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DETERMINATION OF RIFAMPICIN AND ISONIAZID IN PHARMACEUTICAL FORMULATIONS BY HPLC

Yateen Shah, S. Khanna*, K.C.Jindal and V.S.Dighe
Lupin Labs Ltd., MIDC, Chikalthana,
Aurangabad 431210, (M.S) INDIA

ABSTRACT

A liquid chromatographic procedure for the analysis of Rifampicin (RIF) and Isoniazid (INH) in pharmaceutical dosage forms utilizing reverse phase chromatography was developed. Isolation of analytes was carried out under isocratic conditions with a octadecylsilane column and an aqueous mobile phase containing Methanol (75%) and 0.02 M Disodium Hydrogen Orthophosphate (25%) with pH 4.5 adjusted with orthophosphoric acid. The detection was done at 254 nm.

The method is unique in analysing Rifampicin precisely in combination with Isoniazid particularly in liquid formulation. The method is specific and can distinctly isolate the degradation product in suspension.

**For correspondence*

INTRODUCTION

Rifampicin and Isoniazid are the most widely used anti-tubercular drugs. Literature survey reveals several analytical methods including the spectrophotometric, (1) microbiological, (2) and HPLC (3,4). Isoniazid is analysed by various methods such as colorimetry, fluorometry, titrymetry (5,6). Combination products are analysed by HPLC(7,8).

The present work describes in detail the HPLC method for Rifampicin and Isoniazid in combination. The method s advantageous with respect to the use of simple mobile phase, and is unique in analysing Rifampicin precisely in presence of Isoniazid in aqueous preparations where Rifampicin degrades rapidly.

Rifampicin degrades to 3-formylrifamycin SV in acidic solution and to Rifampin quinone in alkaline solution. 3-formyl Rifamycin SV is coloured, microbiologically active compound and interferes in the spectrophotometric assay method (specified by I.P. and B.P.) and microbiological assay of Rifampicin specified by I.P. It was found that the proposed method can effectively isolate Rifampicin from its main degradation products and give consistent accurate assay value. In presence of Isoniazid the degradation products of Rifampicin (in aqueous preparations) are further converted to microbiologically inactive compounds, hence do not interfere in microbiological assay, but still interfere in spectrophotometric assay of Rifampicin (as presented in B.P./I.P).

EXPERIMENTAL

Reagents and Materials

Rifampicin and Isoniazid reference standards were obtained from Central Drug Research Laboratory, Calcutta, India. All reagents used were of HPLC grade. Distilled, deionized water passed through 0.45 micron membrane filter was used throughout.

Chromatographic Instrumentation

The HPLC component system consisted of a dual piston reciprocating pump (Model 510), LC Spectrophotometer (Model 481) and a computing integrator (Model 745) all from Waters Associates, A Rheodyne Model 7125 sampling valve having a 20 micro litre fixed loop. A 15 cm x 3.9 mm ID Novapak C18 Column (Waters Associates) was used. Typical operating conditions were mobile phase flow rate 1.00 ml/min, detector at 254 nm, sensitivity 0.5 AUFS, temperature ambient and chart speed at 0.5 cm/min.

The mobile phase consisted of methanol and 0.02 M disodium hydrogen orthophosphate (75:25) and the pH was adjusted to 4.5 with orthophosphoric acid (85%).

Standard & Sample Preparation

Stock standards of 1mg/ml for Rifampicin and Isoniazid were prepared separately in methanol. Further the solutions were diluted to a concentration of 40 mcg/ml in mobile phase.

The samples of tablets, capsules and aqueous formulation (Plain and combination) were suitably diluted in a similar manner (of stock standard) to obtain a final concentration of 40 mcg/ml of Rifampicin.

Precision,Linearity and Recovery Study

The method precision was evaluated by repeated assays of our commercial formulation over separate periods of one day and one week. The within day precision was determined by performing five consecutive assays within a period of eight hours. The day today repeatibility of the method was determined by analysing the same sample (single operator) on seven consecutive days.

Under the described chromatographic conditions, a linear response was demonstrated for Rifampicin in range of 0-100 mcg/ml and Isoniazid 0-80 mcg/ml.

The accuracy of the procedure was evaluated by several means. Known amount of drug was added to the placebo and were analysed by the proposed method. The recovery data obtained from this study was in the range of 97.5% to 100.45%. The RSD for Rifampicin was 0.437 and the RSD for Isoniazid was 0.9722.

RESULTS AND DISCUSSION

The proposed method is precise, accurate and simple. Moreover, the method is able to separate the compound

TABLE 1

COMPARATIVE ANALYTICAL DATA OF COMMERCIAL FORMULATIONS

PRODUCT	CODE	Assay values, % of declared amount					
		SPECTROPHOTOMETRIC METHOD		MICROBIOLOGICAL METHOD		PROPOSED HPLC METHOD	
		Rifampicin	Isoniazid	Rifampicin	Isoniazid	Rifampicin	Isoniazid
Rifampicin Capsules	A	99.38	-	98.28	-	98.29	-
(450mg/cap)	B	100.39	-	98.99	-	99.09	-
Rifampicin Syrup	C	101.21	-	102.76	-	101.12	-
(100mg/5ml)	D	100.89	-	103.02	-	101.26	-
Rifampicin and Isoniazid Caps.	E	98.76	100.01	99.31	-	99.21	99.26
(RIF 450mg/cap.	F	99.26	99.79	98.96	-	98.92	100.1
INH 300mg/cap)	G	97.79	98.99	97.9	-	98.1	99.1
Rifampicin and Isoniazid Tabs.	H	97.76	100.01	98.6	-	98.31	100.5
(RIF 450mg/tab. INH 300mg/tab.)	I	99.26	101.26	101.12	-	99.3	100.6
RIF & INH Susp. (RIF 100mg/5ml, INH 100mg/5ml)	J	103.26	101.26	68.1	-	71.2	100.6

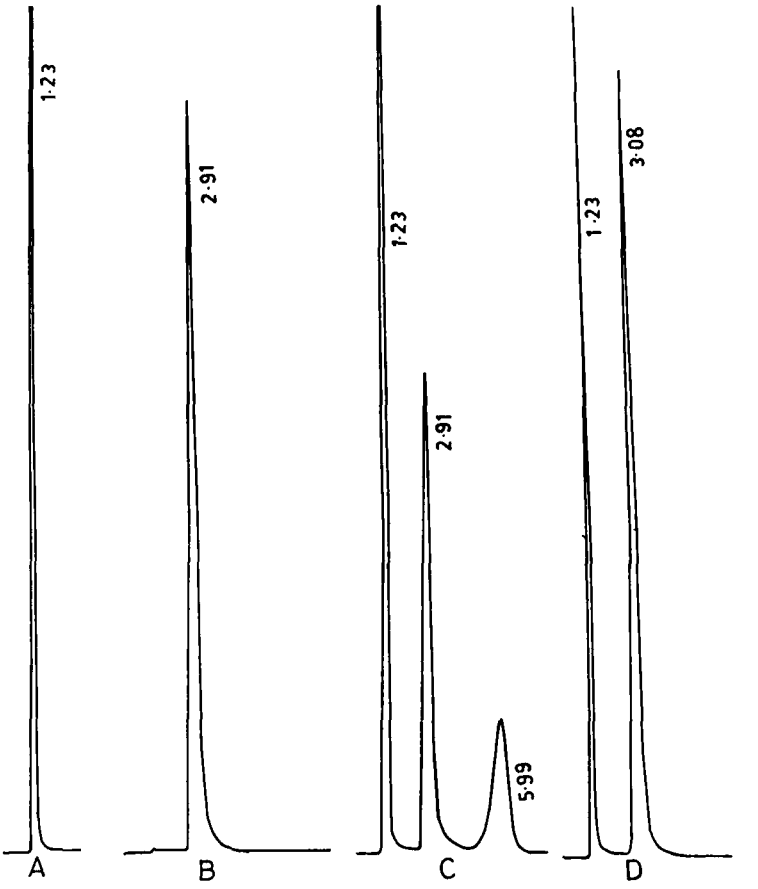

FIGURE-1 : CHROMATOGRAMS OBTAINED UNDER PROPOSED CONDITIONS FOR STANDARD ISONIAZID(A,RT 1.23), STANDARD RIFAMPICIN(B,RT 2.91), RIFAMPICIN & ISONIAZID SUSPENSION (C) & RIFAMPICIN & ISONIAZID CAPSULES(D), THE CHROMATOGRAM 'C' SHOWS DECREASED CONCENTRATION OF RIFAMPICIN & A ADDITIONAL PEAK(RT 5.99) OF DEGRADED PRODUCT FROM RIFAMPICIN

formed due to reaction of Isoniazid and Rifampicin in combination in suspension.

Three samples of Rifampicin and Isoniazid bulk drug obtained from three different sources were assayed by the proposed HPLC method and the results were compared with

the pharmacopoeial methods (IP/BP/USP). The assay results were in the range of 98.5 to 100.2%.

Ten commercial formulations representing five different manufacturers were assayed in triplicate by the proposed procedure. A compilation of the analytical results is presented in Table-1. Except for Rifampicin and Isoniazid Suspension, the assay values for all other products ranged from 97% to 103% of the declared concentration.

None of the chromatograms in this series of samples were found to exhibit interference with the response for Rifampicin and Isoniazid. Typical chromatograms of reference standard and their combination are shown in Figure-1. No interference was observed due to pharmaceutical additives.

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