelled ligand to characterize *in vitro* and *in vivo* serotonin S_2-receptors [4–6]. The major phase I metabolic pathways of ketanserin in mammals are: (a) aromatic hydroxylation at the quinazolinedione; (b) ketone reduction; (c) oxidative N-dealkylation at the piperidine nitrogen (Fig. 1). In rats, (a) and (c) are the main pathways, (b) is the minor one. In humans, (b) and (c) are the main pathways, whereas (a) in only a minor one [7].

Our interests in ketanserin is the application of this agent to the kinetic modeling of serotonin S_2-receptor in rat brain. This requires a sensitive assay for ketanserin in serum and brain tissue in the presence of its metabolites. Various methods for the determination of ketanserin in human plasma have been described; these include a radioimmunoassay [8] and several high-performance liquid chromatographic (HPLC) methods with UV [9–11] and fluorimetric detection [12,13].

We report here a method for determining ketanserin in rat serum by HPLC

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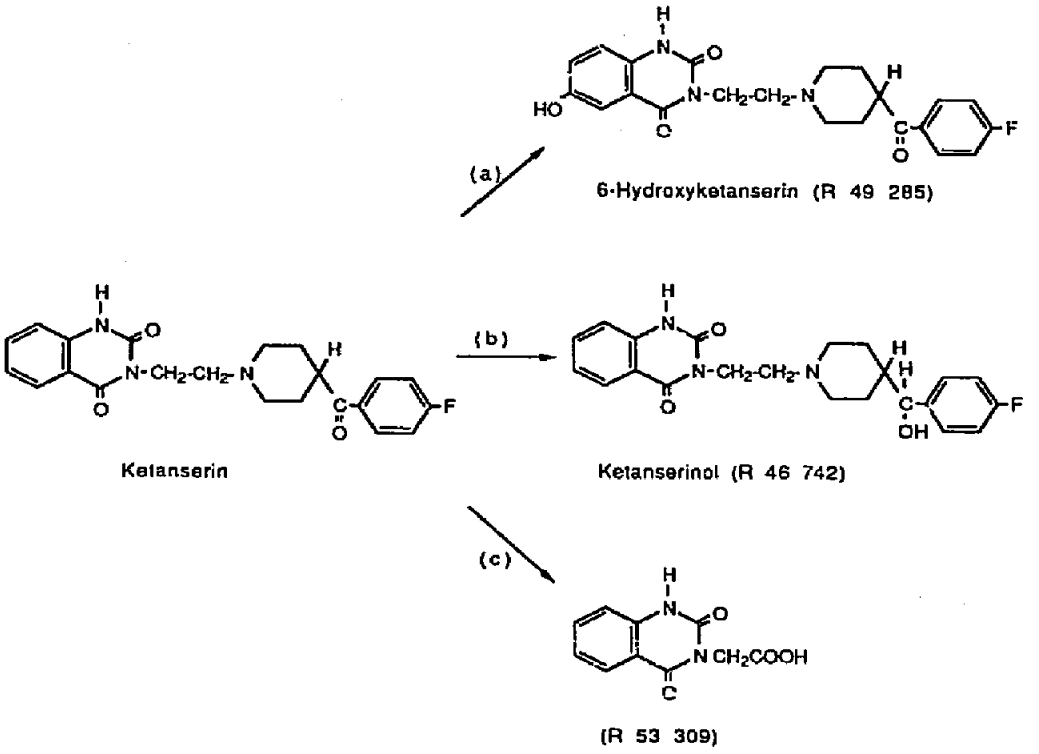

Fig. 1. Major phase I metabolic pathways of ketanserin in mammals.

with fluorimetric detection. The assay is extremely rapid and is as sensitive as other HPLC assays when less than 100 μl of sample is available for analysis. In addition, the assay employs a rapid protein precipitation sample preparation procedure which allows serum samples to be prepared for analysis immediately after collection.

EXPERIMENTAL

Reagents and chemicals

Ketanserin tartrate (R-41,468), ketanserinol (R-46,742) and 6-hydroxyketanserin (R-49,285) were provided as reference compounds by Janssen Pharmaceutica (Beerse, Belgium).

Acetonitrile and methanol were HPLC grade (OmniSolve, MCB Manufacturing Chemists, Cincinnati, OH, USA). Acetic acid and ammonium hydroxide in analytical-grade purity were also from MCB Manufacturing Chemists.

Instrumentation

The chromatograph consisted of a Waters Model 601 pump with a U6K universal injector (Milford, MA, USA) and a Shoeffel Model FS 970 fluorimetric

detector (Krato, Westwood, NJ, USA). Chromatographic peaks were recorded on a Hewlett-Packard Model 3392A integrator (Houston, TX, USA).

The Vortex-Genie 2* mixer and the Fisher Model 235A microcentrifuge were from Fisher Scientific (Pittsburgh, PA, USA).

Chromatographic conditions

Chromatographic analysis was carried out at normal ambient temperature, *i.e.* 23°C. The mobile phase consisted of acetonitrile–acetate buffer (pH 7.0)–distilled water (31:50:19). Acetate buffer (pH 7.0) was prepared by adjusting 2% acetic acid in distilled water to pH 7.0 with ammonium hydroxide. A Waters Nova-Pak C_{18} (4 μm, 15 cm × 0.39 cm) column was used as the analytical column. The flow-rate of the mobile phase was 1 ml/min.

The excitation wavelength was set at 225 nm and the emission was monitored with no emission filter.

Sample preparation

Serum samples were either prepared for assay immediately after collection or stored at −20°C until assay. Each sample was allowed to thaw at room temperature and vortex-mixed for 30 s before an aliquot was removed. Serum (25 μl) was mixed with 50 μl of methanol in a 1.5-ml microfuge tube. The mixture was vortex-mixed for 30 s and centrifuged at 13 600 *g* for 5 min. A 25-μl aliquot of the clear supernatant was injected into the HPLC system.

Calibration plot (peak height *versus* concentration) was constructed using rat serum supplemented with 80, 200, 400, 800 and 1600 ng/ml ketanserin.

Preparation of calibration standards

Ketanserin tartrate, equivalent to 1 mg of ketanserin, was dissolved and made up to 100 ml with methanol to give solution A. A 5-ml volume of solution A was further diluted to 50 ml with methanol to produce solution B containing 1 ng/μl ketanserin. These solutions were freshly prepared before use.

To prepare a standard serum sample containing 80 ng/ml ketanserin, 16 μl of solution B were transferred to a 1.5-ml microfuge tube with a Hamilton syringe and evaporated to dryness under a stream of dry nitrogen. The residue was reconstituted with 200 μl of blank rat serum. The procedure was repeated with 40, 80, 160 and 320 μl of solution B to produce serum standards containing 200, 400, 800 and 1600 ng/ml ketanserin.

RESULTS AND DISCUSSION

The aim of the present study was to develop a simplified method for the determination of ketanserin in rat serum. Initial investigations showed that ketanserin and two of its metabolites could be resolved at pH 7.0 on a reversed-phase C_{18} column. The retention times of ketanserin, ketanserinol and 6-

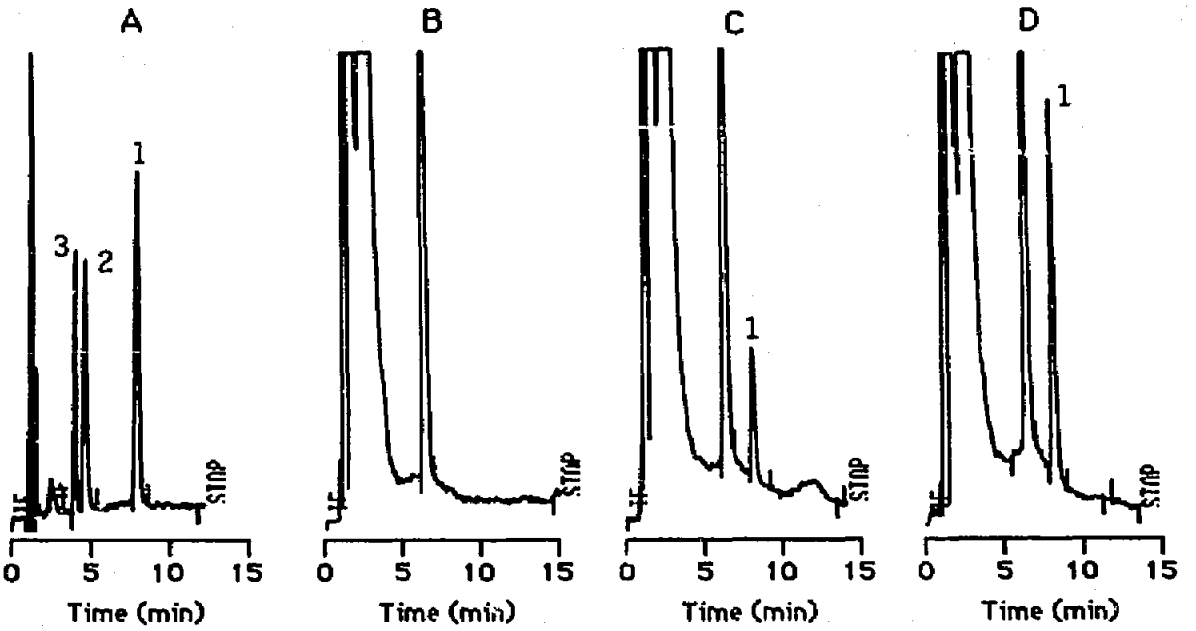

Fig. 2. Representative chromatograms of ketanserin, ketanserinol and 6-hydroxyketanserin. (A) Standard mixture of 2 ng of ketanserin, 4 ng of 6-hydroxyketanserin and 0.5 ng of ketanserinol; (B) blank rat serum sample; (C) blank rat serum sample supplemented with ketanserin at 80 ng/ml; (D) serum sample from a rat 2 h after an intravenous dose of ketanserin at 0.5 mg/kg. Peaks: 1 = ketanserin; 2 = 6-hydroxyketanserin; 3 = ketanserinol.

hydroxyketanserin under the described chromatographic conditions were 7.7, 3.2 and 4.5 min, respectively (Fig. 2A). The acidic metabolite, which is unavailable for evaluation, is not expected to interfere; as a carboxylic acid, this compound would predominantly be in the ionized form at pH 7.0 and is expected to exhibit a very short retention time on a reversed-phase chromatographic system.

The sensitivity of fluorimetric detection depends on careful selection of excitation and emission wavelengths. Although 310 and 270 nm have been used as the wavelengths of excitation for ketanserin [12,13], the detector used in the present study offered the best sensitivity with 225 nm as the excitation wavelength when the emission was monitored with no emission filter. With these detector settings, ketanserin can be detected down to 0.1 ng.

Most HPLC methods for ketanserin published to date include a liquid extraction method with a solvent mixture of pentane and isoamyl alcohol [9–13]. In order to overcome the poor extraction efficiency, the sample is normally extracted twice which is inconvenient. When the sample size available for analysis is less than 100 μl, a simple and more efficient protein precipitation method offers a suitable alternative. The use of this precipitation method has also allowed the assay be run without an internal standard.

Fig. 2D shows a chromatogram resulting from the analysis of a serum sample obtained from a rat 2 h after a single 0.5 mg/kg intravenous dose of ketanserin. A

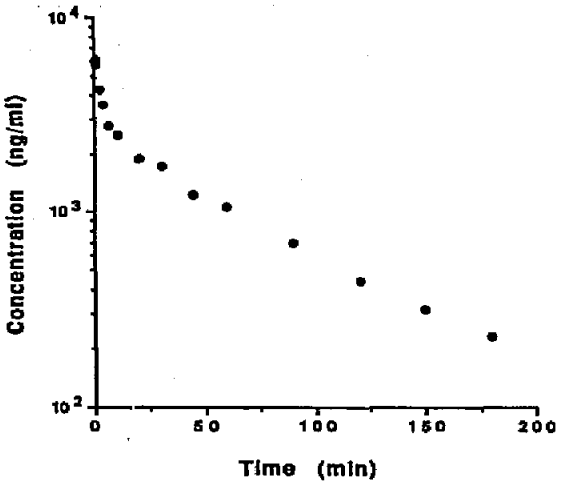

Fig. 3. Serum concentration–time profile of ketanserin in one rat following a single 1.0 mg/kg intravenous dose of ketanserin.

calibration plot of peak height *versus* concentration of ketanserin was linear (range 80–1600 ng/ml) with correlation coefficient (r^2) greater than 0.99. The intra- and inter-assay precision and accuracy were determined by repeated analysis of serum samples supplemented with 200 and 800 ng/ml ketanserin; the results are summarized in Table I. Since the calibration standards for each inter-assay variability study run were prepared using serum from different rats, the inter-assay coefficient of variation reflects the consistent recovery of ketanserin provided by the extraction method. Although the lowest concentration that can be accurately determined is about 40 ng/ml, it can be improved by injecting a larger amount of supernatant in the HPLC system because the chromatograms were essentially free from interferences (Fig. 2).

TABLE I

INTRA- AND INTER-ASSAY ACCURACY AND PRECISION OF THE METHOD FOR DETERMINING KETANSERIN IN RAT SERUM

Theoretical concentration (ng/ml)	Number of determinations (n)	Mean concentration (ng/ml)	Coefficient of variation (%)
Intra-assay			
200	8	203.1	5.6
800	8	808.8	3.0
Inter-assay			
200	8	201.4	5.3
800	8	792.2	3.3

The assay has been applied to determining the elimination kinetics of ketanserin in the rat. Fig. 3 shows a serum concentration–time profile in one rat following a single 1.0 mg/kg intravenous dose of ketanserin.

The method described is developed specifically for ketanserin in rat serum and is as sensitive as other published HPLC methods with fluorimetric detection when less than 100 μl of sample is available for analysis. The major advantage of the method is the simple and rapid sample preparation procedure, which allows samples be processed immediately after collection. In addition, the method is particularly well suited for the analysis of radiolabelled ketanserin in serum when coupled with a radiochemical detector; because of reduced handling in the sample preparation process, radiochemical contamination is substantially reduced.

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