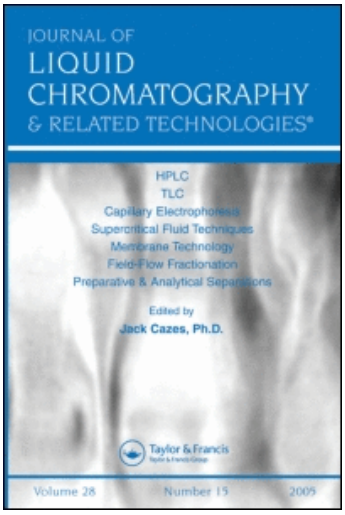

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REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF RIFAMPIN IN THE PRESENCE OF ITS ACID-INDUCED DEGRADATION PRODUCTS

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ABSTRACT

The determination of rifampin in the presence of its acid-induced degradation products by reversed-phase high performance liquid chromatography is described. The method was validated as stability-indicating by forced decomposition of rifampin in acid media. Chromatographic conditions included a Nova-Pak® C18 (3.9 x 150 mm, 5 μm) column and a mobile phase consisting of 0.02M Na_2HPO_4 and acetonitrile (65:35 v/v), apparent pH 4.5 ± 0.2 adjusted by the addition of phosphoric acid. All analyses were done under isocratic conditions at a flow rate of 1 mL/min and at room temperature. The recovery average was 99.39% for rifampin. The method was applied to determine rifampin in commercial capsules and compared with the official method of the USP XXIII with good agreement between the results.

INTRODUCTION

Rifampin is an antibiotic used orally in the treatment of tuberculosis.[1] The main reported impurities of rifampin are 3-formyl rifampin which is formed under acidic conditions, and rifampin quinone formed under alkaline conditions.[2,3] Some analytical methods have been reported for the determination of rifampin including spectrophotometry[4,5] and thin layer chromatography.[6] Some of the reported methods are claimed to be stability-indicating.[5,6]

High performance liquid chromatography (HPLC) has been used for the determination of rifampin and its metabolites in biological fluids.[7,8,9] In this work, a reversed-phase HPLC analytical method, which uses very simple conditions, is described for the determination of rifampin in the presence of its acid-induced degradation products.

The method also permitted the determination of rifampin in pharmaceutical preparations containing isoniazid.

EXPERIMENTAL

Apparatus

HPLC separations were made on a system consisting of a CG solvent delivery pump (Model 480-C) and a CG variable UV detector set at 254 nm connected to a CG integrator (Model CG-200) (Instrumentos Científicos CG Ltda, São Paulo, Brasil). The system was equipped with a Rheodyne 7125 injection valve fitted with a 20 μL loop.

Reagents and Solutions

All reagents and solvents were of analytical grade. Acetonitrile used in the mobile phase was HPLC grade. Distilled water was purified using a Milli-Q system (Millipore, Milford, MA, USA). Solutions and mobile phase were prepared in the moment of use and all solvents and solutions for HPLC analyses were filtered through a membrane filter (Millipore® Durapore hydrophobic filtration membrane, 0.22 μm pore size) and vacuum degassed before use.

Rifampin was kindly donated by a pharmaceutical company and used as standard without further purification. Rifampin standard was dried for 24 h over silica gel before use.

Chromatographic Conditions

The mobile phase used was 0.02 M Na_2HPO_4 and acetonitrile (65:35 v/v) with apparent pH 4.5 adjusted by the addition of phosphoric acid. The analytical column was a Waters Nova-Pak® C18 (3.9 x 150 mm, 5 μm) column. All analyses were done under isocratic conditions at a flow rate of 1 mL/min and at room temperature.

Calibration Curve

Solutions ranging from 2.5 to 120 μg/mL of rifampin were prepared in the mobile phase from a methanolic stock solution of rifampin standard. The calibration curve was constructed by plotting the peak areas against the concentration of rifampin in μg/mL.

Sample Preparation

Commercial capsules were emptied in a mortar and an amount of powder equivalent to 100 mg of rifampin was weighed out into a 100-mL amber volumetric flask. About 50 mL of methanol were added, the flask was placed in an ultrasonicator for 5 min, and then the volume was made up with the same solvent. After filtration through a Whatman no.1 paper filter, a 10-mL aliquot of that solution was transferred to a 50-mL amber volumetric flask and diluted to volume with water. This solution was filtered through a Millipore membrane filter (0.22 μm pore size) and a 5-mL aliquot of the filtered solution was transferred to a 25-mL volumetric flask and diluted to volume with the mobile phase. This solution was injected into the HPLC system.

Commercial rifampin capsules were assayed by the developed HPLC method and by the USP XXIII method as a reference method. Commercial capsules containing rifampin in combination with isoniazid were also analysed by the proposed HPLC method to assess the possible interference of isoniazid in the determination of rifampin.

Recovery of Rifampin in Commercial Samples

The pre-analysed commercial samples (25 μg/mL) were spiked with known concentrations of standard rifampin and subjected to the described HPLC method. Small aliquots (1.0, 2.0, and 3.0 μg/mL) were employed in the recovery study in order to assess the sensitivity of the method to the reduction in the amount of rifampin during the degradation process.

Table 1

Data Obtained in the Recovery Test

Amount of Standard Added (μg/mL)	Average Recovery (%)*
1.0	99.88
2.0	99.12
3.0	99.19

* Each value reported is the mean of three determinations.

Stability-Indicating Validation

The HPLC method was validated as stability indicating by forced degradation of rifampin. Samples were prepared in HCl 0.1 M and placed in a water-bath maintained at 30°C for 60 min in order to obtain the acid-induced degradation products. Kinetic studies were carried out in bulk rifampin to assess the applicability of the method and it was based on the observation of the decreasing concentration of the non-degraded rifampin with time. The kinetic study, using the isothermal stress approach at 52°C, was performed in buffer solutions of pH 1.7 and 5.7. The choice of the stressing temperature used in the experiment was a compromise between time and amount of degradation.

RESULTS AND DISCUSSION

The precision of the results, reported as the relative standard deviation (RSD%), was of 0.73% as determined on 10 replicate injections of an artificial sample. The average recovery was 100.28% and the 95% confidence interval was ± 0.52%. The linearity of response was evaluated by regression analysis and the regression equation ($Y = 11783X - 616$) presented a correlation coefficient of 0.9999. The percentage recovery results are shown in Table 1.

Commercial rifampin capsules were assayed by the developed HPLC method and by the USP XXIII method as a reference method. The results (Table 2) indicated good agreement between the results.

Isoniazid is often coformulated with rifampin in pharmaceutical preparations. This substance was scarcely retained (Rt = 1.08 min) by the reversed-phase system due to its hydrophilic character, and it did not interfere with the analysis of rifampin under the described experimental conditions.

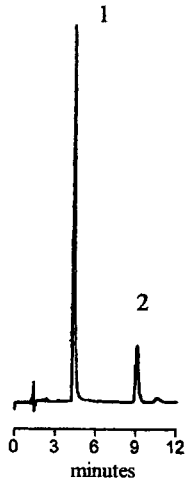

Figure 1. Chromatogram of (1) rifampin and (2) acid-induced degradation product. Chromatographic conditions: Waters Nova-Pack C18 (3.9 x150 mm, 5 µm) column; 0.02 M Na_2HPO_4 : acetonitrile (65:35 v/v) as mobile phase; flow rate of 1 mL/min.

Table 2

Data Obtained from Rifampin Analysis in Commercial Capsules

Sample	Percentage of the Declared Amount* New HPLC Method	USPXIII Method
Commercial Capsules	97.5%	96.98 %

* Each value reported is the mean of three determinations.

Stability investigations were conducted to assess the method specificity for the assay of rifampin without interference from the acid-induced degradation products. The forced degradation experiment described previously yielded a reduction in intact rifampin (retention time ~ 4.5 min) with the formation of a new peak eluting at about 9.0 min (Figure 1).

In the kinetic studies a regular decrease in the concentration of intact rifampin with increasing time intervals was observed and the logarithm of percentage of residual concentration of rifampin against time were plotted.

Table 3

Results of Kinetic Studies of Bulk Rifampin Degradation at 52°C

pH	k x 10^{-2} min^{-1}	Correlation Coefficient (r)
1.7	56.43	0.9955
5.7	6.87	0.9901

k = degradation reaction rate constant.

The linear correlation observed ($r \geq 0.99$) indicated that the degradation rate of rifampin was first-order in all cases (Table 3). The results proved that the proposed HPLC method was specific for determining rifampin without interference from its acid-induced degradation products.

CONCLUSION

The HPLC method described in this work is simple, precise, accurate, and it is useful for stability studies of rifampin. The method is also suitable as an alternative to the official method (USP XXIII) for routine analysis and quality control of rifampin in capsules, either as single or as a mixture in pharmaceutical preparations containing isoniazid.

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