Journal of Chromatography, 146 (1978) 354—360
Biomedical Applications

CHROMBIO. 189

Note

High-pressure liquid chromatographic determination of cimetidine sulphoxide in human blood and urine

R.M. LEE and P.M. OSBORNE

Department of Biochemistry, Smith Kline & French Laboratories Ltd., Research Institute, Welwyn Garden City, Hertfordshire (Great Britain)

(First received January 10th, 1978; revised manuscript received April 21st, 1978)

Cimetidine [1] an H_2-antagonist of histamine, has been introduced recently as Tagamet® for the treatment of gastric and duodenal ulcers. The major identified metabolite of cimetidine is the sulphoxide [2], which is eliminated from the body mainly by renal excretion. A satisfactory method for the determination of cimetidine in blood and urine has been published [3] and this can now be complemented by a method for the determination of the sulphoxide metabolite in blood and urine using high-pressure liquid chromatography (HPLC) to separate the components of the extract and UV extinction to monitor the column effluent. This method differs in several important respects from that published [3] for the assay of cimetidine itself, but can be used for cimetidine analysis by a simple modification of the solvent system.

METHODS

Extraction procedure

All blood samples were heparinized at collection, frozen (—20°) as soon as possible thereafter and stored at this temperature until thawed immediately prior to extraction. Urine samples were frozen within 30 min of collection and similarly stored.

The procedure for extraction of cimetidine sulphoxide was the same for blood and urine samples and is only described for blood samples.

A 3-ml sample of the blood to be assayed was made alkaline (pH 9.0) by the addition of 1 ml of *N* carbonate buffer, which contained a suitable amount (about 2 μg) of internal standard (N-cyano-N′methyl-N″-(3-(4-imidazolyl)-propyl)guanidine; compound SK&F 92374)*. A 4-ml volume of 1-octanol was then added to the samples in 15-ml polythene tubes, which were stoppered and rotated for 15 min on a blood-mixer. After 15 min the octanol layer was

*Available from SK&F Labs., Welwyn Garden City, on request for the analysis of cimetidine sulphoxide.

separated cleanly by centrifugation, removed and stored. A second 4-ml volume of octanol was added and the process repeated, so that the combined octanol extracts (total volume 7 ml) could be re-extracted with 3 ml of 0.02 *N* HCl by the same rotary mixing and centrifugation technique. After removal of the octanol by aspiration, the acid layer (2.8 ml) was transferred to clean tubes, and 200 μl of ethanol added and mixed before saturating the whole with solid potassium carbonate (ca. 5 g). This had the effect of "salting-out" the ethanol into a discrete layer which could be removed after centrifugation and stored at −20° prior to HPLC separation and analysis.

Chromatography

The following HPLC conditions were employed:Column: LiChrosorb Si 60; 5-μm particle size; 25 cm × 3.2 mm I.D. Solvent: acetonitrile—methanol—water (distilled)—ammonium hydroxide (0.88 sp.gr.); (250:20:6:1.5, v/v). Flow-rate: 1 ml/min maintained by a constant flow system (Waters Assoc. 6000). Injection: 10—20 μl of the ethanol extract were introduced onto the column via an Altex variable-volume loop injector. Detection: the UV absorption of the column effluent was monitored by a variable wave-length detector (Perkin-Elmer LC55) set at 228 nm. Recording: peaks were recorded on a conventional chart recorder and the areas under them integrated by means of an Infotronics CRS 309 integrator which compensated for baseline drift. Retention: the retention times of compounds of interest were determined by the chart speed of the recorder, and by the programme of the integrator which was reset at the time of each sample injection.

RESULTS

The relationship between peak height, peak area and the amount of cimetidine sulphoxide applied to the HPLC column is given in Table I; it was found

TABLE I

CORRELATION BETWEEN PEAK AREA OR PEAK HEIGHT AND CIMETIDINE SULPHOXIDE*

Determined by HPLC—UV analysis. Background peak heights were less than 1 mm, and integrator counts associated with background were not greater than 500. The peak height and area values are the means of four observations, together with their standard deviations.

Concentration of cimetidine sulphoxide in ethanol (mg/l)	Cimetidine sulphoxide in 20-μl injection (ng)	Peak height (mm)		Peak area (relative counts)
		Range	Mean	Mean
0.65	13	5—5	5 ± 0	2010 ± 94
1.30	26	9—11	10 ± 1	3970 ± 198
2.60	52	19—21	21 ± 1	8020 ± 301
5.20	104	38—41	40 ± 2	14940 ± 727
10.40	208	76—83	78 ± 4	29520 ± 356
20.80	416	158—162	161 ± 2	62400 ± 1496

*Concentrations up to mg/l.

TABLE II

TEST OF REPRODUCIBILITY OF CIMETIDINE SULPHOXIDE EXTRACTION AND ASSAY FROM BLOOD AND URINE SAMPLES

Injection volume onto column 15 µl from a total of 200 µl.

Sample No. (2 ml)	1 mg/l in Blood				5 mg/l in Urine			
	Sulphoxide		Internal standard		Sulphoxide		Internal standard	
	Peak ht. (mm)	Area (rel. count)	Area (rel. count)	Area ratio	Peak ht. (mm)	Area (rel. count)	Area (rel. count)	Area ratio
1	29	9251	47719	5.1	109	35657	44641	1.25
2	26	8706	45417	5.2	104	33473	38168	1.14
3	24	8822	47148	5.3	112	35341	43867	1.24
4	25	9321	48334	5.2	115	36479	45164	1.24
5	26	8662	45126	5.2	102	31922	37561	1.18
6	29	9294	46732	5.0	92	29554	37244	1.26
7	27	8206	48092	5.9	105	34247	42887	1.25
8	30	9270	48787	5.3	106	32672	40664	1.24
Mean	27	8967	—	5.3	106	33668	—	1.22
Range	24–30	—	—	5.0–5.9	92–112	—	—	1.14–1.26
S.D.	2	—	—	0.3	7	—	—	0.04

to be linear over the range 13–416 ng/20-μl injection, with a minimum reliable estimation of 10 ng. Higher concentrations were not investigated, but the assay was accurate for 200-μl ethanol extracts containing 0.1–4.2 μg of cimetidine sulphoxide.

The results of extracting known quantities of cimetidine sulphoxide from blood and urine samples are shown in Table II. The standard curve was linear for cimetidine sulphoxide blood concentrations between 0.5 and 5.0 mg/l, and the reproducibility (Table II) at 1 mg/l was excellent (S.D. = 5.6% of

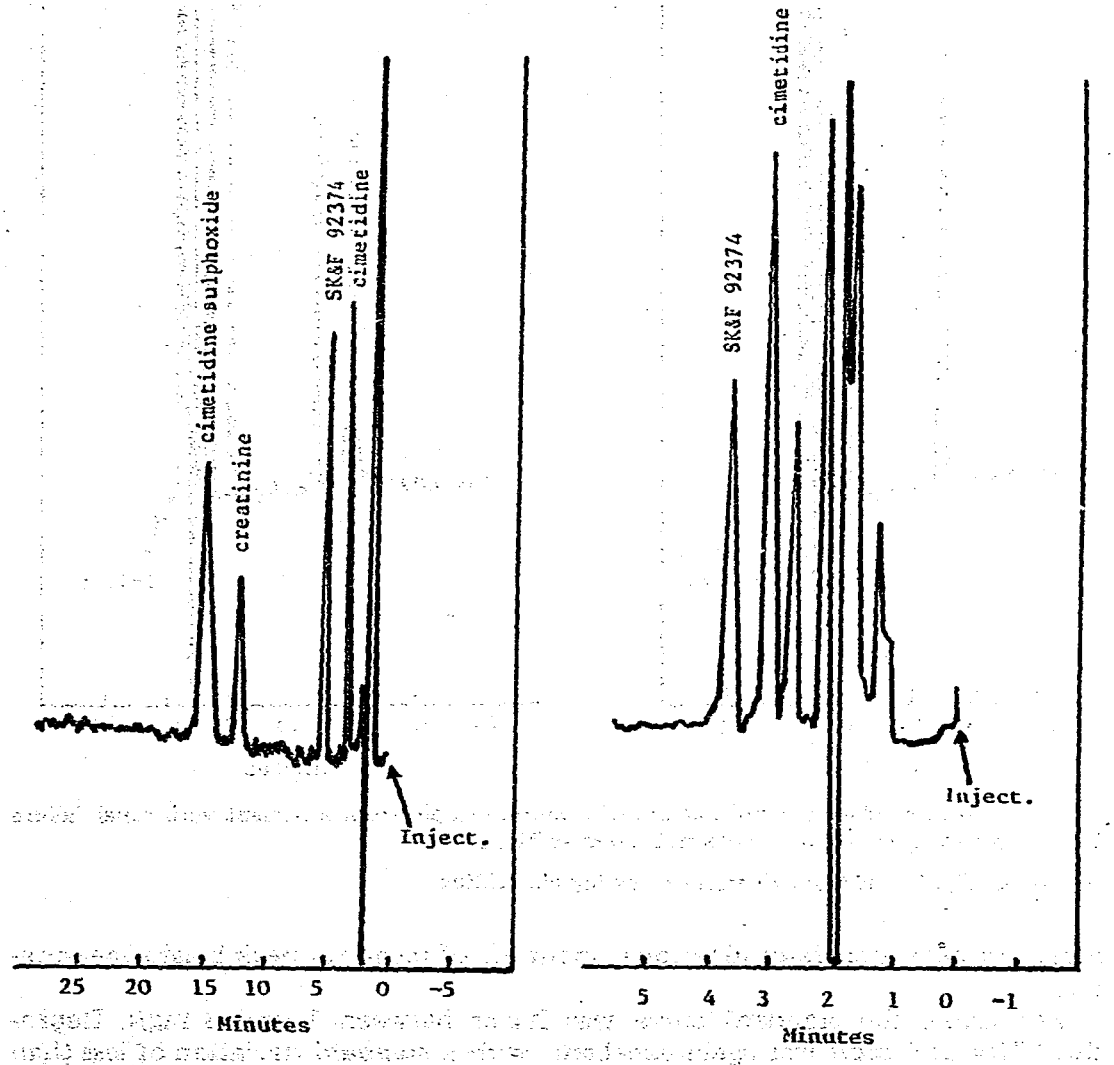

Fig. 1. Injection of a mixture of known quantities of cimetidine, SK&F 92374, creatinine and cimetidine sulphoxide dissolved in ethanol. Solvent system: acetonitrile—methanol—water—0.88g ammonia 250:20:6:1.5, v/v).

Fig. 2. Injection of an ethanol extract of a blood sample from a patient receiving cimetidine, using SK&F 92374 as internal standard. Solvent system: acetonitrile—methanol—water—.88g ammonia 200:30:6:1.5, v/v).

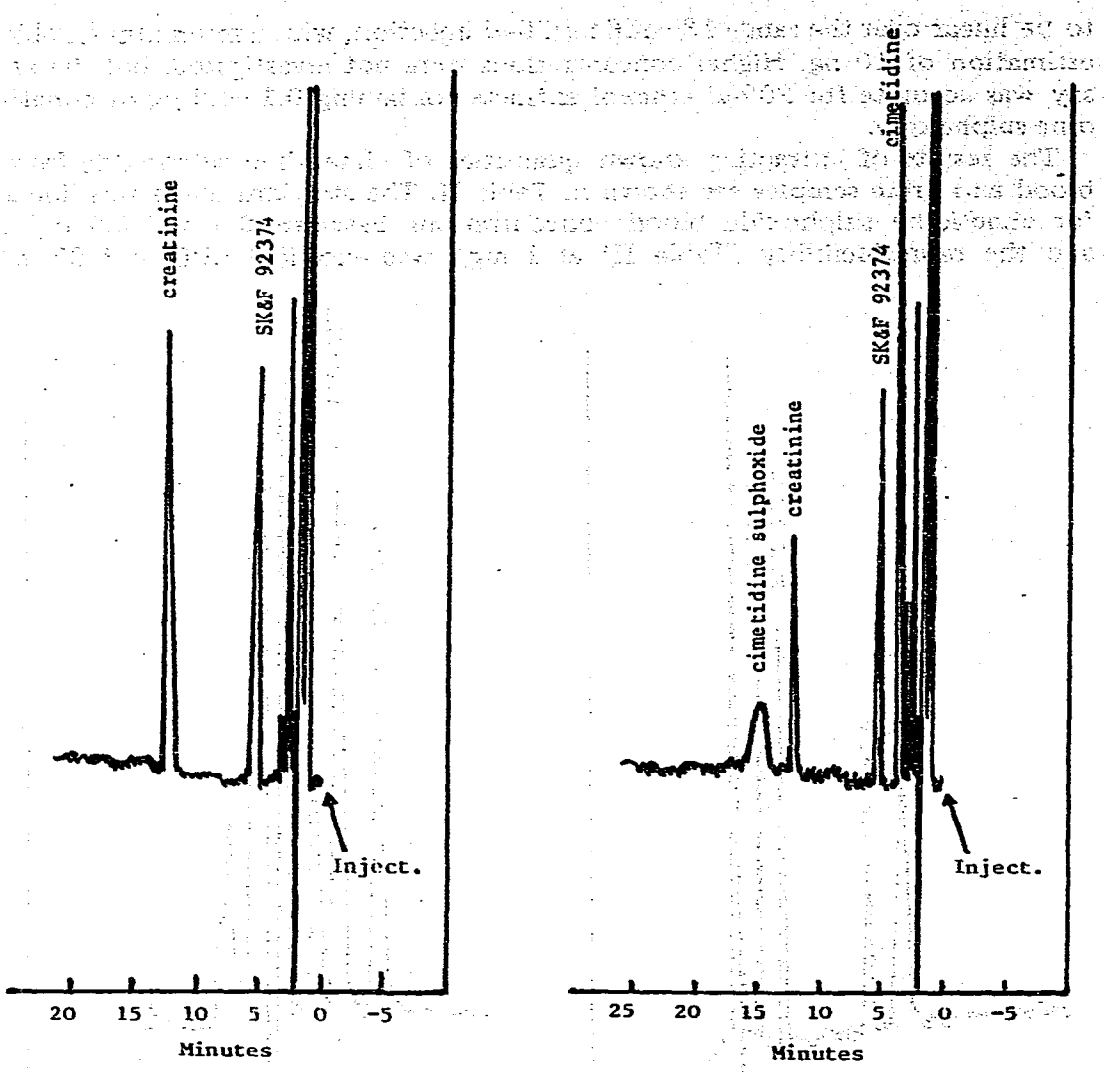

Fig. 3. Injection of an ethanol extract of a blood sample from a patient with renal failure but not receiving cimetidine. Solvent system as Fig. 1.

Fig. 4. As Fig. 2, but from a patient receiving cimetidine.

mean for peak area determinations, about 8% of mean for peak height measurement).

For urine, the standard curve was linear between 1 and 8 mg/l. Reproducibility at 5 mg/l was again excellent, with a standard deviation of less than 4% of the mean for peak area estimations. Theoretically, the complete extraction of 2 μg of cimetidine sulphoxide from a blood sample would result in a concentration of 10 mg/l in the ethanol available for HPLC. A 15-μl injection onto the column would contain 150 ng, giving a peak area of 21,400 counts (Table I). In practice, the count for 8 such samples was an average 8967, giving a recovery of 42%.

The recovery from urine was 31% for the extraction of a 2-ml sample containing 10 μg of cimetidine sulphoxide. With a minimum reliable determination of 10 ng injected onto the column, the assay is limited to concentrations of cimetidine sulphoxide greater than 0.2 μg/ml if 2-ml samples are available for extraction.

The analysis of cimetidine sulphoxide in clinical samples is complicated by the presence of cimetidine itself, and frequently creatinine. The retention times for the HPLC system described herein were 3, 5, 12 and 14 min for

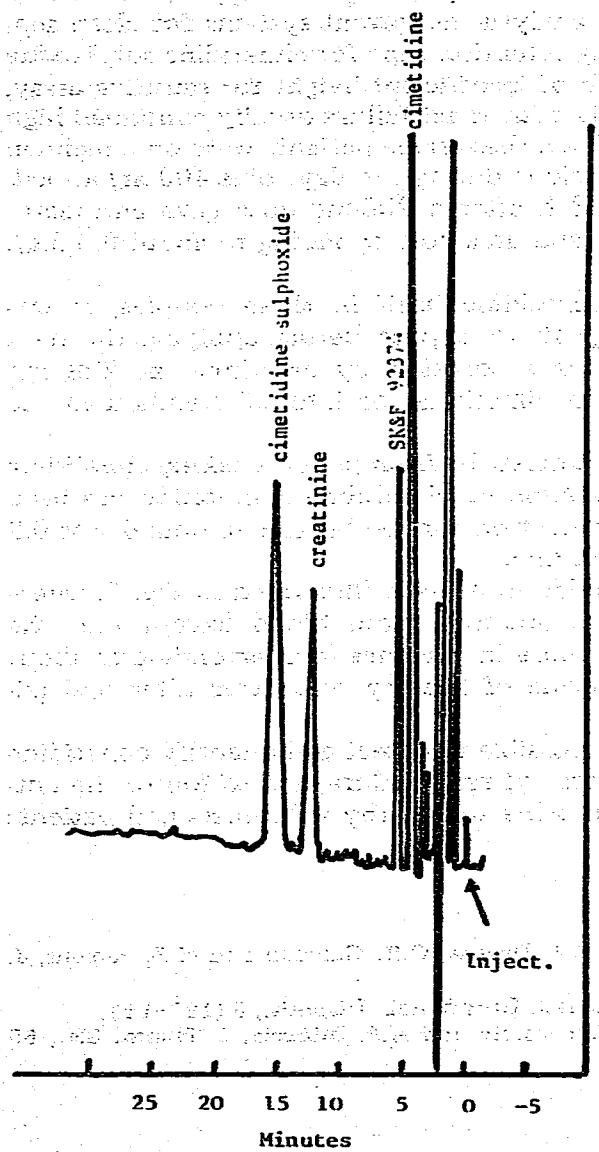

Fig. 5. Injection of an ethanol extract of a urine sample from a healthy volunteer, who had received cimetidine intravenously 2 h previously. Solvent system as Fig. 1.

cimetidine, internal standard, creatinine and cimetidine sulphoxide respectively (Fig. 1).

The separation of cimetidine from indigenous blood components was not always complete under these conditions, and is better performed using the same solvents in the proportions 200:30:6:1.5 with a flow-rate of 1.5 ml/min (pressure approx. 2500 p.s.i. at the pump). This system gave retention times of approximately 3.0 min for cimetidine and 3.5 min for the internal standard (Fig. 2).

The use of metiamide as internal standard [3] is quite inappropriate under the conditions used for sulphoxide analysis, as solvent systems for clean separation of metiamide [3] gave a long retention time for cimetidine sulphoxide which emerged as a wide-based peak of insufficient height for sensitive assay.

Blood samples taken from patients with renal failure usually contained high creatinine concentrations (Fig. 3). When these same patients were on a regimen of cimetidine (200 mg on three occasions during the day, plus 400 mg at bedtime), the blood samples taken 2—3 h after a 200-mg dose gave chromatograms as in Fig. 4, with peak height and area corresponding to about 0.5 mg/l cimetidine sulphoxide.

For the sequential analysis of cimetidine itself in these samples, it was necessary to allow the creatinine peak to appear before applying the next sample to the column, otherwise the absorption by creatinine at 228 nm seriously distorted the peaks due to cimetidine or internal standard in the following sample.

In blood samples from healthy volunteers or from patients taking cimetidine but free from renal failure, no creatinine or cimetidine sulphoxide has been found, although the lower limit of detection for the latter compound was 0.2 mg/l compared to 0.05 mg/l for cimetidine.

The analysis of cimetidine sulphoxide in urine is illustrated in Fig. 5, where the chromatogram is similar to that obtained from blood except that the cimetidine sulphoxide peak is much more in evidence (corresponding to about 3 mg/l) and was observed in the urine of healthy volunteers after oral administration of cimetidine (200 mg).

Using this technique, it would be possible to detect and quantify cimetidine sulphoxide in blood samples from cases of renal failure, and to follow the output of cimetidine sulphoxide in the urine of healthy volunteers and patients with kidney disease.

REFERENCES

1 R.W. Brimblecombe, W.A.M. Duncan, G.J. Durant, C.R. Ganellin and M.E. Parsons, J. Int. Med. Res., 3 (1975) 86.
2 D.C. Taylor, P.R. Cresswell and D.C. Bartlett, Drug Metab. Disposit., 6 (1978) 21.
3 W.C. Randolph, V.L. Osborne, S.S. Walkenstein and A.P. Intoccia, J. Pharm. Sci., 66 (1977) 1148.