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Determination of nizatidine and two of its main metabolites in human serum using high-performance liquid chromatography

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ABSTRACT

A high-performance liquid chromatographic assay has been developed for the determination of nizatidine, a new histamine H2-receptor antagonist, and two of its main metabolites, N-desmethylnizatidine and nizatidine sulphoxide. Drugs were extracted with chloroform–2-propanol (90:10, v/v) from alkalinized samples of serum, using ranitidine as an internal standard. After evaporation of the extraction solvent, the residue was removed and analysed on a LiChrosorb Si60 5-μm column with a mobile phase of acetonitrile–methanol–water–ammonia solution (1000.200:20.5, v/v). The compounds were detected at 320 nm. The lower detection limits were 6–18 ng/ml at a signal-to-noise ratio of 3. This method is simple and specific, and the single-step extraction makes it rapid. It is the first high-performance liquid chromatographic assay to be described for the determination of nizatidine metabolites.

INTRODUCTION

Histamine has been known for ca. 50 years to stimulate the gastric oxyntic cells responsible for the production of acid in the stomach [1–3]. This knowledge did not give immediate therapeutic applications, since conventional antihistaminics were unable to reduce gastric acid secretion significantly [4].

The discovery by Black et al. [5] of a second class of histamine receptor antagonists (H2), specifically blocking the histamine-induced gastric secretion [5], led to the introduction of cimetidine, then ranitidine (respectively marketed in France in 1977 and 1984), which represented an important ad-

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vance in the therapy of gastric and duodenal peptic ulceration and other hypersecretory states.

However, adverse reactions during cimetidine therapy were not rare: myalgia, diarrhoea, rashes and dizziness were the most frequent, but serious side-effects such as acute mental confusion and other kinds of psychotoxicity, antiandrogenic effects, hepatotoxicity and cardiovascular effects were also encountered [6–10]. Moreover, cimetidine is also well known to be an inhibitor of microsomal metabolism in the liver by acting on the cytochrome P-450 enzyme system, which explains its interactions with a large range of drugs [11].

Ranitidine was at first claimed to be free of most of the side-effects exhibited by cimetidine and also to lack its enzyme-inhibition effects; nevertheless, many subsequent case reports indicate that toxicity of ranitidine is similar to that of cimetidine [12–15].

The introduction of nizatidine as a new H2-receptor antagonist signifies a great stride in terms of efficacy and safety. This compound, which is five to ten times more potent than cimetidine under experimental conditions [16], also presents a higher bioavailability and a particularly suitable pharmacokinetic profile [17,18]. The lack of hormonal side-effects [19] and the absence of any interaction with the cytochrome P-450 system [20–22] contribute to its greater safety and ease of use.

After oral administration, 75% of the dose is excreted in the urine as unchanged drug, 25% being metabolized to three main compounds: N-desmethylnizatidine (N1), nizatidine sulphoxide (N2) and nizatidine N-oxide, which is very unstable [17,23].

In a preliminary study, we presented the first high-performance liquid chromatographic (HPLC) method for the determination of nizatidine in plasma and urine [24]. This paper describes a modified procedure that allows the identification and quantification of nizatidine and two of its metabolites, N-desmethylnizatidine and nizatidine sulphoxide.

EXPERIMENTAL

Materials

Nizatidine (free base, MW = 331.5), N1 and N2 were generous gifts from Eli Lilly Labs. (Indianapolis, IN, U.S.A.). Ranitidine hydrochloride was provided by Glaxo (Paris, France). Methanol, acetonitrile, chloroform and 2-propanol were HPLC grade (Merck, Darmstadt, F.R.G.). All other chemicals and reagents were analytical grade (Merck).

Stock solutions of nizatidine, N1, N2 and ranitidine were prepared in methanol at a concentration of 100 μg/ml. They could be stored at 4°C without decay for more than four months. By appropriate dilutions in methanol, standard solutions of nizatidine, N1, and N2 were prepared at concentrations of 20, 50, 100, 250, 500, 1000 and 5000 ng/ml.

The pH 9.5 buffer solution was prepared with a saturated solution of ammonium chloride, diluted 25% with deionized water and adjusted to the desired pH by appropriate addition of 25% diluted ammonia solution. The pH 7.4 buffer solution was prepared with a mixture of 12 mg/ml Na_2HPO_4 and 9 mg/ml KH_2PO_4 (9:1, v/v), adjusted to the desired pH by appropriate addition of 2 *M* hydrochloric acid. The pH 5.5 buffer was prepared with a saturated solution of KH_2PO_4, diluted 50% with deionized water and adjusted in the same manner to the desired pH.

A 20 μg/ml methanolic solution of ranitidine was used as the internal standard (I.S.).

Chromatography

The HPLC system consisted of a pump (Waters 510, Milford, MA, U.S.A.) and an automatic sample injection module (Waters WISP 710 B), which were coupled to a programmable multi-wavelength detector (Waters 490) operated at 320 nm. This detector was monitored with a data and chromatography control station (Waters 840). A LiChrosorb Si60 (Alltech, Interchim, Paris, France) 5-μm column (150 mm×4.6 mm I.D.) was used at ambient temperature (19–22°C). The mobile phase was acetonitrile–methanol–deionized water–25% diluted ammonia solution (1000:200:20:5, v/v). Before analysis, this mobile phase was filtered and degassed through 0.45-μm filters (Durapore GVWP 047, Bedford, MA, U.S.A.) with a Pyrex filter holder (Millipore, Bedford, MA, U.S.A.). The flow-rate was 2.0 ml/min, with an average operating pressure of 8.55 MPa.

At the end of each chromatographic session, the column was washed for 1 h with acetonitrile–deionized water (98:2, v/v).

Procedure

To 1 ml of serum in a 15-ml Pyrex centrifuge tube were added 50 μl of the ranitidine I.S. solution (equivalent to 1 μg), 1.0 ml of pH 9.5 ammonium chloride buffer solution and 5.0 ml of the extracting solvent chloroform–2-propanol (90:10, v/v). After horizontal agitation for 10 min and centrifugation at 2700 *g* for 10 min at 10°C, the lower organic phase was removed and evaporated to dryness at 45°C in a Speed Vac concentrator (Savant Instruments, Hicksville, NY, U.S.A.) connected to a vacuum pump (Yamato Scientific, Tokyo, Japan). The residue was dissolved in 100 μl of methanol, and 60 μl were injected into the column.

Quantification was performed by plotting peak-area ratios (drug/I.S.) against the concentrations of standards to produce standard curves and then comparing the results for the case samples with the curves.

RESULTS AND DISCUSSION

Fig. 1 shows a typical chromatogram obtained following extraction of a healthy volunteer's serum, taken 4 h after ingestion of a single dose (300 mg) of nizatidine. Retention times were 2.65, 4.19, 5.13 and 6.16 min for nizatidine, N1, ranitidine and N2, respectively. As shown on the chromatogram, each compound was clearly separated from the others. In this case, concentrations of 735, 41 and 59 ng/ml were measured for nizatidine, N1 and N2, respectively.

Fig. 2 represents a chromatogram of a mixture of nizatidine, N1, N2 and ranitidine in methanolic solutions; each drug was at a concentration of 1 μg/ml. The systematically observed deformed peak shape for N1 probably results from the presence of two optical isomers.

Fig. 3 shows a chromatogram obtained after extraction of a drug-free serum; no interferences from serum components were noticed at the relevant retention times (arrows).

Ranitidine was chosen as the I.S. for several reasons. First, nizatidine and ranitidine exhibit UV absorption maxima at 325 and 313 nm, respectively, which is not surprising, considering the similarity of their molecular structures; thus, the use of a UV detector operated at 320 nm enhanced the speci-

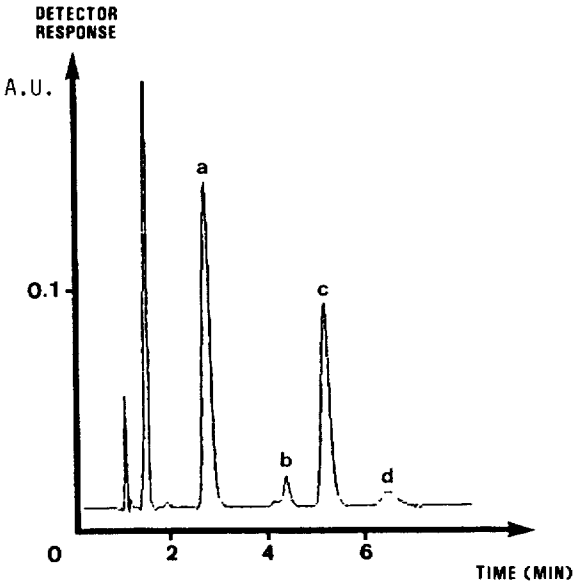

Fig. 1. Chromatogram of a healthy volunteer's serum sample, taken 4 h after ingestion of 300 mg of nizatidine. Peaks: a = nizatidine; b = metabolite N1 (N-desmethylnizatidine); c = ranitidine (I.S.); d = metabolite N2 (nizatidine sulphoxide). Measured concentrations: nizatidine, 735 ng/ml; N1, 41 ng/ml; N2, 59 ng/ml.

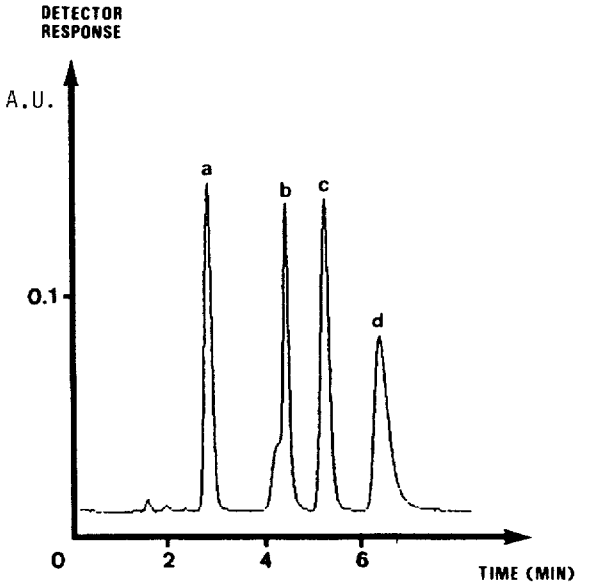

Fig. 2. Chromatogram of a standard mixture (1 μg/ml) of nizatidine, N1, N2 and ranitidine, in methanolic solutions. Peaks as in Fig. 1.

ficity of the method, since most biologically and toxicologically related compounds have little UV absorption at this wavelength; thus, dual-wavelength monitoring was not thought to be useful. In other respects, the simultaneous prescription of two histamine H2-receptor antagonists is not of usual medical practice; therefore, the I.S. quantitation of nizatidine is unlikely to be altered by a concomitant administration of ranitidine.

We investigated a large range of extraction solvents, in order to develop a single-step extraction procedure with sufficient recovery. Absolute recovery was determined at pH 9.5, 7.4 and 5.5, for each compound and each solvent tested, by comparing the representative peak areas of extracted serum samples at a concentration of 500 μg/ml for nizatidine and 50 μg/ml for its metabolites with the peak areas of the methanolic standards at the same respective concentrations. The results are summarized in Table I.

In our preliminary study [24], chloroform–1-propanol (90:10, v/v) was considered to be the solvent of choice for nizatidine extraction, since it produced emulsion-free extracts with an acceptable recovery; however, chloroform–2-propanol (90:10, v/v) was subsequently chosen since it gave a better extraction efficiency for nizatidine metabolites. Alkalinization of the serum with pH 9.5 buffer solution was found to be appropriate for the simultaneous extraction of nizatidine and the I.S. ranitidine, which is in accordance with

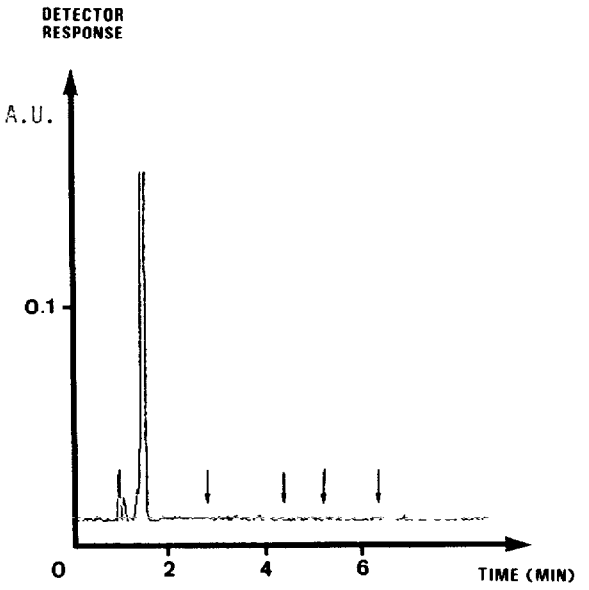

Fig. 3. Chromatogram of a healthy volunteer's drug-free serum; no interferences appeared at the retention times of the drugs studied (arrows).

data in the literature [25,26] describing histamine H2-receptor antagonists as a group of weakly basic compounds.

The seven-point standard curves, constructed by extracting spiked serum samples, showed results that were linear over the ranges 50–5000 ng/ml for nizatidine and N2 (correlation coefficients 0.9952 and 0.9912, respectively) and 25–5000 ng/ml for N1 (correlation coefficient 0.9985).

The accuracy and precision of the method were assessed by carrying six replicate samples at six concentrations of nizatidine (50, 100, 250, 500, 1000 and 5000 ng/ml) through the entire procedure in one day. Results, expressed as relative error to the least-squares equations, are given in Table II. Accuracy and precision were also determined for N1 and N2, and found to be in the same range.

Intra-assay precision was evaluated by multiple analyses of drug-free sera spiked with two different concentrations of nizatidine (100 and 1000 ng/ml); the coefficients of variation (C.V.) were found to be 3.7 and 4.8%, respectively. Inter-assay precision was also studied, using drug-free sera loaded with nizatidine at a concentration of 1 μg/ml. Analyses were performed every day over a period of four weeks. The C.V. was found to be 6.0%.

The lower limits of detection, determined by loading sera with decreasing amounts of the compounds tested until a response equivalent to three times the background noise was observed, were found to be 18, 6 and 15 ng/ml for

TABLE I

ABSOLUTE RECOVERIES OF NIZATIDINE, N1 AND N2 OBTAINED AFTER SINGLE-STEP EXTRACTION FROM SERUM USING VARIOUS ORGANIC SOLVENTS

N = nizatidine; N1 = desmethylnizatidine; N2 = nizatidine sulphoxide. Results are the means ± S.D. of four separate experiments. Each serum was spiked to contain 500 ng/ml nizatidine and 50 ng/ml N1 and N2 each.

Solvent	Drug	Absolute recovery (%)		
		pH 5.5	pH 7.4	pH 9.5
Chloroform	N	20.7 ± 5.2	54.3 ± 5.1	74.4 ± 6.7
	N1	0	16.8 ± 9.2	60.4 ± 6.3
	N2	6.8 ± 2.1	31.4 ± 4.0	55.8 ± 3.9
Ethyl acetate	N	0	0	11.9 ± 4.9
	N1	0	0	0
	N2	0	0	0
Diethyl ether	N	0	0	0
	N1	0	0	0
	N2	0	0	0
Dichloromethane	N	14.8 ± 3.5	29.9 ± 7.5	71.7 ± 8.2
	N1	0	16.4 ± 3.2	55.9 ± 9.8
	N2	2.6 ± 2.0	10.8 ± 4.1	32.6 ± 5.7
Diethyl ether–chloroform–2-propanol (50:25·25, v/v)	N	0	9.8 ± 3.4	11.1 ± 3.1
	N1	0	0	5.2 ± 2.5
	N2	0	13.8 ± 5.1	14.7 ± 4.0
Ethyl acetate–chloroform–2-propanol (33:33·34, v/v)	N	0	6.5 ± 4.3	37.4 ± 5.2
	N1	0	0	12.1 ± 6.5
	N2	0	0	19.1 ± 3.4
Chloroform–1-propanol (90·10, v/v)	N	4.7 ± 2.1	56.1 ± 3.4	84.1 ± 5.7
	N1	0	17.6 ± 3.9	31.2 ± 7.1
	N2	5.9 ± 2.2	22.7 ± 7.4	57.5 ± 6.3
Chloroform–2-propanol (90.10, v/v)	N	3.2 ± 2.5	62.3 ± 5.5	80.7 ± 6.9
	N1	0	15.7 ± 7.9	67.4 ± 7.3
	N2	5.1 ± 2.7	25.6 ± 5.9	75.1 ± 5.2

TABLE II

PRECISION AND ACCURACY FOR THE DETERMINATION OF NIZATIDINE IN SERUM

Concentration added (ng/ml)	Concentration found after extraction (mean ± S.D.) (ng/ml)	Precision (%)	Accuracy[a] (%)
50	52.01 ± 2.9	5.6	+4.2
100	106.37 ± 7.9	7.4	+6.4
250	238.08 ± 26.7	11.2	−4.8
500	477.52 ± 29.1	6.1	−4.4
1000	978.71 ± 55.7	5.7	−2.1
5000	5147.34 ± 216.8	4.2	+2.9

[a]Accuracy = (concentration found − concentration given)/concentration given.

nizatidine, N1 and N2, respectively. These detection limits are adequate for pharmacoclinical experiments.

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