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Short communication

High-performance thin-layer chromatographic method for monitoring degradation products of rifampicin in drug excipient interaction studies

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Abstract

A thin-layer chromatographic assay for the determination of rifampicin and its degradation components in drug–excipient interaction studies is described. The chromatography was performed on thin-layer plates (silica gel) with a mobile phase consisting of chloroform–methanol–water (80:20:2.5, v/v/v). The peaks were quantified by densitometric evaluation of the chromatograms. The method shows a limit of detection of 10 ng per band and good precision and linearity in the range 50–3000 ng per band for rifampicin, 3-formylrifamycin SV, rifampicin N-oxide and 25-desacetylrifampicin and 100–350 ng per band for rifampicin quinone

1. Introduction

Rifampicin, (12*Z*,14*E*,24*E*)-(2*S*,16*S*,17*S*,18*R*,19*R*,20*R*,21*S*,22*R*,23*S*)-1,2-dihydro-5,6,9,17,19-pentahydroxy-23-methoxy-2,4,12,16,18,20,22-heptamethyl-8-(4-methylpiperazin-1-yliminomethyl)-1,11-dioxo-2,7-(epoxypentadeca-1,11,13-trienimino)naphtho[2,1-*b*]furan-21-yl acetate, is a semi-synthetic antibiotic, active against *Mycobacterium* species [1]. The structures of rifampicin, 3-formylrifamycin SV and 25-desacetylrifampicin are shown in Fig. 1. Some physico-chemical properties such as stability of rifampicin in different media have been reported [2].

A liquid chromatographic method using an ODS Permaphase column employing gradient elution with water–methanol as the mobile phase to determine simultaneously rifampicin and its degradation products has been described [3]. In another method, a microPak-NH_2 column was

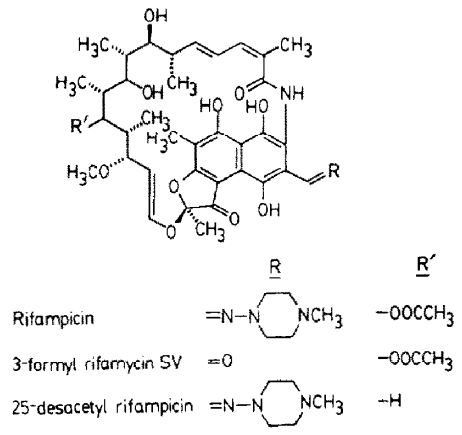

Fig. 1. Structures of rifampicin, 3-formylrifamycin SV and 25-desacetylrifampicin.

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employed to determine rifampicin quinone alone [4], and several liquid chromatographic methods have been reported for the determination of rifampicin metabolites in blood, urine and saliva [5,6]. A qualitative thin-layer and paper chromatographic procedure has been reported for the detection of degradation products but detection was done visually [7–9].

The British Pharmacopoeia specifies a semi-quantitative TLC limit test for rifampicin and related compounds based on visual detection of bands by short-wavelength UV light [1]. The chromatographic purity test for rifampicin described in the US Pharmacopeia is based on liquid chromatography [10]. The method is not sensitive enough to detect some of the degradation products of rifampicin.

In view of the various shortcomings of the tests mentioned above, this study was undertaken to develop a simple and sensitive TLC method making use of the UV absorbance characteristics of rifampicin and its degradation products. The suitability of this method was evaluated on preformulation study samples containing mixtures of rifampicin with various pharmaceutical excipients.

2. Experimental

2.1. Chemicals and materials

Rifampicin was supplied by Lupin Laboratories (Ankleshwar, India). Distilled water was obtained from a Milli-Q system (Millipore, Milford, MA, USA). Solvents, chemicals (analytical-reagent grade) and HPTLC plates were obtained from Merck (Bombay, India). The HPTLC plates (20 cm × 10 cm) were precoated with silica gel 60 (layer thickness 0.25 mm) and a fluorescent indicator. The plates were prewashed in methanol, air-dried and stored under normal conditions.

2.2. Instruments

Solutions of the test samples were applied to the HPTLC plates with a Linomat IV autospotter (Camag, Muttenz, Switzerland). The plates were scanned with a Scanner II (Camag) and data were processed on PC/AT (Zenith, Pune, India) using Cats 3 software (Camag). A Model AE 163 analytical balance (Mettler Instruments, Highstown, NJ, USA) was used for weighing reagents.

2.3. Preparation of rifampicin, 3-formylrifamycin SV, rifampicin N-oxide and 25-desacetylrifampicin standard solutions

Standard solutions containing 10 μg/ml of rifampicin, 3-formylrifamycin SV, rifampicin N-oxide and 25-desacetylrifampicin were prepared separately by dissolution in chloroform. Aliquots of 5, 10, 15, 20, 25 and 30 μl of each standard solution were applied (6 mm band) on TLC plates (4 mm between bands) with a Linomat IV sample applicator to give 50, 100, 150, 200, 250 and 300 ng per band for the determination of linearity. The bands were dried with nitrogen.

2.4. Preparation of rifampicin quinone standard solution

A standard solution containing 25 μg/ml of rifampicin quinone was prepared by dissolution in chloroform. For the determination of linearity 4, 6, 8, 10, 12 and 14 μl of the standard solution were applied (6 mm band) on a TLC plate (4 mm between bands) with a Linomat IV sample applicator to give 100, 150, 200, 250, 300 and 350 ng per band. The bands were dried with nitrogen.

2.5. Preparation of rifampicin test solution

A standard solution containing rifampicin test sample was prepared in chloroform at 1000 μg/ml. A 20-μl volume of this solution was applied (6 mm band) on a TLC plate (4 mm between bands) with a Linomat IV sample applicator. The bands were dried with nitrogen.

2.6. Thin-layer chromatography

The TLC plates were developed in unlined glass twin-trough tanks (Camag) with chloroform–methanol–water (80:20:2.5, v/v/v) as mo-

bile phase [7]. The equilibration time to saturate the tank atmosphere was 1 h. The TLC plates were developed over a distance of 5 cm and finally air dried.

2.7. Densitometric measurement of the chromatograms

The developed plates were quantified by linear scanning at 4 mm/s with a Scanner II equipped with a PC/AT and Cats 3 software. The absorbance mode with a deuterium lamp and a monochromator setting of 330 nm and slit dimensions of 0.2 × 3 mm was used for determination of rifampicin, 3-formylrifamycin SV, rifampicin N-oxide, 25-desacetylrifampicin and other unknown impurities. Rifampicin quinone was determined at 490 nm. The determination of 3-formylrifamycin SV, rifampicin N-oxide, 25-desacetylrifampicin and rifampicin quinone was carred out by comparing the peak areas of the test samples with the peak areas of the respective standards. A 1% equivalent band of rifampicin was used to determine the unknown impurities for which reference standards were not available. The procedure adopted is based on the US Pharmacopeia recommendation.

2.8. Application of the method

The applicability of the method was demonstrated with rifampicin samples drawn from preformulation accelerated stability samples blended with various excipients. Samples were drawn after 1, 2, 3 and 6 months from the accelerated storage conditions at room temperature and 45, 60, 5 and 40°C at 75% relative humidity. All samples were treated in a similar manner to give solutions in chloroform.

3. Results

3.1. Chromatography

Under the conditions described, rifampicin showed no interference from 3-formylrifamycin SV, rifampicin N-oxide, 25-desacetylrifampicin, rifampicin quinone and other degradation components. Representative chromatograms of the pure drug and accelerated stability samples are given in Figs. 2 and 3.

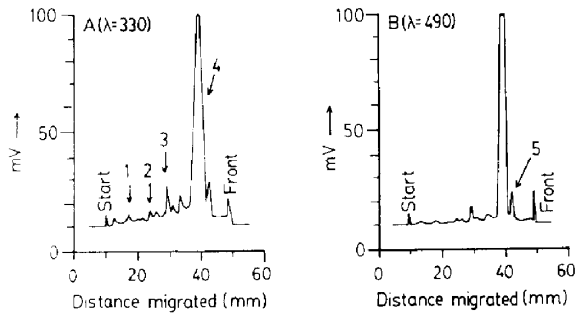

Fig. 2. Representative chromatograms of rifampicin. (A) Detection of degradation components at 330 nm: 1 = rifampicin N-oxide; 2 = 25-desacetylrifampicin; 3 = 3-formylrifamycin SV; 4 = rifampicin (4). (B) Detection of rifampicin quinone (5) at 490 nm.

3.2. Recovery

Recovery studies were performed by spiking a test solution with known concentrations of rifampicin, rifampicin quinone, 25-desacetylrifampicin, rifampicin N-oxide and 3-formylrifamycin SV. For calculation of recoveries, the resulting peak areas were compared with those of rifampicin, rifampicin N-oxide, 25-desacetylrifampicin, 3-formylrifamycin SV and rifampicin reference standards in chloroform. Reference standards and recovery samples had to be

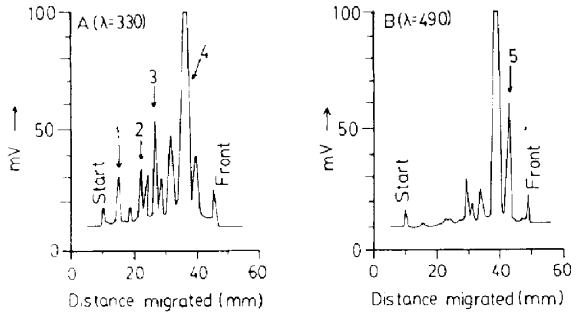

Fig. 3. Representative chromatograms of a rifampicin–sucrose mixture (1:1) kept at 45°C and analysed after 3 months. (A) Detection of degradation components at 330 nm: 1 = rifampicin N-oxide; 2 = 25-desacetylrifampicin; 3 = 3-formylrifamycin SV; 4 = rifampicin. (B) Detection of rifampicin quinone (5) at 490 nm.

prepared in the same solvent in order to run the sample applicator properly. Samples containing three different concentrations of rifampicin, rifampicin N-oxide, 25-desacetylrifampicin, 3-formylrifamycin SV and rifampicin quinone were determined ($n = 6$). The average recoveries were 99.4% for rifampicin, 99.6% for rifampicin quinone, 98.4% for 3-formylrifamycin SV, 99.8% for rifampicin N-oxide and 100.3% for 25-desacetylrifampicin. The results are summarized in Table 1.

3.3. Linearity

The calibration graphs were linear in the range 50–300 ng per band for rifampicin, 3-formylrifamycin SV, rifampicin N-oxide and 25-desacetylrifampicin and 100–350 ng per band for rifampicin quinone. Linearity for rifampicin, 3-formylrifamycin SV, rifampicin N-oxide and 25-desacetylrifampicin were tested with sample bands of 50, 100, 150, 200, 250 and 300 ng per band and rifampicin quinone with sample bands of 100, 150, 200, 250, 300 and 350 ng per band. The correlation coefficients were 0.9995 for rifampicin, 0.9996 for 3-formylrifamycin SV, 0.9989 for rifampicin N-oxide, 0.9976 for 25-desacetylrifampicin and 0.9998 for rifampicin quinone.

3.4. Reproducibility

Reproducibility experiments were carried out for five components at three concentrations ($n = 6$). The results obtained are summarized in Table 1. The relative standard deviations ranged from 0.86 to 1.59% for rifampicin, 1.93 to 2.58% for rifampicin quinone, 1.32 to 1.96% for 3-formylrifamycin SV, 1.72 to 1.88% for rifampicin N-oxide and 1.23 to 1.86% for 25-desacetylrifampicin.

4. Discussion

The method described allows the simple and rapid determination of rifampicin, rifampicin quinone, 3-formylrifamycin SV, rifampicin N-oxide and 25-desacetylrifampicin levels without interference from each other or from other degradation components present in the sample. One of the main advantages of this assay is the very low limit of detection. The detection limit for each component was ca. 10 ng per band,

Table 1
Recovery and precision data

Compound	Concentration (ng per band)	n	Precision recovery (R.S.D.) (%)	Average recovery (%)
Rifampicin	50	6	1.59	99.6	99.4
	150	6	0.86	99.3	
	300	6	1.01	99.4	
Rifampicin quinone	100	6	2.01	98.3	99.6
	250	6	1.93	99.8	
	350	6	2.58	100.7	
3-Formylrifamycin SV	50	6	1.96	98.9	98.4
	150	6	1.32	99.7	
	300	6	1.68	96.8	
Rifampicin N-oxide	50	6	1.88	99.3	99.8
	150	6	1.72	98.9	
	300	6	1.77	101.3	
25-Desacetylrifampicin	50	6	1.54	102.1	100.3
	150	6	1.23	99.3	
	300	6	1.86	99.7	

which was possible because there was no dilution effect by the mobile phase at the time of detection. The HPLC methods described in literature are not suitable for separating all the degradation components simultaneously without any interference [3,10]. The method described here is sensitive, precise and involves a single-step sample preparation. Because almost 50 samples can be analysed within 4 h, the technique may be used for routine raw material purity analysis, satability monitoring studies and dosage form analysis.

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