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Note

Rapid method for determination of cimetidine in biological fluids by high-performance liquid chromatography using Extrelut extraction

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Cimetidine as a specific histamine H_2 receptor antagonist has been introduced recently as Histodil (RG) in Hungary for the treatment of gastric and duodenal ulcers. The degree of inhibition of gastric acid depends on the drug concentration in blood; therefore it was necessary to examine the pharmacokinetics of Histodil and to compare its bioavailability with Tagamet (SKF). Several extraction and high-performance liquid chromatographic (HPLC) methods have been developed for the determination of cimetidine and its metabolites in blood, plasma and urine [1—7] using different organic solvents for extraction (e.g. ethyl acetate, octanol, dichloromethane etc.).

We have developed also a rapid extraction method to study the bioavailability of both Tagamet (SKF) and Histodil (RG) after oral (p.o.) and intravenous (i.v.) administration to healthy volunteers. The HPLC method of Lee and Osborne [2] was used with some modifications. Our method can be used with success for the analysis of cimetidine.

EXPERIMENTAL

Clinical study

This was a balanced three-way cross-over study in which each of six volunteers received each of the following regimens at 2-week intervals. Regimen A: 200 mg of Histodil, i.v. Regimen B: 2 × 200 mg of Histodil. Regimen C: 2 × 200 mg of Tagamet tablets p.o. Heparinized blood samples were collected before and at 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10 and 24 h after the dose.

The blood samples were centrifuged as soon as possible; thereafter the plasma samples were stored at —20° C.

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Extraction procedure

Cimetidine and metiamide (internal standard) can be extracted from plasma into ethyl acetate using an Extrelut (Merck, Darmstadt, G.F.R.) column. Extrelut is a granular support material and the lipophilic compounds can be extracted by organic solvents from aqueous phase. To prepare the column, Pasteur pipettes 14 cm × 0.5 cm I.D. were filled to a height of 9 cm with Extrelut (500 mg). Then 1 μg of metiamide and 0.1 ml of 6 *N* sodium hydroxide were added to 0.8 ml of plasma. This alkaline plasma sample was applied to the top of the column and allowed to soak for 15 min. The compounds were eluted by 5 ml of ethyl acetate (Merck) collected in a tube. The organic phase was evaporated in a stream of nitrogen at 40°C. For analytical purposes 60 μl of plasma extract dissolved in 0.5 ml of eluent were injected onto the analytical column.

Recovery studies were performed at both high and low concentrations (0.03—0.7 mg/l). Percentage recovery was determined by comparing the peak heights of cimetidine and metiamide extracted from plasma samples to standard stock solutions injected directly onto the column. In order to study the intra- and inter-assay variability, a plasma sample was assayed four times on the same day, and the calibration curve was prepared by using 3—5 parallel samples for each point.

Chromatographic conditions

A Hewlett-Packard 1081 B chromatograph equipped with a variable-wavelength UV detector (Labor MIM) and a 3380 S integrator was used. The separation was performed on a 200 × 4.6 mm I.D. HP Si-100 LiChrosorb (10 μm particle size) column. The mobile phase consisted of acetonitrile—methanol—water—25% ammonium hydroxide (250:30:10:0.4, v/v). A flow-rate of 1 ml/min was maintained. The absorbance of the effluent was monitored at 228 nm.

RESULTS AND DISCUSSION

We found that the final recovery of compounds was 75% for metiamide and 95% for cimetidine (S.D. ± 8.65 and ± 6.24, respectively). The recovery of metiamide was lower, but the value proved to be adequate for routine determination of cimetidine in plasma.

The limit for safe quantitation was found to be 0.03 mg/l. The extraction procedure was rapid, the eluate did not contain emulsions and 5 ml of ethyl acetate were enough for quantitative estimation of the drug. We had to analyse the extracts on the same day, because cimetidine and metiamide stored in ethyl acetate at +6°C are not stable after 24 h.

The intra-assay variation was negligible. The calibration curves were linear within the concentration range of interest (30—700 μg/l). The regression line had a correlation coefficient of 0.970885 with a standard error of 1.6×10^{-4}, in the case of 29 samples.

Fig. 1 shows the structure of the compounds. Fig. 2 illustrates chromatograms of three plasma samples. In the chromatogram obtained after extracting 0.8 ml of blank human plasma (Fig. 2a), no additional peaks are seen which

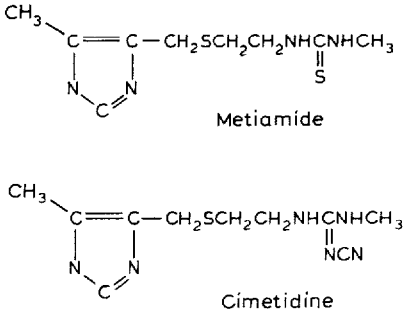

Fig. 1. Structures of cimetidine and metiamide.

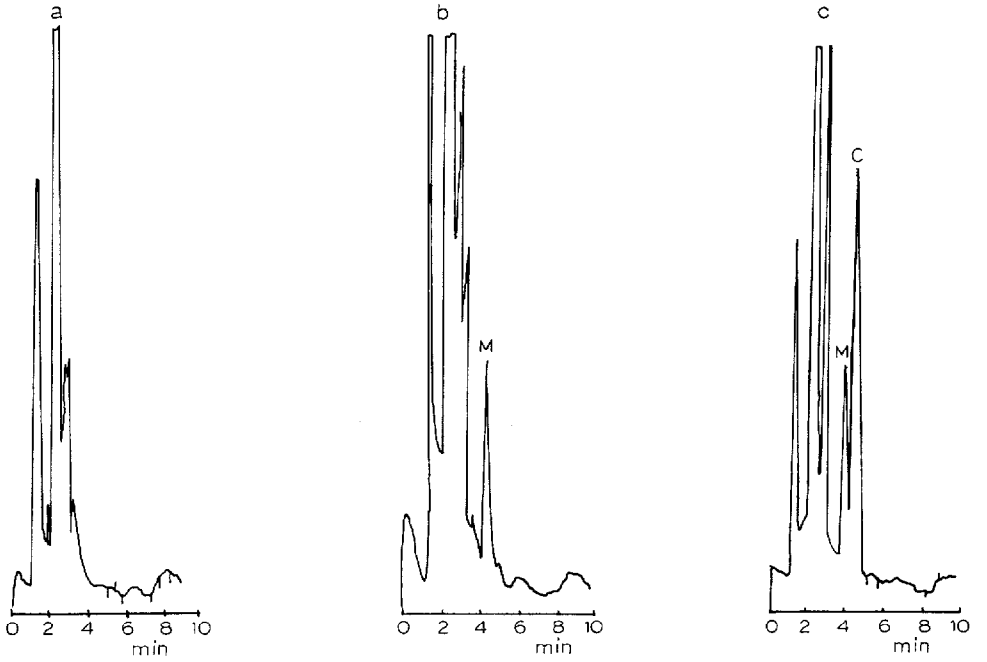

Fig. 2. Chromatogram of plasma extracts: (a) blank human plasma; (b) extract of plasma containing 1 μg of metiamide; (c) plasma extract after the administration of 400 mg of cimetidine p.o.

could interfere with the determination of cimetidine and metiamide. Fig. 2b represents extracts of plasma containing 1 μg of metiamide, and Fig. 2c is a chromatogram obtained after extracting 0.8 ml of plasma from a healthy volunteer 2 h after administration of 400 mg cimetidine p.o. Cimetidine and the internal standard (metiamide) were well resolved with retention times of 4.9 and 4.1 min, respectively.

Fig. 3 shows blood concentration—time curves following both oral and intravenous administration of Histodil tablets and injection, and Tagamet tablets. The analysis of the results was performed by a TPA/i 1001 computer. We used

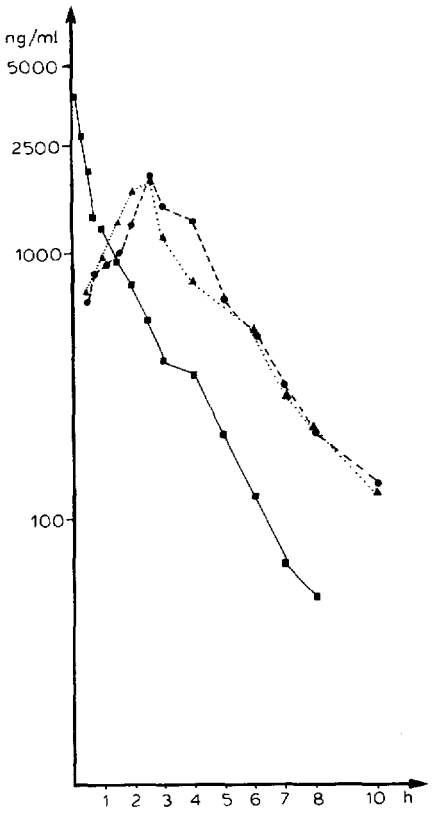

Fig. 3. Blood concentration—time curve following oral administration of 2 × 200 mg of Tagamet and Histodil tablets, and 200 mg of Histodil i.v. The curves show the mean blood plasma levels of cimetidine. (■), Histodil i.v.; (▲), Tagamet p.o.; (●), Histodil p.o.

a two-compartment model to describe the cimetidine plasma concentration kinetics.

Our extraction method with Extrelut has several advantages over methods that incorporate a classic extraction procedure for cimetidine and its metabolites [1—7]. The method is rapid and requires less organic solvent compared to the procedures mentioned above. The eluate does not contain emulsion; therefore there is no need for further purification. The evaporation is carried out in the same vials in which the eluent is collected. The sensitivity (0.03 mg/l) is higher than in the previous procedures [1—7].

Our method was applied to pharmacokinetic investigation of different cimetidine products in humans. The method can be used routinely for clinical monitoring of cimetidine but it does not allow the detection of its metabolites.

REFERENCES

1 N.-E. Larsen, P. Hesselfeldt, S.J. Rune and E.F. Hvidberg, J. Chromatogr., 163 (1979) 57.
2 R.M. Lee and P.M. Osborne, J. Chromatogr., 146 (1978) 354.

3 W.C. Randolph, V.L. Osborne, S.S. Walkenstein and A.P. Intoccia, J. Pharm. Sci., 66 (1977) 1148.
4 J.A. Ziemniak, D.A. Chiarmonte and J.J. Schentag, Clin. Chem., 27 (1981) 272.
5 H.M. Vanderberghe, S.M. MacLeond, W.A. Mahom, P.A. Lebert and S.J. Soldin, Ther. Drug Monit., 2 (1980) 379.
6 Sal Pancorbo Melvin, P. Bubrick, T.W.F. Chin, K.W. Miller and G. Onstadt, Clin. Pharmacol. Ther., 1 (1982) 83.
7 J. Fleitman, G. Torosian and J.H. Perrin, J. Chromatogr., 229 (1982) 255.