INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

NEW RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ISONIAZID AND RIFAMPICIN IN PHARMACEUTICAL DOSAGE FORM

K.GEETHA SOWJANYA*, M.RAM AYYAPPA

Department of Pharmaceutical Analysis, A.K.R.G.College of Pharmacy, Nallajerla, West Godavari, Andhra Pradesh,India.

ABSTRACT

A simple and selective LC method is described for the determination of Isoniazid and Rifampicin in tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 45 volumes of acetonitrile and 55 volumes of mixed phosphate buffer with detection of 225 nm. Linearity was observed in the range 36-84 µg /ml for Isoniazid(r^2 =0.9987) and 30-70µg /ml for Rifampicin (r^2 =0.9977) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim.
The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

KEY WORDS: Isoniazid, Rifampicin, tablet dosage forms, RP-HPLC method

Author for correspondence:
K.Geetha Sowjanya,
Department of Pharmaceutical Analysis, A.K.R.G.College of Pharmacy, Nallajerla, West Godavari, Andhra Pradesh,India.
E mail: sowji.komma@gmail.com

INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical Ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities, assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department. The methods are generally developed in

an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.

Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the "stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time."

A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it.

A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species (1, 2).

High performance liquid chromatography (HPLC) is a separation technique utilizing differences in distribution of compounds to two phases; called stationary phase and mobile phase. The stationary phase designates a thin layer created on the surface of fine particles and the mobile phase designates the liquid flowing over the particles. Under a certain dynamic conditions, each component in a sample has difference distribution equilibrium depending on solubility in the phases and or molecular size. As a result the components move at different speeds over the stationary phase and are thereby separated from each other. The column is a stainless steel (or resin) tube, which is packed with spherical solid particles. Mobile phase is constantly fed into the column inlet at a constant rate by a liquid pump. A sample is injected from a sample injector located near the column inlet. The injected sample enters the column with the mobile phase and the components in the sample migrate through it passing between the stationary and mobile phases. Compound move in the column only when it is in the mobile phase. Compounds that tend to be distributed in the mobile phase therefore migrate faster through the column while compounds that tend to be distributed in the stationary phase migrate slower. In this way each component is separated on the column and sequentially elutes from the outlet. A detector connected to the outlet of the column detects each compound eluting from the column.

Quality investigation plays a very important role in quality specification establishment of chemical drugs. The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs.

Isoniazid is pyridine-4-carbohydrazide used as Anti-Bacterial Agents and Anti-Infective Agents.

Rifamycin -[[(4-methyl-1-piperazinyl) imino]methyl]--5, 6, 9, 17, 19, 21-Hexahydroxy-23 - methoxy-2, 4, 12, 16, 18, 20, 22-heptamethyl-8-[*N*-(4-methyl -1-piperazinyl)formimidoyl]-2,7-(epoxypentadeca [1, 11, 13]trienimino)naphtha[2,1-*b*]furan-1,11-(2*H*)-dione 21-acetate used as Anti-Bacterial Agents and Anti-Infective Agents

Literature review reveals no simultaneous estimation of Isoniazid and Rifampicin was reported so far (3-7). Hence we aim to develop new RP HPLC method for the simultaneous estimation of Isoniazid and Rifampicin in pharmaceutical dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Isoniazid

10 mg of Isoniazid was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard stock solution of Rifampicin

10mg of Rifampicin was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of mixed standard solution

Weigh accurately 60 mg of Isoniazid and 50 mg of Rifampicin in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.From above stock solution 60 µg/ml of Isoniazid and 50 µg/ml of Rifampicin is prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram (8).

Optimized chromatographic conditions

Optimized chromatographic conditions are shown in table-1.

Table-1 Optimized chromatographic conditions

Mobile phase	Mixed phosphate buffer +ACN
Ph	-
Column	Inertsil ODS 3V column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{o}C)
Sample temperature	Room temperature(20-25^{o}C)
Wavelength	225
Injection volume	20 µl
Run time	6 min
Retention time	About 3.420min for Isoniazid and 4.567min for Rifampicin.

Validation (9, 10)

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100%, 150%.

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank.

The amount of Isoniazid and Rifampicin present in the taken dosage form was found to be 99.8 % and 99.6% respectively (Table-2 and fig-1).

Table-2 Assay results of Isoniazid and Rifampicin

	ISONIAZID		RIFAMPICIN	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	295.884	286.448	836.469	833.214
Injection-2	290.743	286.448	839.076	833.214
Injection-3	292.910	291.818	835.627	837.225
Injection-4	293.024	293.805	834.719	833.303
Injection-5	290.900	280.827	829.554	831.491
Average Area	292.692	287.869	835.089	833.689
Standard deviatuion	3.1102		2.1174	
%RSD	1.9		1.2	
Assay(%purity)	**99.8%**		**99.6%**	

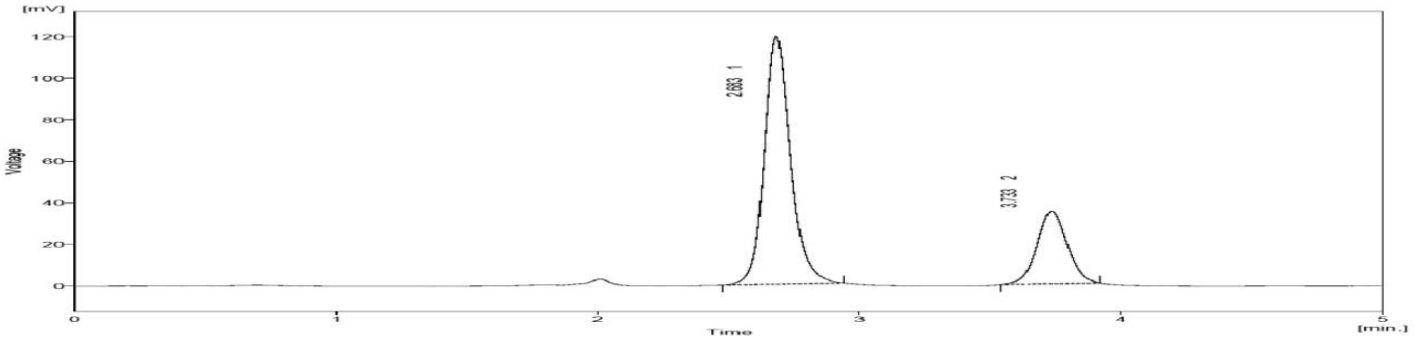

Figure-1 Chromatogram of Assay sample preparation

The % RSD for the retention times and peak area of Isoniazid and Rifampicin were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-3 and 4).

Table-3 Results for system suitability of Isoniazid

Injection	**Retention time (min)**	**Peak area**	**Theoretical plates (TP)**	**Tailing factor (TF)**
1	3.773	295.884	4667	1.111
2	3.733	290.743	4813	1.143
3	3.733	292.910	4813	1.176
4	3.770	293.024	4908	1.206
5	3.733	290.900	4813	1.176
Mean	3.748	292.692	-	-
SD	0.0049	55.704	-	-
%RSD	**0.14**	**0.64**	-	-

Table-4 Results for system suitability of Rifampicin

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor	Resolution
1	4.680	8815.579	2994	1.596	3.247
2	4.837	8708.391	2058	1.627	3.306
3	4.683	8510.447	2436	1.907	3.540
4	4.670	8553.080	2422	1.952	3.531
5	4.680	8815.579	2994	1.596	3.247
6	4.690	8708.391	2058	1.627	3.306
Mean	4.707	8685.245	-	-	-
SD	0.064	128.893	-	-	-
%RSD	**1.36**	**1.48**	-	-	-

The relationship between the concentration of Isoniazid and Rifampicin and area of Isoniazid and Rifampicin should be linear in the specified range and the correlation should not be less than 0.99.

The % recovery of Isoniazid and Rifampicin should lie between 98% and 102% (Fig-2-4).

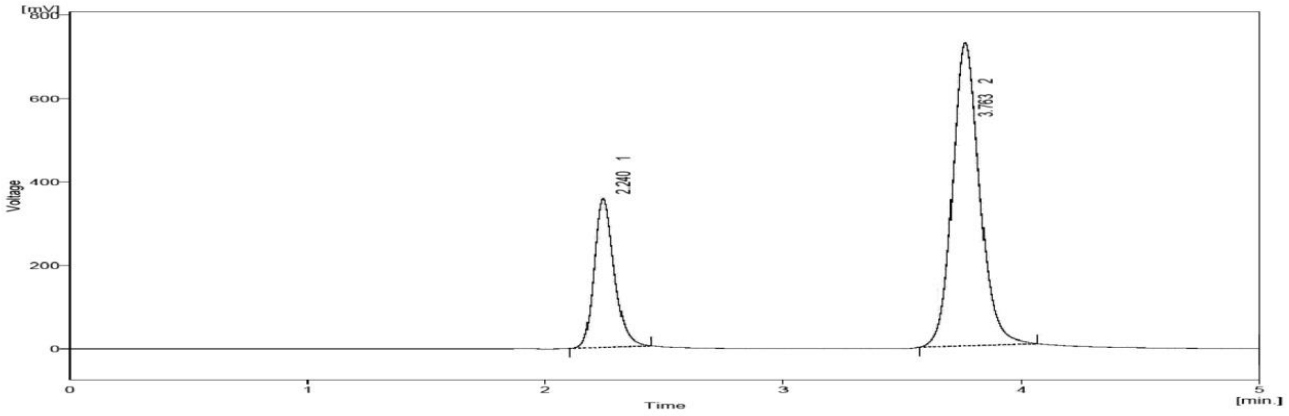

Figure-2 Chromatogram of 50% recovery

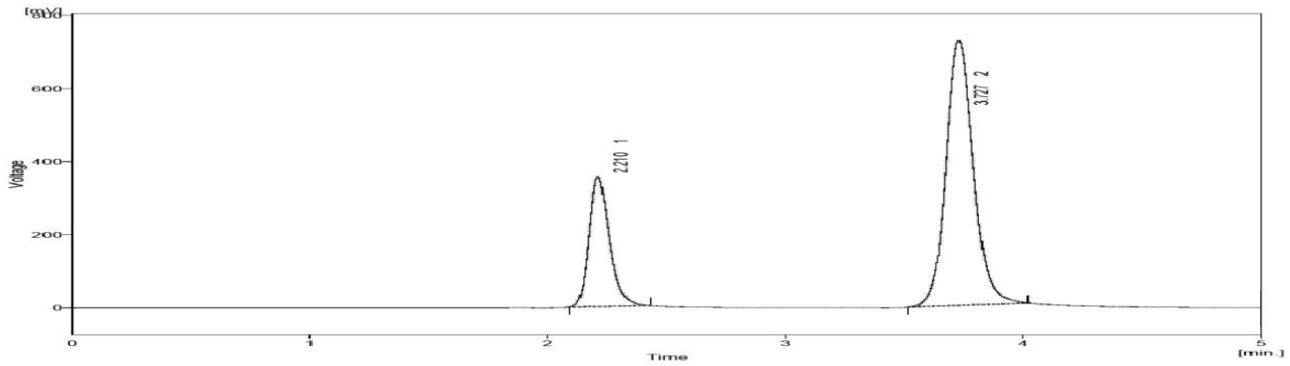

Figure-3 Chromatogram of 100% recovery

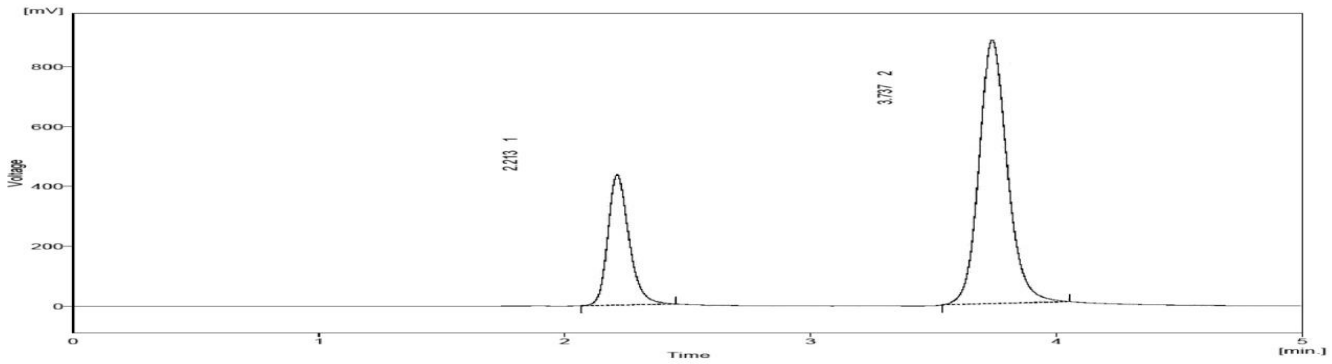

Figure-3 Chromatogram of 100% recovery

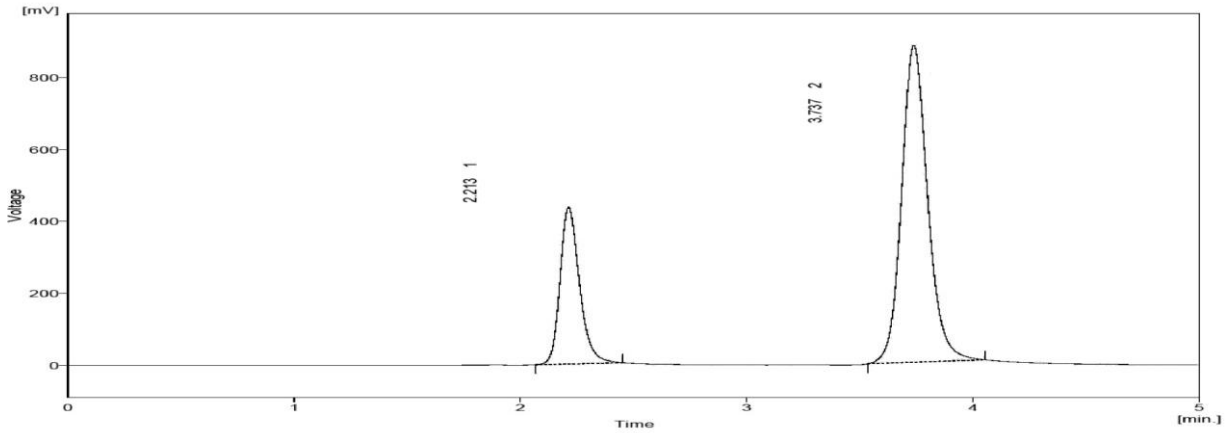

Figure-4 Chromatogram of 150% recovery

The percentage mean recovery of Isoniazid and Rifampicin is 100.93% and 108.93% respectively.

Test results for Rifampicin and Isoniazid are showing that the %RSD of Assay results are within limits.

The LOD for this method was found to be 0.0862 µg/ and 0.0424µg/ml for Isoniazid and Rifampicin.

The LOQ for this method was found to be 0.261µg/ml for Isoniazid and 0.128 µg/ml for Rifampicin.

From the observation the between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation Isoniazid and Rifampicin was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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