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Full Length Research Paper

A NOVEL VALIDATED RP-HPLC METHOD DEVELOPMENT FOR THE ESTIMATION OF RIMONABANT HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORMS

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A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of rimonabant hydrochloride in bulk and tablet dosage forms with greater precision and accuracy. Separation was achieved on C18 column (250X4.6mm i.d., 5µm) in isocratic mode using methanol and water in the ratio 90:10 (v/v) as mobile phase, pumped in to the column at flow rate of 1 ml/min and the detection of eluent from the column was carried out using variable wavelength detector at 280 nm. The total run time was 10 min and the column was maintained at ambient temperature. The retention times of rimonabant hydrochloride and saquinavir mesylate were 5.760 min and 4.657 min, respectively. The standard curves were linear over the concentration range of 0.2-10 µg/ml and the LOD and LOQ values for rimonabant hydrochloride were 0.0113µg/ml and 0.0345µg/ml, respectively. The percentage recovery was found to be 100 to 100.22 and the % RSD of intraday and inter day precision was found to be 0.572 and 0.549, respectively. The percentage amount of two different marketed tablet formulation of rimonabant hydrochloride was found to be 100.4 and 100.5%. The method was validated as per ICH guidelines. Validation studies demonstrated that the proposed RP-HPLC method is simple, specific, rapid, reliable and reproducible. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the routine quality control analysis of rimonabant hydrochloride in bulk and tablet dosage forms.

Key words: Rimonabant hydrochloride, RP-HPLC, saquinavir mesylate, validation, isocratic, ICH guidelines

INTRODUCTION

The endocannabinoid system controls food intake through both central and peripheral mechanisms, and it may also stimulate lipogenesis and fat accumulation (Mazro et al., 2005). Discovery of the cannabinoid receptors has led to the development of rimonabant, a cannabinoid-1 (CB_1) antagonist (Patel et al., 2007).

Rimonabant hydrochloride (RMTH), Acomplia is a specific inhibitor of the endocannabinoid system (Ducobu et al., 2005) and it is the first of a new class of selective cannabinoid receptor-1 blockers. It is a peripherally acting endocannabinoid (CB_1) antagonist (Son et al., 2010) that offers novel therapeutic approach to appetite control, weight reduction, smoking cessation and it was an investigational agent for the management of cardiovascular risk factors (Cox, 2005). It reduces the over activity of the endo cannabinoid system, improving lipid and glucose metabolism and regulating food intake and energy balance (Henness et al., 2006). Cannabinoid

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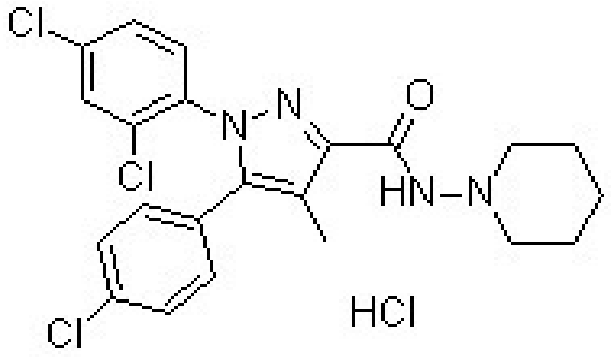

Figure 1. Chemical structure of rimonabant hydrochloride.

drugs exert their effects primarily through activation of cannabinoid CB_1 and CB_2 receptors. Both CB_1 and CB_2 receptors have been implicated in a number of cardiovascular processes, including vasodilation, cardiac protection, modulation of the baroreceptor reflex in the control of systolic blood pressure, and inhibition of endothelial inflammation and the progress of atherosclerosis in a murine model (Ashton et al., 2007).

These effects are mainly mediated through central and peripheral nervous system CB_1 receptors, vascular CB_1 receptors and immune cell CB_2 receptors. Recent data on potential anti-obesity drugs currently undergoing Phase III trials, such as rimonabant and topiramate, demonstrate that these drugs produce greater and more prolonged weight loss (Halford, 2004). It is an anti obesity drug that aid to smoking cessation treatment of alcohol dependency cannabinoid CB_1 antagonist (Sorberal et al., 2005). Chemically, it is 5-(4- Chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide mono hydrochloride (Figure1), with empirical formula of $C_{22}H_{21}C_{l3}N_4O.HCl$ and the molecular weight of 500.29. Literature review reveals that few analytical methods were evoked for the estimation of RMTH in human plasma (Ramakrishna et al., 2007; Melissa et al., 2008; Fouad et al ., 2009), in human plasma and hair (Stanislas Grassin et al., 2008), in rat plasma (Rajesh et al., 2009), in monkey plasma (Javors et al., 2010), identification of endo cannabinoids and related compounds in fat cells by different analytical techniques (Marie Paule et al., 2007), HPLC method for bio analytical application with rimonabant (Uttam et al., 2009), simultaneous estimation of rimonabant with orlistat (Wei et al., 2010) and with four major species in slimy foods (Zhou et al., 2010) Detection and quantification of synthetic drugs in herbal slimming formula (Sofian et al., 2009) , pharmacokinetic studies of RMTH in rats (Zoe et al., 2006), stability indicating assay of RMTH in bulk and pharmaceutical dosage forms by HPLC (Felipe et al., 2010; Satyanarayana et al., 2009), by HPTLC (Rajesh et al., 2010), estimation of RMTH in bulk and pharmaceutical dosage forms using external standard method (Sreekanth et al., 2009) and spectrophotometric estimation of RMTH in marketed formulations (Manas et al., 2009) were reported. In the absence of official RMTH 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. In this report a simple, rapid, accurate and economic RP-HPLC method for the estimation of RMTH in bulk and pharmaceutical dosage forms using saquinavir mesylate as internal standard than the methods existing in the literature are presented.

MATERIALS AND METHODS

Materials

Pure standard of RMTH (purity 99.89%) was obtained as a gift sample from Inventis drug delivery systems, Pvt. Ltd, Hyderabad and pure sample of saquinavir mesylate (IS, assigned purity 99.97%) was obtained from Dr. Reddy'S Laboratories, Pvt, Ltd, Hyderabad. HPLC grade Methanol and water were of HPLC grade obtained from Qualigens, Electronic analytical balance (DONA), Micro pipette (In labs, 10-100µl) and Ribafit tablets (Torrent Pharmaceuticals) Riomont tablets (Cipla Ltd) with the labeled claim of 20 mg were procured from the local market. The chemical structure and purity of the sample obtained were confirmed by TLC, IR, Melting point, DSC studies.

Methods

Instrumentation and chromatographic conditions

A chromatographic system with an Agilent LC 1200 HPLC pump, a variable wavelength detector with deuterium lamp and EZ chrom elite soft ware used for isocratic elution of mobile phase with the contents of methanol and water in the ratio of 90: 10(v/v) at flow rate of 1ml/min was performed on C18 column (250x 4.6 mm i.d., 5µm). 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 eluents were monitored at 280 nm and data were acquired, stored and analysed with soft ware EZ chrom elite. The mobile phase was premixed, filtered through 0.45 µm nylon

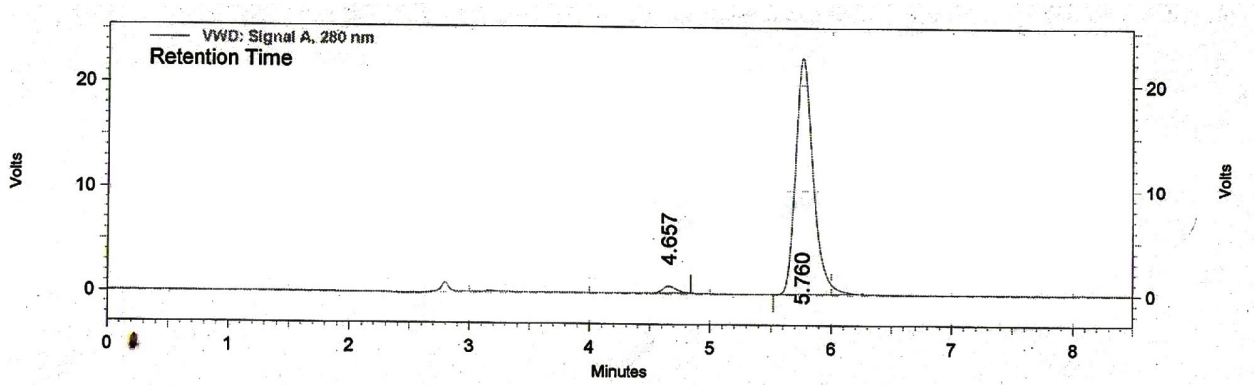

Figure 2. A typical chromatogram of RMTH with saquinavir mesylate (IS).

filter and degassed by sonication

Preparation of mobile phase

The HPLC grade solvents of methanol and water were used for the preparation of mobile phase in the ratio of 90:10 (v/v). The contents of the mobile phase were filtered before use through a 0.45 µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 ml/min.

Preparation of standard solutions

A stock solution of drug and internal standard (IS) was prepared by dissolving 100 mg of RMTH and saquinavir mesylate in a 100 ml volumetric flasks containing 70 ml of methanol (HPLC grade), sonicated for about 15 min and then made up to volume with methanol. Daily working standard solutions of RMTH and saquinavir mesylate were prepared by suitable dilution of the stock solution with the mobile phase. Eight sets of the drug solution were prepared in the mobile phase containing RMTH at a concentration of 0.2 to 10 µg/ml along with a fixed concentration (0.5 µg/ml) of saquinavir mesylate as the internal standard. Each of these drug solutions (20 µl) was injected six times into the column, the peak area and retention times were recorded.

Construction of linearity

The concentrations of analyte were prepared by suitable dilution of stock solution to get concentrations in the linear range of 0.2 to 10 µg/ml. Each of these drug solutions (20 µl) was injected six times into the column, the peak area and retention times were recorded. The calibration curve for RMTH was constructed by plotting the ratio of the peak area of RMTH to the peak area of the internal standard (Y) against concentration (X) and it reveals that there is a good correlation in between concentration and mean peak area ratio.

Procedure for pharmaceutical formulation

Twenty tablets were weighed to obtain the average tablet weight and were powdered by trituration. A sample of the powder claimed to contain 20 mg of the active ingredient was mixed with known amount of methanol and the mixture was allowed to stand for 30 min with intermittent sonication to ensure complete solubility of the drug, then it was filtered through a 0.45 µm membrane filter, followed by addition of methanol to obtain a stock solution of 0.1 mg/ml (100 µg/ml). An aliquot of this solution (1 ml) was transferred to a 10 ml volumetric flask along with 0.5 µg/ml of saquinavir mesylate and made up to a sufficient volume with the mobile phase to give an expected concentration of 10 µg/ml. All determinations were conducted in triplicate.

RESULTS

A RP-HPLC method was developed and validated for the determination of RMTH in bulk and tablet dosage forms using saquinavir mesylate as internal standard (IS) on a column ($C_{18,}$ 250X4.6 i.d., 5µm) with variable wavelength detection at 280 nm. The retention times of RMTH and internal standard saquinavir mesylate was 5.760 min and 4.657 min, respectively. The typical chromatograms of RMTH with internal standard in bulk and tablets were shown in Figures 2 and 3. A standard plot of RMTH was constructed by plotting the ratio of the peak area of RMTH to the peak area of the internal standard (Y) against concentration (X). It was found to be linear with a correlation coefficient (r^2) of 0.9996, the corresponding linear regression equation being y = 3.5451x+1.3975. In the linear range of 0.2-10µg/ml, the coefficients of variation (CV) based on the peak area ratios for six replicate injections, were found to be in between 0.03 to 0.53. The values were shown in table.1. The regression characteristics, such as standard deviation of slope (S_b), the RSD of the slope, the standard deviation of intercept (S_a), regression equation and correlation coefficient (r^2) were calculated. The values were shown in table.2. The precision of the method was determined by repeatability and intermediate precision studies. Repeatability was evaluated by performing six determinations *(n=6)* at the same concentration, during the same day, under the same experimental conditions. Intermediate precision was evaluated by comparing the results of assay on three different days with different analysts. The result revealed the precision with %RSD for intraday and inter day 0.572, 0.549, respectively. The results were shown in Table 3.

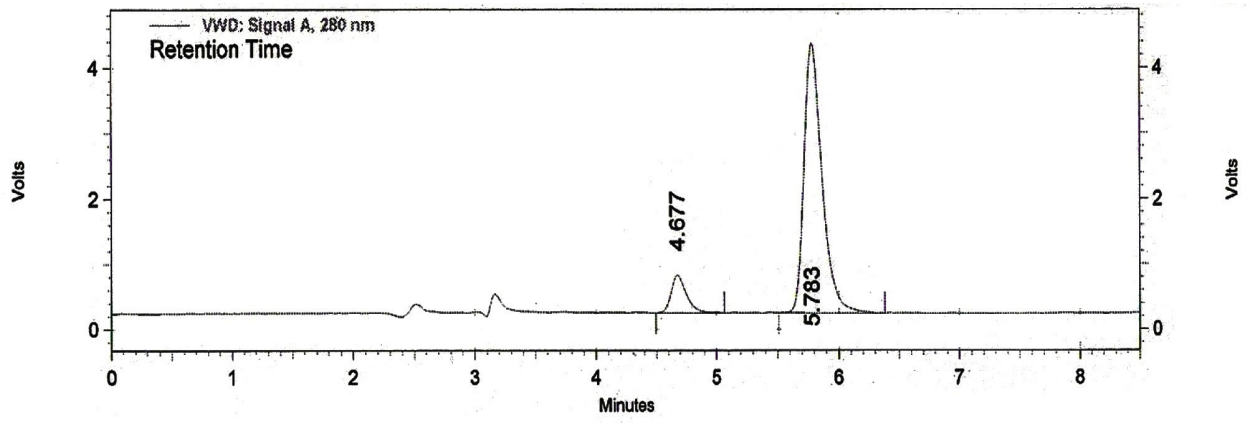

Figure 3. A typical chromatogram of RMTH with saquinavir mesylate (IS) in tablet dosage form.

Table1. Linearity of the method.

Concentration (µg/ml)	Mean peak area ratio*	%CV
0.2	2.33	0.13
0.4	2.72	0.07
0.8	4.2	0.15
1	4.48	0.03
2	8.63	0.45
4	15.7	0.39
6	23	0.53
10	36.62	0.36

*Mean of six values, (r^2 = 0.09996, Y= 3.5451x+1.3975

Table 2. Statistical data of calibration curves of RMTH.

Parameters	RMTH
Linearity	0.2-10 µg/ml
Regression equation	3.5451x+1.3975
Standard deviation of slope	0.214
Relative standard deviation of slope (%)	0.634
Standard deviation of intercept	9.07×10^{-3}
Correlation coefficient (r^2)	0.9996

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 at three different concentrations equivalent to 80, 100, and 120% of the active ingredient, by adding a known amount of RMTH standard to the sample of known concentration and calculated the recovery of RMTH with % RSD and % recovery for each concentration. The mean % recoveries were 100 to 100.22 and the values were shown in Table 4. The high recovery of RMTH indicating that the proposed method for determination of RMTH in tablet dosage forms was highly accurate. The assay of commercial tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The percentage amount of RMTH was found to be 100.4 (Ribafit) and 100.5 (Riomont) of the labeled claim and no interference peaks were found in chromatogram, indicating that estimation of drug free from interference of excipients. The results were shown in the Table 5. To know reproducibility of the method system

Table 3. Intraday and inter day precision of the method.

RMTH	% Recovery		
	Day 1	Day 2	Day 3[a]
1	100.4	100.2	100.2
2	100.5	100.9	101.1
3	100.6	99.9	100.5
4	99.9	99.7	100.4
5	100.2	99.5	101.2
6	99.1	100.4	99.67
Intraday[b] (n=6)	100.11±0.556	100.1±0.509	100.51±0.572
Inter day[b] (n=18)	100.24±0.549		

[a] Different analyst, [b] Mean ± %RSD

Table 4. Accuracy of the method.

RMTH	80% level	100% level	120% level
1	100.4	100.5	100.03
2	99.97	100.2	99.52
3	100.3	99.71	100.2
Mean (n=3)	100.22	100.13	100.00
% RSD	0.22	0.39	0.42

Table 5. Assay of the method.

Brand name	Labeled claim (mg)	Mean amount found*±S.D	% purity
RIBAFIT	20	20.08±0.22	100.4
RIOMONT	20	20.10±0.366	100.5

*Mean of three values

suitability test was employed to establish the parameters such as tailing factor, theoretical plates, resolution, asymmetry factor, and asymmetry (10%), limit of detection and limit of quantification and the values were shown in table-6. The ruggedness of the method was assessed by comparison of intra-day and inter-day results for assay of rimonabant hydrochloride performed by two analysts, by two columns and by two chromatographic systems in the same laboratory. The results were shown in Table 7. The robustness of the method was investigated by making small deliberate changes in the chromatographic conditions at three different levels. The chromatographic conditions selected were flow rate (1, 1.1, and 0.9 ml/min), amount of methanol in the mobile phase (90%, 100%, and 110%) and the column temperatures (28^0C, 30^0C and 32^0C). The System suitability parameters were established and found to be within acceptable limits and the proposed method indicating that the test method was robust for all variable conditions. The results were shown in Table 8**.** The limits of detection (LOD) and quantitation (LOQ) 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 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.

DISCUSSION

The development of HPLC methods for the determination of drugs has received great attention in analytical research because of their importance in the quality control. HPLC is the unique, versatile, universal, basic instrument and well utilized by the researchers because of its ease in the operation, availability and in terms of economy. The main objective of method development was to determine the drug content present in the formulation and its percentage (%) purity (Qualitative and

Table 6. System suitability parameters.

Parameter	Value
Retention time (min)	5.760
Theoretical plates	7584
Tailing factor	0.833
Linearity range (µg/ml)	0.2-10
Limit of detection (µg/ml)	0.0113
Limit of quantification(µg/ml)	0.0345
Resolution	4.448
Asymmetry factor	1.198
Asymmetry (10%)	1.1835

Table 7. Ruggedness of the method.

S.No	Labeled amount (mg)	Analyst 1	Analyst 2	System 1	System2	Column1	Column 2
1	20	20.02	19.56	20.00	19.95	20.42	21.02
2	20	19.97	20.00	19.89	20.23	19.93	19.56
3	20	20.26	20.13	20.67	19.62	20.12	20.23
4	20	20.08	18.93	19.68	19.84	20.53	20.00
5	20	19.53	19.67	19.93	20.83	19.67	19.87
6	20	20.72	20.34	20.03	20.47	20.12	19.81
	Mean ± SD	20.09±0.389	19.77±0.503	20.03±0.335	20.15±0.444	20.13±0.314	20.08±0.509

Table 8. Robustness of the method.

System suitability parameters	Variation in the mobile phase composition			Variation in the flow rate			Variation in the column temperature		
	90%	100%	110%	0.9 ml	1ml	1.1 ml	28°c	30°c	32°c
%RSD	0.15	0.23	0.18	0.38	0.45	0.20	0.18	0.78	0.32

quantitative analysis). In analytical research the time and cost for the method development, validation and the method of quantification was greatly considered. The method was optimized to provide a good separation of RMTH and IS (acceptable theoretical plates and resolution between peaks) with a sufficient sensitivity and suitable peak symmetry (peak tailing factor < 2) in a short run. For this purpose, the analytical column, solvent selection, mobile phase composition, flow rate, and detector wavelength were studied. The use of hydrophobic stationary phases usually provides adequate retention of organic non polar molecules. The chromatographic separation was achieved using an RP C18 column because it was suitable to resolve the degradation products from RMTH with adequate resolution and gave symmetrical peak shapes. Our experiments and data reported in the literature showed that both the methanol and acetonitrile could be used as an organic modifier in the mobile phase. But the tests using methanol was done with water (HPLC grade) as mobile phase was eluted the RMTH in a significant shorter retention time of 5.760 min. We selected methanol and water in the ratio of 90:10 (v/v) as a mobile phase. The method has many advantages, e.g., simplicity, isocratic conditions, usage of internal standard and absence of buffers in the mobile phase that could damage the chromatographic column and equipment. Under these conditions, the retention time of RMTH was 5.760 min, with a good peak (peak symmetry), and the chromatographic analysis time was less than 10 min.

Conclusion

An isocratic RP-HPLC method developed for determination of RMTH in both bulk and tablet dosage form. The validation data demonstrates good precision and accuracy, which proves the reliability of the proposed method. The short runtime and simple extraction procedure are advantageous for analyzing routine quality

control samples of RMTH.

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