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Determination of Impurity Profile in Rifapentine

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***Abstract*: Rifapentine is a semi-synthetic rifamycin antibiotic as same as structure to rifampin. Rifapentine is approved by the US FDA as a first-line drug for once or twice-weekly dosing in the treatment of TB. The developed method is validated as per the guidelines of International Conference on Harmonization (ICH). The chromatographic column of Thermo BDS-Hypersil C18 (250 X 4.6mm, 5µm) and the separation was achieved by gradient elution. Analytical trials were carried by in Waters Alliance 2695 with 2996 PDA detector consisting of a quaternary gradient solvent manager, sample manager and photo diode array detector. Thermo BDS-Hypersil C18 (250mm×4.6mm, 5µm) column at 25°C with a mobile phase containing a gradient mixture of sodium di-hydrogen orthophosphate and acetonitrile with a run time of 55 minutes at monitored wavelength of 254nm. The solubility of rifapentine in methanol, ethanol, acetone, chloroform, acetonitrile, and dichloromethane was measured at temperatures ranging from (278° to 323° K) under atmospheric pressure. The solubility of rifapentine in the above solvents increased in the following order: chloroform, methanol, dichloromethane, ethanol, acetone. The method is developed for identification and quantification of related impurities in the API. The drug has advantage of five time's longer half-life than rifampicin and it is recommended for use in intermittent therapy. Literature survey reveals that only bio-analytical method has been developed for the estimation of Rifapentine in blood, plasma, serum etc.**

***Keywords*: HPLC, Impurity profile.**

1. Introduction

Rifapentine is a rifamycin antibiotic that is similar in structure and activity to rifampin and rifabutin and that is used in combination with other compound to therapy of tuberculosis, particularly in once or twice weekly regimens. Rifapentine is associated with transient and asymptomatic elevations in serum aminotransferase and is a likely cause of clinically apparent acute liver injury [1]-[3].

The API is available in crystalline solid form in brick-red to reddish brown, crystalline powder. It is practically odourless. Its oral bioavailability is increased by ingestion of food. The published crystalline forms of rifapentine are limited to its methanol solvate, which are unsuitable for pharmaceutical use.

It inhibits DNA-dependent RNA polymerase activity in susceptible cells. Especially, it interacts with bacterial RNA polymerase but does not inhibit with a remarkably greater therapeutic efficacy against Mycobacterium tuberculosis and Mycobacterium lepraeine experimental infection. The drug has an advantage of five time's longer half-life than rifampicin and it is recommended for use in spasmodic therapy [4]. Impurity profile is very crucial and critical during the synthesis of drug substances, as it can provide critical data regarding the quality, safety, efficacy, toxicity of drugs, varies limit of detection (LODs) and limit of quantification (LOQs), structures of several organic and inorganic impurities, usually associated with bulk drugs and finished products.

HPLC is a relatively new technique that offers separation capabilities when compared to other classical analytical methods. The literature review showed that no single stability indicating High performance liquid chromatography method for all Rifapentine related impurities. [5]

Rifapentine is a Rifamycin antibiotic that is similar in structure and activity to Rifampin and Rifabutin and that is used in combination with other agents as therapy of tuberculosis, particularly in once or twice weekly regimens. Rifapentine is associated with transient and asymptomatic elevations in serum aminotransferase and is a likely cause of clinically apparent acute liver injur. Rifapentine is an antibiotic drug used in the treatment of TB. It inhibits DNA-dependent RNA polymerase activity in susceptible cells. Specifically, it interacts with bacterial RNA polymerase but does not inhibit the mammalian enzyme. The antimicrobial spectrum of Rifapentine strongly resembles that of its homologue rifapin, with a remarkably greatertherapeutic efficacy against Mycobacterium tuberculosis and Mycobacterium lepraein experimental infection [6]. The drug has an advantage of five time's longer half-life than rifampicin and it is endores for use in intermittent therapy. Literature survey reveals that only bio-analytical method has been developed for estimation of Rifapentine in blood, plasma, serum.

2. Introduction

IUPAC Name:

(7S,9E,11S,12R,13S,14R,15R,16R,17S,18S,19E,21Z)-26-[(1E)-[(4-Cyclopentylpiperazin-1-yl)imino]methyl]-2,15,17,27,29-pentahydroxy-11-methoxy-3,7,12,14,16,18,22-heptamethyl-6,23dioxo-8,30-dioxa-24 azatetracyclo[23.3.1.1[5].0[6]]triaconta-

1,3,5(28),9,19,21,25(29),26-octaen-13-yl-acetate.

Molecular Weight: 877.04

Chemical Name: Rifamycin, 3-[[(4-cyclopentyl-1-piperazinyl) imino] methyl]-; (2) 3-[N-(4-Cyclopentyl-1-piperazinyl) formimidoyl] rifamycin.

Physical Properties:

Log P: 4 [7]

Water Solubility 2.13e-02 g/L [7]

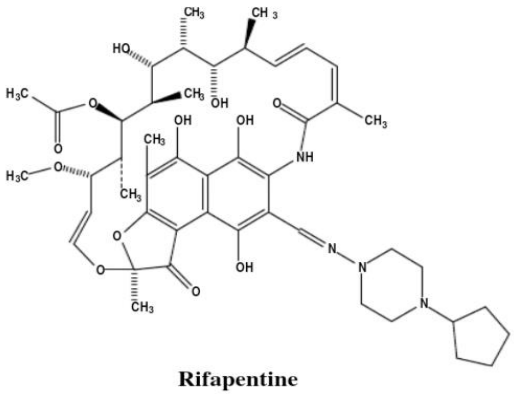
Refractive Index for Rifapentine: 1.6

Solubility:

The solubility of rifapentine is in methanol, ethanol, acetone, chloroform, and dichloromethane

Polymorphism of Rifapentine:

The hydrohalides of rifapentine showed polymorphism depending on the solvent system used for its crystallization and exists in crystalline polymorph form or an amorphous form. Two crystalline forms had been identified. Both show high stability during storage or with handling for the manufacture of the solid dosage unit forms. The crystalline modification of rifapentine mono hydrochloride showing melting point at 192°C has been identified as Form I while the crystalline modification showing a broad endotherm in the temperature range 180-220°C has been identified as form II. Transformation of Form I into the amorphous form requires a prolonged grinding in a mortar while the conversion of Form II into the amorphous form occurs in a shorter amount of time [8].



Rifapentine

3. Material and Method

A. *Chemicals and reagents*

Rifapentine supplied as a gift sample by Lupine Pharmaceuticals Ltd, Aurangabad. Methanol (A. R. Grade) was purchased from Merck Ltd., Worli Mumbai, India.

B. *Equipment*

Analytical trials were carried out in PDA detector consisting of a quaternary gradient solvent manager, sample manager and photo diode array(PDA) detector. The output signal was monitored through Empower software. The photo stability studies were carried out by using UV chamber (Advanced Instruments), Thermal stability studies were performed by using hot air oven. [9]

C. *Chromatoraphic conditions*

The chromatographic column of Thermo BDS-Hypersil C18 (250X4.6mm, 5µm) and the separation was achieved by gradient elution. Mobile phase A contains a mixture of 0.025M sodium dihydrogen orthophosphate buffer with pH adjusted to 7.7 with diluted sodium hydroxide solution and acetonitrile in the ratio of 90:10(v/v); and Mobile phase B contains a mixture of 0.025M sodium dihydrogen orthophosphate buffer with pH adjusted to 7.7 with diluted sodium hydroxide solution and acetonitrile in the ratio of 30:70(v/v). The gradient program (time (min)/%B) was set as 0.00/20, 40.00/80, 45.00/80, 47.00/20 and 55.00/20 with flow rate is 1.0mL/min and injection volume of 20□L. The column temperature was maintained at 25°C and the peaks were monitored at 254nm. Diluent is acetonitrile used for sample preparation.

D. *Preparation of solutions Preparation of standard solution*

A stock solution containing 50g/mL of Rifapentine,100g/mL of Rifapentine N-Oxide,

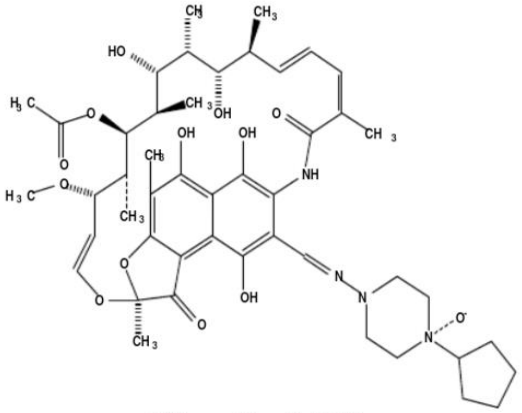
80g/mL of 3-Formyl Rifamycin and 100g/mL of Rifapentine quinone with diluent. From the above stock solution 5g/mL of Rifapentine,10g/mL of Rifapentine N-Oxide, 8g/mL of 3-Formayl Rifamycin and 10g/mL of Rifapentine quinone were prepared with diluent and used for validation.

Preparation of sample solution:

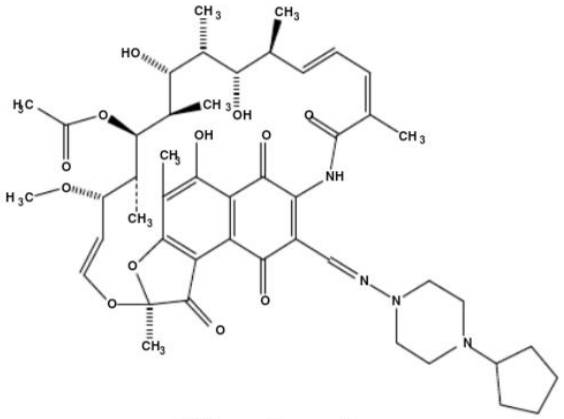
Fifty milli grams of sample was weigh and transferred in to a 50mL volumetric flask, dissolved and diluted up to the volume with diluent [10].

Table 1
Relative Retention Time and Relative Response Factor of Rifapentine and Rifapentine related impurities

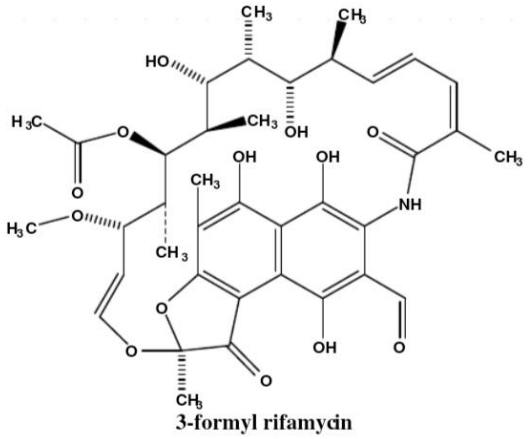
Name of the compound (impurities)	Relative Retention Time (RRT)	Relative Response Factor (RRF)
Rifapentine N-Oxide	0.38	0.38
3-formyl rifamycin	0.82	0.82
Rifapentine Quinone	0.39	0.39
Rifapentine	1.00	1.00



Rifapentine N-Oxide



Rifapentine quinone



3-formyl rifamycin

Fig. 1. Structure of related impurities

4. Result and Discussion

The impurities present in Rifapentine will be calculated by using the following formula. Impurity,

%w/w = Rt/Rs × Cs/Ct × 1/RRF × 100

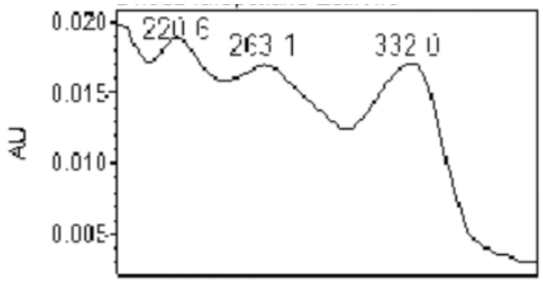
Where, Rt = Peak response of test sample,

Rs=Peak response of impurity standard,

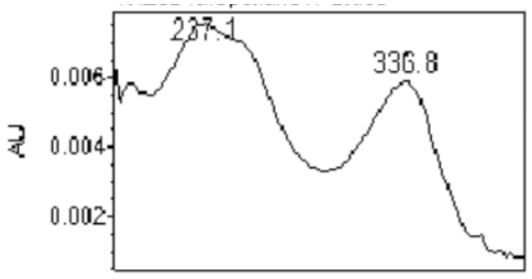
Cs = Concentration of impurity standard and

Ct =Concentration of test sample. (Table 1)

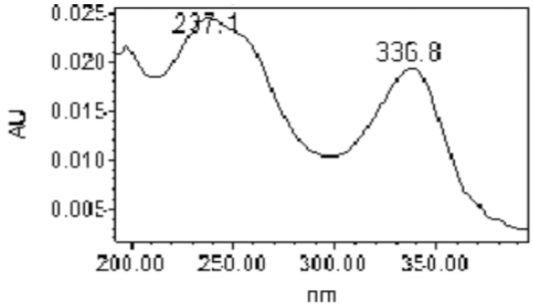
UV spectra of Rifapentine and Rifapentine related impurities showed in (Fig. 2.)



(a) Rifapentine quinone



(b) Rifapentine



(c) Rifapentine N-Oxide

Fig. 2. UV Spectra of Rifapentine and its Impurity

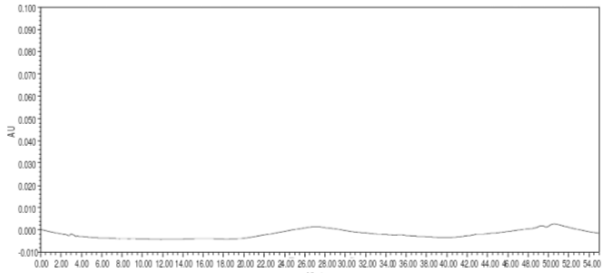


Fig. 3. Diluent

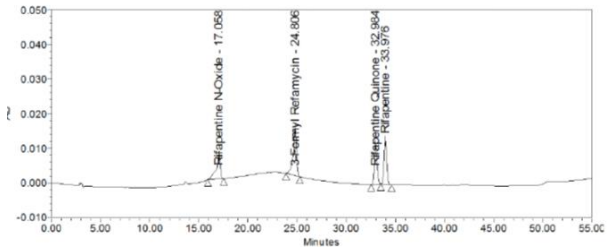


Fig. 4. Rifapentine and its Impurity

Discussion:

Chemical structure of Rifapentine related impurities are shown in Fig. 1. The impurities are labeled as Rifapentine N-Oxide, 3-Formyl Rifamycin and Rifapentine quinone. The Proposed HPLC method is selective for the quantification of Rifapentine related impurities (Rifapentine N-oxide, 3-formyl rifamycin and Rifapentine quinone) present in Rifapentine. (fig. 4).

5. Conclusion

Rifapentine, a drug used in the treatment of tuberculosis in combination with one or more other anti-tuberculosis drugs,

presents several product developments, manufacturing, and regulatory hurdles. Rifapentine is a red-colored dye and, as such, proper cleaning procedures are critical to control and demonstrate prevention of cross-contamination. In practice, this requires that the product be manufactured using dedicated equipment and rooms which can be financially, technically, and operationally challenging for manufacturers interested in such low-margin products.

The Proposed HPLC method is selective for the quantification of Rifapentine related impurities (Rifapentine N-oxide, 3-formyl rifamycin and Rifapentine quinone) present in Rifapentine. The linear curves obtained for Rifapentine and Rifapentine related impurities.

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