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nproved high-performance liquid chromatographic assay for cimetidine sing ranitidine as an internal standard

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The analysis of cimetidine (CMT), an H_2-receptor antagonist, has been eported in the literature by several researchers [1—5]. All methods were inor modifications of the method of Randolph et al. [1]. The methods sed a silica column, and mobile phases containing acetonitrile, methanol, ater, and ammonium hydroxide. Three of the methods [1,3,4] utilized ctanol as the extraction solvent, while Larsen et al. [2] used ethyl acetate, a olvent which is readily hydrolysed at the extremes of pH used during the xtraction. Another method [5] used a complex precipitation procedure. ll the extraction methods [1—5] are time consuming and required the salt- ng out of cimetidine into ethanol or methylene chloride as the final step.

Randolph et al. [1] indicated that an extensive conditioning for 8 h is ecessary for new silica columns. They also indicated that inlet and outlet rits must be replaced to reduce back pressure. Similar problems in these aboratories have manifested as leaks at the pump seals, probably due to the mmonia present in the mobile phase.

The existing methods are difficult to reproduce, technically fragile and equire frequent instrumental maintenance.

The proposed new high-performance liquid chromatographic (HPLC) meth- d utilizes a different column, an entirely different mobile phase, and a single- tep extraction, which is both rapid and efficient.

XPERIMENTAL

nstrumentation

Analysis was performed using a constant-flow high-pressure liquid chroma- ograph (Model 5000, Varian Instruments, Palo Alto, CA, U.S.A.) with a

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variable-wavelength UV detector set at 228 nm (Vari-Chrom, Varian). The alkylnitrile column, Micropak CN-10 (10 μm, 3000 mm × 4 mm I.D.) was supplied by Varian. A manual injection valve with 10-μl loop was used to introduce the samples.

Reagents

The extraction solvents, methylene chloride and isopropyl alcohol were distilled in glass and of chromatographic grade (Burdick and Jackson, Labs., Muskegon, MI, U.S.A.). Acetonitrile, HPLC grade (Fisher Scientific, Fairlawn, NJ, U.S.A.), reagent grade sodium monobasic phosphate and sodium hydroxide were used without further purification. The mobile phase consisted of 50% acetonitrile and 50% 0.01 *M* NaH_2PO_4.

Standard solutions

Stock solutions of CMT (Smith Kline and French Labs., Philadelphia, PA, U.S.A.) and ranitidine (Glaxo Labs., Fort Lauderdale, FL, U.S.A.) each containing 10 mg/ml were prepared in distilled water. Aliquots of CMT stock solution were added to 1.0 ml of plasma so that concentrations of 0.2—2.0 μg/ml of plasma resulted. A 50-μl aliquot of the stock solution of ranitidine, the internal standard, was added to each 1 ml of plasma in the above solutions.

Extraction

The spiked plasma samples were vortexed for a few seconds to ensure adequate mixing. The samples were then made alkaline with 75 μl of 2.5 *N* sodium hydroxide, vortexed for a moment and allowed to stand for 1 min. The extracting solvent, consisting of 5 ml of methylene chloride—isopropanol (90:10), was then added to each test tube. The tubes were shaken 10 min on a vortex mixer then centrifuged at 2000 *g* for 10 min. The aqueous upper layer was aspirated and discarded. The organic layer was transferred to a clean test tube and evaporated to dryness with a stream of dry nitrogen in a water bath at 25°C. The samples were reconstituted with 150 μl of the methylene chloride—isopropanol mixture, vortexed for a few seconds and retained for HPLC analysis. Plasma samples containing unknown amounts of CMT were handled similarly, after spiking with 50 μl of the stock solution of ranitidine.

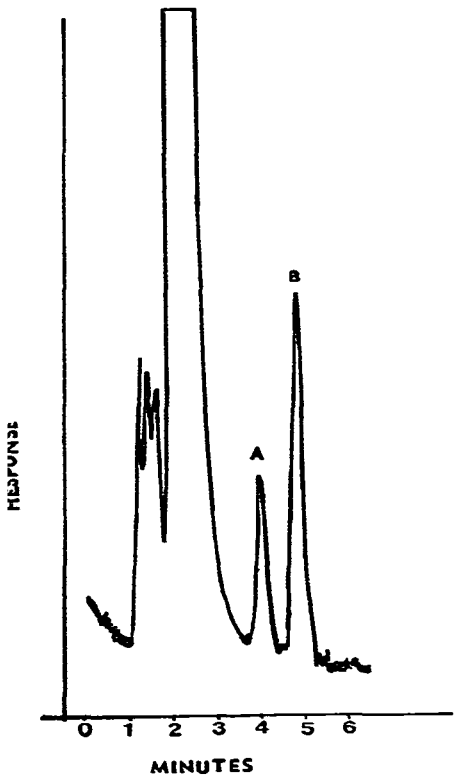
Chromatography

The column was conditioned by flushing firstly with acetonitrile for 15 min at 1 ml/min, then with the mobile phase for 15 min at 1 ml/min. The flow-rate was then increased to 1.6 ml/min and maintained at this rate for a few minutes until a steady baseline was obtained and the analysis performed. Samples were injected and monitored at 228 nm. The peak height ratios of CMT to ranitidine were calculated for the standards and plotted against concentration of CMT. The resulting standard curve was then used to convert peak height ratio of knowns to CMT concentration.

RESULTS AND DISCUSSION

A typical chromatogram of CMT obtained from spiked human plasma at

).2 μg/ml is shown in Fig. 1. There is a good separation between CMT and the nternal standard, ranitidine. Potential interfering peaks from plasma com-ponents are eluted in the first 3 min of the chromatogram, with the detector eaching the baseline before the peaks of interest begin to elute (see Fig. 2). Cimetidine and ranitidine have retention times of 4.0 and 5.0 min, respec-ively, in the system, with no interfering peaks in this region of the chroma-ogram. The sample is completely eluted in 6 min. The response at 0.2 μg/ml s strong and hence the limit of sensitivity for the assay is at least 0.1 μg/ml, nd with care as low as 0.05 μg/ml.



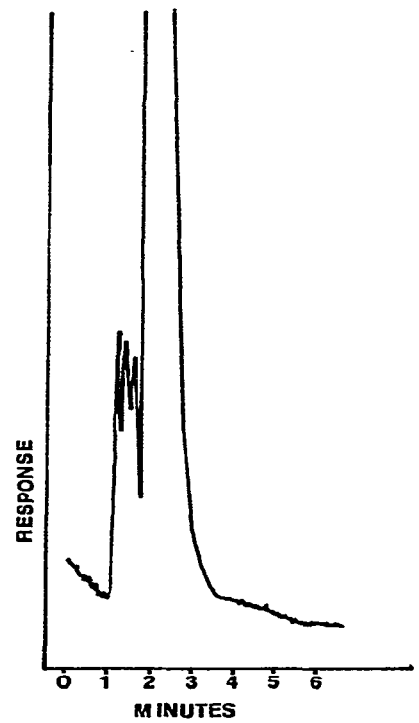


Fig. 1. Typical chromatogram for 0.2 μg/ml cimetidine in human plasma. Peaks: A = cimi-idine; B = ranitidine (0.5 μg/ml).

Fig. 2. Chromatogram of blank human plasma sample.

Table I demonstrates the accuracy at each concentration with coefficients of variation of less than 6% for each of five points on the standard curve. Each point is an average of four replicates. The extraction efficiency ranges from 60 to 71% and is better than that obtained by Chiarmonte and Schentag [4]. The correlation coefficient obtained from the linear regression was 0.9953.

Table II demonstrates the inter-assay precision. Duplicate samples were prepared and run side by side, using two technicians. The coefficient of varia-ion between run 1 and run 2 was determined to be less than 6% demonstrating hat the precision of the analysis did not suffer. The correlation coefficients for runs 1 and 2 were $r = 0.9985$ and 0.9968, respectively.

TABLE I

ACCURACY AND EXTRACTION EFFICIENCY FOR CIMETIDINE IN HUMAN PLASMA

Concentration (μg/ml)	Peak height ratios (± S.D., $n = 4$)	C.V. (%)	Extraction efficiency (%)
0.2	0.440 ± 0.011	2.61	71.1
0.4	0.741 ± 0.028	3.79	70.6
0.8	0.160 ± 0.091	5.71	67.9
1.6	3.310 ± 0.160	4.89	70.0
2.0	3.820 ± 0.093	2.36	60.7

TABLE II

INTER-ASSAY PRECISION

Concentration (μg/ml)	Peak heights ratio		Average ± S.D.	C.V. (%)
	Run 1	Run 2		
0.2	0.474	0.492	0.482 ± 0.028	5.7
	0.442	0.518		
0.4	0.885	—		
	0.869	—		
0.8	1.651	1.852	1.803 ± 0.093	5.2
	1.814	1.897		
1.6	3.750	3.882	3.817 ± 0.067	1.8
	3.750	3.886		
2.0	4.684	4.563	4.59 ± 0.062	1.3
	4.579	4.515		

The proposed analytical procedure offers a robust method to determine plasma levels of CMT in a routine manner. The method is insensitive to small variations of pH, in the ionic strength of the NaH_2PO_4 solution, and hence in minor variations in the composition of the mobile phase. The column needs no unusual conditioning and the extraction is fast and simple. The samples must be run within a few hours after reconstitution, but may be stored dry in a freezer until needed without loss of activity. Plasma samples of cimetidine have previously been reported to be stable when stored in the frozen state [6]. Ranitidine dissolved in 10 mol/l KH_2PO_4 buffer at pH 3 is reported to be stable for several months stored at 4°C [7].

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