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Letter to the Editor

High-performance liquid chromatographic determination of cimetidine in rat plasma, urine and bile

Sir,

Many high-performance liquid chromatographic (HPLC) methods have been reported [1—14], but they sometimes require time-consuming and tedious extraction procedures to eliminate the interference of endogenous substances before determination of cimetidine [1, 3]. On the other hand, an alkaline mobile phase was used in some cases [1—5, 10, 12] and, in these cases, the degradation of the column may be accelerated. Although McPherson and Lee [15] reported that bile does not represent a major route of [^{14}C]cimetidine excretion in rats, no HPLC method for the determination of cimetidine in bile has been published.

The rapid and reliable HPLC procedures that utilize a single-extraction procedure and a low mobile phase pH for analysis of cimetidine in plasma, urine and bile were obtained as follows: to a microcentrifuge tube containing 100 μl of plasma, 100 μl of the internal standard solution (metiamide 50 μg/ml) and 100 μl of 6 *M* sodium hydroxide were added. Ethyl acetate (4.5 ml) was then added and the whole was mixed for 10 min. After centrifugation (1500 *g*, 10 min), 4 ml of the organic phase were transferred to a dry test-tube and evaporated to dryness. The residue was dissolved in 100 μl of distilled water, and 10-μl aliquots of this solution were injected into the chromatograph. Urine samples were treated similarly.

In the case of bile samples, interfering peaks appeared in the chromatogram, and triple-extraction procedures were necessary. After the single basic extraction step (similar to that used in the preparation of plasma samples), 4 ml of the organic phase were transferred to a glass-stoppered tube containing 1 ml of 0.05 *M* hydrochloric acid. The compounds were extracted into the aqueous phase by mixing for 10 min. After centrifugation (1500 *g*, 10 min), 800 μl of the aqueous phase were made alkaline by adding 500 μl of 6 *M* sodium hydroxide. The compounds were extracted into 4.5 ml of ethyl acetate by mixing for 10 min. After centrifugation (1500 *g*, 10 min), 4 ml of the organic phase were transferred into a dry test-tube and evaporated to dryness. The

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residue was dissolved in 100 μl of distilled water and 10-μl aliquots of this solution were injected into the chromatograph.

A Shimadzu LC-3A pump (Shimadzu Seisakusho, Japan) connected to a Model 7125 sample loop injector (fitted with a 20-μl sample loop; Rheodyne) was used. A variable-wavelength UV detector (SPD-2A, Shimadzu Seisakusho) was used to monitor the effluent at 228 nm (sensitivity: 0.01 a.u.f.s.). The chromatographic mobile phase, which consisted of acetonitrile–water–1/30 *M* sodium phosphate buffer (pH 4.9, 40:39:1), was pumped through a Hibar LiChrosorb RP-8 column (250 × 4.0 mm I.D., 10 μm particle size; Merck) at a flow-rate of 1.0 ml/min. The column temperature was kept at 40°C by

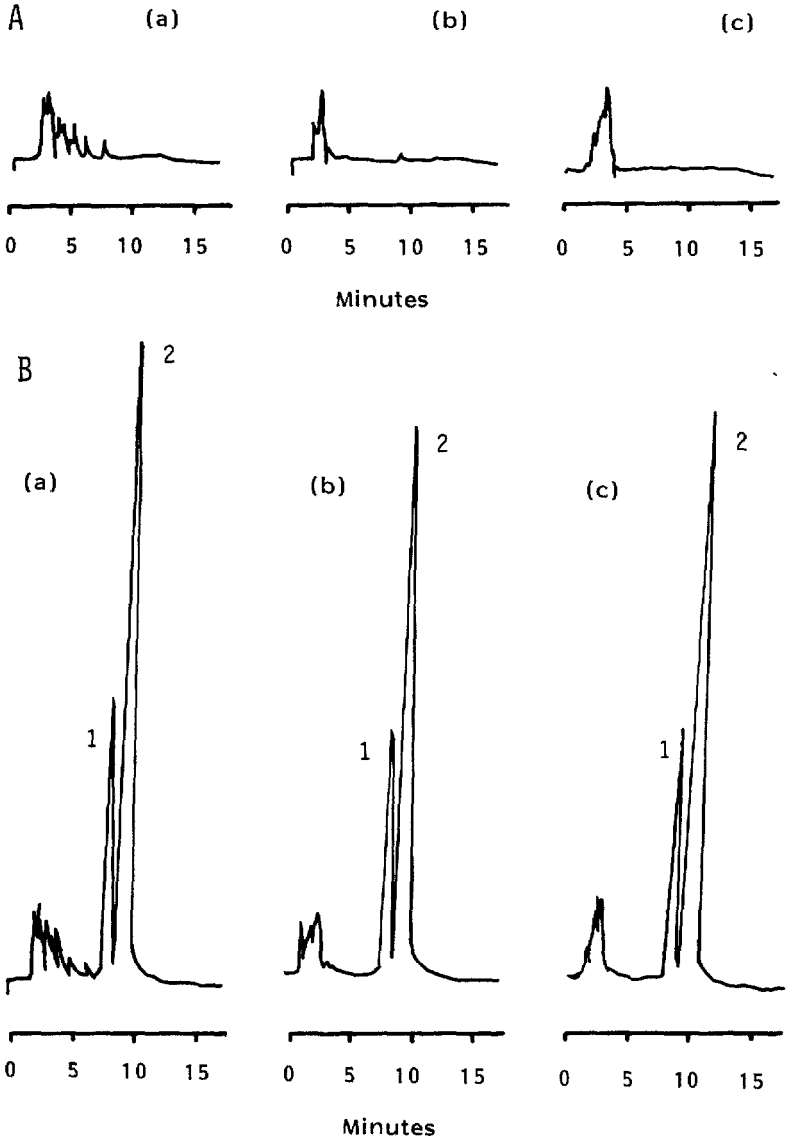

Fig. 1.

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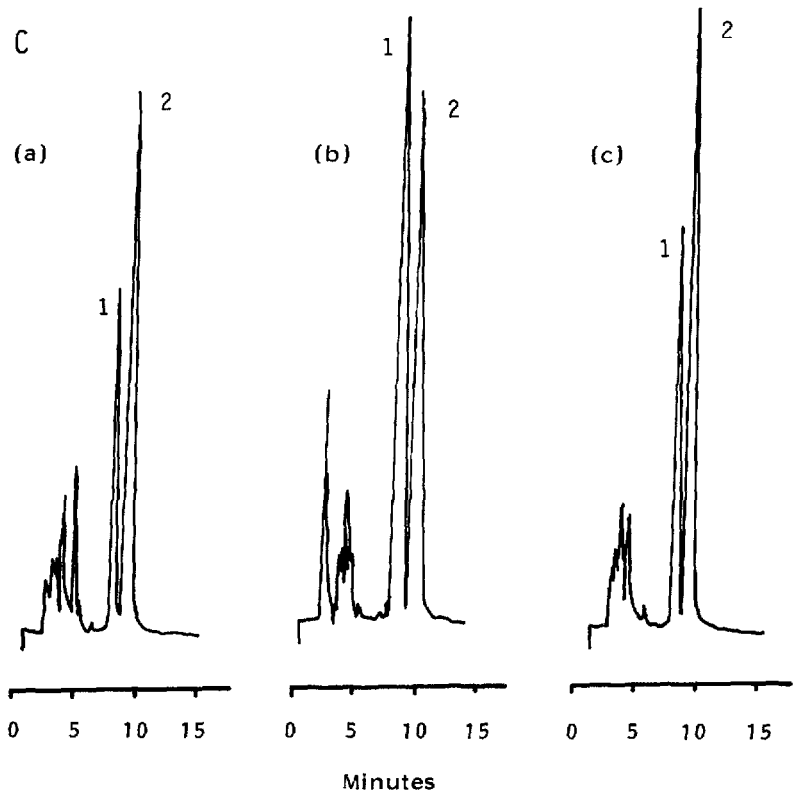

Fig. 1. Chromatograms of extracts from rat plasma (a), urine (b) and bile (c). (A) Blank samples; (B) 10.00 μg/ml cimetidine plus internal standard; (C) collected from rats dosed with cimetidine (intravenously, 100 mg/kg) plus internal standard. Peaks: 1 = cimetidine; 2 = metiamide.

using a CTO-2A column oven (Shimadzu Seisakusho). Peak areas for quantitation of cimetidine were integrated with a Chromatopac C-R1A (Shimadzu Seisakusho).

The interfering peaks of endogenous substances in the chromatogram were eliminated and the peak shapes of both cimetidine and the internal standard are excellent, as shown in Fig. 1. The retention times of cimetidine and the internal standard were 9.5 and 10.5 min, respectively, and one sample could be analysed within 12 min by using this system. As compared with other HPLC methods [1—5, 10, 12], our method offers a lower column degradation rate because of the use of a low mobile phase pH.

The ratios between the peak areas of cimetidine and the internal standard for various cimetidine concentrations in plasma, urine and bile are shown in Table I. The ratio was proportional to the concentration of cimetidine, the correlation coefficient was 1.00 in each case (i.e. plasma, urine and bile samples), and good accuracy was obtained. The coefficient of variation (C.V.) was less than 6% in each case, and good precision was obtained. The detection limit was 0.05 μg/ml if a 0.1-ml sample was available, but it could be lowered with increase of sample volume or injection volume into HPLC.

The assay described in this paper is considered to be sufficiently sensitive

TABLE I

RATIO BETWEEN THE PEAK AREAS OF CIMETIDINE AND THE INTERNAL STANDARD (METIAMIDE) FOR VARIOUS CIMETIDINE CONCENTRATIONS IN PLASMA, URINE AND BILE, WITH RECOVERY DATA

For each concentration, $n = 4$. Theoretical recovery = 100%.

Concentration added (μg/ml)	Ratio	Coefficient of variation (%)	Recovery (%)	S.D.
Plasma				
0.10	0.0022	5.80	95.98	4.72
0.25	0.0058	4.57	94.90	3.29
1.00	0.0171	4.33	96.51	4.36
10.00	0.1443	2.01	87.19	5.10
100.00	1.4959	2.90	90.38	4.87
Urine				
0.10	0.0018	5.56	96.51	7.98
0.25	0.0044	4.40	95.90	4.22
1.00	0.0150	4.67	94.56	5.10
10.00	0.1598	2.64	96.09	2.46
100.00	1.5283	2.72	95.37	2.59
Bile				
0.10	0.0021	6.62	76.84	6.92
0.25	0.0054	4.45	76.84	8.46
1.00	0.0161	3.19	69.67	4.35
10.00	0.1543	1.18	78.11	8.29
100.00	1.3499	2.48	82.60	6.23

and reliable both for detailed pharmacokinetic studies in small animals and for clinical use in patients.

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