METHOD DEVELOPMENT AND VALIDATION OF RIFAMPICINE AND PIPERINE IN THEIR COMBINED DOSAGE FORM

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ABSTRACT

The RP-HPLC method has been developed for simultaneous estimation of Rimfapicin And Piperine in Their Combined Dosage Form. For RP-HPLC method all the standard and sample solutions were prepared in Methanol: Acetonitrile. A stability indicating reversed-phase HPLC method has been developed and subsequently validated for simultaneous estimation of Rimfapicin and Piperine in their combination product. The proposed RP-HPLC method utilizes a Hypersil BDS C18 (25cm x 4.6mm, 5 μm) column, mobile phase consisting of Buffer and Acetonitrile in the proportion of 55 : 45 (v/v) with apparent pH adjusted to 6.8, and UV detection at 341 nm.. The described method was linear over a range of 8-24 μg/ml for Rifampicin and 0.4-1.2 μg/ml for Piperine. The mean recoveries were 99.91 and 100.53% for Piperine and Rifampicine, respectively. Validations of the proposed method were carried out for its accuracy, precision, linearity and range, specificity, LOD and LOQ according to ICH guidelines. The method was validated by evaluation of different parameters. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation.

KEYWORDS

Reverse Phase High Performance Liquid Chromatography, Rifampicine and Piperine.

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INTRODUCTION

Rifampicin (RIFA) is a well known Anti-Tuberculosis drug. Methods for Estimation of Rifampicin is official in IP\textsuperscript{2}, BP\textsuperscript{3} and USP\textsuperscript{4}. Literature survey reveals HPLC\textsuperscript{5}, HPTLC\textsuperscript{6} and Visible Spectrophotometry methods\textsuperscript{7} for determination of RIFA in single and RP-HPLC\textsuperscript{8, 9} Visible Spectrophotometry\textsuperscript{10,11} and HPTLC\textsuperscript{12} methods in combined pharmaceutical dosage forms as well as in biological fluids.

Piperine (PIPE) is a natural alkaloid which is used as Bio enhancer\textsuperscript{13}. Piperine is official in IP\textsuperscript{14}. Literature survey reveals RP-HPLC\textsuperscript{15}, UV Spectrophotometry and HPTLC method for the determination of Piperine in single and in combination with other drugs. The combined dosage forms of RIFA and PIPE along with Isoniazid are available in the market and used as anti tuberculosis drugs.

The simultaneous estimation of Rimfapicin and Piperine in their combined dosage form is not official in any pharmacopoeia; hence no official method is available for the estimation of RIFA and PIPE in their combined dosage forms. Literature survey reveals simple spectrophotometric method for simultaneous estimation of RIFA and PIPE in combined dosage forms. Literature survey may not reveal any RP-HPLC for the estimation of RIFA and PIPE in their combined dosage form.

The proposed RP HPLC method was found to be sensitive, accurate and precise for determination of RIFA and PIPE in capsule dosage form. The method utilizes easily available and cheap solvent for analysis of RIFA and PIPE hence the method was also economic for estimation of RIFA and PIPE from capsule dosage form. Hence it can be conveniently adopted for routine quality control analysis of the drugs in combined pharmaceutical formulation.

EXPERIMENTAL SECTION

Apparatus and instruments

The HPLC system (Shimadzu VP, Japan), consisted of a system controller (SCL-10A\textsubscript{VP}), online degasser (DGU-14A), low-pressure gradient flow control valve (FCV-10AL\textsubscript{VP}), solvent delivery module (LC-10AD\textsubscript{VP}), auto injector (SIL-10 AD\textsubscript{VP}), column oven (CTO-10A\textsubscript{VP}), UV – VIS and PDA detector (SPD-10A\textsubscript{VP}) and CLASS – VP software version 6.14 SP1. Sonicator (5510, Branson Ultrasonics Corporation, USA) was used in the study.

Material

RIFA and PIPE bulk powder was gifted by Cadila Pharmaceuticals Ltd. Dholka, Ahmadabad, and Gujarat, India. The commercial fixed dose combination product was procured from the local market. All other reagents used were of HPLC grade.

Method Development and Optimization

Selection of detection wavelength

The standard solution of Rifampicin and Piperine were scanned over the range of 200 nm to 400 nm wavelengths. The overlay spectra were recorded and the intersection point
wavelength was selected. It was observed at 341 nm. So the wavelength, 341 nm, was selected for the determination of Rifampicin and Piperine.

**Selection of mobile phase**

The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. So no. of trials was taken for the selection of mobile phase. Generally As per I.P’2007 method for Rifampicin and various combination of Rifampicin, a combination of Buffer (1.4 gm Disodium Hydrogen Orthophosphate in 1000 ml water, pH 6.8 adjusted with dilute Phosphoric acid) with Acetonitrile was used as Mobile phase. So also for this combination of Rifampicin and Piperine, the same mobile phase in different ratio was used to optimize best result. Also different solvent mixtures were used to optimize best result.

**Selection of pH**

In mildly basic aqueous solutions (pH 8.2, 20-22°C) in the presence of air, Rifampicin is converted to rifampin quinine. Under basic conditions Rifampicin undergoes desacetylation at 22°C forming the 25-desacetylrifampin. Rifampicin decomposes rapidly in acidic or alkaline conditions at 25°C but slowly in neutral conditions so it is best to prepare aqueous solutions with oxygen-free solvent and at neutral pH. So pH 6.8 was adjusted with dilute Phosphoric acid.

**Selection of column**

For HPLC, various columns are available, but C_{18} column was preferred over other columns. Hypersil BDS C_{18} column (250 mm x 4.6 mm i.d., 5 µm particle size) was chosen to give good peak shape, good lifetime and high resolution compared to other C_{18} columns.

**Selection of column temperature**

The column temperature (30°C) has minimized variation of retention and made the peak sharp. It has also shortened the run time.

**Chromatographic Conditions**

- **Column** – Hypersil BDS C18 (25cm x 4.6mm, 5 µm)

- **Column temp.** - 25°C

- **Sample temp.** - 10°C

- **Buffer**- 1.4 gm Disodium Hydrogen Orthophosphate in 1000 ml water, pH 6.8 adjusted with dilute Phosphoric acid

- **Mobile Phase** - Buffer : Acetonitrile (55 : 45)

- **Solvent Mixture** - Methanol : Acetonitrile ( 50 : 50 )

- **Standard Solution** - (A) Piperine – 50 mg → dissolve and dilute upto 100 ml with solvent mixture
- (B) 20 mg Rifampicin → Dissolve in 70 ml Solvent mixture → 2 ml solution A → Dilute up to 200 ml with solvent mixture (Rifampicin 100 μg/ml & Piperin 5 μg/ml)

- **Flow Rate** – 1.5 ml per minute

- **Detector Condition** – 341 nm

- **Injection Volume** – 20 μl

### Buffer Preparation

Dissolve 1.42 g of disodium hydrogen phosphate anhydrous in 1000 ml of water. Adjust pH to 6.8 with orthophosphoric acid.

### Diluent

Prepare filtered and degassed mixture of methanol and acetonitrile in ratio (50:50).

### Piperine standard stock preparation:

Accurately weigh and transfer 50.0 mg of Piperine working standard of known potency into 100 ml volumetric flask. Add 70 ml of diluent and sonicate to dissolve. Dilute up to mark with diluents and mix.

### Standard Solution preparation

Accurately weigh and transfer 20.0 mg of Rifampicin working standard of known potency into 200 ml amber colored volumetric flask and add 70 ml of diluent. Sonicate to dissolve, add 2.0 ml of Piperine standard stock solution and make up to mark with diluent and mix.

### Figure 1. Chromatograph of standard RIFA and PIPE

### Table 1. Data for system suitability test for Rifampicin and Piperine

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Rifampicin ± RSD (n = 6)</th>
<th>Piperine ± RSD (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention time (min)</td>
<td>2.75 ± 0.1%</td>
<td>8.11 ± 0.2%</td>
</tr>
<tr>
<td>Tailing factor</td>
<td>1.1 ± 0.3%</td>
<td>1.0 ± 0.6%</td>
</tr>
</tbody>
</table>
Assay Preparation

Weigh 20 capsules and determine average net content of blend. Reserve Isoniazid tablet for assay of Isoniazid. Accurately weigh and transfer quantity of capsule contents equivalent to about 200.0 mg of Rifampcin into 200 ml amber colored volumetric flask. Add 100 ml of diluent and sonicate for about 20 minutes. Dilute to volume with diluent and mix. Filter resulting solution with 0.45 μm PVDF filter discarding first few ml of filtrate. Further dilute 5.0 ml of this solution to 50.0 ml with diluents and mix.

Chromatographic Parameters

Liquid chromatograph is equipped with PDA detector or variable wavelength UV detector, an injector and data processor.

- **Column** – Hypersil BDS C18 (25cm x 4.6mm, 5 μm)
- **Column temperature** - 25ºC
- **Sample temperature** - 10ºC
- **Flow Rate** – 1.5 ml per minute
- **Detector Condition** – 341 nm
- **Injection Volume** – 20 μl
- **Retention time** – Rifampicin: About 3.5 minutes. Piperine: About 7.0 minutes.

**RESULTS AND DISCUSSION**

Selection of Wavelength

The detection was carried out in the UV region and wavelength selected for detection was 341 nm in mobile Phase.

Validation of RP-HPLC Method

As per the ICH guidelines\(^\text{16}\), the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation.

**Linearity and Range**

The linearity of the method was determined for the formulation at five concentration levels ranging from 8 to 24 μg/ml for RIFA and 0.4- 1.2 μg/ml PIPE. The equation for regression
line was \( y = 23308X - 1898.2 \) (\( R^2 = 1 \)) for RIFA and \( y = 105066X - 372.4 \) (\( R^2 = 0.999 \)) for PIPE.

Figure 4. Calibration curve for Rifampicin

\[
y = 23308x - 1898.2 \\
R^2 = 1
\]

Figure 5. Calibration curve for Piperine

\[
y = 105066x - 372.4 \\
R^2 = 0.9999
\]

Table 2. Linearity of Rifampicin and Piperine by RP-HPLC method

<table>
<thead>
<tr>
<th>Linearity level, Concentration (µg/ml)</th>
<th>Mean area (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pangampicin</td>
<td>Piperine</td>
</tr>
<tr>
<td>Level-1 (50%)</td>
<td>8</td>
</tr>
<tr>
<td>Level-2 (75%)</td>
<td>12</td>
</tr>
<tr>
<td>Level-3 (100%)</td>
<td>16</td>
</tr>
</tbody>
</table>
The results show that an excellent correlation exists between response factor and concentration of drugs within the concentration range indicated above.

**Accuracy and Precision**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out at three levels of 50, 100 and 150% and the percentage recovery was calculated and presented in Table 3.

**Table 3. Recovery studies of Rifampicin (RIFA) and Piperine (PIPE)**

<table>
<thead>
<tr>
<th>Level of % Recovery</th>
<th>% Mean Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RIFA</td>
<td>PIPE</td>
</tr>
<tr>
<td>50</td>
<td>100.1</td>
<td>99.97</td>
</tr>
<tr>
<td>100</td>
<td>100.2</td>
<td>100.1</td>
</tr>
<tr>
<td>150</td>
<td>100.1</td>
<td>100.2</td>
</tr>
</tbody>
</table>

**Table 4. Intraday precision data for analysis of Rifampicin and Piperine**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μg/ml)</th>
<th>Mean area ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>50</td>
<td>1153497 ± 0.054</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2320522 ± 0.049</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>3491052 ± 0.051</td>
</tr>
<tr>
<td>Piperine</td>
<td>2.5</td>
<td>260639 ± 0.071</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>529052 ± 0.056</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>788648 ± 0.062</td>
</tr>
</tbody>
</table>
Table 5. Interday precision data for analysis of Rifampicin and Piperine

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (μg/ml)</th>
<th>Mean area ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin</td>
<td>50</td>
<td>1152086 ± 0.153</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2318678 ± 0.078</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>3490432 ± 0.172</td>
</tr>
<tr>
<td>Piperine</td>
<td>2.5</td>
<td>260608 ± 0.108</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>528969 ± 0.080</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>788593 ± 0.184</td>
</tr>
</tbody>
</table>

RSD value ranges for Rifampicin it is 0.049 – 0.054% for intraday, 0.078 – 0.172% for interday and for Rifampicin is 0.056 – 0.071% for intraday, 0.080 – 0.184% for interday. The results obtained accurately fall within the limit of acceptance criteria. Hence, the method can be termed as precise.

**Limit of detection and limit of qualification**

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated:

\[
\text{LOD} = 3.3 \times \frac{\sigma}{S}
\]

\[
\text{LOQ} = 10 \times \frac{\sigma}{S}
\]

Where, \( \sigma \) = the standard deviation of the response,

\( S \) = slope of the calibration curve.

LOD and LOQ for Rifampicin are found to be 0.498 μg/ml and 1.51 μg/ml respectively. LOD and LOQ for Piperine are found to be 0.081 μg/ml and 0.246 μg/ml.

**CONCLUSION**

From the results obtained, it is obvious that the proposed method is applicable for the determination of Rifampicin and Piperine without interference and with good sensitivity. The results obtained indicate that the proposed method for the estimation of Rifampicin and Piperine is simple, specific, rapid, linear, accurate, precise, robust, sensitive and suitable for intended use. These merits suggest the use of the proposed method in routine and quality control analysis of Rifampicin and Piperine without interference from commonly encountered excipients and additives.
REFERENCES


