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Note

New rapid assay of cimetidine in human plasma by reversed-phase high-performance liquid chromatography

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Cimetidine is a potent inhibitor of gastric acid secretion and is used worldwide with increasing frequency in the treatment of duodenal and stomach ulcers [1, 2]. Earlier dosage directions of 1000 mg of cimetidine per day have now been reduced to values of 400 mg twice daily. It is therefore important to note not only the clinically demonstrable effect, but also the therapeutically effective serum levels. So far, high-performance liquid chromatographic (HPLC) data have been published only for normal-phase chromatography [3—7], three papers on reversed phase [8—10], and one paper using a weak polar phase [11].

The aim of this present publication is the elaboration of a rapid assay for cimetidine, which provides not only an easy procedure and a short analysis time, but also a high recovery rate, making it the method of choice for the determination of cimetidine in biological material, where this is linked with pharmacokinetic examinations.

MATERIAL AND METHODS

Reagents

Methanol, absolute, was from J.T. Baker (Deventer, The Netherlands); ammonium carbamate, for analysis, was from E. Merck, Darmstadt, G.F.R.; Sep-Pak C-18 cartridges were from Waters Assoc. (Milford, MA, U.S.A.); disposable extraction columns were from J.T. Baker (Phillipsburg, NJ, U.S.A.); cimetidine and the internal standard (N-cyano-N'-{2-[5-methyl-1H-imidazol-4-yl)-methylthio] ethyl}-S-methyl-isothiourea), a cimetidine synthesis precursor, were obtained from Gerot Pharmazeutika (Vienna, Austria).

To prepare the internal standard solution for use with Sep-Pak cartridges,

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1 mg of the internal standard was dissolved in 500 ml of water. For use with the disposable columns from Baker, 1 mg of the internal standard was dissolved in 50 ml of water.

HPLC unit

A high-pressure liquid chromatograph Type SP 8000 from Spectra-Physics (Santa Clara, CA, U.S.A.), with a Spectro-Monitor (LDC, FL, U.S.A.) as UV detector, was used to separate the substances. A LiChrosorb RP-18 column (5 μm, 250 × 4 mm; Hibar, Merck) thermostatted at 45°C was used.

Detector operation took place at 0.02 A, at a wavelength of 220 nm. A mixture of 40% of methanol and 60% of 0.01 mol/l ammonium carbamate solution (pH 8.9) was used for the mobile phase, at a flow-rate of 1.2 ml/min. The unit was fitted with a 25-μl loop.

Plasma extraction

To 1 ml of plasma in a small glass tube 1 ml of internal standard and 1 ml of water were added, vortexed for 20 sec, and passed through a conditioned Sep-Pak cartridge by means of a 5-ml syringe. The glass tube and syringe were rinsed afterwards with 2 ml of water and the rinse also passed through the cartridge. After rinsing the cartridge with 9 ml of water, residual water was removed by vigorous shaking. Cimetidine and the internal standard were then eluted with 5 ml of methanol; the eluent was subsequently evaporated until 1 ml of largely aqueous solution remained; 100—200 μl of this solution were used to rinse and fill the 25-μl loop. For conditioning of new or used Sep-Pak cartridges they must first be rinsed with 5 ml of methanol and then with 5 ml of water. For rinsing and elution a constant flow of 1 ml within 2—3 sec should be maintained.

To avoid the large volumes obtained with the above procedure, we used Baker octadecyl extraction columns with encouraging success. A 1-ml aliquot of plasma and 0.1 ml of internal standard solution were thoroughly mixed and put through a Baker C-18 1-ml column by means of short centrifugation. After twice rinsing with 1 ml of water the Baker column was changed to a clean glass tube and the substances eluted with 500 μl of methanol, always with the aid of the centrifuge. The 25-μl loop was rinsed and filled with the solution obtained.

RESULTS

The calibration curve was linear within the concentration range 0.2—15.84 μmol/l ($Y = 0.87326X + 0.0504$; $r = 0.9996$, $n = 16$). The reproducibility of the method has been determined over a period of approx. two years using seven samples with a mean quotient value of 1.64 ± 0.164 (= ± 10%). The recovery rate with Sep-Pak cartridges is shown in Table I. The absolute recovery for cimetidine was 96.2 ± 3.4% and for the internal standard 94.3 ± 4.0%.

TABLE I

RECOVERY OF THE METHOD USING SEP-PAK CARTRIDGES

Quantity added (μmol/l)	Plasma quotient (n = 5)	Water quotient (n = 3)
0.79	0.2231 ± 0.0189 (= ± 8.5%)	0.1626 ± 0.0014 (= ± 0.9%)
3.96	0.9313 ± 0.0395 (= ± 4.2%)	0.8380 ± 0.0160 (= ± 1.9%)
15.84	3.541 ± 0.0735 (= ± 2.1%)	3.525 ± 0.1271 (= ± 3.6%)

Chromatograms of human plasma before and after intake of 600 mg of cimetidine are shown in Fig. 1. Under the above-mentioned conditions, cimetidine showed a retention time of 4 min, the internal standard 6.4 min.

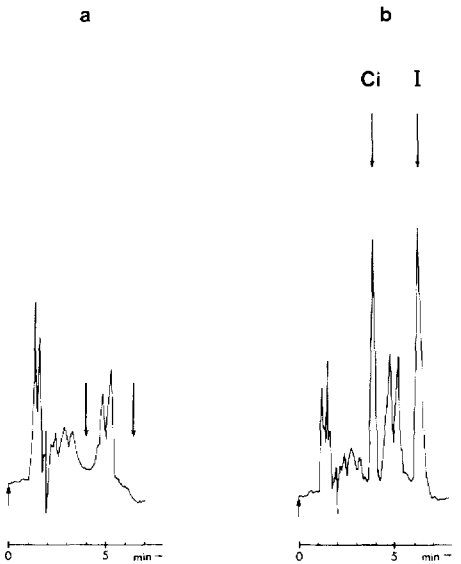

Fig. 1. Chromatograms of extracts of human plasma after oral intake of 600 mg of cimetidine. (a) Blank plasma, (b) 4.12 μmol/l cimetidine (Ci). I = internal standard.

DISCUSSION

The HPLC plasma assay described is characterized by a clearly reduced analysis time thus improving on the methods previously reported [3—11]. Previous cimetidine assays required single or multiple extraction steps prior to chromatography while, in contrast, the assay described involves only passage through an extraction column, thus greatly reducing sample preparation time and manipulation errors.

Naturally, there is always the possibility of working with only 0.1—0.2 ml of plasma, where clinical controls are undertaken in which therapeutic cimetidine concentrations are to be expected, because the sensitivity of the method is very good (0.08 μmol/l). This method can also be used for urine assay although so far no experiments have been undertaken in this direction.

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