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Method Development and Validation for the Quantitative Estimation of Rimonabant in pharmaceutical preparation by RP- HPLC

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ABSTRACT

A Simple, selective, rapid, precise and economical reverse phase HPLC method has been developed and validated for the Quantitative Estimation of Rimonabant in pharmaceutical preparation. Isocratic separation was accomplished using C18 column (250 mn x 4.6 mm, 5 µm particle size) with mobile phase consisting of acetonitrile: 0.1% Formic acid (90:10, v/v), flow rate was 1.0 ml/min. and the detection wavelength was 268 nm. The proposed method has permitted the quantification of Rimonabant in the linearity range of 10- 50µg/ml .The column was maintained at ambient temperature and analytical run time of approximately 10 min and it was eluting at approximately 6.4 min.The percentage recovery was found to be in between 96.30 to 99.12 and the % RSD of system and method precision was found to be 1.27. The percentage amount of marketed commercial brand of Rimonabant was found to be 99.63. The method was validated for linearity, accuracy, precision, specificity, Robustness, Ruggedness, solution stability, the assay may be applied to a routine analysis in industries.

Keywords: Rimonabant; RP-HPLC; Assay method; Quantitative estimation; Method validation

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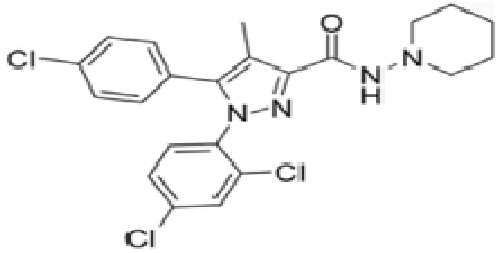
INTRODUCTION

Rimonabant, [5-(4-chlorophenyl)-1-(2, 4-dichloro-phenyl) - 4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide, Fig.1] is a neurokinin-3 antagonist and selective cannabinoid (CB1) receptor antagonist used for the management of obesity [1, 2]. Rimonabant reduces the food intake and increases the energy expenditure. These effects are due to inhibition of the CB receptors situated in the mesolimbic area. These modulate the neurochemical activation of hypothalamic neurons and the state of relative energy balance. Rimonabant also inhibits the enzymes involved in lipogenesis [3]. Rimonabant has good oral bioavailability and long duration of action (8 hours). In healthy adults with a body mass index of 18 to 28 kg/m^2 receiving once-daily doses of rimonabant 20 mg, the half-life ranged from 6-9 hours. In obese individuals with a body mass index of >30 kg/m^2, the half-life was longer (16 hours), due to the larger peripheral volume of distribution [4, 5].



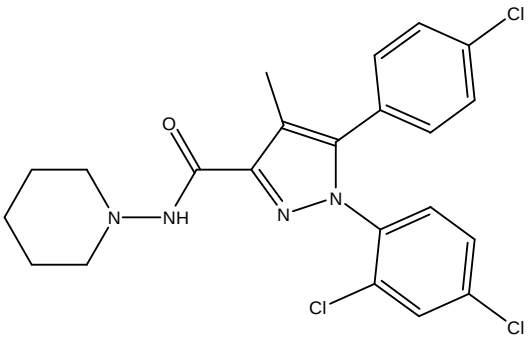


Fig.1 Molecular Structure of Rimonabant

MATERIALS AND METHODS

Chemicals and reagents

All the reagents were of analytical-reagent or HPLC grade unless stated otherwise.Rimonabant was kindly supplied by Cadila Healthcare Limited, Ahmedabad (India).Acetonitrile (Qualigens Fine Chemicals), Methanol (Rankem).Formic acid (Hi Media) triple distilled water.

Instrumentation

HPLC analysis were performed on YoungLin system equipped with quaternary SP930D gradient pump, a vacuum degasser & mixer, an UV730D UV/VIS detector and a rheodyne injector holding 20 µl loop. The signals were acquired and analyzed using Windows XP based YoungLin Autochro-3000 software.

Preparation of Stock solutions

Accurately weighed 50mg Rimonabant was transferred into 50 ml volumetric flask and dissolved in Acetonitrile, then volume was made up to 50 ml with mobile phase to get a concentration of 1000µg/ml (Stock-A). 5 ml of stock-A was taken in 25 ml volumetric and diluted up to 25ml to get concentration of 200µg/ml (Stock-B). Finally from stock-B solution different of, 10, 20, 30, 40 and 50µg/ml were prepared for analysis.

Chromatographic Conditions

Before the mobile phase was delivered into the system, mobile phase were filtered through 0.45 µm filter and degassed using vacuum. For analysis of samples, the homogeneity was expressed in terms of peak purity and was obtained directly from the special analysis report obtained using the above mentioned software. The chromatographic conditions used for the analysis were given below.The separation of the compound was made on a nucleosil-C 18 column (250 mn x 4.6 mm, 5 µm particle size) using isocratic elution. Wavelength: 268 Nm, Injection volume: 20 µl, Flow rate: 1.0 ml/min, Column temperature: 25ºC, Run time: 9 min [Fig.2]

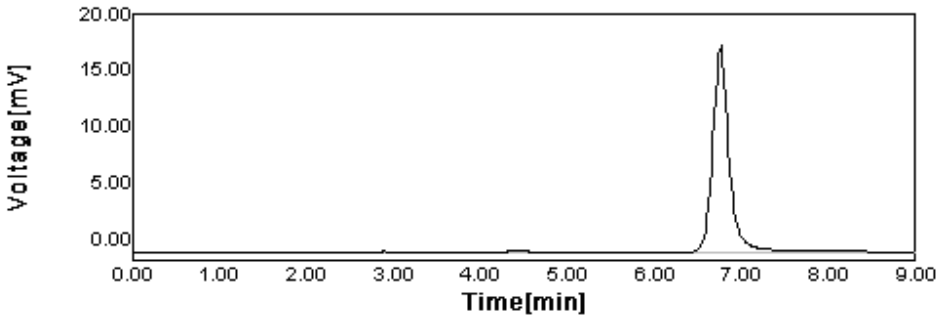


Fig.2 Chromatogram of Rimonabant

System Suitability Parameters

Separation variables were set and column was allowed with the mobile phase at a flow rate of 1.0ml/min. After complete saturation of column, six replicates of standard of Rimonabant (50µg/ml) were injected. Peak report and column performance are given in Table 1 for Rimonabant

Table 1 Result of system suitability parameters

System suitability Parameter →	RT	AUC	Tailing factor
Mean	6.64	4738.72	1.30
S.D.	0.0232	0.584	0.0175
%CV	0.0348	0.012	1.347

RESULTS AND DISCUSSION

In order to confirm the validity of the method, laboratory and Tablet samples containing Rimonabant were prepared in the range of 10µg/ml – 50µg/ml. The amount of drug present in the standard and Test solution was calculated by using the selected linearity equation and the results are tabulated in the Table 2

Table 2. Results of Laboratory and Tablet samples Analysis

Parameters	Laboratory samples	Tablet samples
% Found	98.79	99.63
SD	0.929	0.590
% RSD	0.9403	0.592
Acceptance criteria	NMT 2.0%	NMT 2.0%

Method Validation

The optimized chromatographic conditions were validated by evaluating specificity, linearity, precision, accuracy, robustness and system suitability in accordance with ICH guidelines

Linearity

The curve proved to be linear over a concentration range of 10-50 µg mL-1 (Fig3). Standard solution were prepared at five concentrations (10, 20,30,40,50 µg mL-1) were injected in triplicate. Linear regression of concentration Vs peak area resulted in an average coefficient of determination (R2) 0.9998. The Regression equation is Y= 94.792X+18.504 (Fig.3). For establishing the linearity range samples of five concentration of rimonabant in the range of 10-50 µg mL-1 was prepared and analyzed the response ratio for each concentration. The curve was plotted between response ratios Vs. Concentration. (Fig4).

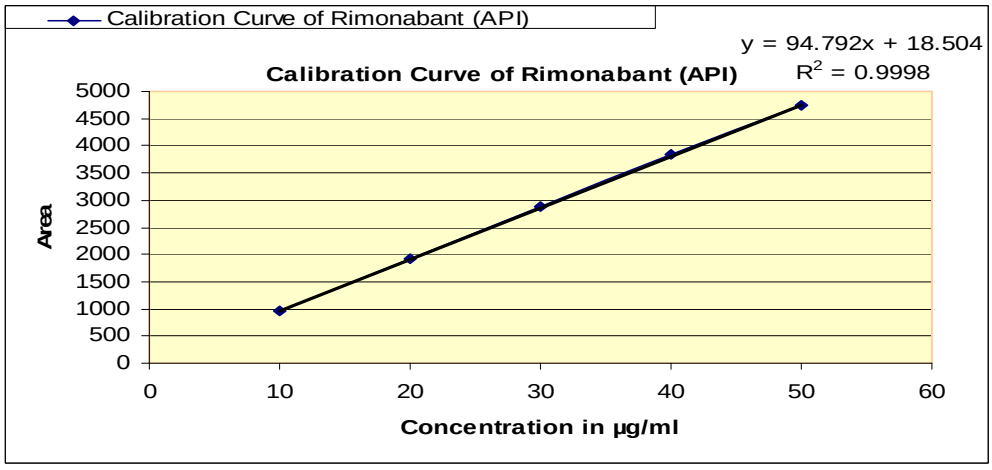


Fig.3 Calibration Curve of Rimonabant

Precision

The precision of the method was evaluated by carrying out six independent assays of test samples of Rimonabant. The precision of the method was also evaluated using two different analysts, different LC systems. The results shown in Table.3, indicates that the method is reproducible.

Table 3. Results of Precision

Precision	% Found	% RSD	Acceptance criteria
Repeatability	98.25	0.273	NMT 2.0%
Intermediate Precision	99.15	0.342	NMT 2.0%

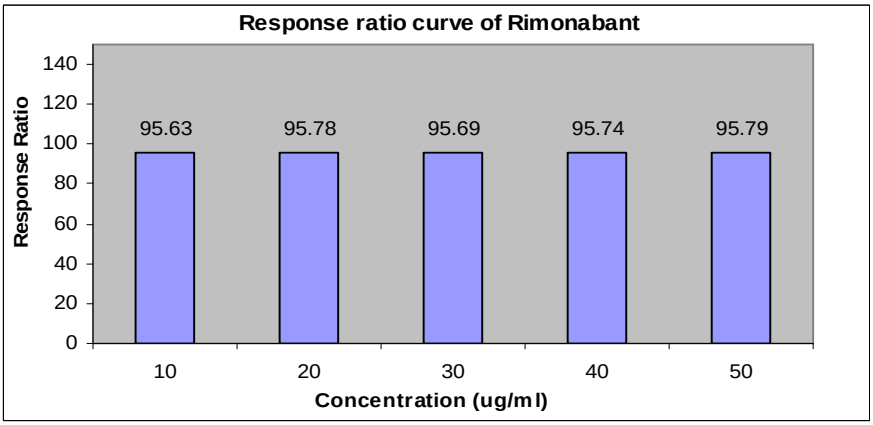


Fig. 4 Response ratio curve of Rimonabant

Accuracy

Accuracy was calculated as the percentage recovery of the known added amount of Rimonabant reference substance in the sample solutions using three concentration levels (50%, 100%, and 150%). covering the specified range (10%, 20%, and 40 µg mL-1). The accuracy of the method ranged from 98.25-99.15% indicating that this assay is reliable (Table 4).

Table 4. Results of Recovery Study

Percentage Level	% Recovery	RSD (%)	Acceptance criteria
50	96.30	1.046	NMT 2.0%
100	98.12	1.016	NMT 2.0%
150	99.12	1.273	NMT 2.0%

Robustness

To determine the robustness of the developed method, experimental conditions were purposely altered. The ratio of mobile phase was change 90:10 by ± 2 to 92:8 and 88:12 (Acetonitrile: 0.1% Formic Acid **v**/v) and Changed flow rate by ± 0.1 ml/minute [use flow rate 0.9 ml and 1. 1 ml]. While the other parameters were held constant in chromatographic condition. The RSD was not more than 2% in both conditions (Table 5).

Table 5. Results of Robustness study

Robustness study when	Changed Ratio & Flow rate	Observation	% RSD	Acceptance criteria
Mobile Phase Composition Change	92:08	The cumulative % RSD of assay values	0.0101	NMT 2.0%
	88:12		0.0073	
Flow rate Change	0 .9 ml/min	The cumulative % RSD of assay values	0.0049	NMT 2.0%
	1.1 ml/min		0.0092	

Stability in Analytical Solution

Sample and standard solution were prepared and injected and assay value calculated. After storing at 25ºc it was run against the freshly prepared standard solution at 4 hrs, 8 hrs, and 12 hrs. The % RSD was not more than 2%.

Analysis of Tablet Formulation

Tablet formulation that was used for analysis (Slimona) contains 20mg Rimonabant per tablet. Mfg. by Cadila Healthcare Limited, Ahmedabad (India) for analysis, 20 tablets were taken, accurately weighed there average weight was determined and crushed into powder. From the crushed mass, powder equivalent to 25mg of Rimonabant was accurately weighed and transferred to a 25ml volumetric flask and made up to the mark with the solvent (acetonitrile). This solution was sonicated for 20 min and filtered through whatman filter paper to get a solution of

1000µg/ml. Further diluted samples (3 replicates) in the range of 10-50µg/ml were prepared and injected to the HPLC system after filtering through 0.22µ syringe filter. Corresponding peak areas of chromatograms obtained by UV/VIS detection at 268nm were used to calculate the amount of Rimonabant present in the sample solution by using the selected linearity equation.

CONCLUSION

The method showed good specificity, sensitivity, linearity, precision and accuracy over the entire range of significant, thereby the assay may be applied to a routine analysis. The developed method can also be used for routine analysis in industries because the linearity found nearly to 1 i.e. not a component lies below 0.9998 which shows the good regression.

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