Journal of Chromatography, 221 (1980) 419—424
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 668

Note

High-performance liquid chromatographic determination of sulphathiazole in human plasma and urine

A. SIOUFI*, J. GODBILLON and F. CAUDAL

Ciba-Geigy, Centre de Recherche Biopharmaceutique, B.P. 308, 92506 Rueil-Malmaison Cedex (France)

(First received March 10th, 1980; revised manuscript received July 17th, 1980)

Gas chromatographic procedures [1, 2] that have been described for the assay of sulphonamides in body fluids were found to be cumbersome for the assay of sulphathiazole as they require a derivatization step. High-performance liquid chromatographic (HPLC) methods have been also described [3—8]. Sharma et al. [3] described a method for the analysis of sulphathiazole in pure solutions as well as in cattle urine, but as the retention time depends on the nature of the sample (water or urine), it was not applied to plasma. Vree et al. [6] described chromatographic conditions suitable for sulphathiazole but the dilution of the plasma in the sample preparation step limits the sensitivity. A range of sulphonamides in pure solutions are well separated under the conditions described by Cobb and Hill [8] but sulphathiazole is eluted within 20 min.

This paper describes a simple HPLC method for the assay of sulphathiazole in plasma and urine. Plasma samples are injected after protein precipitation by acetonitrile, urine samples after dilution with water.

EXPERIMENTAL

Chemicals

Sulphathiazole, sulphadiazine, sulphamerazine, sulphapyridine, acetonitrile, and pH 4 and 5 buffers (Titrisol) were purchased from Merck (Darmstadt, G.F.R.). Standard solutions of sulphathiazole were made up in distilled water alkalinized with a few drops of 0.1 *N* sodium hydroxide. These solutions were stable for more than two months at 4°C.

The acetyl derivative of sulphathiazole was prepared by acetylation with acetic anhydride—pyridine (1:0.1, v/v) and recrystallization in dioxane—water (1:1, v/v).

0378-4347/80/0000—0000/$02.25

Chromatographic instrumentation and conditions

Chromatography was performed on a Hewlett-Packard 1084A high-performance liquid chromatograph equipped with a fixed-wavelength (254 nm) UV detector. Stainless-steel columns were used; a 12 cm × 7.5 mm I.D. column filled with LiChrosorb RP-8 (5 μm) for plasma, and a 25 cm × 4.7 mm I.D. column filled with LiChrosorb RP-8 (10 μm) for urine. They were packed using the balanced-density-slurry packing technique. The column compartment was at room temperature.

Analytical procedure for plasma and urine

Precipitation of proteins from plasma

In a 10-ml glass centrifuge tube were introduced 500 μl of plasma, 1500 μl of acetonitrile, and 50 μl of distilled water (or calibration solutions). The tube was stoppered and shaken for 30 sec on a Vortex mixer. After a 15-min centrifugation at 2100 *g*, 20 μl were injected onto the column.

Urine dilution

In a 10-ml stoppered glass centrifuge tube were mixed 50 μl of urine, 2400 μl of distilled water, and 50 μl of distilled water or calibration solution; 20 μl of this solution were injected.

Chromatography

Plasma. A precolumn filled with Whatman Co:Pell ODS (10 cm × 4.7 mm I.D.) was used to protect the separation column. The degassed mobile phase, pH 5 buffer—acetonitrile (80:16, v/v), thermostated at 40°C, was used at a flow-rate of 2 ml/min. The column-top pressure was about 80 bars.

Urine. The degassed mobile phase, pH 4 buffer—acetonitrile (92:8, v/v), thermostated at 40°C, was used at a flow-rate of 2.5 ml/min. The column-top pressure was about 100 bars.

Calibration curves. Aliquots of 50 μl of different aqueous solutions of sulphathiazole were added to 500 μl of plasma to produce reference samples in the range of concentrations 250—20,000 ng/ml of plasma. Urine calibration samples were obtained by adding 50 μl of different aqueous solutions of sulphathiazole to 50 μl of urine and 2400 μl of distilled water. The range of concentrations was 2.5—100 μg/ml of urine. The calibration solutions were stable for one week at 4°C.

RESULTS AND DISCUSSION

Sensitivity, reproducibility and accuracy

Various spiked plasma and urine solutions were prepared and analysed several times. The results, summarized in Tables I and II, show that the proposed procedure permits the accurate determination of sulphathiazole at concentrations down to 250 ng/ml of plasma and 2.5 μg/ml of urine. Lower concentrations could be determined with a lower accuracy.

TABLE I

PRECISION AND RECOVERY OF THE HPLC DETERMINATION OF SULPHATHIAZOLE IN SPIKED HUMAN PLASMA SAMPLES

Amount added (ng/ml)	Mean amount found (n = 6) (ng/ml)	Precision C.V. (%)	Recovery (%)
250	255	4.8	102
500	515	2.1	103
1000	908	6.2	91
1600	1546	1.0	97
5000	4961	2.0	99
10000	10302	1.1	103
			99 ± 5.5

TABLE II

PRECISION AND RECOVERY OF THE HPLC DETERMINATION OF SULPHATHIAZOLE IN SPIKED HUMAN URINE SAMPLES

Amount added (ng/ml)	Mean amount found (n = 6) (ng/ml)	Precision C.V. (%)	Recovery (%)
2.5	2.7	5.8	107
10.0	10.1	1.8	101
20.0	20.8	2.2	104
50.0	51.6	1.5	103
			104 ± 3.7

Stability of sulphathiazole

Sulphathiazole remains stable for at least one month in human plasma if the samples are stored at —20° C.

Specificity

Using different columns for plasma and urine, the components of these biological materials do not interfere in the assay of sulphathiazole (Fig. 1 and 2). The method is specific in presence of sulphadiazine, sulphamerazine and sulphapyridine. The metabolite acetyl-sulphathiazole is suitably separated from the parent drug (Fig. 3). However, the method is not reliable for the simultaneous determination of sulphathiazole and its acetylated metabolite.

Application

The method described above was applied to plasma and urine samples from two subjects given orally one experimental tablet of Formo-Cibazol® containing 1 g of formo-sulphathiazole. The results are shown in Table III.

CONCLUSION

The sensitivity of the method permits the determination of sulphathiazole in plasma and urine at the concentrations generally found in pharmacokinetic studies in animals [9, 10] and man.

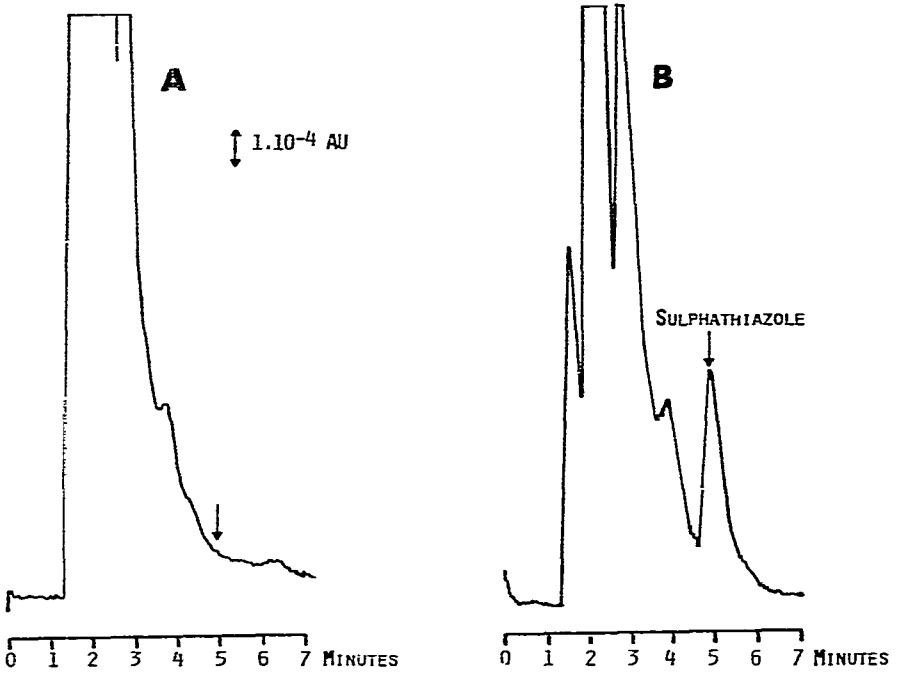

Fig. 1. Chromatograms of blank plasma (A) and human plasma containing 1600 ng/ml sulphathiazole (B).

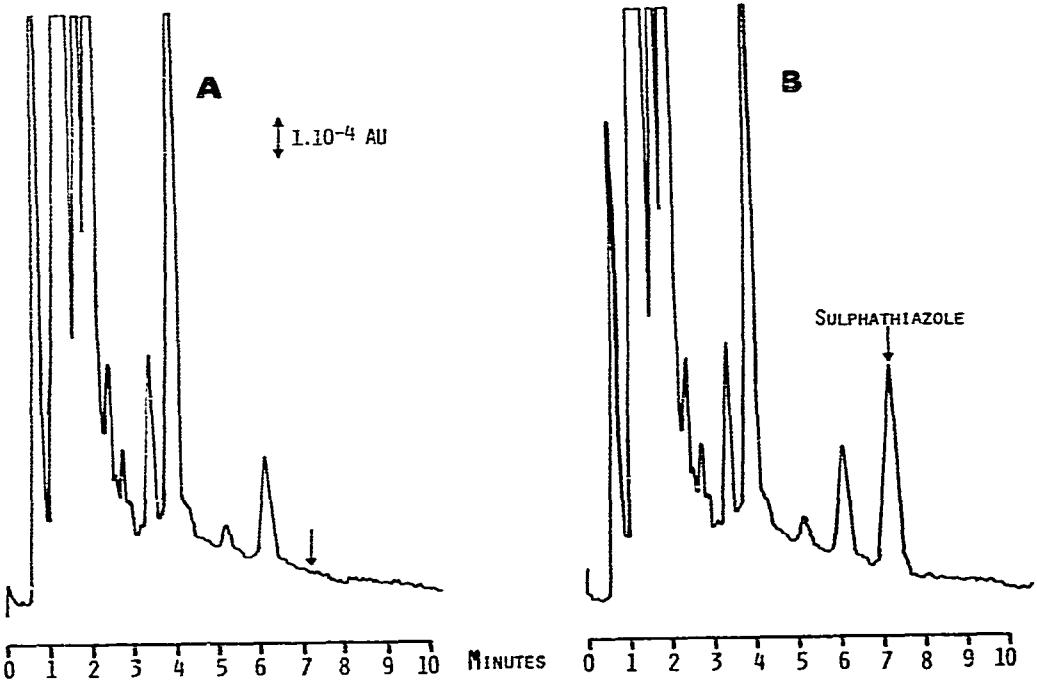

Fig. 2. Chromatograms of blank human urine (A) and human urine containing 20 μg/ml sulphathiazole (B).

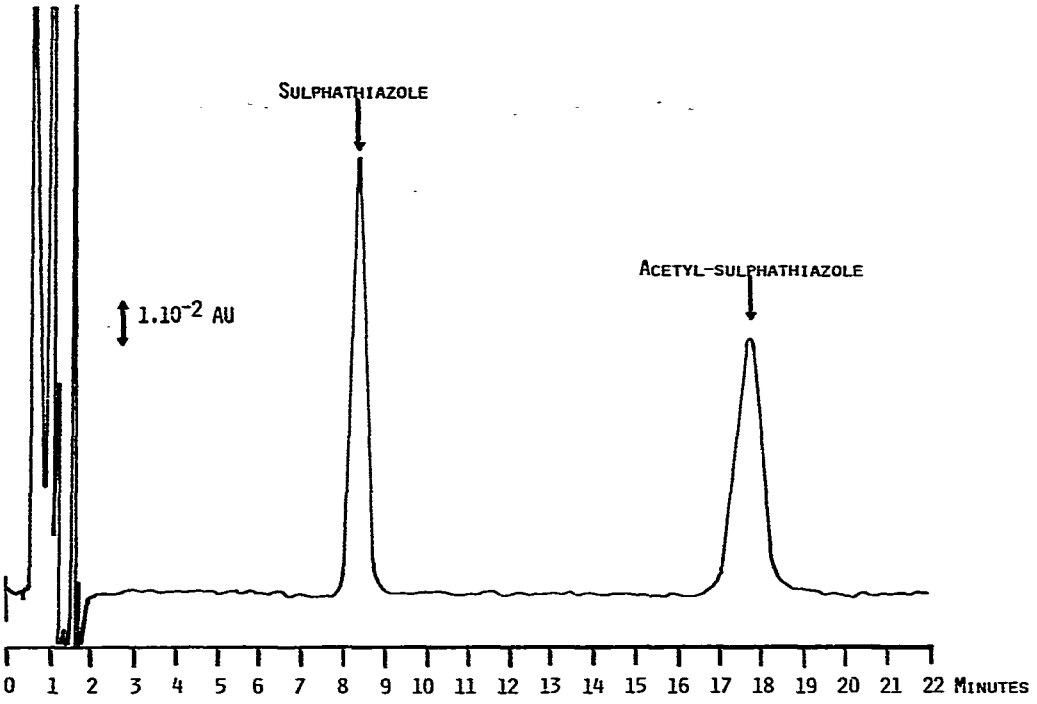

Fig. 3. Chromatogram of an aqueous solution of sulphathiazole and acetyl-sulphathiazole (chromatographic conditions described for urine). Each peak represents 20 μg of each synthetic compound.

TABLE III

PLASMA CONCENTRATIONS AND URINARY EXCRETION OF SULPHATHIAZOLE IN TWO SUBJECTS AFTER THE ORAL ADMINISTRATION OF ONE 1-g TABLET OF FORMO-SULPHATHIAZOLE

Time (h)	Sulphathiazole plasma concentrations (ng/ml)		Intervals of time (h)	Sulphathiazole cumulative urinary excretion (% of the dose)	
	Subject 1	Subject 2		Subject 1	Subject 2
0	ND*	ND*	0—8	0.18	0.42
0.5	122	129	8—24	0.41	0.27
1	223	251			
2	252	342	0—24	0.59	0.69
4	266	357			
6	216	114			
24	346	198			

*ND = Not detected.

REFERENCES

1 O. Gyllenhaal and H. Ehrsson, J. Chromatogr., 107 (1975) 327.
2 N. Nose, S. Kobayashi, A. Hirose and A. Watanabe, J. Chromatogr., 123 (1976) 167.
3 J.P. Sharma, E.G. Perkins and R.F. Bevill, J. Pharm. Sci., 65 (1976) 1606.
4 K. Lanbeck and B. Lindström, J. Chromatogr., 154 (1978) 321.
5 C. Fischer and U. Klotz, J. Chromatogr., 146 (1978) 157.
6 T.B. Vree, Y.A. Hekster, A.M. Baars, J.E. Damsma and E. Van der Kleyn, J. Chromatogr., 146 (1978) 103.
7 T.J. Goehl, L.K. Mathur, J.D. Strum, J.M. Jaffe, W.H. Pitlick, V.P. Shah, R.I. Poust and J.L. Colaizzi, J. Pharm. Sci., 67 (1978) 404.
8 P.H. Cobb and G.T. Hill, J. Chromatogr., 123 (1976) 444.
9 R.F. Bevill, G.D. Koritz, L.W. Dittert and D.W.A. Bourne, J. Pharm. Sci., 66 (1977) 1297.
10 D.W.A. Bourne, L.W. Dittert, G.D. Koritz and R.F. Bevill, J. Pharmacokinet. Biopharm., 6 (1978) 123.