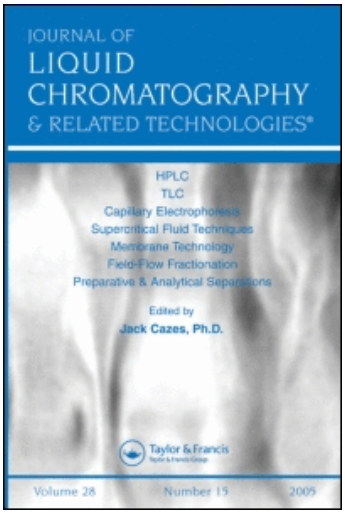

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P. F. Carey[a]; L. E. Martin[a]
[a] Biochemical Pharmacology Department, Glaxo Group Research Ltd., Ware, Herts, U.K.

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A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF RANITIDINE IN PLASMA

P.F. Carey and L.E. Martin

Biochemical Pharmacology Department
Glaxo Group Research Ltd.
Ware, Herts. U.K.

ABSTRACT

Ranitidine hydrochloride is a highly potent H_2 receptor antagonist of value in the treatment of peptic ulceration. A method is described for the determination of ranitidine in biological fluids. The drug is extracted from plasma prior to determination by reverse phase high performance liquid chromatography. The method is sensitive enough to determine 10ng ranitidine per ml in plasma. The h.p.l.c. method has been automated so that analysis can be carried out without attention over a 24 hour period. The automated h.p.l.c. method has been used for studying the pharmacokinetics of oral and intravenous doses of ranitidine hydrochloride administered to man.

INTRODUCTION

The H_2 antagonist, ranitidine hydrochloride (AH 19065) (Figure 1) is a highly potent inhibitor of gastric secretion of value in the treatment of patients who have peptic ulceration.

As part of the safety evaluation studies in animals, and the clinical efficacy studies of the drug in man, it was necessary to develop a method for determining ranitidine in body fluids.

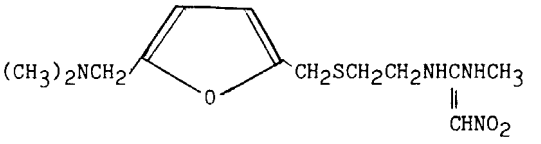

Figure 1. Ranitidine Hydrochloride

Ranitidine hydrochloride is four to five times as potent as cimetidine in inhibiting gastric acid secretion (1) and the therapeutic oral dose of ranitidine hydrochloride was expected to be about 80mg. From metabolic studies in animals give ^{14}C labelled ranitidine it was estimated that this dose of drug would give a peak plasma level in the sub-microgram per ml range and therefore a highly senstitive analytical method for the determination of the drug in biological fluids was required. Attempts to develop a g.l.c. method for direct analysis were unsuccessful nor could any derivatives suitable for quantitative g.l.c. be prepared. Ranitidine is not fluorescent and attempts to develop a method based on the formation of an ion pair complex with a fluorescent anionic detergent were unsuccessful. The nitro group in ranitidine could be reduced electro-chemically but because of interference from endogenous material in plasma and urine it was not possible to develop a polarographic method for the determination of ranitidine. The u.v. spectrum of a methanolic solution of ranitidine shows absorption peaks at 230 and 320 nm. The expected low plasma concentration of ranitidine and the variation in amounts of endogenous u.v.

absorbing material in plasma, prevented the direct determination of the drug by u.v. analysis.

High pressure liquid chromatography using a u.v. detector appeared to be a method of choice for determining ranitidine in biological fluids. A reversed phase rather than an adsorbent system was chosen because it would allow better separation of the drug from the more polar metabolites. The determination of ranitidine in large numbers of plasma samples was required as part of the clinical pharmacological studies and therapeutic trials of ranitidine. These analyses had to be completed as rapidly as possible, therefore, an automated analytical system was essential.

This paper describes a method for the extraction of ranitidine from plasma and its determination using a reversed phase high performance liquid chromatography system fitted with an automated injector and a computerised data processing system. Unattended, the system can analyse samples throughout the day and night. The method is sensitive enough to determine 10ng ranitidine per ml plasma.

MATERIALS

Dichlorodimethylsilane [DCMS] (Aldrich Chemical Co. Ltd. London), toluene, disodium hydrogen phosphate, octan-1-ol and methanol were all of 'Analar' grade and obtained from British Drug Houses, Poole, Dorset.

AH 20480, Figure 2 was synthesised in the Chemistry Division.

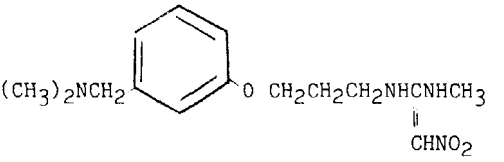

Figure 2. AH 20480, the internal standard

Ranitidine base was used as the analytical standard and ranitidine hydrochloride tablets were used in the clinical studies. Both preparations were supplied by the Pharmacy Division.

INSTRUMENTATION

The liquid chromatograph was assembled from separate commercially available units. The eluent, 60% methanol and 40% 0.05 M phosphate buffer pH 7.0 was pumped at a flow rate of 0.9ml per minute using a pulseless dual-piston reciprocating pump (740B, Spectra Physics, St. Albans, Hertfordshire.)

A Waters Intelligent Sample Processor (WISP), which is a micro processor controlled fully automated injection system, was used to introduce the sample on to the column. The WISP can be programmed to inject from 1µl to 2 ml of any individual sample. Normally 100µl of an extract from plasma was injected.

The chromatography columns were 100mm x 5mm i.d. stainless steel (Shandon Southern, Runcorn, Cheshire) containing 5µ Spherisorb ODS reverse phase material (Phase Separations Ltd.

Queensferry, Clwyd) packed by a slurry technique using a pneumatic pressure intensifier (Haskel Eng. and Supply Co., Burbank, California.)

All columns were maintained at a constant temperature of 40°C in a column oven (A.C.S., Luton, Bedfordshire.)

A Pye LC3 detector (Pye Unicam Ltd. Cambridge) set at 320nm was used to monitor the eluate.

There could be changes in the baseline value of a chromatogram during the eight hour period which it takes to analyse the forty eight samples in the WISP carriage. To avoid having to manually correct for changes in baseline, a drift correction circuit was designed and constructed by Mr. R. Hartley of the Bio-Engineering Unit. The principle on which it works is that at the start of the chromatographic analyses the circuit measures and stores the detector base line voltage signal, V_R. Then it uses V_R as a reference value for the normalisation of all subsequent detector signals.

Following the command from the WISP to inject, the circuit allows a ten second delay and then measures the baseline signal V_B immediately prior to the elution of the first peak. The drift correction is V_D which equals $V_B - V_R$. This value is then subtracted from the total detector signal V_O, over the ten minute period which it takes for each chromatographic analysis and the resultant analogue signal processed by the Spectra Physiscs 4000 system. This drift correction procedure is carried out after each injection.

Silanisation of Glassware

The extraction tubes were soaked overnight in 5% DCMS in toluene and then allowed to drain dry prior to immersion in methanol for several minutes. The methanol was removed and the tubes placed in an air oven at 110°C until completely dry.

Extraction Procedure

Analysis of Ranitidine in Human Plasma

One hundred µl of a solution containing 200ng ranitidine and 600ng AH 20480 per ml were injected and the retention times of each substance determined.

Standard solutions of ranitidine in human plasma over the range 0-200ng ranitidine per ml plasma were prepared by adding with a calibrated 10µl syringe (Hamilton, Switzerland) measured amounts of an aqueous solution containing 10µg ranitidine per ml to each ml plasma.

One ml volumes of the standards and test samples of plasma were pipetted into clean silanised glass tubes (16 x 100mm, Sovirel, France) and 1 ml of 0.1 M disodium hydrogen phosphate, pH 9.0 was added from an 'Oxford' dispenser (Boehringer Corporation (London) Limited.) Thirty µl of an aqueous solution containing 10µg AH 20480 per ml was added to each sample as an internal standard, using a S.M.I. micro-pettor (Alpha Laboratories, Greenford) and all tubes were agitated for two seconds on a 'Whirlimixer' (Jencons, Hemel Henstead, Hertfordshire).

Five ml of octan-1-ol were added to each solution via an 'Oxford' pipettor and the tubes were rotated on a tube rotator

Stuart Scientific Co. Ltd. Croydon, Surrey) at speed 3.5 for 5 minutes. The tubes were removed and centrifuged at 1200g or 15 minutes in a Mistral 6L centrifuge (M.S.E. Crawley, urrey).

4.5 ml of each upper octanol layer was transferred to a lean dry tube using a disposable 5ml pipette, and 500µl of .1 M phosphate buffer, pH 6.0 was added to each tube. The anitidine and internal standard were re-extracted into the uffer solution by gentle rotation on the Stuart rotator for 5 minutes and the tubes were centrifuged at 1200g for a 15 inute period on a Mistral 6L centrifuge.

The upper octan-1-ol layer was removed using a Pasteur ipette attached to a water vacuum pump and 400µl of the queous phase from each tube was transferred to a low volume lass insert contained in a standard size 4ml bottle which as fitted with a P.T.F.E. septum and screw cap. The bottles ere placed in the carriage of the WISP which was set to nject 100µl of the extract.

RESULTS

The retention times of ranitidine and AH 20480 were espectively 225 seconds and 350 seconds. Figure 3 is a ypical chromatogram for a plasma sample spiked with 103ng anitidine per ml and with the internal standard. It is lear that the peaks of interest are well resolved. Fig 4 nows the linear relationship between the peak area ratios ranitidine to the internal standard), and ranitidine

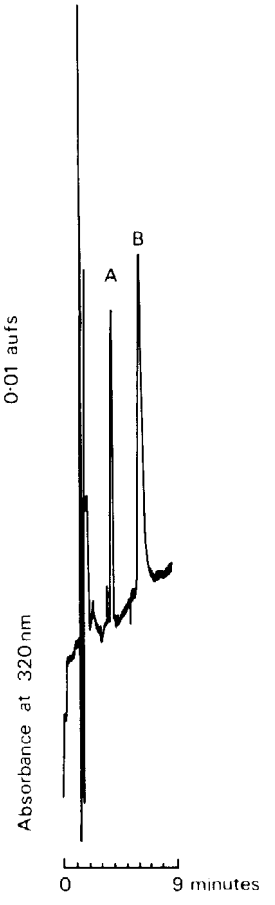

Fig 3. A chromatogram of an extract from a plasma standard containing 103 ng ranitidine/ml. Injection volume, 100µl. Peak A is ranitidine and Peak B AH 40480, the internal standard.

concentration in plasma. The efficiency of extraction of ranitidine from plasma as calculated from the analysis of twelve standards was 50 ± 5%. Figure 5 shows a chromatogram of a sample of plasma obtained at 0.25hr. from a subject given an intravenous dose of 0.25mg ranitidine hydrochloride/kg.

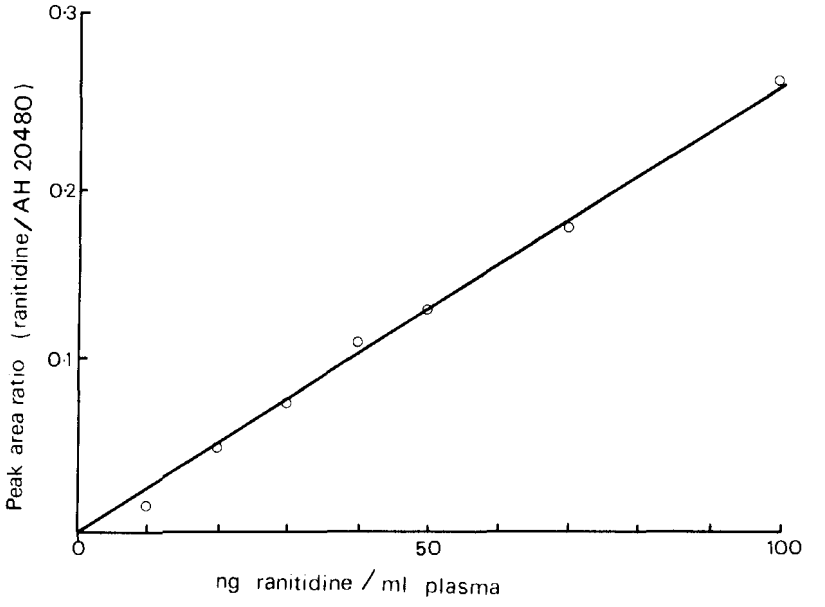

Fig. 4. Standard line showing the ratio of the peak area of ranitidine to the internal standard, against the concentration of ranitidine (ng/ml plasma).

The plasma concentrations of ranitidine obtained after separate oral doses of 20, 40 and 80 ng ranitidine hydrochloride to one subject are shown in Figure 6. There is a good correlation between the dose of ranitidine hydrochloride and the concentration of ranitidine in plasma.

DISCUSSION

A sensitive and rapid method for the determination of ranitidine in plasma is described. The drug and the internal standard are extracted from plasma into octan-1-ol at pH 8.2 and then re-extracted at pH 6.0 into a 0.1 M. phosphate

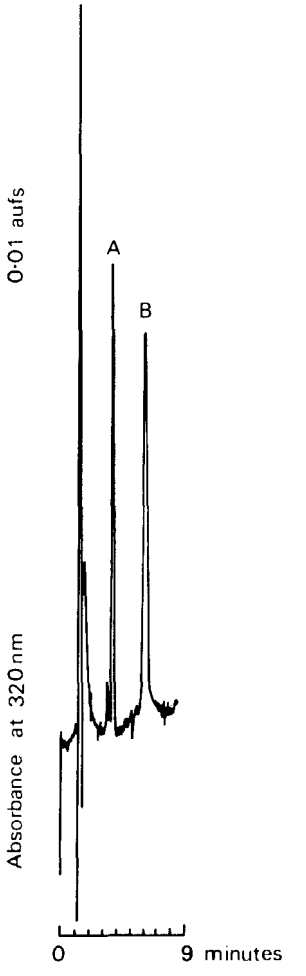

Fig 5. A chromatogram of an extract from a plasma sample collected 0.25 hours after an intravenous dose of 0.25 mg ranitidine per kg. Injection volume 100µl. Peak A is ranitidine, peak B AH 20480, the internal standard.

buffer. The concentration of ranitidine in the aqueous solution is determined by reverse phase h.p.l.c. using absorption at 320nm for the quantitation of the drug.

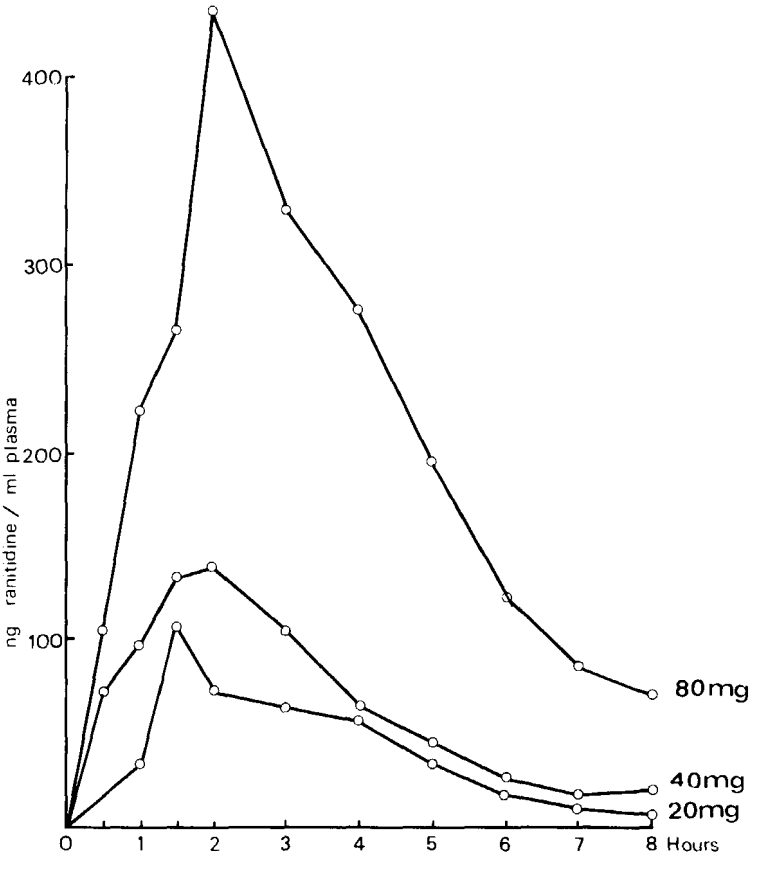

Fig. 6. Plasma concentrations of ranitidine in a subject given on separate occasions oral doses of 20, 40 and 80 mg ranitidine hydrochloride.

In order to analyse the large number of plasma samples arising from clinical trials of the drug, a fully automated h.p.l.c. system has been developed. A WISP fully automatic injection system was coupled to the high performance liquid chromatograph to process the samples. The injection system was reliable, there was no carry over between the samples

and the variable injection volume facility was useful when injecting samples containing different concentrations of the drug.

The time to complete the analysis of each sample was ten minutes. The carriage of the WISP holds 48 samples and therefore it takes eight hours to complete the analysis of a carriage filled with samples. The work schedule was arranged so that at the beginning of the working day the carriage was loaded with samples of plasma which had been processed the previous day and the analysis started.

Eight hours later on completion of the analysis, the carriage was refilled with another 48 samples and these were analysed unattended during the night. The low concentration of ranitidine in plasma samples necessitated that the u.v. detector was used at 0.01 a.u. full scale deflection. When operating over an eight hour period at this high sensitivity it was observed that base line changes can occur. The electronic drift correction circuit automatically compensated for any of these changes and maintained the signal within the range of the Spectra Physics 4000 data system.

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hydrochloride, the staff of the Chemistry Division for synthesising AH 20480 and to Mr. L. Hysom and Mr. D. Knight for technical assistance.

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