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Note

Determination of cisapride in plasma and animal tissues by high-performance liquid chromatography

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Cisapride, (±)-*cis*-4-amino-5-chloro-N-[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide monohydrate (I, Fig. 1), is a new gastrointestinal prokinetic agent, which most likely facilitates the release of acetylcholine at myenteric plexus sites [1,2]. In humans, cisapride is extensively metabolized, primarily by oxidative N-dealkylation and aromatic hydroxylation; renal excretion of the parent drug is less than 1% [3].

In this paper we describe a high-performance liquid chromatographic (HPLC) method for the determination of the compound in biological samples. The procedure has been applied to plasma and animal tissues and thus enabled the study of the pharmacokinetics of cisapride in both humans and experimental animals.

EXPERIMENTAL

Standards and reagents

Cisapride (R 51 619) and the internal standard (R 54 680), *cis*-4-amino-5-chloro-N-[1-[5-(4-fluorophenoxy)pentyl]-3-methoxy-4-piperidinyl]-2-methoxybenzamide monohydrate (II, Fig. 1), were obtained as reference compounds from the Janssen Life Sciences Products Division (Beerse, Belgium).

Stock solutions, corresponding to 0.1 mg/ml methanol, were prepared for both compounds. Standard solutions were obtained by diluting the cisapride stock solution to concentrations down to 0.020 μg/ml. The internal standard stock solution was diluted to final concentrations of 1 and 2 μg/ml.

Spectrophotometric-grade acetonitrile, *n*-heptane and methanol were used. Diethylamine and isoamyl alcohol were of analytical grade. The inorganic reagents were prepared in doubly distilled water.

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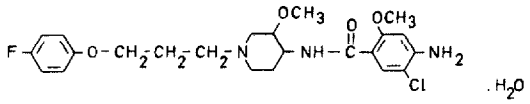
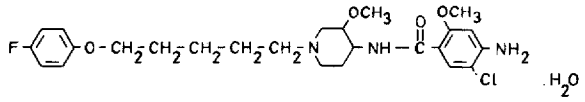


I



II

Fig. 1. Chemical structures of cisapride (I) and the internal standard (II).

Apparatus

The analyses were performed on a Varian (Walnut Creek, CA, U.S.A.) Vista 5500 liquid chromatograph, equipped with a Perkin-Elmer (Überlingen, F.R.G.) ISS-100 autosampler and a Varian UV-200 variable-wavelength detector operating at 276 nm. The separations were achieved on a reversed-phase column (15 cm × 2.1 mm), packed with 5 μm particle sized ODS-Hypersil (Shandon, Cheshire, U.K.) by the balanced-density procedure by means of an air-driven fluid pump (Haskel, Burbank, CA, U.S.A.). The samples were eluted at ambient temperature with water–acetonitrile (56:44) at a constant flow-rate of 0.8 ml/min. To suppress the ionization of the basic functions of the investigated compounds, 0.02% diethylamine was added to the solvent system. Area integrations, calculations and plotting of the chromatograms were carried out by a Varian Vista 402 chromatography data system.

Extraction procedures

Plasma (2 ml) was pipetted into a 15-ml glass test-tube, spiked with 100 ng of internal standard and made alkaline with 0.5 ml of 1 *M* sodium hydroxide. The samples were extracted with 6 ml of heptane–isoamyl alcohol (95:5, v/v) using a rotary mixer at 10 rpm for 10 min. The organic layer was separated after centrifugation at 1000 *g* for 5 min, back-extracted with 3 ml of 0.05 *M* sulphuric acid and removed after centrifugation. The remaining acidic phase was then made alkaline with 150 μl of concentrated ammonia and re-extracted with 4 ml of heptane–isoamyl alcohol. The separated organic layer was evaporated to dryness under a gentle stream of nitrogen in a metal heating-block at 55 °C and analysed by HPLC.

Animal tissues, ground in a Waring commercial blender, were homogenized (1:3, v/v) in a 0.01 *M* sodium–potassium phosphate buffer (pH 7.4) containing 1.15% potassium chloride, using an Ultra-Turrax homogenizer. Aliquots (1 ml) of the homogenates were then spiked with 200 ng of internal standard and submitted to the plasma extraction procedure.

The various extraction residues were redissolved in 120 μl of the elution solvent and transferred to 0.2-ml glass microvials, and 40-μl aliquots were injected onto the HPLC column.

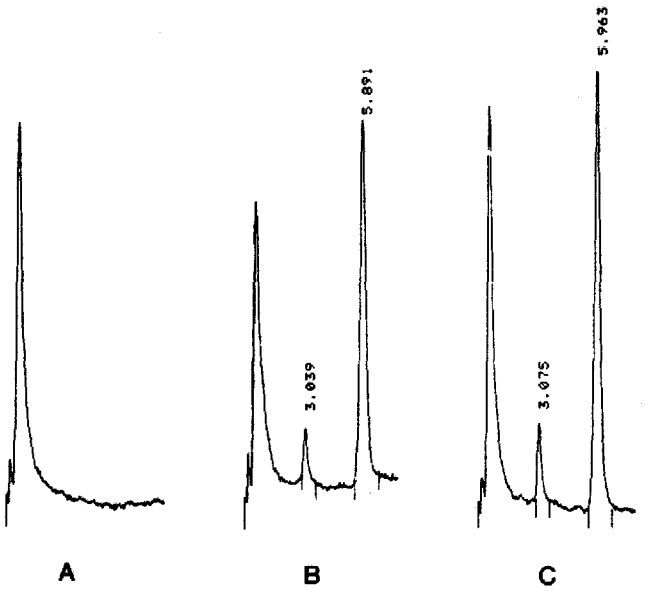


Fig. 2. Chromatograms of extracts from (A) blank control plasma, (B) control plasma spiked with 5 ng/ml cisapride (retention time, $t_R = 3.039$ min) and 50 ng/ml internal standard ($t_R = 5.891$ min) and (C) plasma from a healthy subject, 12 h after oral intake of 10 mg as a suspension, containing 5.6 ng/ml cisapride ($t_R = 3.075$ min) and 50 ng/ml internal standard ($t_R = 5.963$ min).

Calibration procedure

Using 100-μl aliquots of the standard solutions, samples of blank plasma (2 ml) were spiked with cisapride at concentrations ranging from 1 to 100 ng/ml, and with the internal standard at fixed concentrations of 50 ng/ml. Blank tissue homogenate (1 ml) was spiked at concentrations ranging from 0.008 to 8 μg/g, and with the internal standard at 0.800 μg/g. All calibration samples were taken through the extraction procedure.

Calculations

Final sample concentrations were calculated by determining the peak-area ratio of cisapride related to the internal standard, and comparing this ratio with the standard curve, obtained after analysis of the calibration samples. For quantification of lower-concentrated samples, peak-height ratios were also used.

RESULTS AND DISCUSSION

The extractability of cisapride, a lipophilic (log $P = 3.96$) weak base ($pK_a = 7.83$), from plasma and animal tissues was calculated from comparison between directly injected standards and standards subjected to the extraction procedure. The optimum compromise between chromatographic purity and extraction recovery was obtained at pH 13 using heptane–isoamyl alcohol (95:5, v/v). Over the 5–100 ng/ml plasma concentration range, the recovery amounted to $74.1 \pm 2.5\%$ (mean $\pm$ S.D., $n = 5$). For various spiked tissue homogenates (200 ng/ml), the extraction recovery ranged from 69 to 73%.

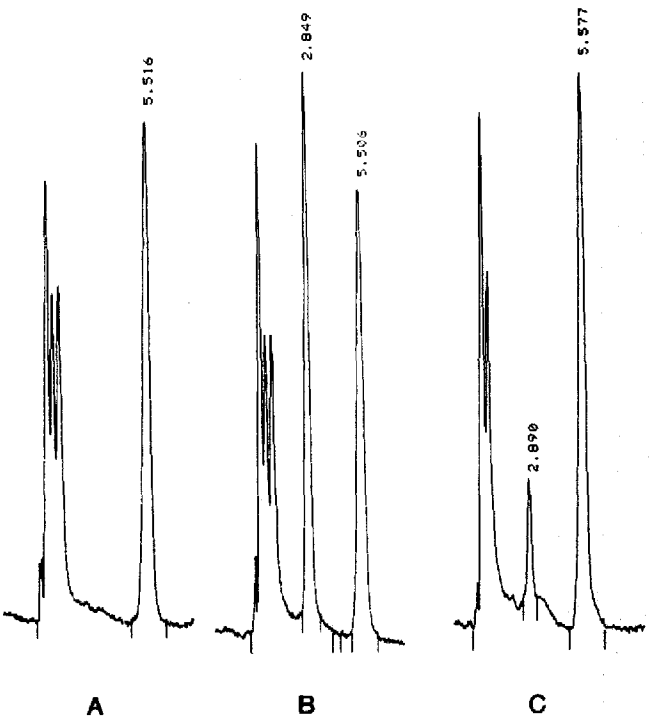


Fig. 3. Chromatograms of extracts from (A) blank rat liver spiked with 0.800 μg/g internal standard (t_R=5.516 min), (B) control rat liver spiked with 0.800 μg/g of both cisapride (t_R=2.849 min) and internal standard (t_R=5.506 min) and (C) liver from a rat, 24 h after termination of a repeated oral dosing schedule of 10 mg/kg per day for seven days, containing 0.152 μg/g cisapride (t_R=2.890 min) and 0.800 μg/g internal standard (t_R=5.577 min).

Fig. 2 shows some chromatograms of blank human plasma (A), control plasma spiked with cisapride and the internal standard (B) and plasma from a healthy subject after intake of cisapride (C). Chromatograms of extracts of liver tissue from rats are depicted in Fig. 3. All samples were spiked with the internal standard at a concentration of 0.800 μg/g. The chromatogram in Fig. 3C was obtained from the liver extract of a rat that had been chronically treated at 10 mg/kg per day for seven days. All chromatograms are free of interferences at the retention times of cisapride (3.0 min) or its internal standard (5.7 min), and both compounds eluted as completely resolved peaks.

Linear relationships were found when the peak-area or peak-height ratios of cisapride to the internal standard were plotted versus the cisapride plasma concentrations (1–100 ng/ml) on a log–log scale. Table I summarizes the mean regression parameters obtained for sixteen standard curves, used for studying the bioavailability and dose-proportionality of the drug in humans [4]. A separate standard series was analysed together with each series of study samples.

The accuracy and reproducibility of the method was investigated by replicate analysis of quality-control samples. The results are given in Table II. Reliable results are obtained, even at the lower limit of detection, which appeared to be

TABLE I

REGRESSION PARAMETERS FOR CISAPRIDE STANDARD CURVES IN PLASMA

The regression equation was $\log y = a \log x + b$ where y = peak-area or peak-height ratio (cisapride to internal standard) and x = cisapride plasma concentration (ng/ml). Values are mean ± S.D.

Calculation method	a	b	r	n
Peak-area measurements	1.012 ± 0.041	−1.672 ± 0.073	0.9993 ± 0.0004	16
Peak-height measurements	0.992 ± 0.044	−1.524 ± 0.075	0.9994 ± 0.0004	16

TABLE II

ACCURACY AND REPRODUCIBILITY OF THE HPLC METHOD FOR THE DETERMINATION OF CISAPRIDE IN PLASMA SAMPLES

Cisapride added (ng/ml)	Cisapride found (mean ± S.D., n = 7) (ng/ml)	C.V. (%)	Relative error (%)
1.0	1.0 ± 0.1	6.1	+1.0
2.0	2.0 ± 0.1	4.2	+0.5
5.0	5.3 ± 0.3	6.0	+6.0
10.0	9.8 ± 1.1	10.8	−2.4
20.0	19.3 ± 1.1	5.6	−3.7
50.0	48.8 ± 1.2	2.4	−2.4
100	95.8 ± 6.0	6.2	−4.2

ca. 1 ng/ml. Wherever possible, peak-area measurements by the integration system were used for calculations. At lower concentrations (1–10 ng/ml), however, manual peak-height measurements proved to be more reliable. More reproducibility data were obtained from duplicate analyses performed on patient plasma samples. A mean coefficient of variation (C.V.) of 4.8% (n = 121) was found. In spiked rat liver homogenates, similar performance characteristics were obtained. The standard curve, covering a 0.008–8 μg/g concentration range, was linear (r = 0.9993). Relative errors were within ± 10% and the C.V. ranged from 3 to 12% (mean 6.9%).

The stability of the drug in deep-frozen plasma could be studied from laboratory quality-control charts set up for the drug at concentrations of 11.6 and 48.2 ng/ml, respectively. Mean analytical recoveries found after 1, 2, 4, 6, 7, 8 and 9 months were 113, 106, 108, 107, 90, 99 and 105%, respectively. The drug thus appeared to be stable in plasma when stored at −20°C for at least 9 months.

The method described has been used extensively to study the pharmacokinetics of cisapride in laboratory animals [3,5], volunteers [4,6] and patients [7]. The method could also be applied to urine and milk samples, and was therefore used to study the urinary excretion in volunteers [3] and the secretion in human breast milk [7].

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