Journal of Chromatography, 183 (1980) 378—382
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 616

Note

Determination of cinnarizine in plasma by high-performance liquid chromatography

H.K.L. HUNDT*, L.W. BROWN and E.C. CLARK

Department of Pharmacology, University of the O.F.S., Bloemfontein 9301 (South Africa)

(Received February 11th, 1980)

To be able to carry out a bioavailability comparison between a generic and an innovator's brand of cinnarizine (Stugeron®, Janssen Pharmaceuticals, Beerse, Belgium), an analytical method for its determination had to be devised since no assay method could be traced despite a thorough literature survey. A gas—liquid chromatographic method has since been published [1]. This paper describes a high-performance liquid chromatographic method for the determination of cinnarizine in plasma. The assay is sufficiently sensitive to follow reliably plasma levels of cinnarizine for a period of four half-lives after a therapeutic dose of 50 mg.

EXPERIMENTAL

Reagents

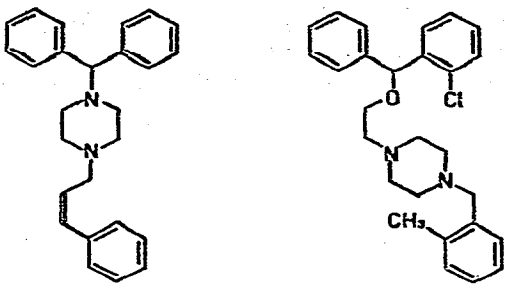
The chemical structures of cinnarizine and the internal standard, chlorbenoxamine, are shown in Fig. 1. Cinnarizine was obtained from Labethica (Bethlehem, South Africa) and chlorbenoxamine from Lennon (Port Elizabeth, South Africa).

Stock solutions of cinnarizine and chlorbenoxamine were made up in 0.05 *M* sulphuric acid and methanol, respectively, and stored at 4°C. Methanol and ammonium dihydrogen phosphate used for the mobile phase were guaranteed reagent grade (Merck, Darmstadt, G.F.R.) and were used as received. Diethyl ether used for the extractions was of anaesthetic grade (Natal Cane By-products, Merebank, South Africa) and was freshly distilled over sodium hydroxide pellets before use.

Apparatus

An M6000 pump and a WISP autosampler (Waters Assoc., Milford, MA, U.S.A.) were coupled to a 30 cm × 4 mm I.D. stainless-steel column packed

0378-4347/80/0000-0000/$02.25



1 2

Fig. 1. Chemical structures of cinnarizine (1) and chlorbenoxamine (2).

with Spherisorb 5 ODS (Phase Separations, Queensferry, Great Britain). A Waters variable-wavelength detector Model 450 was used to measure absorbance of the eluate. The results were processed on a Waters 730 data module.

Other apparatus used consisted of glass centrifuge tubes with B24 and B19 ground-glass joints and stoppers, a variable-speed reciprocating shaker (Kötterman, Hänigsen, G.F.R.), a Damon CRU-5000 refrigerated centrifuge (Needham Heights, MA, U.S.A.), a Reactitherm heating module (Pierce Chemical Company, Rockford, IL, U.S.A.), 5-ml glass ampoules, high-purity nitrogen and a 100-μl Hamilton syringe.

Stock solutions

A cinnarizine stock solution was prepared by making 20 mg (accurately weighed) of cinnarizine up to 1 l with 0.05 *M* sulphuric acid. The internal standard solution was 40 mg of chlorbenoxamine made up to 100 ml with methanol.

Extraction procedure

To 5 ml of plasma, in a B24 ground-glass centrifuge tube, were added 100 μl of internal standard solution, 1 ml of 0.25 *M* NaOH and 10 ml of diethyl ether. The stoppered tubes were shaken for 10 min on a reciprocating shaker at the rate of 180 strokes/min. The tubes were centrifuged for 5 min at 900 *g* at 5°C. The supernatant ether layer was then transferred to 20-ml B19 ground-glass centrifuge tubes containing 2 ml of 0.05 *M* sulphuric acid. After shaking and centrifuging as before, the ether layer was aspirated off and discarded. The aqueous acidic solution was alkalinised by adding 0.5 ml of 0.5 *M* NaOH and extracted with 5 ml of diethyl ether as before and centrifuged. As much of the supernatant ether layer as possible was transferred to 5-ml ampoules in which the ether was evaporated at 40°C under a gentle stream of high-purity nitrogen.

The residue in the ampoules was redissolved in 120 μl of the mobile phase used for chromatography and 100 μl of this solution were injected for analysis.

Chromatography

The mobile phase consisted of 850 ml of methanol plus 150 ml of aqueous 0.05 *M* ammonium dihydrogen phosphate. A constant flow-rate of 1.0 ml/min was maintained with a pressure of 1500 p.s.i. at ambient temperature through

a 30 cm × 4 mm I.D. stainless-steel column containing Spherisorb ODS (5 μm). The absorbance of the eluate was monitored at 285 nm and 0.04 a.u.f.s.

Retention times of cinnarizine and chlorbenoxamine were 6.2 and 8.7 min, respectively.

RESULTS AND DISCUSSION

Fig. 2 shows representative chromatograms obtained and demonstrates the lack of interfering endogenous compounds.

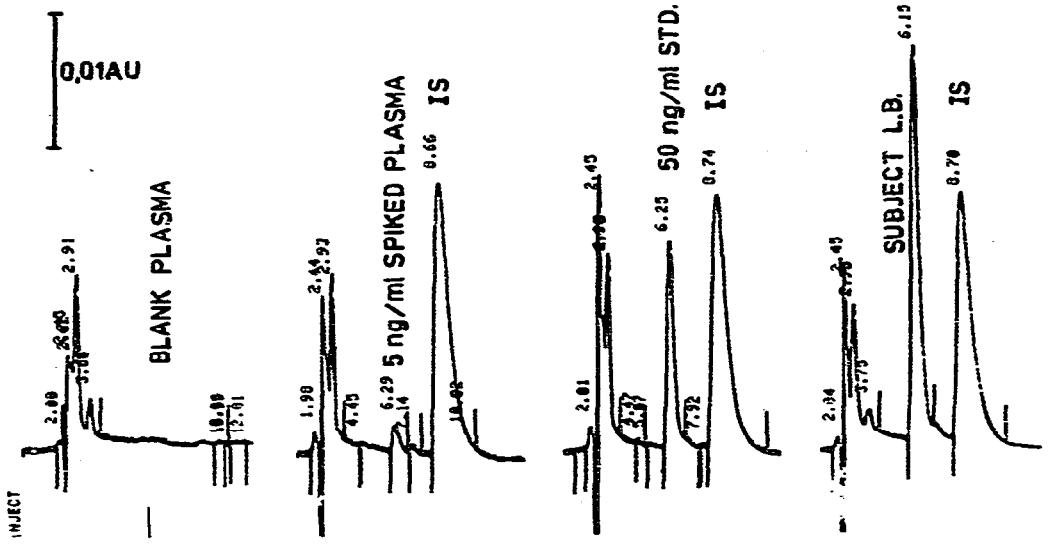


Fig. 2. Typical chromatograms of plasma samples.

Linear calibration curves of cinnarizine peak heights/internal standard peak heights vs. plasma concentrations were obtained with plasma standards containing 20—100 ng/ml. The lines passed very close to the origin and the slopes remained relatively constant as can be seen from the following equation

$$y = (47.00 \pm 1.90)x + (0.44 \pm 1.47)$$

This equation represents the average equation of ten calibration curves (obtained by linear regression analysis) constructed during a period of two weeks while the assays of cinnarizine in actual plasma samples of the bioavailability trial were being carried out. Correlation coefficients for these linear regressions were consistently greater than 0.999 (n=4) making one-point calibration feasible.

A summary of the results obtained with spiked samples during the trial using a single plasma standard concentration of cinnarizine to calibrate the data module is presented in Table I.

The limit of sensitivity for this method, defined as 2 × S.D. obtained at zero concentration from the intercept of a straight line plot of S.D. of the mean vs. plasma concentration of replicate spiked samples, was found to be 2 ng/ml.

TABLE I

RECOVERY OF CINNARIZINE IN SPIKED PLASMA SAMPLES

Concentration spiked (ng/ml)	Mean concentration found (ng/ml)	C.V. (%)	*N*
26.0	25.7	8.4	6
52.0	52.7	4.3	6
78.0	76.2	6.3	5
104.0	107.8	5.7	5

Only chloroform and diethyl ether were evaluated for their extraction efficiencies and ability to separate cinnarizine from impurities in the plasma. Direct chloroform extraction of cinnarizine from ten buffer solutions ranging from pH 1 to pH 10 and with cinnarizine concentrations of 20 μg/ml revealed a remarkable consistency in the extraction efficiency over the whole pH range investigated. This was not the case with diethyl ether as can be seen from Fig. 3.

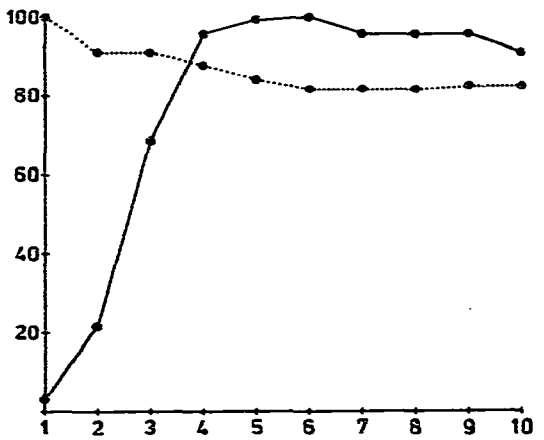


Fig. 3. Effect of pH on extraction of cinnarizine from buffer solutions with chloroform (•· · · · · •) and diethyl ether (•———•). *Y* axis: relative extraction efficiency. *X* axis: pH of solution.

Even more remarkable is the fact that cinnarizine, being a basic compound, extracts best into chloroform at the lowest pH values; a circumstance which ncessitated the addition of 1 ml of 1 *N* sulphuric acid by Morrison et al. [1] in order to obtain good recoveries for extractions from 1 ml of plasma.

Since further purification of the extract from plasma was required before injection into the liquid chromatograph, diethyl ether was chosen as extraction solvent because cinnarizine could be back-extracted from it into acid medium.

The absolute extraction yield of cinnarizine carried through the whole extraction procedure with spiked plasma samples containing 100 ng/ml cinnarizine and using chlorbenoxamine as an external standard was 67%. That of chlorbenoxamine at 8 μg/ml, using cinnarizine as external standard, was 55%.

The low absolute extraction yield of cinnarizine was compensated for by the purity of the extract allowing the injection of the entire extract from 5 ml of plasma into the chromatography column.

TABLE II

PHARMACOKINETIC PARAMETERS FOR ORAL ABSORPTION OF 50 mg OF CINNARIZINE AS TABLETS IN TWO DIFFERENT FORMULATIONS

Values are means (± S.D.) in six normal trial subjects.

	Mean peak plasma conc. (ng/ml)	Mean time to peak plasma conc. (h)	Mean AUC* (0—24 h) (ng·h/ml)	Mean plasma half-life (h)
A	73.7 ± 34.5	2.3 ± 0.4	583 ± 180	4.4 ± 1.0
B	88.6 ± 42.3	2.4 ± 1.1	721 ± 268	5.3 ± 1.6

* AUC, Area under the curve.

Since this method was used only during a bioavailability trial during which time the participating volunteers were instructed to refrain from the ingestion of medicines, no drugs were tested for interference with this assay.

The results obtained in the bioavailability trial are summarised in Table II.

REFERENCE

1 P.J. Morrison, I.D. Bradbrook and H.J. Rogers; Brit. J. Clin. Pharmacol., 7 (1979) 349.