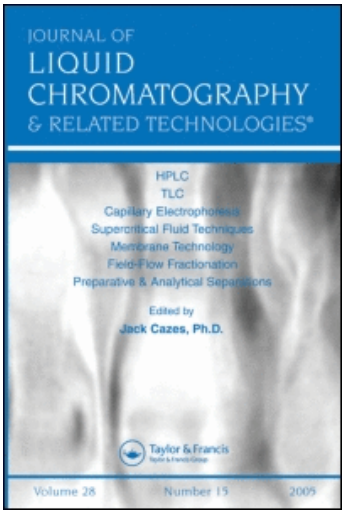

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High Performance Liquid Chromatographic Analysis of Rifampin and Related Impurities in Pharmaceutical Formulations

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF RIFAMPIN AND RELATED IMPURITIES IN PHARMACEUTICAL FORMULATIONS

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ABSTRACT

High pressure liquid chromatography was employed for the assay of rifampin in capsules. A reverse phase RP-2 column and a mobile phase of 48% methanol, 5% tetrahydrofuran and 47% 0.05 M ammonium formate (pH 7.3) were used with detection at 254 nm. Rifampin was separated from all its major degradation products and quantitated.

INTRODUCTION

Rifamycins are a family of semi-synthetic antibiotics (Figure 1). The major fermentation product is rifamycin B which, in aqueous solution containing dissolved oxygen, is converted to rifamycins O and S(1). Reduction of rifamycin S produces rifamycin SV, which can then be transformed to 3-formylrifamycin SV. Several derivatives of 3-formylrifamycin SV have been prepared, the most therapeutically important being the 3-(4-methylpiperazinoiminomethyl) derivative rifampin (2).

Degradation during formulation or upon storage, and incomplete separation from its precursors results in impurities in rifampin. Rifampin is hydrolyzed to form 3-formylrifamycin SV under acidic conditions (2). Rifampin quinone is formed upon oxidation of ri-

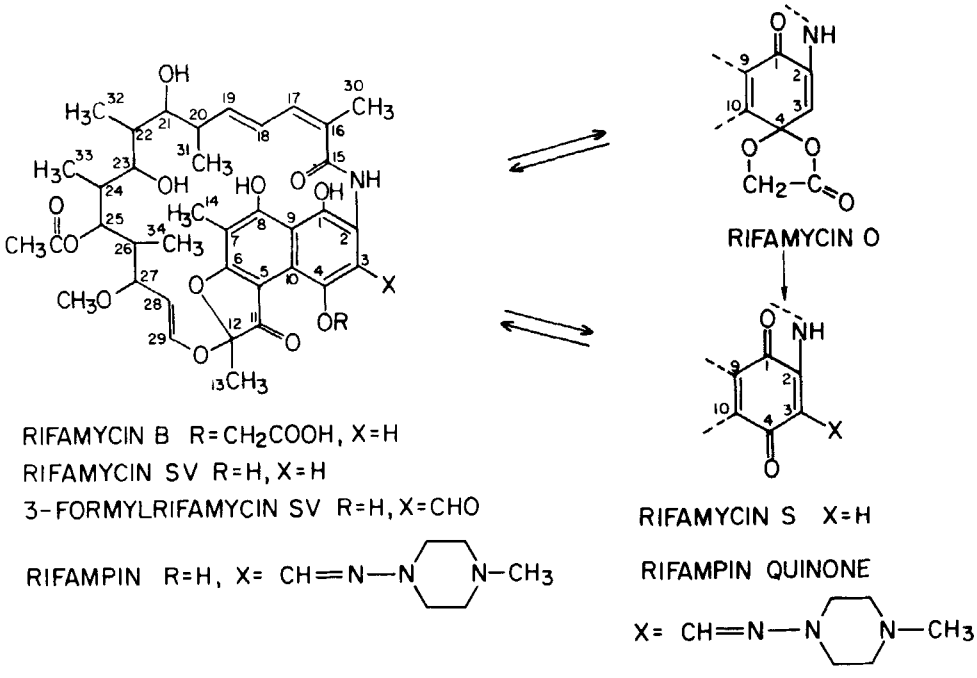

FIGURE 1. Structures of the Rifamycins.

fampin in solutions of pH greater than 7 in the presence of atmospheric oxygen (2). The major metabolite in man (3), 25-desacetylrifampin, could also be present as an impurity in rifampin arising from solvolysis. Since the metabolites and impurities either have similar absorption spectra or are biologically active, they interfere in the microbiological and spectrophotometric rifampin assay techniques (4,5). There have been several high performance liquid chromatographic (HPLC) methods reported (6,7) but these require the prior separation of formulation excipients from the sample (6) or the use of a non-commercially available column (7).

This paper describes a simple HPLC assay procedure for rifampin as well as the possible impurities, 25-desacetylrifampin, 3-formylrifamycin SV, and rifampin quinone.

EXPERIMENTAL

Chemicals & reagents: The rifampin degradation products, 25-desacetylrifampin, 3-formylrifamycin SV, and rifampin quinone, were obtained from Ciba (Dorval, Canada). The rifampin bulk drug substance was a house standard (893 µg/mg versus USP Rifampin).

The diphenyl phthalate which was used as internal standard was obtained from Pfaltz & Bauer Inc. (Flushing, N.Y.). The ammonium formate was from Aldrich Chemical Company Inc. (Milwaukee, Wis.). HPLC grade methanol and acetonitrile were obtained from Fisher Scientific Company (Fair Lawn, N.J.). The tetrahydrofuran (THF) was obtained from Burdick & Jackson Laboratories Inc. (Muskegon, Mich.).

Apparatus: A liquid chromatograph equipped with a Waters 6000A pump and a Waters U6K injector, Waters Associates (Milford, Mass.) was used. The column was a reverse phase RP-2, 10 µ Brownlee Labs. (Santa Clara, Ca.). A variable wavelength detector, Model 155-40, Altex Scientific Inc. (Berkeley, Ca.) was operated at 254 nm and a range setting of 0.05 Aufs. Peak areas and calculations were computed using an SP4000 chromatography data system, Spectra Physics (Santa Clara, Ca.).

Chromatographic Parameters: The mobile phase was prepared by adding 480 ml methanol to 470 ml 0.05 M ammonium formate and degassed by vacuum filtration through a reeve angel glass fiber filter Whatman Inc. (Clifton, N.J.). Any loss on filtering (due to evaporation under vacuum) was replaced by methanol. Then 50 ml of THF was added and the apparent pH adjusted to 7.3 using approximately three drops of 2 N ammonium hydroxide. A flow rate of 2 ml/min (1200 psi) was used and injection volumes were 5 µl.

Internal standard solution: A solution of diphenyl phthalate (10 mg/ml) was prepared in methanol (Solution A) or acetonitrile (Solution B). These solutions should be prepared fresh weekly.

Rifampin reference standard solution: The rifampin House Standard was used as a working standard. An accurately weighed quantity of the House Standard or USP Rifampin Reference Standard equivalent to about 30 mg rifampin was weighed into a 5.0 ml volumetric flask and made to volume with internal standard solution A. This solution was prepared fresh daily.

Impurity standard solutions: An accurately weighed quantity (10 mg) of 25-desacetylrifampin, 3-formylrifamycin SV and rifampin quinone were each weighed into separate 10.0 ml volumetric flasks and made to volume with internal standard solution A or internal standard solution B (3-formylrifamycin SV). For determination of the standard curve, suitable aliquots of these solutions were further diluted with the appropriate internal standard solution to give a concentration range of about 0.01 mg/ml to 1.0 mg/ml.

Sample Solution: An accurately weighed quantity of a representative portion of capsule contents equivalent to about 60 mg of rifampin was made to a volume of 10.0 ml with internal standard solution A.

RESULTS AND DISCUSSION

Optimum baseline separation of rifampin, 25-desacetylrifampin, 3-formylrifamycin SV, rifampin quinone and the internal standard, diphenyl phthalate, was achieved using an RP-2 column (Figure 2) and the mobile phase specified above. Other reverse phase columns, RP-8, RP-18 and Microbondapak C-18 were evaluated. However, these columns required a gradient solvent system to produce efficient separation and to elute the rifampin quinone within a reasonable time.

Table 1 indicates the retention times of rifampin, 25-desacetylrifampin, 3-formylrifamycin SV, rifampin quinone and the internal standard, diphenyl phthalate on the RP-2 column.

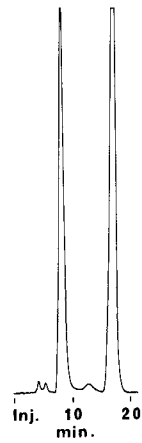

FIGURE 2

Separation of Rifampin (32 µg), impurities (0.3 µg) and internal standard (50 µg) on an RP-2 column with a mobile phase of methanol: tetrahydrofuran: 0.05 M ammonium formate, 48:5:47 and a flow rate of 2.0 ml/min. Detection was performed at 254 min. See Table 1 for peak assignments.

TABLE 1
Retention Times of Rifampin and Impurities

Compound	Retention Times (min.)
25-desacetylrifampin	4.4
3-formylrifamycin SV	5.8
rifampin	8.4
rifampin quinone	13.3
diphenyl phthalate	17.2

The linearity of the HPLC system was confirmed by the injection of seven solutions of rifampin (0.35 - 8.8 mg/ml) dissolved in internal standard solution A. A graph of the ratio of the area counts of the rifampin peak divided by the area counts of the internal standard peak versus the concentration of rifampin (mg/ml) yielded a straight line, coefficient of correlation 0.9999, ($y = 0.229 x + 0.002$).

Similarly, quantitation of 25-desacetylrifampin, 3-formylrifamycin SV and rifampin quinone was found to be linear (Table 2) over a range of about 0.05 - 6 µg injected which at a level of 30 µg rifampin injected represents a range of about 0.15 - 20%.

The solutions of 3-formylrifamycin SV were prepared in acetonitrile (internal standard solution B) as a rapid (approximately 30 min.) degradation of this compound occurred in methanolic solutions probably due to oxidation to the quinone. The impurities listed in Table 2 chromatographed as single peaks on HPLC.

The precision of the HPLC was determined by injection of ten aliquots of the rifampin reference standard solution. A relative standard deviation of the ratio of the area of the rifampin peak to the internal standard peak of 0.35% was obtained.

The House Standard was assayed by HPLC against USP Rifampin Reference Standard (labelled 98.8%) and was found to have a potency of 89.3%. In comparison, the CFR (4) absorptivity test yielded a value of 91.3% rifampin. The USP Reference Standard and the House Standard showed no trace of 25-desacetylrifampin. The former was found to contain 0.3% and 0.2% and the latter to contain 0.3% and 0.4% 3-formylrifamycin SV and rifampin quinone respectively.

Table 3 shows the results obtained for five capsule formulations by both the HPLC and CFR absorptivity methods of analysis.

Excellent agreement between the two methods was obtained. The amounts of the three degradation products quantitated were less than 1% for the formulations tested. The ranges of 3-formylrifamycin SV and rifampin quinone were 0.2 - 0.5% and 0.1 - 0.5%

TABLE 2
Determination of Linearity of Rifampin Impurities

Compound	No. of solutions	Coefficient of correlation	Equation y=
25-desacetylrifampin	8	1.0000	0.235x
3-formylrifamycin SV	10	0.9999	0.207x+.002
rifampin quinone	7	0.9998	0.189x

TABLE 3
Analysis of Rifampin Capsules

Sample	Dosage (mg)	% Label Claim	
		HPLC	CFR
1	150	100.7, 100.7	100.9
2	150	99.7, 99.1	97.8
3	300	95.4, 96.2	95.4
4	150	100.2, 101.5	101.3
5	300	102.3, 102.3	101.7

respectively. The 25-desacetylrifampin was found to be present at less than 0.1% levels. All formulations contained at least detectable (0.05%) amounts of all three degradation products.

This HPLC method is a rapid, precise and accurate method for the determination of rifampin and allows for the simultaneous quantitation of 3-formylrifamycin SV, rifampin quinone and 25-desacetylrifampin in bulk drug substance and formulations.

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