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A Validated stability indicating RP-HPLC method for the determination of Rimonabant in Bulk and Pharmaceutical dosage forms

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Abstract: A sensitive, selective, precise and stability indicating high-performance liquid chromatographic method of analysis of rimonabant both as a bulk drug and in formulations was developed and validated. The chromatographic separation was achieved on a Microbondapak C_{18} (50 cm × 4.6mm, 5µm) column using Methanol: Water: Acetonitrile (70: 23: 7 v/v) as a mobile phase. Solution concentrations were measured on a weight basis to avoid the use of an internal standard. The detection wavelength was 220nm. This system was found to give the sharp peak for rimonabant (Rt at 11.86 ± 0.05 min.). Rimonabant was subjected to acid and alkali hydrolysis, oxidation, dry heat, wet heat and photo degradation. The drug underwent degradation under acidic and basic conditions. The degraded products were well resolved from the pure drug with significant difference in their Rt values. The response of the drug was found to be linear in the range of 5-80 µg/ml (r^2 > 0.997). The method was validated for the precision and recovery. The limit of detection and quantitation were 0.395 and 1.659 µg/ml, respectively. As the method could effectively separate the drug from its degradation products, it can be employed for analysis of stability samples.
Keywords: Rimonabant, Stability indicating, Degradation, Validation and RP-HPLC.

Introduction:

Rimonabant,5-(4-Chlorophenyl)-1-(2,4-dicholoro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide[1](fig.1) is an anorectic anti-obesity drug. It is a CB1 cannabinoid receptor inverse agonist[2]. Its main avenue of effect is reduction in appetite. Rimonabant is the first selective CB1 receptor blocker to be approved for use any where in the world. In Europe, it is indicated for use in conjunction with diet and exercise for patients with a body mass index greater than 30 kg/m^2 or patients with a BMI greater than 27 kg/m^2 with associated risk factors, such as type II diabetes or dyslipidaemia. Rimonabant reduced resumption of cocaine-seeking responses triggered by two of the three most common triggers of relapse in humans, priming and cues. It may also reduce ethanol and opiate seeking behavior[3]. Rimonabant may improve short-term memory. Indeed in animal studies it significantly improved the performance of rats to encode information in the short-term memory[4]. Rimonabant is not official in any pharmacopoeia. Although some pilot data on the Spectrophotometry [5], HPLC [6, 7], LC-MS [8, 9] and LC-MS/MS [10] of rimonabant have already been reported, no simple and economic study on the drug has been performed.

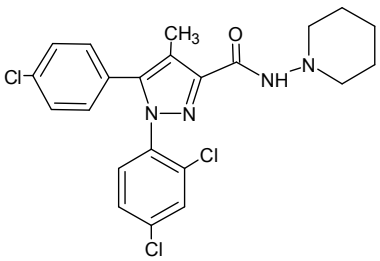

Fig.1: Chemical structure of rimonabant

The scientific novelty of the present work is that the method used are simple, rapid, sensitive, less expensive and less time consuming compared with other published HPLC methods. This paper also deals with the assay of rimonabant in presence of degradation products by HPLC.The International Conference on Harmonization (ICH) guideline entitled 'Stability testing of new drug substances and products[11], requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substances, several reports in literature highlight on this aspect. Susceptibility to oxidation is one of the required tests. Also the hydrolytic and photolytic stability are required. An ideal stability indicating method is one that quantifies the drug and also resolves its degradation products. The aim of the present study was to develop an accurate, specific, reproducible and stability indicating method for the determination of rimonabant in presence of its degradation products and for assessment of purity of bulk drug and stability of its dosage forms. Stability study of rimonabant will provide the pieces of information which will help in formulation and storage and the characterization of the degraded compounds will open a new scope of research on toxicity study of degraded components. The findings of toxicity study will help in scrupulous determination expiry, adverse effects etc.The proposed method was validated as per ICH guidelines [12, 13].

Materials and Methods

Rimonabant Active Pharmaceutical Ingredient (API) was provided by Ranbaxy Research Laboratories, Gurgaon, India. It was used without further purification. All chemicals and reagents used were of HPLC grade and were purchased from Merck (India) Ltd., Mumbai. The mobile phase was ultra sonicated for 10 min and filtered through 0.45 microns membrane filter. Also, the prepared sample solutions were filtered through 0.45 micron membrane filter before use.

The HPLC system employed was Waters (India) 510 model with UV Waters 486 tunable absorbance detector set as 220 nm. The sample was injected through a 20 µl loop injector at ambient temperature. The mobile phase used was Methanol: Water: Acetonitrile (70:23:7 v/v), flowing at the rate of 0.5/ml min. The Millennium[32] software was used for quantification and data processing.

The standard and sample stock solutions of rimonabant were prepared separately in a solvent mixture of methanol: water (48:52 v/v) at a concentration of 100 µg/ml.Working standard solutions were prepared by diluting the stock solution with above solvent to give solutions concentration in the range of 5-80 µg/ml. 20 µl of each working standard solution was injected six times and chromatograms were obtained at 220 nm using a UV detector.

Method validation:

Linear calibration plot for assay method was obtained over the calibration ranges tested, i.e. 5 µg/ml to 80 µg/ml. The peak area versus concentration data was performed by least-squares linear regression analysis.

The accuracy of the assay method was evaluated by injected six times at three concentration levels i.e. 16 µg/ml, 20 µg/ml and 24 µg/ml in bulk drug and formulation samples. The % recoveries were calculated from the slope and Y-intercept of the calibration curve obtained during linearity study of assay method.

Repeatability of measurement of peak area was carried out using six replicates of the same sample (40 µg/ml of rimonabant). The intra and inter day variation for the determination of rimonabant was carried out at three different concentration levels, 50, 60 and 80 µg/ml.

The robustness was tested by introducing small changes in the mobile phase composition. Mobile phase having different compositions of methanol: water: acetonitrile (70:23:7 ± 0.5 v/v) were tried and chromatogram was obtained. Also, the operating temperature, detection wavelength and relative humidity were varied in the range of ± 5%. Robustness of the method was done at two different concentration levels of 20 and 50 µg/ml.

In order to estimate the limit of detection (LOD) and lower limit of quantitation (LOQ), blank methanol: water: acetonitrile (70:23:7 v/v) was sampled six times. The signal to noise ratio was determined. LOD was considered at an S/N ratio of 3:1 and LOQ at an S/N ratio of 10:1.

The specificity of the method was ascertained by analyzing standard drug and sample. The retention time (Rt) of rimonabant was confirmed by comparing the Rt with that of the standard fig. 3(b). The use of placebo like calcium carbonate, starch and talc in different concentration was also studied by spiking the standard rimonabant solution and no interference was observed in the chromatogram.

Analysis of marketed formulation:

To determine the content of rimonabant in conventional tablets (label claim: 20 mg/tablet), the tablets were powdered and the powder equivalent to 10

mg of rimonabant was taken in a 100ml volumetric flask and add approximately 80 ml of suitable diluent (methanol and water 48:52 v/v). The content of the flask were then allowed to stand for 15min with intermittent sonication to ensure complete miscibility of the drug and then filtered through 0.45µm membrane filter. Final volume was made upto 100ml with above diluent to get a stock solution of 100 µg/ml. 2.0 ml of the resulting solution was transferred into a 10ml volumetric flask and diluted to the mark with diluent. A sample of 20µl of this solution was directly injected. The average drug content (Table 2) of the formulations was determined either from the calibration graph or using the corresponding regression equation.

Accelerated degradation of Rimonabant:

All degradations were done at a drug concentration of 80 µg /ml. For acid and alkali decomposition studies, the drug solution was mixed with 0.1(N) HCl and 0.1(N) NaOH separately. These mixtures were refluxed on a water bath for 4 h at 60°. The forced degradation in acidic and basic media was performed in the dark in order to exclude the possible degradative effect of light. The resulting solution was neutralized by base and acid, respectively to avoid any interference of acid or base and suitably diluted with diluent to obtain solution of concentration of 80 µg/ml.20 µl of resulting solution were injected into HPLC and the chromatograms were recorded. rimonabant was subjected to oxidative degradation by treating with 3% v/v hydrogen peroxide solution for 24h at ambient temperature. The powdered drug was stored in an oven at 60˚ for 72 h to study dry heat degradation. Also the test solution was refluxed for 6 h on a water bath set at 60˚ for wet heat degradation study. The photo chemical stability of the drug was also studied by exposing the drug to direct UV light (254nm) for 24h and the chromatogram were obtained. In all degradation studies the average peak area of rimonabant of six replicates were obtained.

Table 1: Summary of validation parameters

Parameter	Data
Linearity range (mcg/ml)	5-80
Linear regression equation	Y=28291X-28197
Correlation coefficient	0.9975
Limit of detection (mcg/ml)	0.395
Limit of quantitation (mcg/ml)	1.659
Recovery	96.89±1.20
Precision (% R.S.D.)	
Inter day*	1.21
Intra day*	0.97
Robustness	Robust
Specificity	Specific
Theoretical plate	5187
Tailing factor	1.13

***Each data represent the average of 6 reading**

Table 2: Assay of rimonabant in dosage form

Brand name of Tablets	Labeled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method* (n = 6)	% mean (± SD) labeled amount* (n = 6)
Slimona	20	18.600 (±0.42)	93.030 (±0.88)
Rimoslim	20	19.120 (±0.36)	95.600 (±0.43)

***Each data represent the average of 6 reading**

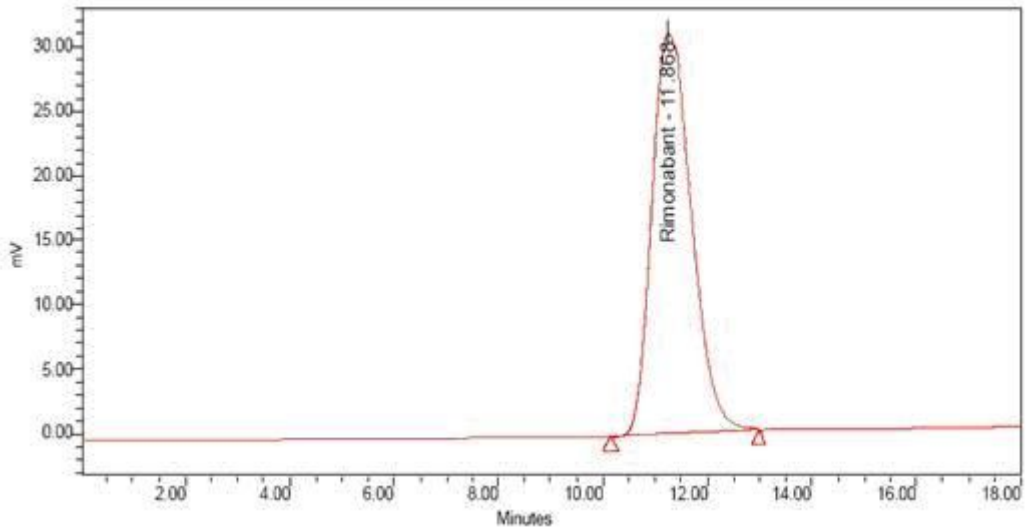

Fig. 2: Chromatogram of rimonabant tablet (20 µg/ml) at 220 nm

Table 3: Recovery studies

Amount of drug added (µg) to solution of tablet formulation	Recovery from Tablet Formulation	
	Mean (±SD) amount (µg) found* (n=6)	Mean (±SD)% recovery*(n=6)
16	15.503 (±1.32)	96.893 (±1.20)
20	16.352 (±0.84)	81.760 (±0.12)
24	20.101 (±0.68)	83.754 (±0.06)

***Each data represent the average of 6 reading**

Result and Discussion

The HPLC procedure was optimized with a view to develop a stability indicating assay method. Pure drug chromatogram was run in different mobile phase systems containing methanol, water and acetonitrile in different ratios. The retention time and the tailing factor were calculated for each chromatogram. Finally the mobile phase consisting of methanol: water: acetonitrile (70: 23: 7 v/v) was selected which gave a sharp and symmetrical peak with minimum tailing. Calibration graph was found to be linear and in adherence to Beer's law over the concentration range of 5- 80 µg/ml ($r^2 \pm$ C.V. = 0.9975, 0.929)

The repeatability of sample application and measurement of peak area were expressed in terms of % R.S.D. and found to be 0.92. The % R.S.D. for within and day to day analysis was found to be less than 2% in all the cases (Table 1). The standard deviation and % RSD of peak area calculated for each parameter was less than 2 %, indicating robustness of the method. The LOD with a S/N ratio of 3:1 was found to be 0.395 µg /ml. The LOQ with S/N ratio of 10:1was found to be 1.659 µg/ml. The peak purity of rimonabant was assessed by comparing the chromatogram of standard and sample solutions. Good correlation (r^2= 0.9975) was obtained between standard and sample spectra of rimonabant.

A single peak at Rt 11.86(fig.2) was observed in the chromatogram of the drug samples extracted from the tablets. There was no interference from the excipient commonly present in tablets. The drug content was found to be 95.60% with a SD of ± 0.43. It may therefore be inferred that degradation of rimonabant had not occurred in the marketed formulations that were analyzed by this method (Table 2**).** The low SD

value indicated the suitability of this method for routine analysis of rimonabant in pharmaceutical dosage form. The proposed method when used for extraction and subsequent estimation of rimonabant from pharmaceutical dosage form after spiking with additional drug provided recovery of 82-97% (Table 3).

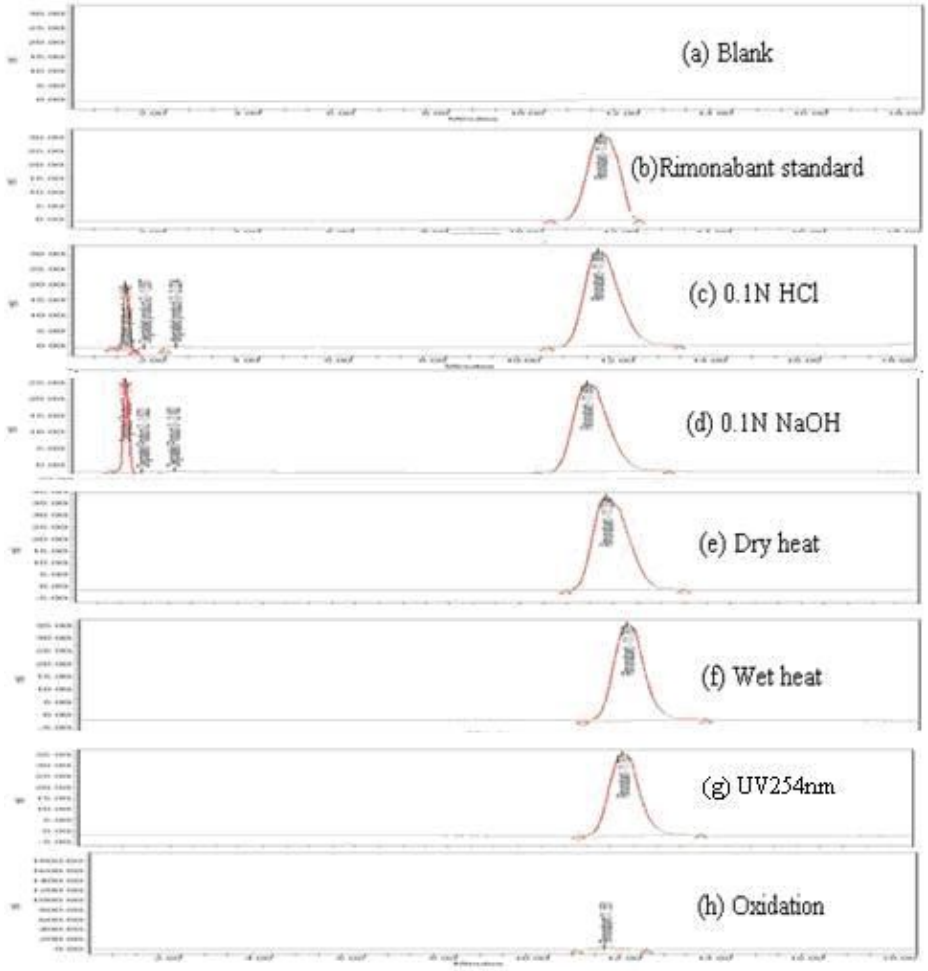

Fig. 3: HPLC chromatogram of rimonabant under different stress conditions

3a : only solvent system (methanol : water : acetonitrile 70 : 23 : 7 v/v) injected, 3b: standard chromatogram of rimonabant (20 µg/ml), 3c: hydrolysis under acidic condition, 3d: hydrolysis under basic condition, 3e: upon exposure to dry heat, 3f: upon exposure to moist heat, 3g: upon exposure to light, 3h:subjected to oxidative condition (3% H_2O_2, 24h).

Table 4: Degradation of rimonabant

Stress condition	Time (h)	% Assay of active substance	% Assay of degraded products	Mass Balance* (%)
Acid Hydrolysis (0.1N HCl)	4	68.362	26.7	95.050
Basic Hydrolysis (0.1NNaOH)	4	75.366	18.669	94.035
Dry Heat (60^0)	72	93.141	-	93.141
Wet Heat (60^0)	6	91.930	-	91.930
UV (254nm)	24	91.020	-	91.020
Oxidation (3% H_2O_2)	24	95.200	-	95.200

***It is the summation of assay of active substance and degraded products**

The chromatogram of the acid degraded sample for rimonabant showed the peak at Rt 1.14, 1.55, 2.22 and 11.86 fig.3(c). The chromatogram of the base degraded sample showed the peaks at Rt 1.14, 1.52, 2.18 and 11.86 fig.3 (d). The areas of the degraded peaks were found to be lesser than the area of standard drug concentration 80 µg/ml indicating that rimonabant underwent degradation under acidic and basic conditions, as listed in (Table 4). The peak of degraded products were well resolved from the drug peak. When rimonabant was subjected to dry heat degradation by stored in an oven at 60˚ for 72 h, wet heat degradation by refluxed for 6 h on a water bath set at 90^{o} and oxidative degradation by treating with 3% v/v hydrogen peroxide solution for 24 h at ambient temperature, no degradation was observed. Test solution of rimonabant prepared in suitable diluent was subjected to photolytic degradation by exposing the same to UV light for 24h; no degradation was observed.

Degradation studies there by indicate that the drug is susceptible to acid and basic hydrolysis with maximum degradation was observed in acid hydrolysis.

The developed HPLC technique is precise, specific, accurate and stability indicating. Statistical analysis proved method was repeatable and selective for the analysis of rimonabant as bulk drug and in pharmaceutical formulation without any interference from the excipient. Also the above result indicate the suitability of the method for acid, base, oxidative, dry heat, wet heat and photo degradation studies. As the method separates the drug from its degradation products, it can be employed for analysis stability samples.

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