Journal of Chromatography, 525 (1990) 495–497
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 5053

Letter to the Editor

High-performance liquid chromatographic assay of rifampicin in human serum

Sir,

Rifampicin is widely used in the treatment of tuberculosis [1]. There has been an increasing demand for a suitable method for the assay of rifampicin in the serum of patients taking this drug. However, at present only a few assay methods of rifampicin using high-performance liquid chromatography (HPLC) are available [2,3] and these involve cumbersome and time-consuming extraction procedures. We thought that the assay could be simplified and improved by the use of a new extraction procedure. This led us to establish this fast, simple, selective and sensitive method for the assay of rifampicin in biological samples.

Rifampicin was obtained from the United States Pharmacopeia (USP) as a reference standard (Rockville, MD, U.S.A.). Methanol (HPLC grade) was obtained from J.T. Baker (Phillipsburg, NJ, U.S.A.). Ammonium formate (G.R. grade) was obtained from Loba-Chemie (Bombay, India). All other chemicals were of analytical-reagent grade. A stock standard solution (1 mg/ml) of rifampicin was prepared in methanol, freshly on the day of analysis. A 20-μl volume of stock standard solution was added to 980 μl of drug-free serum and 200 μl of this solution were used as a standard specimen and treated in the same way as described in the experimental procedure below.

The procedure was as follows. A 200-μl volume of serum containing rifampicin was placed in an Eppendorf microcentrifuge tube (1.5-ml capacity) and 300 μl of acetonitrile were added, followed by mixing on a vortex mixer for 1 min prior to centrifugation at 1130 g for 3 min at 25°C. A 25-μl volume of the organic layer was injected into the HPLC system (Waters Assoc., Milford, MA, U.S.A.), consisting of a Model 6000A pump, a Model U6K universal injector with capacity for injection volumes from 2 to 2000 μl, a Lambda Max-481 variable-wavelength UV detector and stainless-steel column (30 cm $\times$ 3.9 mm I.D.) of μBondapak C_{18} (10 μm). A guard column was used (Waters Assoc., Part No.

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84550) to prevent obstruction by minute particles. Rifampicin was eluted with methanol–0.05 *M* ammonium formate (65:35, v/v) at pH 7.3 with a flow-rate of 1.6 ml/min. Samples were injected into the column and the detector was set at 254 nm with attenuation 2.0. The retention time of rifampicin was 3.4 min under these conditions. The results were calculated with a Waters Assoc. Model 745 data module.

The calibration graph for rifampicin was linear over the range 0.8–80 μg/ml. Linear regression analysis gave a correlation coefficient (r) of 0.9990, indicating excellent agreement. The method was found to be precise and selective. The inter-assay precision of the method was assessed by analysis of serum samples containing 5 μg/ml rifampicin. The inter-assay coefficient of variation of rifampicin was 5.5% for 5 μg/ml and 5.0% for 10μg/ml ($n=10$).

Known amounts of rifampicin were mixed with drug-free serum, then the compound was isolated as described above. Fig. 1 shows the HPLC elution pattern of rifampicin in human serum.

We found that another antituberculosis drug, isoniazid [1], did not interfere with the assay of rifampicin, even if they were administered together.

Using the above method, we measured the rifampicin levels in serum from ten healthy human subjects given a single dose of 600 mg of rifampicin orally. The blood samples were collected via an intravenous canula at various time intervals as shown in Table I. The maximum concentration of rifampicin in serum was 11.17±5.66 μg/ml 1.5 h after administration of the drug.

This method has several advantages over the previously reported HPLC

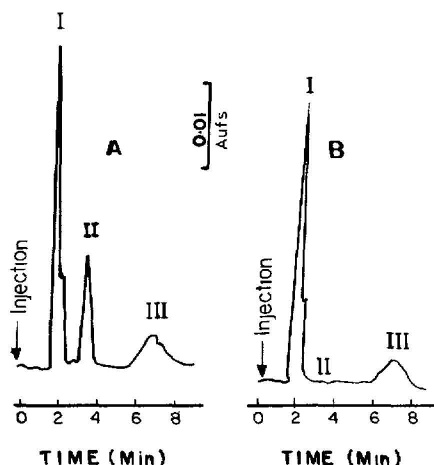

Fig. 1. (A) Chromatogram of rifampicin in human serum. Each of the injection volumes (25 μl) contained 0.2 μg of rifampicin at attenuation 2. Peak II represents rifampicin with a retention time of 3.4 min and peaks I and III represent unknown materials in the serum. (B) Chromatogram of serum from an untreated subject where peaks I and III represent the same unknown materials as in (A).

TABLE I

HUMAN SERUM LEVELS OF RIFAMPICIN AFTER A SINGLE ORAL DOSE OF 600 mg OF RIFAMPICIN

Time of blood collection (h)	Concentration (mean ± S.D., $n=10$) (μg/ml)	Time of blood collection (h)	Concentration (mean ± S.D., $n=10$) (μg/ml)
0.00	0.00 ± 0.00	3.00	9.17 ± 4.68
0 25	0.00 ± 0.00	4.00	7.55 ± 3.54
0.50	1.72 ± 3.00	5.00	6.92 ± 3.90
0.75	5.87 ± 5.40	7.00	6.46 ± 3.66
1.00	8.43 ± 7.15	8.00	6.45 ± 3.48
1.50	11.17 ± 5.66	12.00	2.97 ± 2.70
2.00	10 39 ± 4.90	24.00	0.00 ± 0.00

methods for rifampicin [2,3]. The sample preparation technique is very fast and simple, the method is accurate and the chromatographic peaks of rifampicin are very sharp in comparison with those of previous methods [2,3].

Rifampicin is about 80% bound to serum protein, so the drug was freed from serum protein by protein precipitation with acetonitrile. The recovery of rifampicin for the assay was 95.5 ± 4.40% ($n=5$). The recovery of the drug from the column was nearly 100% as assessed by using known amounts of rifampicin. The selectivity of this method was such that no endogenous materials from the biological samples interfered with the assay of rifampicin. This assay technique could be useful for the measurement of rifampicin levels in biological samples, such as serum, urine and pharmaceutical preparations.

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(First received April 17th, 1989; revised manuscript received September 21st, 1989)