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Note

Thin-layer chromatographic identity and purity test for rifampin

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The rifamycins (Fig. 1) are a family of antibiotics obtained by fermentation and chemical modification whose structures consist of a naphthoquinone moiety spanned by an aliphatic ansa bridge[1]. Rifamycin B (the major fermentation product) has the unusual property that in aqueous solutions containing dissolved oxygen it changes spontaneously into microbiologically more active substances such as rifamycin O and rifamycin S[2]. Rifamycin SV, obtained from rifamycin S by mild reduction, has been employed as a parenteral anti-tubercular drug since 1962. 3-Formylrifamycin SV, prepared by oxidizing Mannich bases of rifamycin SV, has yielded several derivatives with remarkable biological activity. The most therapeutically important of these is the 3-(4-methylpiperazinoiminomethyl) derivative, rifampin[3], a broad-spectrum antibiotic used orally in the treatment of tuberculosis.

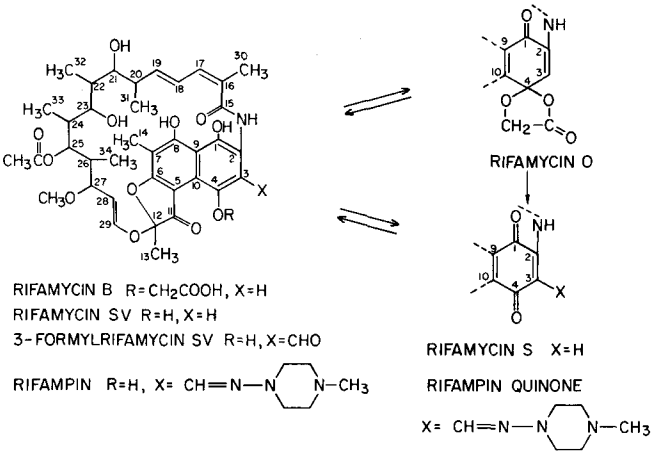

Fig. 1. Structures of rifamycins.

Impurities in rifampin can arise from incomplete separation of the drug from its precursors and from degradative changes during formulation and storage[3]. Under acidic conditions, rifampin is hydrolyzed to form 3-formylrifamycin SV. At pH values greater than 7 and in the presence of atmospheric oxygen, rifampin under-

goes oxidation to rifampin quinone and to an N-oxide. The major metabolite in man[4] 25-desacetylrifampin, can also form 25-desacetylrifampin quinone.

Rifampin is available in Canada from a variety of commercial sources. It is listed in the USP[5] and governed by regulations published in the Code of Federal Regulations[6]. USP specifications include identity controls for rifampin bulk drug material by infrared spectroscopy. No identity or purity tests are stipulated for finished products. In Canada, rifampin products are controlled under Division 8 of the Food and Drug Regulations and quality standards for raw materials and finished dosage forms are contained in the New Drug Submission[7].

Under drug quality assessment programs operating in this country, large numbers of samples are tested for identity, potency and purity, necessitating the use of reliable and time-saving methods. The present paper describes a thin-layer chromatographic (TLC) method for the identification of rifampin and for its resolution from the above-mentioned possible degradative and synthetic by-products. Direct comparison of spot intensities with known quantities allows the semi-quantitative determination of the more common impurities, rifampin quinone, 3-formylrifamycin SV and rifampin N-oxide.

EXPERIMENTAL

Chemicals and equipment

Authentic samples of rifamycin B, rifamycin O, rifamycin S, 3-formylrifamycin SV, 25-desacetylrifampin, 25-desacetylrifampin quinone, rifampin, rifampin quinone and rifampin N-oxide were generously provided by Ciba (Dorval, Canada) and Dow Chemical (Richmond Hill, Canada). USP rifampin reference standard, Lot F, was used as the working standard for purity comparisons. 1-Amino-4-methylpiperazine was purchased from ICN Pharmaceuticals (Plainview, N.Y., U.S.A.).

Commercially available precoated silica gel 60F (Merck, Elmsford, N. Y., U.S.A.) plates (EM Brand 20 × 20 cm, 0.25 mm thickness) were used. The solvent system consisted of chloroform–methanol–water (80:20:2.5).

Solutions for spotting

Standard solutions. Solutions (1.5 mg/ml) of each of the above rifamycins (Table I) were prepared in chloroform. An additional more concentrated solution of the USP rifampin reference standard (15.0 mg/ml) was prepared in chloroform.

Sample solutions. For the rifampin bulk drug substance and capsules, solutions containing 15 mg/ml were prepared by placing an accurately weighed portion of powder equivalent to 150 mg rifampin in a 10-ml volumetric flask, shaking thoroughly, then diluting with chloroform to volume.

Chromatographic procedure

Identity test. For identification purposes, standard solutions (1 μl) of all the rifamycins were spotted on the plate. In addition, a 10-μl aliquot of the sample solution representing 150 μg of rifampin was also applied. The plate was inserted into a filter paper-lined chromatographic chamber which had been saturated with solvent vapour for 1 h prior to use. The plate was developed to a height of 15 cm (*ca.* 45 min), removed from the chamber and allowed to dry at room temperature. The rifamycins

appear as coloured spots ranging from yellow to purple on a white background. Visualization may also be obtained by viewing the plate under short-wavelength ultraviolet light. In this case, the rifamycins appear as dark spots on a bright green background. The identity test is positive when the major spot in the sample solution appears at the same R_F as that of the spot from the USP rifampin reference standard solution.

Purity test. For a semi-quantitative determination of the amounts of impurities present in the sample solution, 1- and 2-μl aliquots of the impurity in question (as identified by the above TLC test), representing 1.5 and 3.0 μg, respectively, were applied to the plate. Ten-microliter aliquots of the sample solutions (equivalent to 150 μg of rifampin) were also spotted. For comparison purposes, a 10-μl aliquot of the more concentrated USP rifampin reference standard solution (15 mg/ml) was spotted as well. The plates were developed as described above and the level of impurity was evaluated by comparing the intensity of the impurity spot in the sample with that of the standards.

RESULTS AND DISCUSSION

In Table I are listed the R_F values, minimum detectable amounts and colours of the spots of the various rifamycins when chromatographed in this system. All are resolved except for rifamycin S and O; however, these two can be distinguished by their different colours. Other TLC systems have been published, but these usually dealt only with rifampin alone[3], its metabolites[4] or other rifamycins[8,9]. Therefore, this report includes TLC data on all the rifamycins listed in Table I. The 1-amino-4-methylpiperazine was not visible unless sprayed with a reagent such as ninhydrin, but all other compounds exhibited coloured spots without spraying. The minimum detectable quantities listed in Table I are the amounts of these coloured spots visible without any further treatment. Increased sensitivity is possible by fluorescence quenching when the spots are viewed under short-wavelength ultraviolet light.

TABLE I

R_F VALUES OF RIFAMYCINS ON SILICA GEL 60

Solvent system: chloroform–methanol–water (80:20:2.5).

Compound	*Minimum detectable amount (μg)*	*R_F Value**	*Spot colour*
Rifamycin O	0.75	0.83	yellow
Rifamycin S	0.75	0.81	brown
Rifampin quinone	0.30	0.75	purple
25-Desacetylrifampin quinone	0.15	0.66	purple
Rifampin	0.30	0.60	orange
25-Desacetylrifampin	0.30	0.41	orange
3-Formylrifamycin SV	0.75	0.27	pink
Rifamycin B	0.75	0.15	yellow
Rifampin N-oxide	0.15	0.09	yellow
1-Amino-4-methylpiperazine	0.15**	0.05**	brown

* Average of five plates.

** Visible only after spraying with ninhydrin.

Fig. 2 shows a chromatogram of commercial formulations, representative of those available on the Canadian market, in which the levels of rifampin quinone, 3-formylrifamycin SV and rifampin N-oxide are quantitated. From a comparison of the spot intensities these impurities generally were found to be present at less than 1 % levels except in the case of product C (an expired lot, spot 5) where the rifampin quinone level was about 2 %. The USP rifampin reference standard (spot 6), which has a labelled potency of 98.8 %, was shown to contain rifampin quinone and N-oxide but at less than the 1 % impurity level.

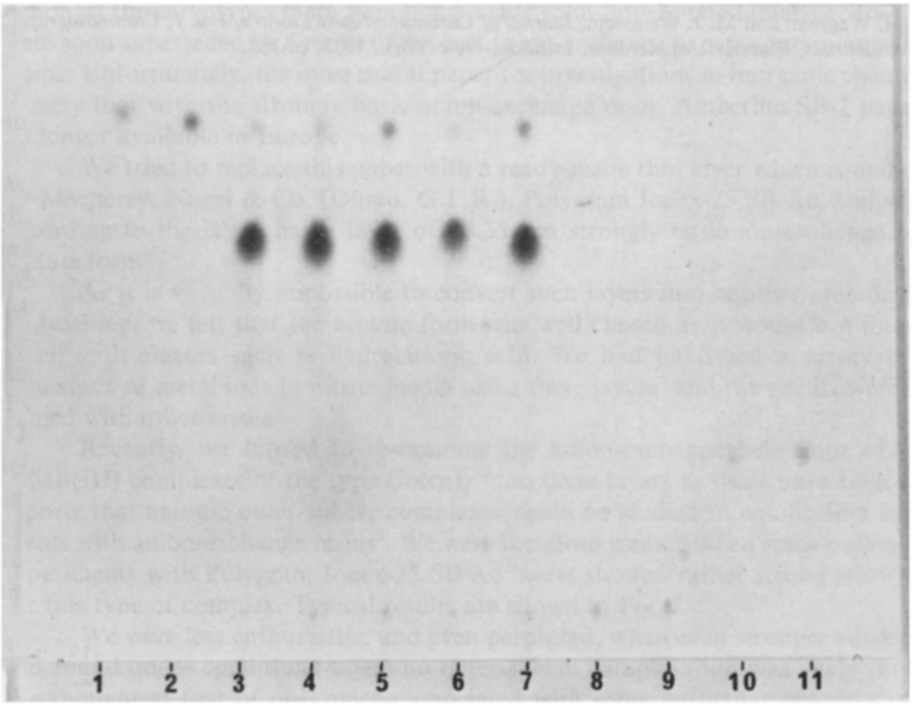

Fig. 2. TLC chromatogram of rifampin formulations and impurities on silica gel. 1 = 1.5 μg of rifampin quinone; 2 = 3.0 μg of rifampin quinone; 3 = 150 μg of product A; 4 = 150 μg of product B; 5 = 150 μg of product C; 6 = 150 μg of USP rifampin reference standard; 7 = 150 μg of product D; 8 = 1.5 μg of rifampin N-oxide; 9 = 3.0 μg of rifampin N-oxide; 10 = 1.5 μg of 3-formylrifamycin SV; 11 = 3.0 μg of 3-formylrifamycin SV.

Although amounts of rifampin equivalent to 150 μg were spotted, levels of up to 300 μg could be chromatographed without spot distortion or streaking. With the minimum detectable amount of rifampin N-oxide being 0.15 μg, the application of 300 μg of rifampin would allow the detection of 0.05 % levels of this impurity by this TLC procedure.

The proposed TLC method is a simple procedure suitable for the identification of rifampin and as a limit test for the known related impurities.

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