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Journal of Chromatography B, 681 (1996) 412–415

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Short communication

# High-performance liquid chromatography assay of rifapentine in human serum

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Received 20 September 1995; revised 28 December 1995; accepted 8 January 1996

**Abstract**

A high-performance liquid chromatographic method for the determination of rifapentine in human serum was developed. The method utilized a Spherisorb $C_{18}$ column, ultraviolet detection (336 nm), rifampin as internal standard and a calibration curve ($C=7.010\ A_s/A_{in}\pm0.156$, $r=0.999$) with reproducibility studies which yield a coefficient of variation (C.V.) of intra-day and inter-day assays lower than 10%. The average recovery of rifapentine from serum in the concentration range of 0.5 to 30 $\mu$g/ml was 92.93±9.704%.

*Keywords:* Rifapentine

## 1. Introduction

Rifapentine is a semisynthetic antibiotic belonging to the rifamycin group. It has an antibacterial spectrum similar to rifampicin but its antituberculosis power is nine-fold stronger than that of rifampicin, its orally toxic reaction is lower and its half-life time is longer. Because of its strong bacteriostatic activity to *Staphylococcus aureus*, it has also been used for some tolerant *S. aureus* infections recently and has great potential.

There has been an increasing demand for a suitable method for the assay of rifapentine in the serum of patients taking this drug. However, the usual microbiological assay of rifapentine is not sensitive and specific. This prompted us to establish this simple, sensitive, efficient method for the assay of rifapentine in biological samples with reference to some articles on assaying other rifampicins [1–3].

## 2. Experimental

### 2.1. Materials

Rifapentine standard was obtained courtesy of China Pharmaceutical University. Rifampin (China Pharmaceutical University) was used as internal standard (I.S.). Other solvent reagents were of analytical grade.

### 2.2. Analytical instruments

The high-performance liquid chromatographic (HPLC) system used consisted of a pump (Shimadzu LC-6A, Shimadzu, Kyoto, Japan), a column packed

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0378-4347/96/$15.00 
*PII* S0378-4347(96)00021-7

Table 1
Extraction recovery of rifapentine

| Added ($\mu$g/ml) | Found ($\mu$g/ml) | Recovery (%) |
|---|---|---|
| 0.5 | 0.409±0.026 | 81.8 |
| 5.0 | 4.87 ±0.13 | 97.4 |
| 10.0 | 9.96 ±0.28 | 99.6 |

with Spherisorb $C_{18}$ (5 $\mu$m, 250×4.6 mm), a column oven (Shimadzu CTO-6A) operated at 28°C, a spectrophotometric detector (Shimadzu SPD-6AV) operated at 0.02 AUFS and at a wavelength of 336 nm and an integrator (Shimadzu CR-4A Chromatopac).

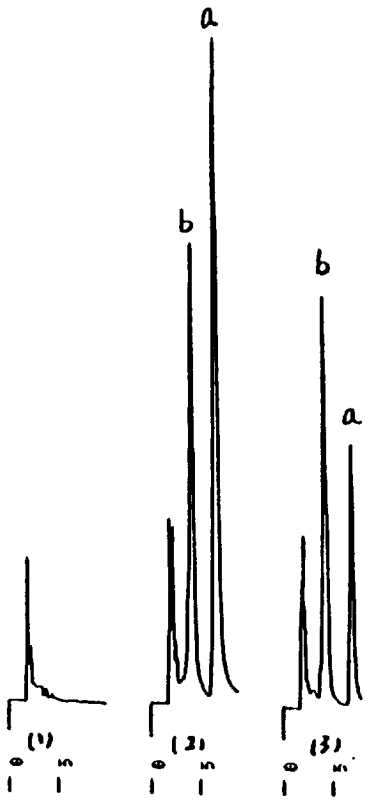


Fig. 1. Chromatogram: (1) HPLC trace of a blank serum extract; (2) standard serum solution containing rifapentine and rifampin; (3) serum extract from a healthy volunteer receiving 450 mg of rifapentine. Peaks: (a) rifapentine; (b) rifampin (I.S.).

### 2.3. Mobile phase

The mobile phase consisted of 0.01 mol/l $NaH_2PO_4$ (pH 5.5) and methanol (32:68, v/v) and was pumped at a constant flow-rate of 1 ml/min.

### 2.4. Extraction and assay procedures

The sample for analysis was prepared as follows: An aliquot of 1 ml of sample serum was placed in a 10-ml tube, followed by 400 $\mu$l phosphate buffer (1 mol/l, pH 4.0), 40 $\mu$l ascorbic acid solution (10 mg/ml) and 50 $\mu$l rifampin solution (100 $\mu$g/ml) as an I.S. After vortexing for 10 s, 4 ml ethyl acetate was added to the tube. The mixture in the tube was again vortex-mixed for 5 min, then centrifuged at 1800 *g* for 10 min; 3 ml of the upper aqueous phase were transferred to a new tube and evaporated under a stream at 100°C. The residue was reconstituted with 500 $\mu$l methanol, vortex-mixed for 1 min and a 20-$\mu$l aliquot of the solution was injected onto the HPLC system.

### 2.5. Calibration standard

Standard solution of rifapentine was prepared by weighing 10.0 mg rifapentine accurately, transferring

Table 2
Intra-day reproducibility of the assay

| Concentration ($\mu$g/ml) | $A_s/A_{in}$ ($n$=5) | C.V. (%) ($n$=5) |
|---|---|---|
| 0.5 | 0.0549±0.00335 | 6.11 |
| 5.0 | 0.6858±0.0189 | 2.75 |
| 10.0 | 1.406 ±0.041 | 2.92 |

Table 3
Inter-day reproducibility of the assay

| Concentration ($\mu$g/ml) | $A_s/A_{in}$ ($n$=5) | C.V. (%) ($n$=5) |
|---|---|---|
| 0.5 | 0.464 ±0.0040 | 8.62 |
| 5.0 | 0.7017±0.319 | 4.55 |
| 10.0 | 1.544 ±0.144 | 9.33 |

it to a volumetric flask, dissolving it in 1 ml ethanol and adding water to 100 ml to produce a 100 $\mu$g/ml stock solution. A 10.0-mg amount of rifampin was also accurately weighed, transferred to a 100-ml volumetric flask, dissolved in 1 ml ethanol and added with water to 100 ml to produce a 100 $\mu$l/ml stock solution (I.S.). Both stock solutions were stable for at least one month at −20°C. Control blank serum samples were spiked with rifapentine in the concentration range of 0.5 to 30.0 $\mu$g/ml and subjected to the treatment procedure described above. Peak-area ratios of rifapentine/rifampin ($A_s/A_{in}$) versus the concentration of rifapentine ($\mu$g/ml) were subjected to linear regression. The concentration of rifapentine in samples was then obtained from the regression equation.

### 2.6. Samples from healthy volunteers

Blood (2 ml) was sampled in a heparinized dry tube with 40 $\mu$l ascorbic acid solution (10 mg/ml) to prevent oxidation of rifapentine. The samples were obtained from 10 healthy volunteers by oral administration of 450 mg of rifapentine. The samples were centrifuged for 5 min at 1800 *g* and treated according to the method described above.

## 3. Results and discussion

A standard calibration curve was constructed by plotting the peak-area ratios of rifapentine/rifampin ($A_s/A_{in}$) against the concentration of rifapentine. A linear regression analysis revealed a standard curve of $C=7.010\ A_s/A_{in}\pm0.156$, $r=0.999$.

The absolute recovery of rifapentine was calculated by comparing the peak area obtained from the theoretical amount of rifapentine with that obtained after injection of the extracts from the serum samples. Table 1 shows that the average recovery of rifapentine was 92.93±9.704%.

Fig. 1 shows a chromatogram of rifapentine and rifampin. Fig. 1(1,2) shows that the serum components did not interfere with rifapentine and the rifampin (I.S.) and Fig. 1(3) shows a chromatogram of serum extracted from a healthy volunteer treated with rifapentine. The retention times of rifapentine and rifampin were 6.83 min and 4.33 min, respectively.

Table 2 and Table 3 show the good reproducibility and accuracy of the assay method. The C.V. was less than 10% for both inter- and intra-day assays for rifapentine. The low limit quantitation of rifapentine was 5 ng.

The method described was applied to the de-

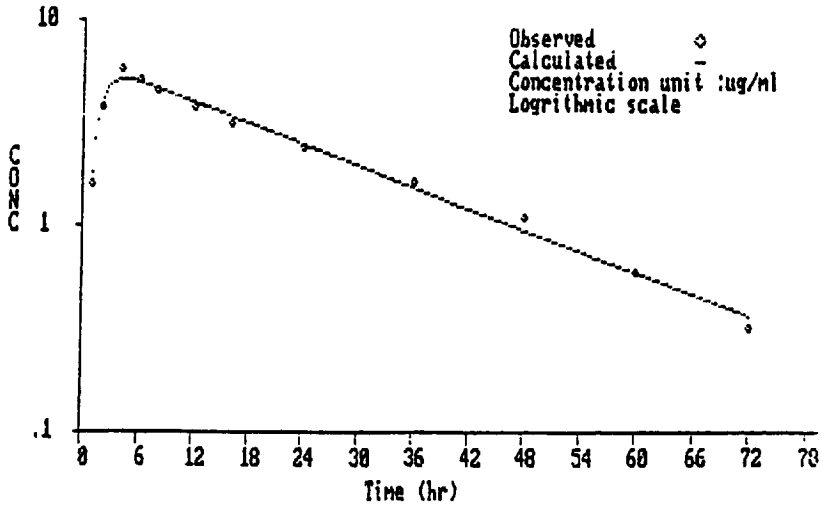


Fig. 2. Concentration of rifapentine in serum after oral administration of 450 mg rifapentine capsules.

termination of rifapentine in the serum of healthy volunteers following oral administration of 450 mg of rifapentine. Fig. 2 shows the serum level determined. Its $t_{1/2}=17.1\pm1.8$ h, $C_{max}=5.07\pm1.82$ $\mu g/ml$ and $t_{max}=4.53\pm0.83$ h.

Rifampicins are easily bound to serum protein [1] so the rifapentine was freed from serum protein by protein precipitation with ethyl acetate. The average recovery of rifapentine was nearly $92.93\pm9.704\%$ ($n=5$). The rifampicins are easily oxidized [2] so ascorbic acid was added to the sample to protect rifapentine from oxidative degradation in order to raise the recovery. Rifampin is a suitable I.S. because of its good separation from the rifapentine, sharp chromatographic peak and the short retention time (4.33 min).

This method has several advantages over the usual microbiological assay of rifapentine [4,5]. The sample preparation technique is very fast and simple and the method is accurate. This assay technique could be utilized for measuring rifapentine in serum and is also suitable for pharmacokinetic studies of rifapentine.

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