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ANALYSIS OF A NEW H_2 RECEPTOR ANTAGONIST, 3-AMINO-5-[3-[4-(1-PIPERIDINOINDANYLOXY)]PROPYLAMINO]-1-METHYL-1H-1,2,4-TRIAZOLE, IN HUMAN PLASMA AND URINE BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

A high-performance liquid chromatographic (HPLC) method for the determination of a new H_2 receptor antagonist, 3-amino-5-[3-[4-(piperidinoindanyloxy)]propylamino]-1-methyl-1H-1,2,4-triazole (I), in human plasma and urine was developed. The method employs liquid–liquid extraction of the analyte and an internal standard and chromatographic separation using an alkylphenyl-bonded HPLC column. The total time of chromatography was less than 10 min. Sensitivity was 10 ng/ml for the plasma analysis and 1 μg/ml for the analysis of I from urine. The coefficients of variation, based on interpolated concentrations, were less than 10%. The method was used for more than 5000 samples during clinical pharmacokinetic studies.

INTRODUCTION

A new histamine H_2 receptor antagonist, 3-amino-5-[3-[4-(1-piperidinoindanyloxy)]propylamino]-1-methyl-1H-1,2,4-triazole (I; RG W-2568; Fig. 1), has been shown in clinical trials to suppress gastric acid secretion at lower doses than similar drugs [1–4]. The drug is well absorbed, has a long biological half-life, low intersubject variability, and a linear pharmacokinetic profile between doses of 50 and 400 mg.

This report describes the high-performance liquid chromatographic (HPLC) method used in the analysis of I from plasma and urine samples. The method has been validated in two laboratories for clinical studies of more than 5000 samples. It employs an extraction procedure with internal standard method quantitation. The method can be used to quantitate from 10 ng/ml to 5 μg/ml I using a 1-ml plasma sample or from 1 μg/ml to 500 μg/ml using 100 μl of urine.

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EXPERIMENTAL

Apparatus

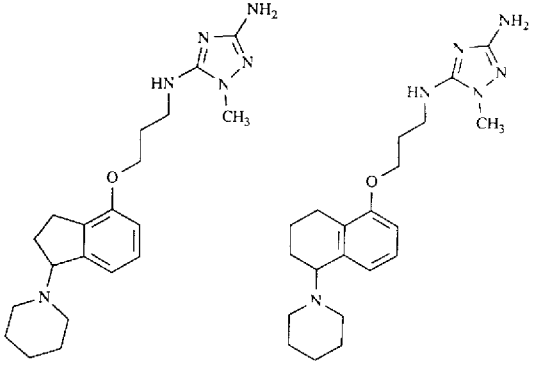
A Waters Assoc. (Milford, MA, U.S.A.) Model 510 pump and a WISP 710B autoinjector were used in the assay. The detector was a Spectroflow 783 monitor from Kratos Analytical Instruments (Ramsey, NJ, U.S.A.). Either a Hewlett-Packard (Avondale, PA, U.S.A) Model 3390A or a Spectra-Physics (San Jose, CA, U.S.A.) SP4100 integrator was used. The analytical columns were 150 mm×4.5 mm, 5 μm particle size bonded phenyl purchased from Phase Separations (Queensferry, U.K.).

Reagents

The internal standard used was 3-amino-5-[3-[5-[[1-(N-piperidyl)]-1,2,3,4-tetrahydronaphthyloxy]]propylamino]-1-methyl-1H-1,2,4,-triazole (II; RG W-2516; Fig. 1) supplied by Rorer Central Research (Ft. Washington, PA, U.S.A.). Methanol, acetonitrile, triethylamine, and concentrated phosphoric acid were all HPLC grade. Monobasic sodium phosphate, 1 *M* hydrochloric acid, ethyl acetate, and 1 *M* sodium hydroxide, were certified ACS grade (Fisher Scientific, Pittsburgh, PA, U.S.A.). Distilled water was obtained from Millipore (Bedford, MA, U.S.A.).

Chromatography

The mobile phase was prepared by combining 0.02 *M* monobasic sodium phosphate (adjusted to pH 3.0 using 50% phosphoric acid) with methanol and acetonitrile in the following proportions: plasma samples were analyzed using a mobile phase with a volume ratio of 60:16:24 and urine samples were analyzed using a volume ratio of 60:20:20. Triethylamine was added (0.01% of the total) and then the mobile phase was filtered through a 0.047-μm membrane filter (HVLP, Millipore). The mobile phase flow-rate was 1.5 ml/min for both plasma and urine



I II

Fig. 1. Structures of compounds I and II.

analyses, and the effluent was monitored at a wavelength of 214 nm. All analyses were done under ambient temperature conditions.

Preparation of standards

Plasma standards of I ranging from 0.010 to 5.00 μg/ml were prepared by adding appropriate aliquots of either 10 or 100 μg/ml aqueous stock solution to plasma. Sample aliquots were stored at −20°C until the time of the assay. Urine standards were prepared similarly using a 1 mg/ml aqueous solution and diluting with urine, to prepare 1.00–500 μg/ml standards.

Extraction procedure

*Plasma.*To each 15-ml polypropylene centrifuge tube (Corning) containing 1.0 ml of plasma sample, standard, or control, 50.0 μl of the internal standard (10 μg/ml II in distilled water) and 100 μl of 1 *M* sodium hydroxide were added. After mixing, the aqueous samples were extracted with 5 ml of ethyl acetate by shaking (approximately 120 times/min) for 10 min. The organic phase was transferred to a 15-ml polypropylene tube containing 200 μl of 0.01 *M* hydrochloric acid and vortexed for 1 min. After centrifugation for 5 min at 1800 *g*, the ethyl acetate was aspirated off and the aqueous phase was transferred to a limited-volume insert (glass, 100 μl, Waters Assoc.) for HPLC analysis. A 50-μl aliquot was injected onto the HPLC column.

*Urine.*To each labeled, 1-ml glass minivial (Waters Assoc.), 100 μl of urine sample, standard, or control and 100 μl of internal standard (40 μg/ml II in water) were added and diluted to 1.0 ml with distilled water. The samples were vortexed briefly. A 40-μl aliquot was injected onto the column.

Calculation of results

The peak-height ratios of I to internal standard were calculated using the integrator determinations for peak height. Calibration curves were constructed by plotting the peak-height ratios versus concentration for the standards. Weighted ($1/\text{concentration}^2$) linear regression parameters were generally used to determine plasma sample concentrations. Alternatively, in some studies, the standard curves were broken into two parts; a low curve (0–20 μg/ml) and a high curve (20–500 μg/ml).

RESULTS AND DISCUSSION

Chromatograms of an extracted plasma blank and standards are shown in Fig. 2, and chromatograms of a diluted urine blank and standards are shown in Fig. 3. Fig. 4 shows chromatograms of plasma and urine samples drawn from subjects after administration of I. Resolution between the drug and internal standard, and between analytes and endogenous components, was satisfactory. Because retention times varied from column to column, the acetonitrile concentration in the mobile phase was adjusted (within 2% limits) to maintain separation and relative retention times. The retention times for plasma analyses were approximately 5.8 min for the analyte and 7.7 min for the internal standard. The retention times during the urine assays were approximately 5.7 and 8.0 min for the analyte and

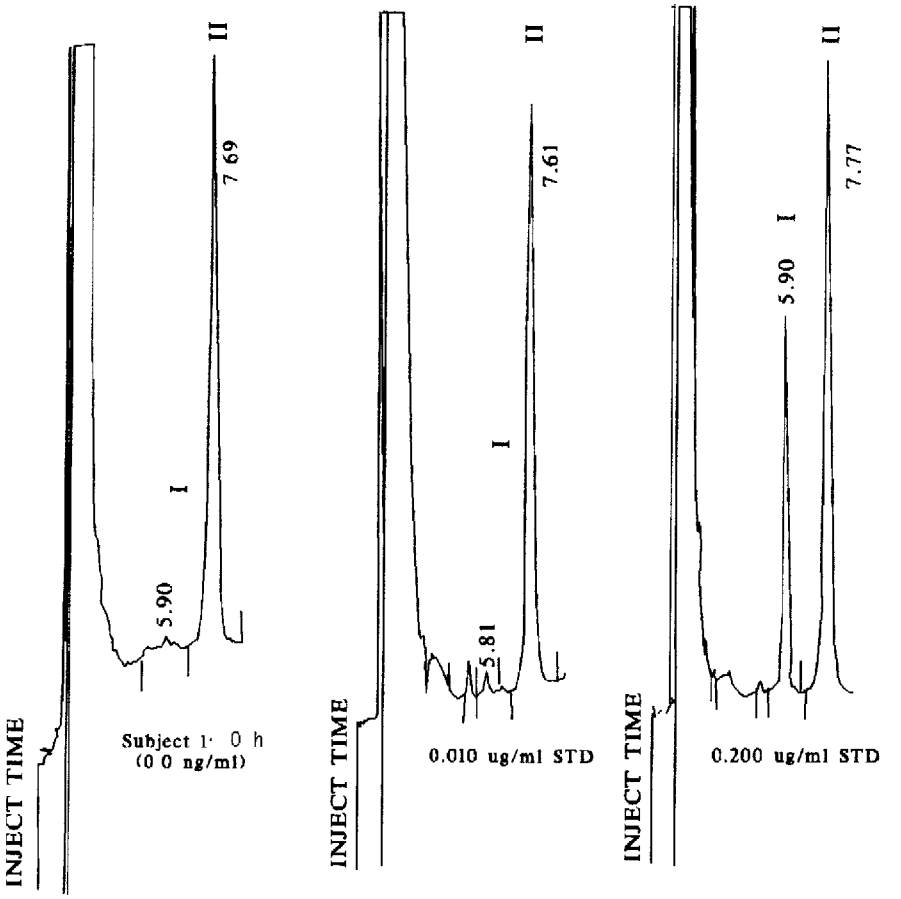


Fig. 2. Sample chromatograms (from 3 to 8 min) of plasma spiked with 0, 10 (MQL), and 200 ng/ml I.

internal standard, respectively. No interferences were found in either plasma or urine blanks, nor in predose samples from more than 100 subjects assayed during clinical pharmacokinetic studies.

Linearity and sensitivity

Calibration curves were linear over the ranges studied. Correlation coefficients (r^2) were generally greater than 0.99 for standard curves. Table I includes linear regression parameters generated during plasma analyses. Table II illustrates data from the urine analyses. The limits of sensitivity (minimum quantitatable level, MQL), based on a signal-to-noise ratio of 5 or greater, were 10 ng/ml for the plasma analyses and 0.2 μg/ml for urine. However, the MQL generally used was 1 μg/ml for urine because there was no need to extend the assay to lower limits. The coefficient of variation of interpolated concentrations at the MQL was 7.4% in plasma and 1.9% in urine during the validation runs.

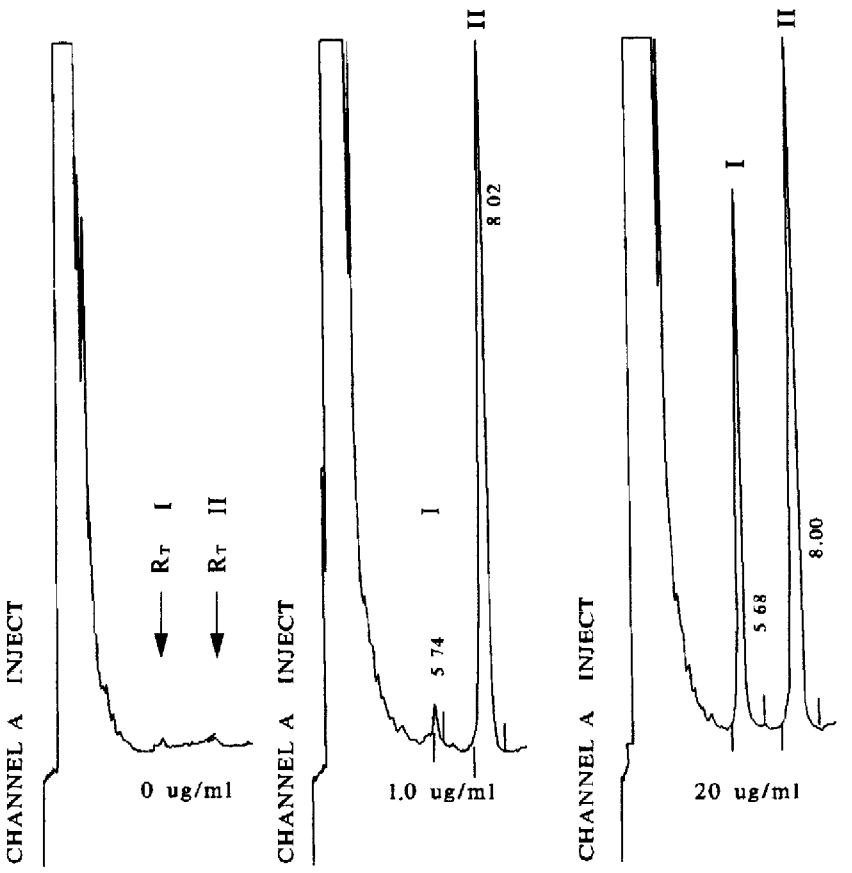


Fig. 3. Sample chromatograms (from 3 to 8 min after injection) of urine spiked with 0 (no internal standard), 1 (MQL), and 20 μg/ml I.

Precision and reproducibility

The interpolated concentrations of standards and controls from validation studies and from clinical studies were used to assess within-day and between-day variability of the analysis. During a pharmacokinetic study in which plasma samples were analyzed for 27 days (35 runs), the coefficients of variation for the controls (30, 300, and 3000 ng/ml) were less than 6%, and the interpolated concentrations were within 6% of the nominal values (Table I). During the prestudy validation, within-day precision was represented by coefficients of variation of 10% ($n=8$) or less. Similarly, during the urine analysis of the same study (Table II) the coefficients of variation of control samples over 11 analysis days (14 runs) was less than 8.6% at concentrations ranging from 2.6 to 398 μg/ml. The difference from the nominal value was less than 5% for any of the control concentrations.

Selectivity

During the analyses from four pharmacokinetic studies, no indication of metabolic interference was observed from the assessment of peak width or peak

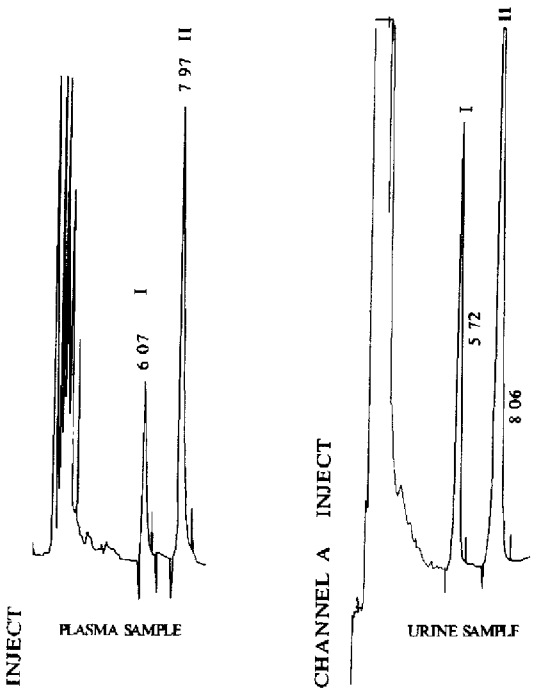


Fig. 4. Chromatograms of a plasma sample and a urine sample obtained during a pharmacokinetic study. The actual interpolated concentration of I in the plasma sample was 162 ng/ml, and the urine sample concentration was 20 μg/ml.

TABLE I

MEAN LINEAR REGRESSION PARAMETERS FOR STANDARD CURVES AND INTERPOLATED CONTROL CONCENTRATIONS FOR PLASMA SAMPLES

	Linear regression analysis		
	Slope	Intercept	Correlation coefficient
Mean	2.864	0.005	0.998
S.D.	0.111	0.004	0.002
C.V. (%)	3.88	Not applicable	0.20
n	35	35	35
	Interpolated control sample concentration (μg/ml)		
	0.030	0.300	3.00
Mean	0.031	0.318	2.981
S.D.	0.002	0.013	0.171
C.V. (%)	5.7	4.2	5.7
n	35	34	34

TABLE II

MEAN LINEAR REGRESSION PARAMETERS FOR LOW AND HIGH STANDARD CURVES AND INTERPOLATED CONTROL CONCENTRATIONS FOR URINE SAMPLES

The standard curve was analyzed in two groups using non-weighted linear regression analysis.

	Linear regression analysis					
	Low curve			High curve		
	Slope	Intercept	Correlation	Slope	Intercept	Correlation
Mean	0.0366	0.004	0.9985	0.0326	0.2289	0.9977
S.D.	0.0021	0.01	0.0027	0.0041	0.1265	0.0020
C.V. (%)	5.74	Not applicable	0.27	12.58	Not applicable	0.20
n	13	13	13	11	11	11
	Interpolated control sample concentrations (μg/ml)					
	2.5	100	400			
Mean	2.614	104.6	392.0			
S.D.	0.223	5.3	21.4			
C.V. (%)	8.53	5.07	5.46			
n	14	12	12			

shoulders. In support of this, no major metabolites have been isolated from dog and rat plasma, and in humans about 70% of the drug is excreted unchanged. Endogenous plasma or urine components were not observed to interfere in any of the pre-dose samples analyzed without internal standard added, nor were there any interferences in more than five plasma or urine pools after extraction and chromatography.

Stability

Stability of the drug in the plasma and urine has been studied to one year with no apparent degradation occurring. Extracted samples which are left at room temperature in injection vials for 72 h, likewise, indicate no degradation and display linearity and peak shape comparable to freshly extracted sample. Plasma samples left at room temperature for 2 h under UV-filtered fluorescent lights were also stable.

Data from a pharmacokinetic study

Typical plasma concentration profiles are illustrated for two subjects after administration of 200 mg of I by intravenous infusion for 1 h (Fig. 5) and oral administration in the form of a capsule (Fig. 6). Peak plasma concentrations of 2.8 μg/ml (intravenously) and 2.1 μg/ml (orally) were measured for these subjects. The method was sufficiently sensitive to enable the drug in plasma to be quantitated after the oral or intravenous infusion dose consistently to 24 h and generally to 48 h after doses of 100 mg or more. An initial elimination phase had a half-life of approximately 5–6 h and a terminal (24–48 h) half-life of approximately 12 h was determined for all dose levels. Plasma concentration and areas

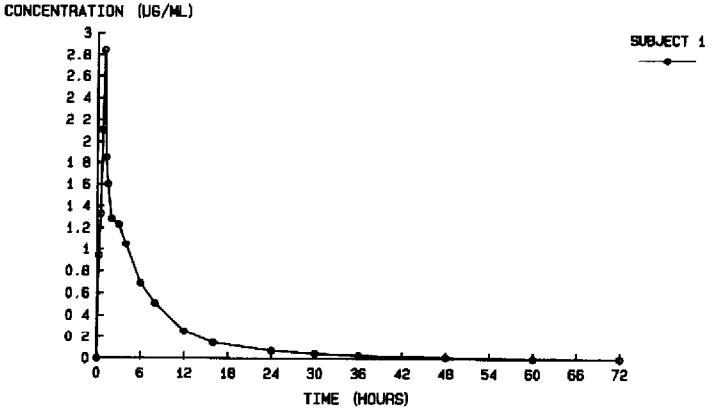


Fig. 5. Plasma elimination curve of I after a 200-mg intravenous infusion for 1 h.

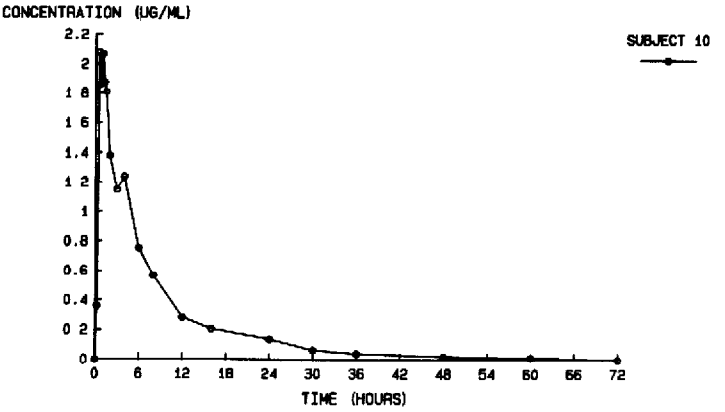


Fig. 6. Plasma elimination curve of I after a 200-mg oral capsule dose

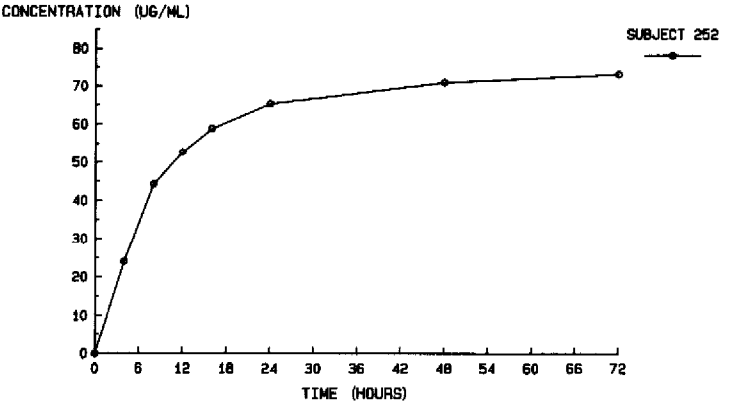


Fig. 7. Urinary excretion plot following a 200-mg oral dose of I.

under the curve were directly proportional to dose over the range of 50–300 mg. Bioavailability was found to be 98% based on plasma area under the curve data.

Excretion data from the urine analyses of one subject is shown in Fig. 7. The method was sufficiently sensitive to quantitate urine drug concentrations for 72 h after dosing.

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