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Note

Rapid high-performance liquid chromatographic assay of cinnarizine in human plasma

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Cinnarizine belongs to the category of antihistamines, more exactly to the sub-category of H_1 receptor blockers. It is used in the treatment of cerebral and peripheral circulatory disturbances. Clinical experience of the use of cinnarizine goes back over a period of more than ten years [1—6]. There is, however, little documentary material relating to pharmacokinetic studies and only a few publications have appeared on methods for cinnarizine assay in biological fluids [7—10]. The present study describes a method of cinnarizine assay in serum or plasma employing high-performance liquid chromatography. The analysis can be carried out rapidly, it produces accurate results and it is thus especially suitable for routine examinations such as are involved in pharmacokinetic studies.

EXPERIMENTAL

Equipment

The analyses were carried out on an SP 8000 chromatograph (Spectra Physics, Santa Clara, CA, U.S.A.). A LiChrosorb RP-8 (5 μm) column (250 × 4 mm, Hibar; E. Merck, Darmstadt, G.F.R.) was used, connected by a short steel capillary and Swagelok joints to a pre-column (40 × 3 mm; Altex, Berkeley, CA, U.S.A.) which was filled with LiChrosorb RP-2 (30 μm). Both columns were placed in a column heater at a temperature of 60°C. The detector used was a variable-wavelength detector Model SF 770 (Schoeffel Instruments, Westwood, NJ, U.S.A.), which was run at 250 nm and 0.02 a.u.f.s. The mobile phase consisted of 85% methanol and 15% buffer (820 mg of sodium acetate and 3 ml of 1 *N* HCl per l of distilled water, pH approximately 5.2). The flow-rate was 2.0 ml/min. The SP 8000 was fitted with a 50-μl sample loop.

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Extraction of the plasma sample

To 1 ml of plasma is added 0.15 ml of 0.5 *N* HCl and thoroughly mixed. The mixture should then have a pH value of between 3.5 and 5; under no circumstances should it exceed 5. After the addition of 5 μl of internal standard solution it is necessary to mix once more thoroughly. The extraction is now made with 0.5 ml of carbon tetrachloride, shaking for 1 min and then centrifuging for 10 min at 1500 *g*. Once the phase separation has been carried out, the greater part of the organic phase is transferred to a small container by means of a 500-μl Hamilton syringe and evaporated under a nitrogen stream. The residue is dissolved in about 100 μl of 85% methanol by rotating, and the 50-μl loop of the chromatograph is then rinsed and filled with the solution.

Internal standard solution

The amount of 0.6 mg of DPA 14 [7-chloro-3-dipropyl-acetoxy-5-(2'-fluorophenyl)-1,3-dihydro-2H-1,4-benzodiazepin-2-one] is dissolved in 10 ml of carbon tetrachloride. We used this substance because its retention time is well suited to the system, while the recovery rate is around 100%. There is, however, no structural similarity with cinnarizine.

For measurements when only very small concentrations of serum are expected, an internal standard solution can be used containing only 0.2 mg of DPA 14 in 10 ml of carbon tetrachloride.

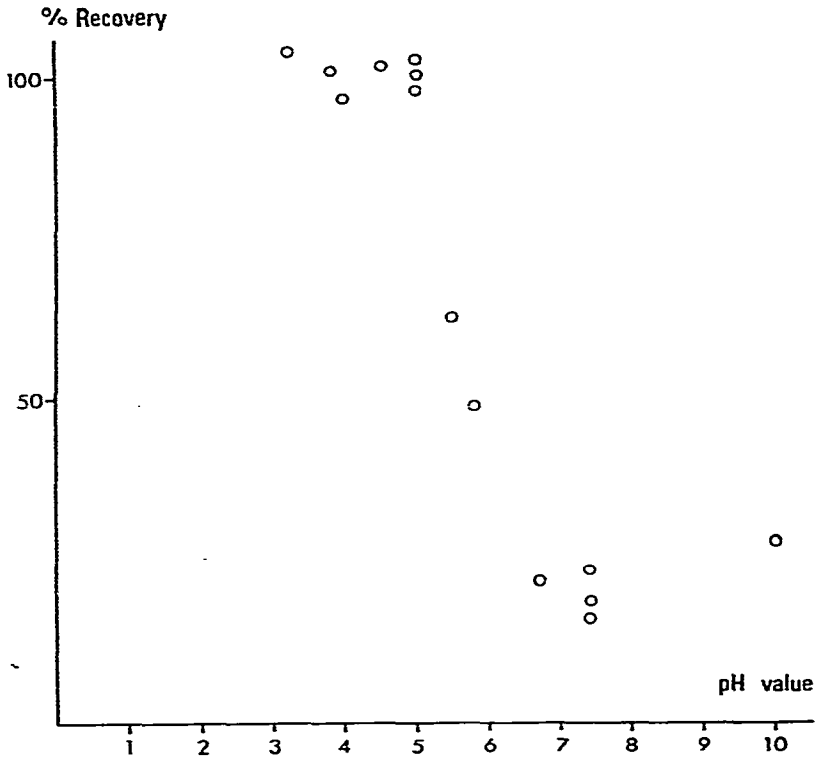

Fig. 1. Recovery of cinnarizine in plasma as a function of the pH (100 ng of cinnarizine were added to 1 ml of plasma).

Evaluation

The chromatographic evaluation was carried out on the basis of peak heights, by computing the quotient of the cinnarizine peak height with the peak height of the internal standard, or by computing the quotient of the areas. Calibration curves were drawn by adding known quantities of cinnarizine to a sample of serum: $y = 0.0043868x + 0.0137$, $r = 0.99964$ at five reference points.

Recovery

Since cinnarizine is much more soluble in non-polar than in polar solvents, it seemed reasonable to carry out a preliminary cleansing and concentration using a fluid—fluid distribution. The appropriate pH range for these tests was neutral to weak alkaline. As Fig. 1 (giving the recovery as a function of the pH value) shows, however, the recovery was only about 50%. Further tests with different pH values suggested that the best pH range was 3.5—5. Within this range the recovery rate was 101.5% with a standard deviation of 2.9% ($n = 6$). Thus, in carrying out the extractions we were careful to remain within the pH limits 3.5—5. The absolute recovery for cinnarizine was 85 ± 7.9% ($n = 5$).

RESULTS

The chromatograms shown in Fig. 2 are the result of plasma determinations after the oral administration of 75 mg of cinnarizine. They represent selected results of analyses carried out at different times. The figure shows a maximum concentration, an intermediate concentration, a concentration near the detec-

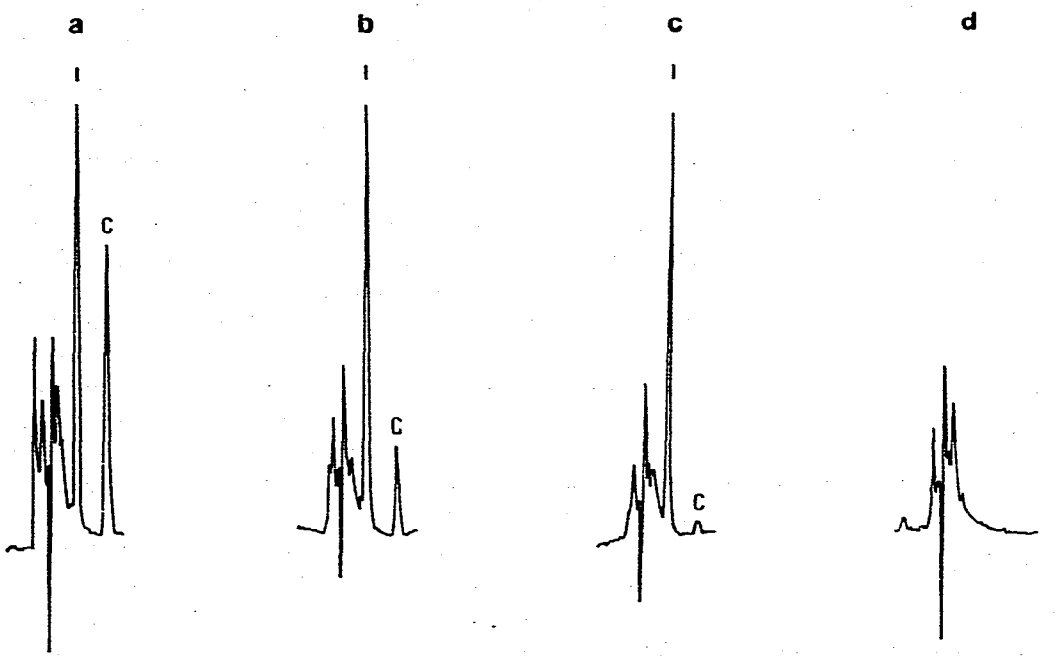

Fig. 2. Chromatograms of plasma samples at various times after oral administration of 75 mg of cinnarizine. I = Internal standard. C = cinnarizine. (a) 370 ng/ml; (b) 110 ng/ml; (c) 12 ng/ml; (d) blank plasma.

tion limit, and a blank plasma sample. This latter chromatogram shows no irregular peaks within the range of the cinnarizine and internal standard peaks, so this method has a very low detection limit — around 2 ng/ml of plasma. The calibration curve on which the evaluation is based is linear in the expected therapeutic range of 20—220 ng/ml, and it passes through the origin.

This method has a good reproducibility. In multiple tests the standard deviation for samples containing approximately 230 ng/ml was ± 4% (n = 8), while for samples containing approximately 130 ng/ml the standard deviation was ± 4.7% (n = 10). With 15 ng/ml the standard deviation obtained was ± 8.5% (n = 5).

Under the given conditions the retention times obtained for the active substance and the internal standard were 180 and 130 sec, respectively.

DISCUSSION

The methods for cinnarizine determination described in the medical literature are confined to the four publications referred to in the introduction. Dell and Fiedler [9] detected only a metabolite of cinnarizine in the urine of rabbits. This method can thus be applied to cinnarizine only within certain limits. The publication by Akada and Setuko [8] describes the detection of cinnarizine by means of gas chromatography, but it is difficult to glean specific details from this Japanese study. The gas chromatographic method outlined by Morrison et al. [7] produces good results; on the other hand, the low recovery of 60% at the most suggests an unsatisfactory extraction of the active substance from the serum, which considerably raises the detection limit; moreover, he has to work in the step-drop range of the solvent peak, which can lead to inaccuracies.

In contrast to the method described by Hundt et al. [10], we take our measurements at 250 nm; why Hundt takes his measurements at 285 nm — a wavelength at which cinnarizine displays only about 7% of the absorption at 250 nm — we fail to understand. Moroever, the mobile phase was not ideally selected, because — especially with the internal standard — strong tailing occurs. For the analysis we use only 1 ml of plasma, while Hundt uses 5 ml. When this latter quantity is considered in the context of pharmacokinetic studies, it can be of considerable significance in that it involves the patients in withdrawing large quantities of blood. What is more, our absolute recovery for cinnarizine is 85% (n = 5), while Hundt's is 67%, although both methods can compensate for this by using an internal standard. However, one important advantage of our method is that it saves time in work and analysis. We require only approximately one third of the time involved in Hundt's method, an important factor when carrying out routine analysis.

Our method envisages the use of a pre-column, which is necessary to protect the analysis column, since biological extracts are employed with no additional cleansing, and the life expectancy of the separation column would be considerably shorter without a pre-column.

The advantage of using a higher temperature (60° C) is that it makes a faster flow-rate for the mobile phase possible, thus reducing the analysis time.

We considered using a buffer solution for the extraction, but this has the dis-

advantage of involving an undesired increase in the volume of both the non-organic and the organic phase. The result would have been to lengthen the analysis time by a further analysis step, i.e. reduction of the large extraction volume.

REFERENCES

1 O. Wilcke, Med. Welt, 27 (1966) 1472.
2 V. Schuermans, Drug Res., 21 (1971) 1541.
3 K. Alexander, Med. Welt, 49 (1970) 2132.
4 J.B. Toledo, Drug Res., 22 (1972) 448.
5 J.A. Yesavage, Arch. Gen. Psychiat., 36 (1979) 220.
6 S. Voina, Praxis, 32 (1970) 1165.
7 P.J. Morrison, I.D. Bradbrook and H.J. Rogers, Brit. J. Clin. Pharmacol., 7 (1979) 349.
8 H. Akada and M. Setuko, Eisei Kagaku, 22 (1976) 291 (in Japanese).
9 H.D. Dell and J. Fiedler, Z. Anal. Chem., 284 (1977) 126.
10 H.K.L. Hundt, L.W. Brown and E.C. Clark, J. Chromatogr., 183 (1980) 378.