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Note

High-performance liquid chromatographic assay for nizatidine, a new H_2 blocker, in human plasma and urine using disposable solid-phase extraction columns

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Nizatidine (N-[2-[[[2-[(dimethylamino)methyl]-4-thiazolyl] methyl]-thio]ethyl]-N′-methyl-2-nitro-1,1-ethenediamine, Fig. 1) is a recently developed histamine H_2 receptor blocker. Because of its high potency, evaluated on a weight basis versus other H_2 receptor antagonists, nizatidine has been widely studied for the treatment of peptic ulcers. After oral administration, peak plasma concentrations are reached within 1–3 h, with a limited proportion of protein binding (32–35%) [1]. Nizatidine is largely excreted unchanged in the

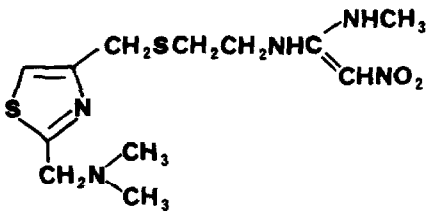

Fig. 1. Structure of nizatidine.

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urine by both glomerular filtration and tubular secretion. Following administration of a 150-mg oral dose of ^{14}C-labelled nizatidine, over 90% of the dose is recovered in urine and ca. 6% in the faeces within three days [2]. Three metabolites have been identified in urine: N-2-monodesmethylnizatidine, nizatidine N-2-oxide and nizatidine sulphoxide, representing ca. 7, 6 and 6% of the dose, respectively [4–7].

In order to extend pharmacokinetic studies and to assess patient compliance, a method for evaluation of the drug in biological fluids is required. Two chromatographic methods have been developed for the determination of nizatidine in serum and urine [3,4] using different organic solvents for extraction. The aim of this study was to develop a high-performance liquid chromatographic (HPLC) assay for nizatidine using solid-phase extraction (SPE) columns.

EXPERIMENTAL

Chemicals

Nizatidine and the internal standard, ranitidine, were supplied by the Department of Pharmacology of the University of L'Aquila. Methanol (HPLC grade), potassium dihydrogenphosphate and sodium hydroxide were purchased from Farmitalia Carlo Erba (Milan, Italy). Acetonitrile (HPLC grade) was supplied by Merck (Darmstadt, F.R.G.). All water used in this assay was distilled, treated by a Milli-Q water purification system (Millipore, Bedford, MA, U.S.A.) and subsequently filtered through a 0.45-μm pore Nylon filter.

Chromatographic system and conditions

The liquid chromatographic system consisted of an M6000A pump, a U6K injector and a Lambda Max Model 481 LC variable-wavelength absorbance detector connected to a Model 740 Data Module integrator (Waters Assoc., Milford, MA, U.S.A.). The analysis was performed using a Viosfer ODS reversed-phase column (25 cm $\times$ 4.6 mm I.D., 5 μm particle size) (Violet, Rome, Italy) connected to a disposable Pelliguard 20 mm $\times$ 4.6 mm I.D. (40 μm particle size) precolumn (Supelco, Bellefonte, CA, U.S.A.). The mobile phase, 0.05 *M* potassium dihydrogenphosphate (pH 7)–methanol (40:60, v/v), was prepared daily and delivered at a flow-rate of 1.0 ml/min. The extraction apparatus was a Supelco SPE manifold equipped with a drying attachment. Bond-Elut silica SPE cartridges (3.0 ml capacity with 500-mg packings) were purchased from Analytichem International (Harbor City, CA, U.S.A.)

Standard solutions

Stock solutions (1.0 mg/ml) of nizatidine and the internal standard ranitidine were prepared in methanol. Standard solutions containing between 0.1 and 7.0 μg/ml nizatidine were prepared by diluting the stock solution with

methanol. The internal standard solution was diluted with methanol to a final concentration of 5 μg/ml. Human plasma control pools were prepared by diluting the nizatidine stock solution to 10.0 μg/ml with control human plasma. This solution was further diluted with control human plasma to yield control pools containing 3.0, 2.0 and 1.0 μg/ml. For urine identically solutions were prepared.

Sample preparation

Plasma. Heparinized blood samples from various patients were centrifuged and plasma was collected and frozen at −20°C. Samples were thawed just before the extraction procedure, thoroughly agitated and centrifuged at 800 *g* for 15 min. The Bond-Elut cartridges were placed in a luer that fitted the top of the Supelco vacuum manifold, which may be loaded with up to twelve cartridges. A vacuum of 250–500 Torr was applied to the manifold to carry out the various steps of the extraction. A 1-ml rinse of methanol followed by 2 ml of HPLC-grade water served to activate the cartridges prior to introduction of plasma samples. Then 1 ml of plasma added to internal standard was run through the cartridge followed by 2 ml of water. The effluent was discarded, and 2 ml of acetonitrile were then applied to the cartridge, and the eluate was collected. This fraction was finally centrifuged, filtered through a Millipore HV filter (0.45 μm) and evaporated to dryness with a nitrogen stream under vacuum with the Supelco drying attachment. The samples were then reconstituted to 200 μl with the mobile phase and mixed on a vortex agitator. Aliquots of each sample (10 μl) were chromatographed with the mobile phase. The column eluate was monitored at 320 nm.

Urine. Urine samples were stored frozen (−20°C) until required for assay. Samples were thawed just before the extraction procedure, thoroughly agitated, added to the internal standard and vortex-mixed. After cartridge activation, a sample of 0.5 ml of urine was passed through it, followed 2 ml of water. The effluent was discarded. A 2.0-ml volume of acetonitrile was then applied to the cartridge, and the eluate was collected and evaporated to dryness. The sample were reconstituted to 100 μl. An aliquot of 10 μl was injected into the chromatograph.

RESULTS AND DISCUSSION

The retention times for nizatidine and the internal standard were 2.9 and 4.2 min, respectively. Fig. 2 illustrates typical chromatograms from control human plasma and urine and from human plasma and urine of a treated patient. No endogenous plasma components or metabolites were observed near the retention time corresponding to nizatidine or the internal standard. The assay was validated by analysing eight nizatidine standards (plasma and urine) in triplicate over three consecutive days. Peak-height ratios were pro-

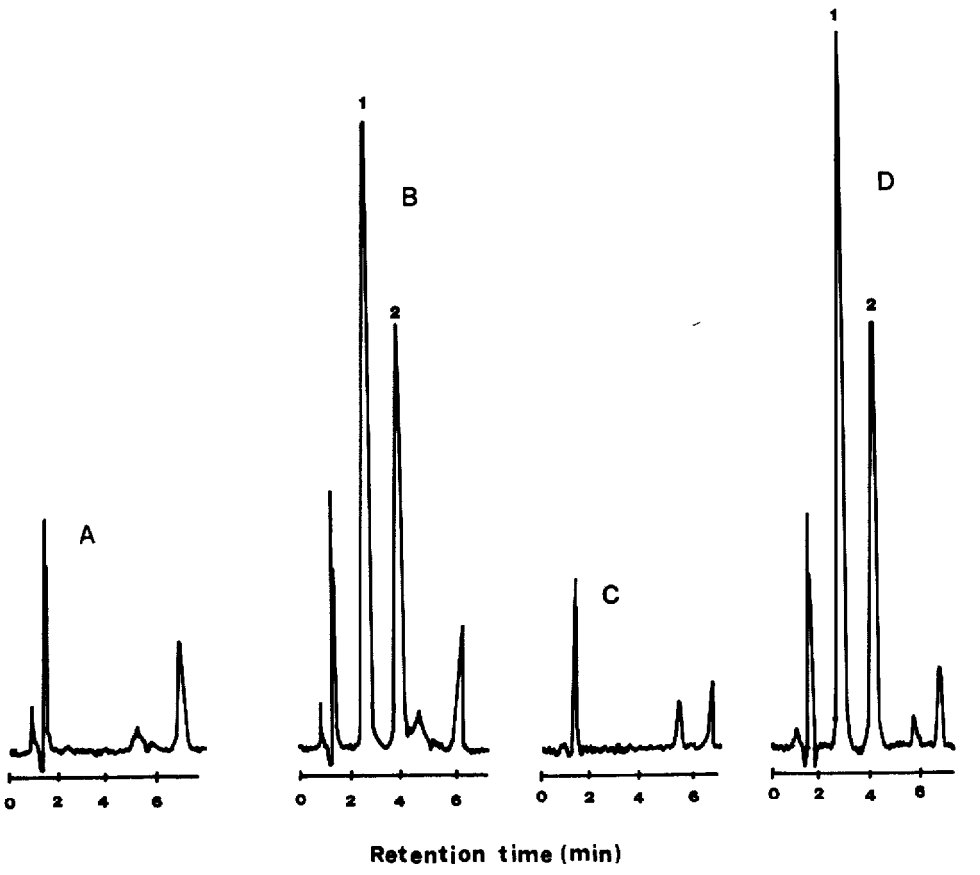

Fig. 2. Chromatograms of (A) human control plasma, (B) human plasma from a nizatidine-treated patient, (C) human control urine and (D) human urine from a nizatidine-treated patient. Peaks: 1 = nizatidine; 2 = ranitidine (internal standard).

portional to nizatidine concentration over the ranges 0.1–7.0 μg/ml for human plasma and 0.05–10 μg/ml for urine. The best-fit line was determined daily by least-squares regression analysis using a weighting factor of 1/concentration squared [8]. The result of a typical regression for human plasma was: peak-height ratio = 0.772 × nizatidine concentration + 0.004 (r = 0.999); for urine it was: peak-height ratio = 0.651 × nizatidine concentration + 0.003 (r = 0.988).

The accuracy and precision of the calibration curves were determined from the variation of the standards from the regression line. The precision for the human plasma calibration standard ranged from 2.6 to 5.4% (relative standard deviation) with relative errors of 6.1–3.7%. The precision for urine calibration standards ranged from 1.6 to 3.1% with relative errors of 4.7–2.5%.

The assay precision and accuracy were determined by analysing three control pools of human plasma and urine in triplicate on three consecutive days.

The extraction efficiencies of nizatidine added to human plasma at concentrations of 1.0 and 3.0 μg/ml were 95.4 ±1.2 and 97.0 ±2.3%, respectively, and the extraction efficiencies from human urine were 96.7±1.3 and 98.7±2.1% at identical concentrations. The extraction efficiency of the internal standard was 96.5±3.1%. These values were determined by comparison with a methanolic solution of nizatidine, treated in the same way as the plasma or urine solutions. The detection limits (signal-to-noise ratio of 3) for nizatidine were 0.1 μg/ml in plasma and 0.05 μg/ml in urine.

The method described in this paper has been used extensively for measuring nizatidine in patients' plasma and urine, for assessing patient compliance in assuming prescribed nizatidine regimes and for examining the relationship between the nizatidine concentration in plasma and antisecretory effects. Twelve samples can be processed and prepared for analysis in ca. 10 min.

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