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Research Article

RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF RIMONABANT HYDROCHLORIDE IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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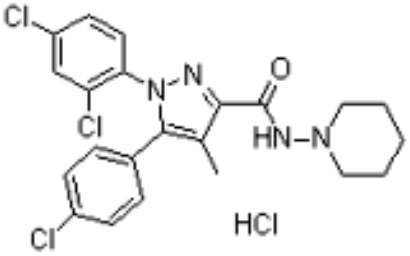
ABSTRACT

A simple and accurate RP-HPLC method has been developed for the estimation of Rimonabant hydrochloride in bulk and pharmaceutical dosage forms, using C_{18} column 250 x 4.6 mm i.d, 5µm particle size in isocratic mode, with mobile phase comprising of buffer (pH 6.8) and Acetonitrile in the ratio of 30:70 v/v. The flow rate was 0.5ml/min and the detection was monitored out by UV detector at 205nm. The retention time for Rimonabant hydrochloride was found to be 3.873min.The proposed method has permitted the quantification of Rimonabant hydrochloride over linearity in the range of 10-100 µg/ml and its percentage recovery was found to be 100.05%. The intra day and inter day precision were found 0.26% and 0.19%, respectively.

Key words: Rimonabant HCL, HPLC, Isocratic.

INTRODUCTION



Rimonabant hydrochloride is an endocannabinoid (CB_1) antagonist offer novel therapeutic approach to appetite control and weight reduction and smoking cessation. It is clinically recognized as an anorectic anti obesity drug and known as Acomplia, Bethin, Monaslim, Remonabent, Riobant, Slimona, Rimoslim, and Zimulti. Chemically it is 5-(4-Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide monohydrochloride, with empirical formula of $C_{22}H_{21}Cl_3N_4O.HCl$ and the molecular weight of 500.29. It elicits the pharmacodynamic response by blocking endogenous cannabinoid binding to neuronal CB_1 receptors in which Activation of these receptors by endoegenous cannabinoids, such as anadamide, increases the appetite. On other view the drug has the opposite effects of cannabinoid receptor agonists such as tetrahydrocannabinol, a neuro protective against excitotoxicity so as to theorize that Rimonabant promotes the development of neurodegenerative diseases of the central nervous system such as Multiple sclerosis, Alzheimer's disease, Amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Huntington's disease in persons who are susceptible. Literature review reveals that few analytical methods were evoked for the estimation of Rimonabant hydrochloride in human plasma by modern analytical instrument like LC-MS/MS[1-3] and pharmacokinetic studies of Rimonabant hydrochloride in rats[4.] In the absence of official Rimonabant hydrochloride monograph in the pharmacopoeias, including the European Pharmacopoeia, British Pharmacopoeia, and United States

Pharmacopoeia, development of such a method may prove all the more useful. We here in report a simple and reliable RP-HPLC for the estimation of Rimonabant hydrochloride in bulk and pharmaceutical dosage forms.

EXPERIMENTAL

Reagents& materials

Pure standard of Rimonabant Hydrochloride (99.85%) was obtained as gift sample from Inventis drug delivery systems Pvt. ltd, Hyderabad along with certificate of analysis (COA). HPLC grade Acetonitrile (Qualigens), HPLC grade water, Potassium dihydrogen phosphate (S.D.Fine Chemicals) H_3PO_4 (Qualigens), RIBAFIT tablets (Torrent Pharmaceuticals), RIOMONT tablets (Cipla), Electronic analytical balance (DONA), Micro pipette (In labs, 10-100µl) were employed in the study. All the glassware employed in the work cleaned with hot water followed acetic anhydride then acetone and dried in hot air oven when ever required. Working environment was maintained in between 18-22^0C. How ever, the chemical structure and purity of the sample obtained were confirmed by TLC, IR, Melting point, DSC, and XRD studies.

HPLC apparatus and chromatographic conditions

The HPLC system (Shimadzu co, Tokyo, Japan) consisted of a Shimadzu model LC-10 ATVp, a Shimadzu model SPD-6AV variable wavelength detector (Possessing deuterium lamp with a sensitivity of 0.005 AUFs and adjusted to an absorbency of 205nm), a Shimadzu model C-R5A chromatograph integrator module (chart speed at 10mm/min and an attenuation 0), a Shimadzu model SIL-6A auto injector and a Shimadzu module SCL-6A system controller. Isocratic elution of mobile phase (30:70 v/v of buffer pH 6.8 and acetonitrile) with flow rate of 0.5 ml/min was performed on C_{18} ODS analytical column (thermo hypresil, 5µm; 250x4.6mm i.d with C_{18} insert (100 A^o, waters limited) as pre column to protect the analytical column from strongly bonded material. Integration of the detector out put was performed using the Shimadzu class Vp soft ware to determine the peak area. The contents of the mobile phase were 30:70 v/v Buffer pH 6.8 and Acetonitrile. They were filtered through 0.45 µm membrane filter and degassed by sonication before use. The flow rate of mobile phase was optimized to 0.5 ml / min which yield a column back pressure of 110-112 kg/cm^2. The run time was set at 10 min and column temperature was maintained at ambient. The volume of injection was 20 µl, prior to injection of analyte, the column was equilibrated for 30-40 min with mobile phase. The eluent was detected at 205 nm.

Preparation of mobile phase

Buffer pH 6.8 and Acetonitrile in the ratio of 30:70 v/v were employed as a mobile phase and Buffer solution was prepared as directed by the procedure of Indian pharmacopoeia (1996).

Preparation of stock solution of Rimonabant hydrochloride

A stock solution was prepared by dissolving 20mg of Standard Rimonabant hydrochloride in a 100 ml volumetric flask containing 70 ml of methanol (HPLC grade) and sonicated for about 15 min and the volume made to the mark with methanol. Daily working standard solutions of Rimonabant hydrochloride were prepared by suitable dilution of the stock solution with the mobile phase where, ten sets of analyte solution were prepared in the

mobile phase containing Rimonabant hydrochloride at a concentration of 10-100 µg/ml. Each of the these dilutions (20µl) was injected six times in to the column, with flow rate of 0.5 ml/min and peak area of each of the drug concentrations, retention times were recorded.

Construction of linearity

The concentrations of analyte were prepared from the stock solution by taking suitable volume (0.5 - 5 ml) and diluted up to 10 ml to get the desired concentrations for linearity in the range of 10-100 µg/ml. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected five times into the column. The calibration curve for Rimonabant hydrochloride was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis). It was found to be linear in the concentration range 10-100 µg/ml with good correlation in between concentration and mean peak area.

Estimation of Rimonabant hydrochloride in Tablet dosage forms

20 tablets were weighed to obtain the average tablet weight and were powdered by trituration. A sample of the powdered tablets claimed to contain 20 mg of active ingredient, was mixed with 50 ml of methanol. The mixture was allowed to stand with intermittent sonication to ensure complete solubility of drug. Further the resulting solution was passed through 0.45 µm membrane filter followed by adding of methanol to obtain a stock solution of 0.2mg/ml. an aliquote of this solution (1 ml) was transferred to a volumetric flask and made up to a sufficient volume with mobile phase to get desired concentration of 20 µg/ml. The prepared dilution was injected five times in to the column to obtain chromatogram. From that peak area, the drug content in the tablets was quantified.

RESULTS AND DISCUSSIONS

Method development

Buffer pH 6.8 and Acetonitrile in the ratio of 30:70 v/v were employed as a mobile phase.

The present RP – HPLC method for the quantification of Rimonabant hydrochloride in bulk and pharmaceutical dosage forms, revealed as simple, accurate and precise method with significant shorter retention time of 3.873min. The typical chromatograms of Rimonabant hydrochloride was shown in Fig.1 and Fig.2.

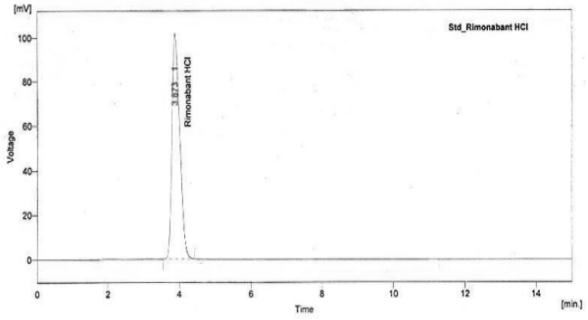


Fig.1.A Typical Chromatogram of Rimonabant Hydrochloride

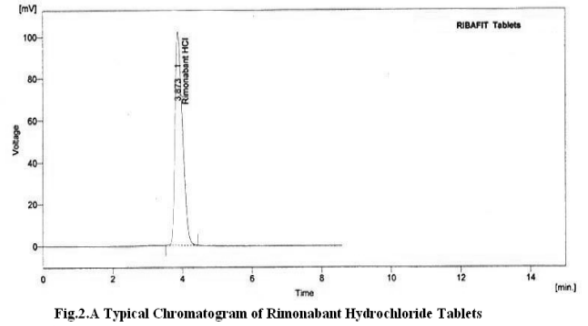


Fig.2.A Typical Chromatogram of Rimonabant Hydrochloride Tablets

Method validation

Linearity

The linearity for the detection of Rimonabant hydrochloride was 10-100µg/ml with (R^2=0.999; Y=19.11x-3.545) the coefficients of variation based on mean peak area for five

replicate injections were found to be 0.07% to 0.73%. Results were shown in **table-1** and statistical data of calibration curves were shown in **table-2**.

Table 1: Concentration Vs Mean Peak area of Rimonabant hydrochloride

Concentration (µg / ml)	Mean Peak Area*	%RSD
10	193	0.73
20	386	0.31
30	582	0.26
40	766	0.18
50	931	0.10
60	1100	0.73
70	1341	0.09
80	1519	0.07
90	1736	0.08
100	1921	0.06

*Mean of five values, Regression equation (y= 19.11x-3.545), R^2= 0.999

Table 2: Statistical data of calibration curves of Rimonabant hydrochloride

Parameters	Rimonabant hydrochloride
Linearity	10—100µg/ml
Regression equation	19.11x-3.545
Standard deviation of slope	0.013
Relative standard deviation of slope (%)	0.495
Standard deviation of intercept	0.215
Correlation coefficient (r^2)	0.999

Precision of the method

The intraday and inter-day variations of the method were determined using five replicate injections of three concentrations and analysed on the same day and three different days over a period of two weeks. The result revealed the precision with %RSD (0.26% and 0.19%) respectively for intra day and inter day. Results were shown in **table–3**.

Table 3 : Intra and inter day precision of Rimonabant hydrochloride

Concentration (µg/ml)	Observed Concentration*			
	Intra day	%RSD	Inter day	%RSD
10	10.02	0.26	10.04	0.19
20	20.03	0.14	19.98	0.17
30	29.98	0.13	30.04	0.16

*Mean of five values

Accuracy of the method

To ensure the reliability and accuracy of the method, the recovery studies were carried out by adding a known quantity of drug with pre-analysed sample and contents were reanalyzed by the proposed method. Accuracy was evaluated by injecting five times at three different concentrations equivalent to 80, 100, and 120% of the active ingredient, by adding a known amount of Rimonabant hydrochloride standard to a sample of known concentration and calculating the recovery of Rimonabant hydrochloride with RSD (%), and % recovery for each concentration. The mean % recoveries were in between 99.9-100.5% and were given in **table -4**.

Table 4: Recovery studies of Rimonabant hydrochloride

Amount Added (mg)	Amount Present (mg)	Amount Found* ±SD	%Recovery* ±SD
16	36	35.93±0.245	99.8±0.12
20	40	40.02±0.340	100.5±0.14
24	44	43.95±0.315	99.9±0.13

*Mean of five values

Estimation of Rimonabant hydrochloride in tablet formulation

The assay for the marketed tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The average drug content was found to be 100.15% of the labeled claim. No interfering peaks were found in chromatogram, indicating that the estimation of drug free from inference of excipients. The results were shown in **table-5**.

Table 5: Amount Rimonabant hydrochloride in tablet dosage forms

Tablet Brand Name	Labeled Claim (mg)	Mean Amount ±SD	%Purity ±SD
RIBAFIT	20	20.03±0.02	100.15±0.14
RIOMONT	20	19.98±0.03	99.90±0.16

*Mean of five values

System suitability

To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, limit of detection and limit of quantification and the values were shown in table-6.

Table 6 : System Suitability Parameters

Retention time	3.873
Theoretical Plates	4226
Tailing factor	1.78
Linearity Range (µg/ml)	10-100
Limit of Detection (LOD) (µg /ml)	0.090
Limit Of Quantitation (LOQ) (µg /ml)	0.302
Relative standard deviation (RSD)	0.55

Ruggedness

Ruggedness of the method (intermediate precision) was estimated by preparing six dilutions of the Rimonabant hydrochloride as per the proposed method and each dilution injected in duplicate using different column and analyst on different days. The results were shown in **table-7**.

Table-7: Ruggedness (Method Precision)

S.No	Labeled Claim (mg)	Amount estimated*(mg)	Mean ±SD	%RSD
Set-1	20	20.06	20.06±0.258	1.3
Set-2	20	19.93	19.93±0.265	1.4

*Mean of six values

Robustness

Robustness of the proposed method was estimated by changing mobile phase composition from buffer: Acetonitrile 30:70v/v to buffer: Acetonitrile 25:75 v/v, changing the column brand, changing the flow rate from 1.5 ml to 1.7ml/min, changing the pH (±0.2), changing the temperature ($\pm 5^0$c) and changing the wave length (±5nm) and System suitability parameters were found to be within acceptable limits. Results were shown in table-8 indicating that the test method was robust for all variable conditions. Hence the method was sufficiently robust for normally expected variations in chromatographic conditions.

Table 8: Robustness

Parameter	Variation	System suitability		
		Theoretical Plates	Tailing Factor	%RSD
Standard	-	4226	1.54	0.2
Flow	+0.2	3247	1.75	0.3
Wave length	-5nm	6365	1.73	0.4
	+5nm	6439	1.72	0.1
Mobile phase	30:70 to 25:75	3549	1.89	0.1
Temperature	-5°C	3325	1.86	0.5
	+5°C	3569	1.88	0.3
pH	-0.2 units	5493	1.32	0.4
	+0.2 units	5002	1.29	0.1

Detection and quantification limits

Limits of Detection (LOD) and Quantification (LOQ)**,** the limits of detection and quantitation were calculated by the method based on the standard deviation (σ) and the slope (S) of the calibration plot, using the formulae LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$.

Specificity

The specificity test of the proposed method demonstrated that the excipients from tablets do not interfere in the drug peak.

Furthermore, well shaped peaks indicate the specificity of the method.

CONCLUSION

The results of the study reveal that the proposed RP-HPLC method for the estimation of Rimonabant hydrochloride is simple and accurate in bulk and pharmaceutical dosage forms.

Acknowledgement

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REFERENCES

1. Ramakrishna N, Vishwottam K, Koteshwara M, Devender Liquid chromatography tandem mass spectrometry method for the quantification of rimonabant, a CB1 receptor antagonist, in human plasma. Bio.med. Chrom 2007; 22(5): 469 – 477.
2. Melissa M, Xiang Z, Yan X, Steve B, Linda S Determination of endocannabinoid receptor antagonist SR141716 (rimonabant) in plasma by liquid chromatograph tandem mass spectrometry. j.chrom b 2008; 863 (2): 258-65.
3. Marie Paule G, Laurence H, Franck F, Isabel M, Marta V, Sandrine Bes H, Claude R, Christine Robert Da S, Serge C, Christian Lefebvre d, Maya C, Vincenzo Di Marzo, Regis R. Identification of Endocannabinoids and Related Compounds in Human Fat Cells. Obesity 2007; 15: 837–845.
4. Zoe D. Thornton J, Guy A, Karen R , Dean F, Anil M ,Daniel M. Sellwood , Steven P and Peter G. The cannabinoid CB1 receptor inverse agonist, rimonabant, modifies body weight and adiponectin function in diet-induced obese rats as a consequence of reduced food intake.Pharmacol. biochem and behavi 2006; 84: 353–359.