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Note

High-performance liquid chromatographic method for the simultaneous determination of cimetidine and antipyrine in plasma

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Traditionally drug—drug interaction studies have been performed by monitoring the kinetics of only one of the interacting drugs. This experimental design does not allow one to study the potential mutual interaction between the drugs and, of equal importance, the mechanism of the interaction [1]. In order to investigate these questions it is necessary to set up assay methodology capable of measuring both drugs and possibly their metabolites. It would be preferable if one could analyse all relevant species simultaneously.

We have been interested in the interaction between cimetidine and antipyrine. Cimetidine is well known to inhibit the microsomal metabolism of a number of drugs [2—4] and interfere with renal secretion [5] but the pharmacokinetic nature of the mechanism has not been studied in vivo. Therefore, we have developed an assay method capable of simultaneously measuring cimetidine and antipyrine in microlitre quantities of plasma. Although there are existing assays for cimetidine [6—11] and antipyrine [12—14] there is no published account of the simultaneous assay of the two compounds. Our method is a modification of the methods of Ziemniak et al. [7] and Mihaly et al. [9] for cimetidine. The assay has been applied to a preliminary study in the rat.

EXPERIMENTAL

Instrumentation

Chromatography was performed using a Waters Assoc. Model 6000A pump, a Rheodyne Model 7125 valve injector, a Pye-Unicam variable-wavelength UV detector (LC3 UV) and a Phillips Model PM 8251 single-pen recorder.

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Reagents

Cimetidine, cimetidine sulphoxide and internal standard (I.S.), N-methyl-N'-[3-(imidazol-4-yl)propyl]cyanoguanidine (SK&F 92374), were generous gifts from SK&F Labs. (Welwyn Garden City, U.K.). Sodium chloride (BDH), potassium dihydrogen phosphate (BDH), HPLC grade acetonitrile (Rathburn Chemicals), analytical-reagent grade dichloromethane (Fisons Scientific Equipment), analytical-reagent grade methanol (Fisons) and antipyrine (Sigma) were all used without further purification.

Standard solutions

Stock solutions of cimetidine and antipyrine were prepared in acetonitrile. Standard solutions (0.1—50 mg/l) in plasma and buffer were prepared by spiking drug-free blank plasma or buffer with cimetidine and antipyrine stock solution. Stock solution (30 mg/l) of the I.S. was prepared in acetonitrile.

Chromatography

The column used was a commercially pre-packed silica column (Hichrom, 5-μm Spherisorb, 25 cm × 4.9 mm I.D.). The mobile phase composed of acetonitrile—water—ammonia solution (95:5:0.2) was pumped at a flow-rate of 1.0 ml/min and the eluent monitored at 228 nm. The column was washed after each day with methanol for about 30—45 min.

Extraction

I.S. (50 μl), 50 μl of 2 *M* sodium hydroxide and 5 ml of methylene chloride were added to 100 μl of plasma. The mixture was vortexed for 10 s and then extracted on a rotary mixer for 10 min. It was then centrifuged at 2000 *g* for 5 min after which the upper aqueous layer was removed by aspiration. The organic layer was evaporated to dryness in a warm water bath under a stream of air and reconstituted in 100 μl of mobile phase prior to injection of 20 μl onto the column.

Interaction experiments in rats

Experiments were performed by administration of cimetidine (40 mg/kg) and antipyrine (50 mg/kg) both individually and together intraperitoneally to different rats which had been cannulated in the jugular vein the previous day. Serial blood samples (250 μl) were taken over a period of 4 h. The blood samples were centrifuged (2000 *g* for 10 min) and 100 μl of plasma taken and extracted as previously described. Concentrations were determined from standard calibration curves obtained from standard solutions extracted under the same conditions.

RESULTS AND DISCUSSION

There were no interfering peaks in the chromatograms that were obtained from either plasma or buffer. The metabolites were also well resolved from the parent compounds. A typical chromatogram is shown in Fig. 1. No deterioration in column performance was noticed, considering the alkalinity of the mobile phase being used, over a period of about nine months of intensive

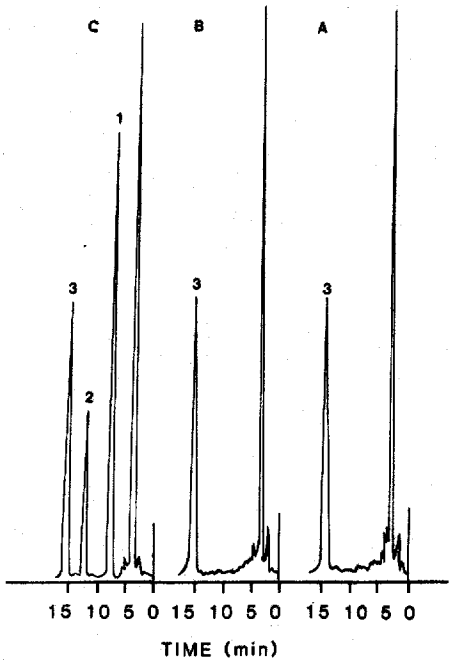


Fig. 1. Chromatogram obtained from (A) extracted blank plasma, (B) blank buffer and (C) rat plasma after cimetidine and antipyrine administration (cimetidine concentration 9.3 mg/l, antipyrine concentration 24.8 mg/l). Peaks: 1 = antipyrine; 2 = cimetidine; 3 = internal standard.

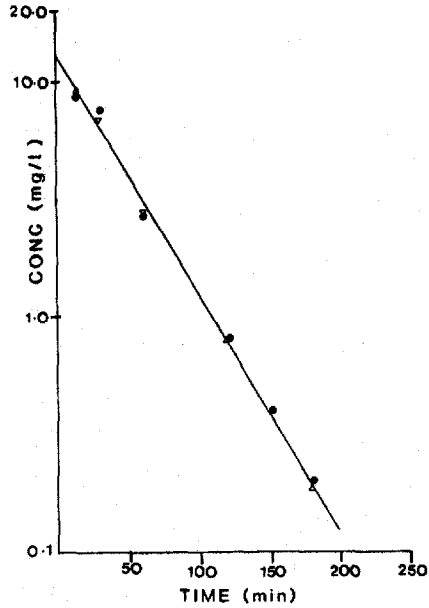
use. This was perhaps due to thorough washing of the column each day.

Extractions from phosphate-buffered saline B.P. (pH 7.4) were identical to those from rat plasma. Consequently, standards were subsequently prepared in this medium, avoiding the use of large quantities of blank rat plasma.

The method of extraction reported here is quicker than the method of Ziemniak et al. [7]. The extraction efficiency of cimetidine was found to be about 70% over the entire concentration range within the linear region and this compares favourably with that found with the method of Ziemniak et al. [7]. The inclusion of antipyrine in the assay did not alter these results and the extraction efficiency for antipyrine was about 80—90%.

The cimetidine assay was found to be linear up to 50 mg/l beyond which there appears to be saturation of the extraction. For antipyrine, the assay was linear up to 100 mg/l. Cimetidine could be detected down to a concentration of 0.1 mg/l and a coefficient of variation of 20% or less was obtained for a cimetidine concentration of 1.0 mg/l or greater. These values are comparable to those obtained by Mihaly et al. [9]. The limit of detection for the antipyrine assay was 0.05 mg/l and a coefficient of variation of less than 10% was obtained for concentrations of 1.0 mg/l or greater. These limits could be reduced by the use of a greater plasma volume, which can be obtained in human studies.

The intra- and inter-day variabilities of the assay were found to be less than 10% for concentrations of 10 and 50 mg/l and less than 15% for a concentration of 1 mg/l. Concentrations of cimetidine and antipyrine in plasma samples



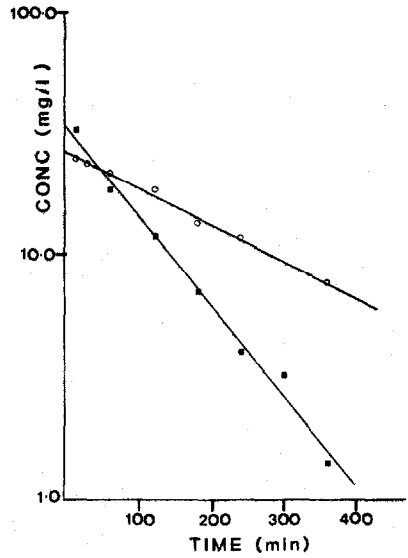


Fig. 2. The concentration—time profile of cimetidine when administered alone (•) and in combination with antipyrine (△).

Fig. 3. The concentration—time profile of antipyrine when administered alone (▪) and in combination with cimetidine (○).

from rats were measured using the assay described above. The capability of the assay to measure both compounds simultaneously from in vivo experiments was demonstrated. The results shown in Figs. 2 and 3 show that cimetidine affects the kinetics of disposition of antipyrine while antipyrine does not seem to have any effect on cimetidine kinetics. The effect of cimetidine on antipyrine is manifested by an increased half-life (from 80 to 205 min, $p < 0.01$) and reduced clearance (from 0.76 to 0.38 l $h^{-1}kg^{-1}$, $p < 0.01$) of antipyrine in the presence of cimetidine.

CONCLUSION

The present report has demonstrated that careful modifications of available assays can improve their use especially in the area of drug interactions. The ability to monitor two interacting compounds simultaneously will undoubtedly improve our ability to understand the mechanism of the interaction. The adaptation of the assays for monitoring two compounds simultaneously does not necessarily mean a sacrifice of the efficiency of the assay in terms of variability, linearity and limits of detection. The animal experiments have shown that cimetidine does affect antipyrine kinetics by prolonging its elimination half-life and reducing its clearance from the body. The assay described here will be used in future studies to elucidate the nature of the kinetic interaction between cimetidine and antipyrine in the rat and possibly man.

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