Journal of Chromatography, 224 (1981) 513—518
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 897

Note

High-performance liquid chromatographic determination of free resorcinol in plasma and in urine

DAVID YEUNG*, SERGIO NACHT and EUGENE H. GANS

Vick Divisions Research and Development, Richardson-Merrell Inc., One Bradford Road, Mt. Vernon, NY 10553 (U.S.A.)

(First received December 29th, 1980; revised manuscript received March 20th, 1981)

Resorcinol is used in some topical antiacne preparations to produce keratolysis. Because the agent can penetrate human skin [1], this study was done in order to develop a suitable method for the analysis of free (unchanged) resorcinol in plasma and in urine. Such a method was considered to be essential to studies of the absorption and subsequent metabolic disposition of topically-applied resorcinol.

Previously reported techniques for the determination of resorcinol [2—4] lack specificity and sensitivity; in particular, the high-performance liquid chromatographic (HPLC) procedure for this agent in pharmaceutical dosage forms [5] is not sensitive enough for pharmacokinetic studies.

This paper describes a HPLC technique, which requires only a relatively simple extraction procedure. It permits sensitive and speedy determination of free resorcinol in human plasma and in urine at concentrations as low as 0.5 μg/ml. Recoveries are more than 90% of theoretical, with good reproducibility. So far as we know, this is the first report of an assay for resorcinol in biological fluids.

EXPERIMENTAL

Materials

Analytical grades of resorcinol and orcinol (internal standard) were obtained from Sigma (St. Louis, MO, U.S.A.). Acetonitrile, HPLC grade, was obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). Glass-distilled water was filtered through Type EG 0.22 micrometer filter paper (Millipore, Bedford, MA, U.S.A.) before use. All other chemicals and solvents used were ACS grade or better.

0378-4347/81/0000—0000/$02.50

Apparatus

A high-performance liquid chromatograph (Waters Assoc., Milford, MA, U.S.A.) composed of a M6000 pump, a Model 450 variable-wavelength UV detector, and a U6K injector was used. A WISP (Waters Assoc.) automatic sample injection system was used in the analysis of large numbers of samples. Chromatography was performed on a 250 mm × 4.6 mm I.D., 5-μm particle size, Zorbax ODS C_{18} reversed-phase column (Dupont, Wilmington, DE, U.S.A.), preceded by an Altex 5 cm × 2.1 mm ODS pre-column. All analyses were performed isocratically at a flow-rate of 1.5 ml/min, with a mobile phase consisting of 15% acetonitrile in a pH 6.6 phosphate buffer. The detector wavelength was set at 280 nm. A Hewlett-Packard 5830A electronic integrator was used for data processing.

Extraction procedure

The plasma or urine sample (1.0 ml) was pipetted into a 16 × 125 mm culture tube provided with a PTFE-lined screw cap. Five ml of diethyl ether containing a known amount of internal standard (orcinol) were added. The closed tube was shaken for 3 min and then centrifuged at 2000 rpm for 3 min. The organic layer was transferred into another tube and the aqueous layer was again washed with 5 ml of diethyl ether. The pooled organic extracts were evaporated to dryness at 30°C under a gentle stream of nitrogen. The residue was taken up into 1 ml of mobile phase and 20 μl were injected into the liquid chromatograph.

Calibration and recovery

Calibration curves were constructed with plasma and urine samples containing added concentrations (0.5—10.0 μg/ml) of resorcinol. To all samples, 5 μg of orcinol were added as internal standard and the samples then were extracted and analyzed as described. The ratio of the height of resorcinol peak to the height of orcinol peak was plotted against the concentration of resorcinol. The concentrations of unknown samples were subsequently determined from the standard curve.

The efficiency of the extraction procedure was assessed by adding known amounts (0.5—10 μg) of resorcinol to 1.0-ml aliquots of plasma and urine. The samples were extracted as previously described and reconstituted into 1 ml of the mobile phase containing 5 μg of orcinol as internal standard.

Recovery and reproducibility

Good recovery and reproducibility of this assay were confirmed by replicate analyses of plasma and urine samples fortified with 1.0 and 5.0 μg/ml of resorcinol, respectively.

RESULTS AND DISCUSSION

Various chromatographic conditions (different columns and mobile phases) for the analysis of resorcinol in plasma and urine were investigated. Under the experimental conditions described, no interferences from the endogenous constituents of plasma or urine were observed. Chromatograms obtained

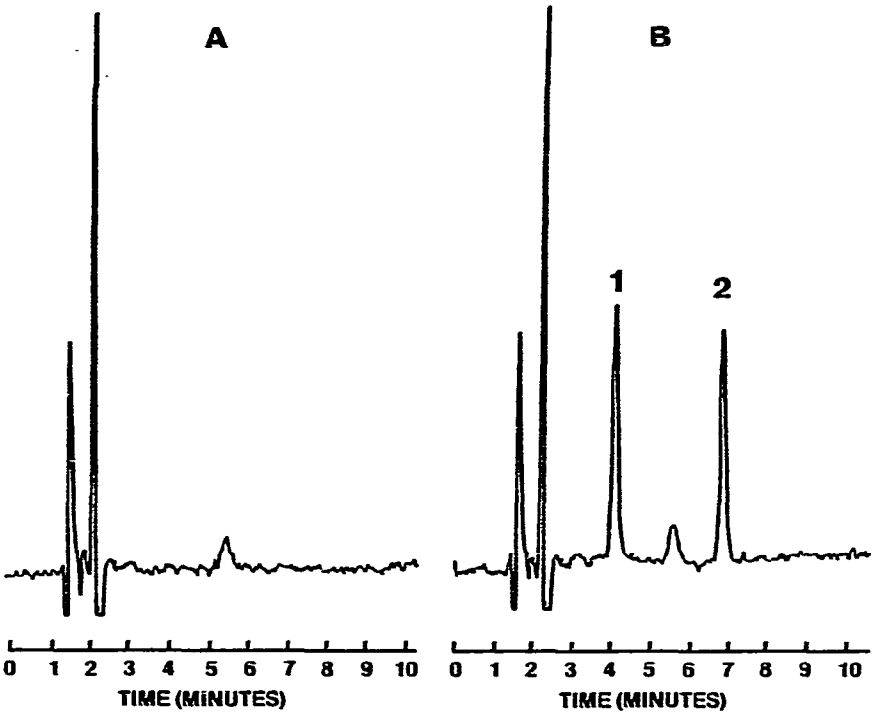

Fig. 1. Chromatograms of (A) blank plasma and (B) plasma spiked with 1.0 μg/ml resorcinol and orcinol (internal standard). Peaks: 1 = resorcinol; 2 = orcinol.

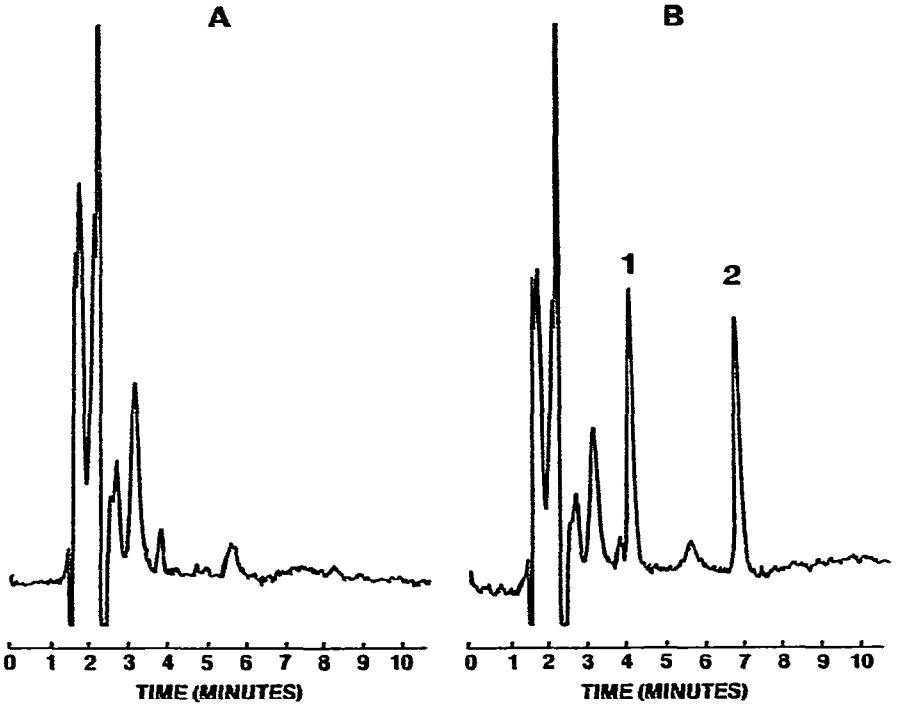

Fig. 2. Chromatograms of (A) blank urine and (B) urine spiked with 1.0 μg/ml resorcinol and orcinol (internal standard). Peaks: 1 = resorcinol; 2 = orcinol.

from the analysis of plasma and urine blanks, as well as those from samples spiked with resorcinol and orcinol, are shown in Figs. 1 and 2. These compounds were eluted with retention times of 4.19 and 7.04 min, respectively. The total time required for the analysis of each sample was 10 min.

The resorcinol calibration curves in plasma and urine were linear over the concentration range studied. Least-square regression analysis of the data resulted in good linearity in the range of 0—10 μg/ml with correlation coefficients of 0.9999 for plasma and 0.9993 for urine. The limit of detection of resorcinol with this method is 0.5 μg/ml.

The efficiency of the extraction procedure was confirmed by analyzing plasma and urine samples spiked with known amounts of resorcinol, ranging in concentrations from 0.5—10 μg/ml. Five replicates at each concentration were analyzed. As shown in Table I, the recovery of resorcinol in plasma throughout the concentration range studied varied from 87.6—98.3%, with

TABLE I

RECOVERY OF RESORCINOL FROM SPIKED BIOLOGICAL FLUIDS

Five trials for each level of resorcinol.

Biological fluid	Amount of resorcinol spiked (μg/ml)		Recovery (%) (Mean ± S.D.)	Coefficient of variation (%)
Plasma	0.5		87.6 ± 2.08	2.37
	1.0		94.3 ± 5.28	5.60
	2.0		93.9 ± 4.58	4.88
	3.0		98.3 ± 3.11	3.16
	5.0		96.3 ± 2.25	2.34
		Mean	94.1	3.67
Urine	0.5		89.6 ± 1.82	2.03
	1.0		88.8 ± 10.03	11.30
	2.0		96.0 ± 1.76	1.83
	3.0		98.0 ± 3.35	3.42
	5.0		94.6 ± 2.85	3.01
		Mean	93.4	4.32

TABLE II

REPRODUCIBILITY OF THE ANALYSIS

Sample	No. of trials	Conc. of resorcinol (μg/ml)	Standard deviation	Coefficient of variation (%)
Plasma	8	1.0	±0.019	2.4
	8	5.0	±0.088	3.9
			Mean	3.2
Urine	9	1.0	±0.029	4.0
	7	5.0	±0.056	2.5
			Mean	3.3

TABLE III

PLASMA AND URINARY LEVELS OF RESORCINOL OF THREE SUBJECTS AFTER 2 WEEKS OF DAILY TOPICAL RESORCINOL ADMINISTRATION (800 mg/day)

Subject	Plasma	24-h urine	
		mg	% dose
Untreated Control	0*	0	0
1	0	3.75	0.47
2	0	12.65	1.58
3	0	22.98	2.87

*Less than 0.5 μg/ml.

a mean recovery of 94.1%. In urine, the recovery ranged from 88.8—98.0%, with a mean recovery of 93.4%.

The mean measured concentration, standard deviation and coefficient of variation of the assay are shown in Table II. In plasma, the coefficient of variation was 2.4% at the lower concentration of 1.0 μg/ml, and 3.9% at 5.0 μg/ml. In urine, the coefficients of variation for samples of low and high resorcinol concentration were 4.0 and 2.5%, respectively.

Biological results

The percutaneous absorption and metabolic disposition of resorcinol was investigated following highly exaggerated repeated topical application of a 2% resorcinol in a hydroalcoholic vehicle to three volunteers. After 2 weeks of treatment, an average of 1.64% (range 0.47—2.87%) of the administered dose was excreted in 24-h urines (Table III). No resorcinol could be detected in any of the blood samples collected after 1, 2, 3 and 4 weeks of drug application.

CONCLUSION

The method described is simple, rapid, and specific; it has good recovery for the determination of free resorcinol in plasma and in urine. Moreover, the assay is sensitive — the detection limit is approximately 0.5 μg/ml — and reproducibility is very acceptable. In contrast, the more commonly used technique of deproteinization of plasma with acetonitrile, followed by the direct analysis of the filtrate, results in peaks with retention times similar to that of resorcinol, which interfere with the analysis. Even greater sensitivity might be achieved with this method by increasing the volume of plasma or urine extracted or by increasing the injection volume. The nondestructive nature of this technique allows the isolation and subsequent identification of free resorcinol and its metabolites in animal or human pharmacokinetics studies.

ACKNOWLEDGEMENT

The authors thank Mr. M.C. Tillery, Manager of R&D Publications, for editorial assistance.

REFERENCES

1 M.S. Roberts, R.A. Anderson and J. Swarbrick, J. Pharm. Pharmacol., 29 (1977) 677.
2 P. Roza, Acta Pharm. Hung., 37 (1967) 145.
3 D.C. Garrott, The Quantitative Analysis of Drugs, 3rd ed., Chapman and Hall, London, 1964, pp. 513.
4 V. Das Gupta and L.A. Cates, J. Pharm. Sci., 63 (1974) 93.
5 V. Das Gupta, J. Pharm. Sci., 65 (1976) 1707.