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STABILITY INDICATING HPLC METHOD FOR DETERMINATION OF RIMONABANT

Y. PADMAVATHI*, P. HARI KRISHNA and B. MADHAVA REDDY

G. Pulla Reddy College of Pharmacy, Mehdipatnam, HYDERABAD – 500028 (A.P.) INDIA

ABSTRACT

A simple, inexpensive and rapid, stability indicating isocratic HPLC method has been developed for the quantitative determination of rimonabant, an anti-obesity drug. Degradation studies were performed on the bulk drug by heating to 105°C, exposure to UV light at 254 nm, acid (0.1 N hydrochloric acid), base (0.1 N sodium hydroxide) and aqueous hydrolysis and oxidation with 3.0 % *v/v* hydrogen peroxide. Considerable degradation was observed under oxidation conditions. Good resolution between the peaks corresponding to impurities produced during synthesis, degradation products and the analyte was achieved on Ace-C18, 150 x 4.0 m.m column using a mobile phase consisting of a mixture of aqueous potassium dihydrogen phosphate and acetonitrile. Validation of the method was carried out as per ICH requirements.

Key words: HPLC, Degradation studies, Rimonabant, Method validation.

INTRODUCTION

Rimonabant is an anti-obesity drug. Chemically, rimonabant is 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(piperidin-1 yl)-1-H-pyrazole-3-carboxamide. (Molecular formula: $C_{22}H_{21}N_4Cl_3O$). Literature review reveals that few analytical methods were evoked for the estimation of rimonabant in human plasma by modern analytical instrument like LC-MS/MS[1-3] and pharmacokinetic studies of rimonabant in rats[4]. In the absence of official rimonabant monograph in the pharmacopoeia, including the European Pharmacopoeia, British Pharmacopoeia and United States pharmacopoeia, development of such a method may prove all the more useful. We herein report a simple and reliable stability indicating RP-HPLC for the estimation of rimonabant in bulk and pharmaceutical dosage forms.

*Author for correspondence; E-mail: pgundlapalli@yahoo.com

EXPERIMENTAL

Material and methods

The separation was carried out on Shimadzu HPLC LC 2010 AP Series with Agilent 1100 series software with UV detector. The column used was Ace – C 18 (150 x 4.0 mm, particle size 5 μ).

Chemicals and reagents

Rimonabant, is a gift sample from Hetero Pharmaceuticals. Acetonitrile HPLC grade and Milli-Q purified water were used. Ammonium acetate, triethylamine, trifluoroacetic acid, hydrogen peroxide and sodium hydroxide of AR grade were procured from S.D Fine Chemicals.

Chromatographic conditions

The column used was a C 18, 150 x 4 mm, stainless steel column with particle size 5 μ. The high performance liquid chromatograph was operated at ambient temperature. The flow rate of the mobile was maintained at 1.0 mL/min. Detection was carried out at 250 nm and the injection volume was 20 μL.

Preparation of mobile phase

Accurately weighed of ammonium acetate (3.854 g) was dissolved in 1000 mL of water and 1 mL of triethylamine and 1 mL of trifluoroacetic acid were added the contents were dissolved. A mixture of 300 mL of buffer and 700 mL of acetonitrile was prepared. The contents were mixed and the solution was filtered through 0.45 μ membrane filter and the mixture was degassed.

Working standard solution of drug

Accurately weighed, 25 mg of pure rimonabant was transferred into 50 mL volumetric flask. About 30 mL of diluent (mobile phase) was added and than it was, sonicated for 10 min The volume was made up with diluent. 5 mL of this solution was diluted to 50 mL in a 50 mL volumetric flask with the diluent to give 50 μg/mL solution.

Estimation of rimonbant in tablets

20 commercially available tablets of rimonabant were taken, weighed and finely powdered. Powder quantity equivalent to 25 mg of rimonabant was placed in 100 mL of

clean and dry volumetric flask. The contents were dissolved in 70 mL of diluent by sonication and the volume was made up to the mark with the mobile phase. It was filtered through Whatmann filter paper. 5 mL of this solution was diluted to 25 mL in a volumetric flask with mobile phase. Then the solution was filtered through 0.45µ nylon membrane filter. 20 µL of the standard and sample solution was injected into the chromatograph. Chromatograms were recorded and peak areas were measured.

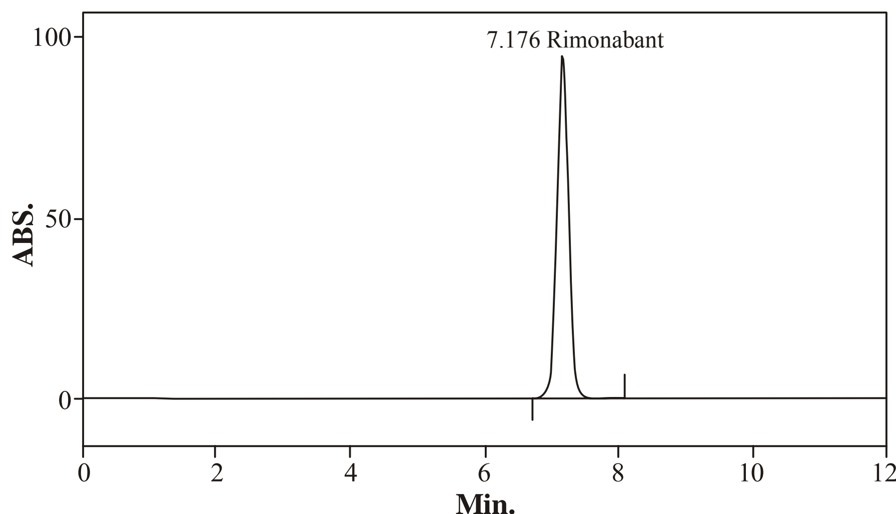

Fig. 1: A typical chromatogram of rimonabant (t_R – 7.175)

Drug content per tablet was calculated using following formula -

$$\frac{\text{Sample}}{\text{Avg. Std. area}} \times \frac{5}{50} \times \frac{100}{\text{Sample}} \times \frac{25}{5} \times \frac{P}{100} \times \text{Avg. wt.} = \text{mg/tablet}$$

Were P = Purity of rimonabant working standard.

Method validation

The method of analysis was validated as per the recommendations of ICH[9] and USP[10] for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness. The accuracy of the method was determined by calculating percentage recovery of rimonabant. Recovery studies were carried out by applying the method to drug sample to which known amount of rimonabant corresponding to 50, 100 and 150% of label claim had been added (standard addition method). At each level of the amount, six determinations were performed and the results obtained were compared.

Table 1: Assay of rimonabant in tablet dosage form by the proposed method

Pharmaceutical formulations	Labeled amount (mg)	Amount obtained by proposed method	% Recovery of proposed method
Ribafit	20	20.03 ± 0.02	100.15 ± 0.14
Riomont	20	19.98 ± 0.03	99.90 ± 0.16

Intra-day and inter-day precision study of rimonbant was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for the concentration of rimonabant.

System suitability tests are an integral part of any chromatographic analysis method, which is used to verify reproducibility of the chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution to check the reproducibility of the system and the results are shown in Table 2.

Table 2: Summary of validation and SST parameters

Parameters (Units)	Rimonabant
Linearity range (ppm)	20- 80 ppm
Correlation coefficient	0.99992
Recovery (%)	100.6
Precision (%RSD)	
Inter-day	0.75
Intra-day	0.308
Robustness	Robust
Theoretical plates	8176.36
Tailing factor	1.03

SST-System suitability parameters

For robustness evaluation of HPLC method, a few parameters like flow rate (± 10), wavelength (± 5), mobile phase composition (± 2 %), temperature and pH of mobile phase (0.2 units) were deliberately changed. One factor was changed at one time to estimate the

effect. Each factor selected was changed at three levels (-1, 0, + 1) with respect to optimized parameters. Robustness of the method was done using standard solution as per the test method and injected in five replicates at different variable conditions and the results are shown in Table 2.

Table 3: Robustness data

Parameter	% RSD of retention time
Flow rate 0.9 mL/min	0.075
1.1 mL/min	0.576
Temperature 35°C	0.368
Mobile phase variation	
Buffer : Acetonitrile	
314 : 686	0.049
286 : 714	0.113

Forced degradation studies

Forced degradation studies of rimonabant was carried out under conditions of hydrolysis, dry heat, oxidation, UV light and photolysis. Rimonabant solutions were prepared as per the test procedure and used for the degradation studies.

Forced degradation in basic media was performed by refluxing 10 mL of stock solution in 0.1N NaOH for 30 min. Forced degradation in acidic media was performed by keeping the drug in contact with 0.1N HCl for upto 30 min at ambient temperature as well as heating for up to 8 h at 70°C in dark. Degradation with hydrogen peroxide was performed by refluxing rimonabant in 3 % hydrogen peroxide solution for 1 hr. For thermal degradation, solid drugs were kept in petri dish in an oven at 105°C for 48 h. The photo stability was also studied by exposing stock solution of the drug to direct sunlight in summer days for 5 h on a wooden plank. For UV degradation study, the stock solution of the drug was exposed to UV radiation of a wavelength of 254 nm

For HPLC analysis, all the degraded sample solutions were diluted with mobile phase to obtain final concentration of 50 μg/mL. Besides, solution containing 50 μg/mL of drug was also prepared without performing the degradation. Then 20 μL solution of above

solutions were injected into the HPLC system and analyzed under the chromatographic analysis conditions described earlier.

Table 4: Summary of degradation studies for rimonabant

Degradation mechanism/Condition	% Degradation	Peak purity
Protected sample	NA	0.99741
Water reflux – 30 min	4.5	0.99216
Acid degradation 0.1N HCl – 30 min	9.5	0.99914
Base degradation 0.1N NaOH – 30 min	22.7	0.99598
Peroxide degradation		
3.0 % H_2O_2 Reflux – 1.0 hrs	2.5	0.99803
Thermal degradation –105°C 48 hrs	0.4	0.99829
Photolytic degradation at 254 nm – 24 hrs	2.9	0.99849

RESULTS AND DISCUSSION

Development of HPLC methods for the determination of drugs has received considerable attention in recent years because its importance in quality control of drugs and drug products. The aim of present work was to develop a simple, precise and rapid RP-HPLC method for quantitative analysis of rimonabant in its pharmaceutical dosage forms.

Different mobile phases were tried, but good symmetrical peak was obtained with mobile phase, buffer: acetonitrile 30 : 70. A series of solutions were prepared using Rimonabant working standard at concentration levels from 40% to 160% of target concentration. The linearity of the curve was obtained by plotting the peak area against concentration over the range of 20 - 80 ppm. (y = -9144.929, m = 56938.829, r^2 = 0.99985).

The retention time of rimonabant was found to be 7.176 min. (Chromatogram shown in Fig. 1). The results for analysis of commercial samples are shown in Table 1. The percentage recovery studies reveal that the recovery levels lie between 99 % to 100.9 %. Recovery results demonstrated that test method has acceptable level of accuracy.

The repeatability (intra-day and inter-day) of sample analysis and measurement of intensity are expressed in terms of % RSD and it was found to be less than 2 %.

The peak purity data of rimonabant peak at every degradation sample shows that the rimonabant peak is homogenous and there are no co-eluting peaks indicating that the method is stability indicating and specific. The summary of degradation studies of rimonabant is given in Table 4.

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