Eurasian Journal of Analytical Chemistry

Eurasian J Anal Chem 7(3): 115-122, 2012

RP-HPLC Method Development and its Validation for Quantitative Determination of Rimonabant in Human Plasma

Shravan K Bankey[a], Surendra K Jain[b], Pawan K Basniwal[c] and Deepti Jain[a*]

[a] School of Pharmaceutical Sciences, Rajiv Gandhi Technological University, Gandhi Nagar, Bhopal, MP 462 036, India
[b] Sagar Institute of Research and Technology, Bhopal, 462 036, MP, India
[c] LBS College of Pharmacy, Jaipur, Rajasthan, 302 004, India

Received: *22/06/2010****; Accepted:*** *02/08/2012*

Abstract

A simple, accurate and precise HPLC method was developed and validated for determination of rimonabant in human plasma. Following liquid-liquid extraction, chromatographic separation was accomplished using C18 column with mobile phase consisting of acetonitrile: water (90:10, v/v), drug was detected at 260 nm. The LOD and LOQ were 3.3 and 9.9 ng mL^{-1}, respectively. The method is linear in range 50-1000 ng mL^{-1}. The average extraction recovery of drug from plasma was found 78.45%. The precision of method was found less than 2.8%, and accuracy was found between 97.12-101.71%. The assay may be applied to a pharmacokinetic and bioequivalence study of rimonabant.

Keywords:

Rimonabant, RP-HPLC, Human Plasma, Pharmacokinetic study

1. Introduction

Chemically, rimonabant [N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichloro phenyl)-4-methyl-3-pyrazole-carboxamide] is a selective cannabinoid-1 receptor (CB1) antagonist used for treatment of obesity (Fig. 1)[1]. Initially it was intended as anti-obesity and smoking cessation dual-purpose drug but later program has been discontinued [2,3]. The anti-obesity potential of rimonabant was demonstrated in a phase 3 trial in obese patients, where significant reduction of body weight and triglyceride levels was observed in a 2-year study [4].

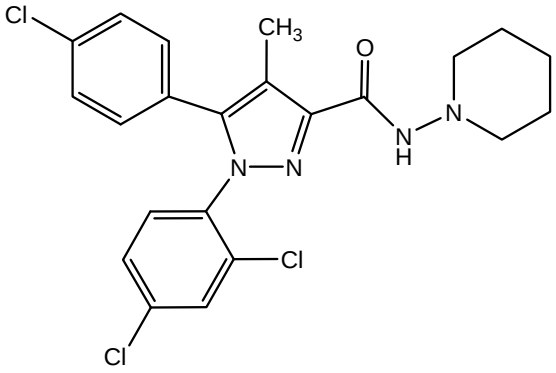

Fig. 1. Structure of rimonabant.

* **Corresponding Author**
E-mail: deepti2515@yahoo.com
ISSN: 1306-3057

The drug is believed to interact with the G-protein coupled CB1 receptor in the brain and produces several biochemical consequences, including inhibition of Ca^{+2} channel [5]; activation of K^{+} channels [6]; inhibition of adenylate cyclase [7]; and activation of mitogen-activated protein kinase [8]. The effect on Ca^{+2} and K^{+} ion channels results in a reduction of neuronal excitability and a suppression of neurotransmitter release. Rimonabant has been reduced the rewarding/reinforcing behaviors of several drugs of abuse in rodents like heroin [9], cocaine [10], etc. So rimonabant may be used in the treatment of addiction. Literature reveals a very few methods have been published for the quantification of rimonabant in plasma. According to the best of our knowledge, no analytical method using HPLC/PDA has been reported for the determination of rimonabant in plasma. Determination of rimonabant in human plasma by high performance liquid chromatography–tandem mass spectrometry (LC/MS/MS) [11] and a stability-indicating LC method for rimonabant were reported [12]. Only a few analytical methods using LC/MS/MS have been reported for the determination of rimonabant in rat and mouse plasma [13]. However, these techniques are not suitable because this equipment is not available in all clinical laboratories due to its high cost. In the present study, a simpler method has been developed and validated for the determination of rimonabant in human plasma by HPLC with PDA detection.

2. Experimental

2.1. Instruments

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. Milli-Q system (YoungLin Basic 370 series) was used for the purification of water.

2.2. Chemicals and reagents

Rimonabant was kindly supplied by Cadila Healthcare Limited, Ahmedabad (India). HPLC grade acetonitrile and methanol were purchased from Qualigens Fine Chemicals, India and Rankem, RFCL Limited India, respectively. Purified water was prepared in house. The blank human plasma with EDTA-K3 anticoagulant was collected from local blood bank.

2.3. Chromatography

The chromatography was performed on a nucleosil-C18 column (250 mm x 4.6 mm, 5 µm) by isocratic elution at flow rate of 1 mL min^{-1} with UV detection (260 nm). The mobile phase composed of acetonitrile and water in the ratio of 90:10.

2.4. Method development & validation

The developed method has been intensively validated as per bioanalytical guidelines [14], using validation parameters viz system suitability, linearity, LOQ (limit of quantitation), accuracy, precision, extraction recovery and freeze thaw and bench top stability. LOQ is the minimum analyte concentration that can be accurately and precisely quantify by the method.

2.4.1. Standard preparation

Standard stock solution of 1000 µgmL^{-1} was prepared by dissolving appropriate amounts drugs in acetonitrile. A homogenous mixed plasma stock of 10 µg mL^{-1} was prepared by spiking 0.1 mL of respective standard stock solutions. Standard calibration solutions were prepared by further dilution of mixed plasma stock with blank plasma to get final concentrations ranging from 50- 1000 ng mL^{-1} of rimonabant.

2.4.2. Plasma sample preparation

To 1 mL of plasma 3 mL of acetonitrile was added, mixed thoroughly and vortexed for 5 min at room temperature. Solution was then centrifuged at 5000 rpm for 16 min at room temperature. The clear supernatant liquid was removed, filtered through 0.45 μ syringe filter and injected directly into HPLC system.

2.4.3. Specificity

To evaluate the specificity of the method, drug free plasma sample was carried through the assay procedure and retention times of the endogenous compound in the plasma were compared with those of rimonabant. Specificity of the method was assessed to test the matrix influence between different plasma samples.

2.4.4. LOD & LLOQ

The limit of detection (LOD) and lower limit of quantitation (LLOQ) of method were determined by calculating the standard deviation of the response of lowest standard on the calibration curve and the slope of calibration curve of analyte. The limit of detection and limit of quantitation were calculated by LOD = 3.3σ/S and LLOQ = 10σ/S respectively, (σ = the standard deviation of the response, S = the slope of the calibration curve).

2.4.5. Extraction recovery

Recovery of rimonabant was evaluated by comparing the mean peak areas of six extracted low, medium and high quality control samples with the mean peak areas of six neat reference solutions containing the same amount of the test compound.

2.4.6. Precision & accuracy

The intra-day, inter-day and analyst to analyst precision and accuracy of the developed method were evaluated in plasma samples spiked with rimonabant. Intra-day, inter-day and analyst to analyst precisions were carried out at nominal concentration of 50, 400 and 1000 ng mL^{-1}. The intra-day precision was evaluated five times in day, inter-day precision was evaluated on five consecutive days and analyst-to-analyst precision was evaluated by five different analysts.

2.4.7. Stability

The stability of the rimonabant in matrix at ambient temperature should be evaluated over a time period equal to the typical sample preparation, sample handling, and analytical run times. Blank samples were spiked with appropriate aliquots of diluted stock solutions to prepare LQC (low quality control, 100 ng mL^{-1}), MQC (medium quality control, 400 ng mL^{-1}) and HQC (high quality control, 1000 ng mL^{-1}) samples. These samples were kept to evaluate different stability parameters such as stock solution stability at room temperature for six hours, bench top stability at room temperature for 12 hours, post processing stability over the maximum time, i.e. from sample work-up to data collection, freeze and thaw stability at -20ºC and long-term storage stability (at -20ºC during the entire period of the study, i.e. from the first day of sample preparation to the last day of sample analysis). For each concentration and storage conditions six replicates were analyzed. The concentration of rimonabant after each storage period was related to the initial concentration as determined for the samples that were freshly prepared and processed immediately.

3. Results and discussion

During method development, number of variables was optimized to get early elution and symmetric peaks with good resolution.

3.1. Optimization of precipitating agent

Organic solvents like methanol, ethanol, acetonitrile and aqueous salt solutions (sodium sulphite, zinc sulphate solution of various strengths) were tried for precipitation of plasma proteins. Perchloric acid solution as precipitating agent has found to be cause turbid supernatant. Sodium sulphite solution upto 21% concentration requires very low temperature of 2°C while zinc sulphate solution requires high speed of rotation 20000 rpm. These astringent conditions are difficult to maintain during the whole experimentation, therefore methanol and acetonitrile were tried. Acetonitrile and methanol gives complete precipitation but recovery of analytes was better with acetonitrile. Thus based on completeness of precipitation, drugs stability, peak characteristics and efficient recovery acetonitrile was preferred over other precipitating agent.

3.2. Mobile phase optimization

The selection of solvent for method development was based upon the solubility, stability and elution characteristics of the drug. Acetonitrile: water combinations were used to elute the rimonabant effectively with acceptable limits of system suitability parameters. Increased retention time with higher tailing factor was observed with higher content of water (20%) due to greater hydrophobic interaction between the bonded alkyl stationary phase and the drug. Rimonabant was best eluted by acetonitrile-water in the ratio of 90:10 (v/v) at flow rate 1 mL min^{-1} (Fig. 2A). Peaks of plasma content were found in chromatogram of plasma sample without drug (Fig. 2B) and these peaks were well separated with high resolution by the optimized mobile phase (Fig. 2C) without interference of sample matrixes.

3.3. System suitability parameters

System suitability parameters were analyzed to ascertain the system performance consistency for number of theoretical plates tailing factor, height equivalent to theoretical plates (HETP) and retention time (RT). The % RSD values for these parameters were found far less than 15%, which indicates acceptance of system performance (Table 1).

Table 1. Validation parameters for rimonabant estimation in plasma

Parameters	Values[a] ± SD[b], %RSD[c]
System Suitability	
RT[d]	6.66 ± 0.01, 0.17
No. of Theoretical Plates	8053 ± 101.01, 1.25
Tailing Factor	1.20 ± 0.01, 0.74
HETP[e]	0.031 ± 0.008, 0.032
Linearity	50-1000 ng mL^{-1}
Regression equation	AUC = 0.1212 X - 0.1655
Correlation coefficient	r^2 = 0.9992 ± 0.32, 0.04
Response ratio	0.1207 ± 0.0008, 0.6628
LLOQ[f]	9.93 ng mL^{-1} ± 1.86, 0.075
LOD[g]	3.28 ng mL^{-1} ± 2.23, 0.056
Robustness	
Mobile phase composition (±2%)	98.59 ± 1.33, 1.35
Temperature (±5%)	101.02 ± 1.84, 1.53
Specificity	Ascertained by analyzing standard drug and equivalent concentration of the plasma samples

[a] mean of six replicates; [b] Standard deviation; [c] % Relative standard deivation; [d] Retention time; [e] Height equivalent to theoretical plates; [f] Lower limit of quantitation; [g] Limit of detection

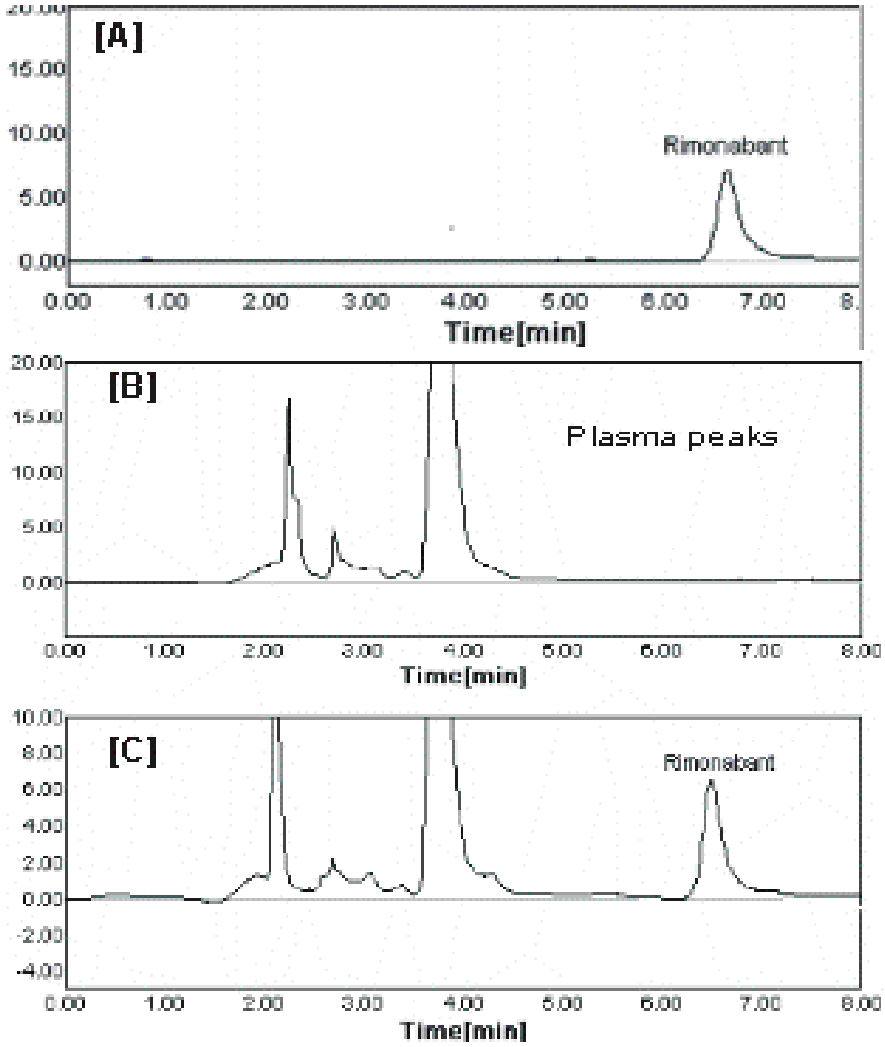

Fig. 2. Chromatogram of rimonabant (A), plasma sample without rimonabant (B) and plasma sample with rimonabant (C).

3.4. Specificity

Comparison of the retention time and UV spectrum of the peak from the sample with those of the peak from the standard, and assessment of peak purity for the drug confirmed the specificity of the method. It was ascertained by analyzing standard drug and equivalent concentration of the drug sample from plasma which showed same type characteristic on the chromatograph. No interfering peaks at the retention time of the drug were found in the chromatogram obtained from blank plasma, as the area of analyte did not differ in case of successive analysis of matrix from different samples.

3.5. Linearity, Limit of Detection and Lower Limit of Quantification

The regression equation of calibration curve was AUC = 0.1212 X - 0.1655 (r^2 = 0.9992). The correlation coefficient showed good linearity (50-1000 ng mL^{-1}). As per the guidelines the acceptance criteria for a validated method is ≤ 20% deviation of the LLOQ from nominal concentration and ≤ 15% deviation of standards other than LLOQ from nominal concentration[14]. The LOD and LLOQ were found 3.28 ng mL^{-1} and 9.93 ng mL^{-1} respectively (Table 1). Thus, the developed method may be applied for the bioequivalence study of the rimonabant as it has higher value of C_{max} (192±28 ng mL^{-1}) as compared to LOD and LLOQ.

3.6. Accuracy and Precision

The intra-batch, inter-batch, inter-day and analyst to analyst precision showed a relative standard deviation (RSD %) of 0.04-0.14, 0.01-0.16, 0.36-2.8 and 0.08-2.29% respectively. The accuracy of proposed method was found between 97.12 to 106.71%. The data proved good precision of the developed method. The results of intra-batch, inter-batch, inter-day and analyst to analyst are illustrated in table 2.

Table 2. Precision, accuracy and recovery data for method.

Nominal concentration (ng mL^{-1})	Concentration found (Mean[a] ± SD[b])	% RSD[c]	% Accuracy
Intra-day (n=5)			
[d]LQC (40)	48.88 ± 0.07	0.14	97.75
[e]MQC (400)	402.19 ± 0.49	0.12	100.55
[f]HQC (1000)	999.43 ± 0.36	0.04	99.94
Inter-day (n=5)			
LQC (50)	51.34 ± 0.08	0.16	102.67
MQC (400)	426.82 ± 0.34	0.08	106.71
HQC (1000)	1012.88 ± 0.14	0.01	101.13
Analyst to analyst (n=5)			
LQC (50)	51.34 ± 0.08	0.16	102.67
MQC (400)	426.82 ± 0.34	0.08	106.71
HQC (1000)	1012.88 ± 0.14	0.01	101.13

[a] mean of five replicates; [b] Standard deviation; [c] % Relative standard deviation; [d] Low quality control; [e] Medium quality control; [f] High quality control

3.7. Extraction recovery

Extraction of drug from plasma was carried out by organic solvents as precipitating agent viz. methanol, acetonitrile and different concentrations (12.6, 15.8 and 21%) of salt solutions (sodium sulphite). In case of salt solutions, as precipitating agent till 21% salt concentration followed by centrifugation at 10000 rpm for half an hour, clear supernatant was not observed. The recovery with methanol was comparable to acetonitrile but 3 ml of methanol was required for complete precipitation of 1 ml of plasma, which diluted the drug concentration in sample to half as compared to acetonitrile. Therefore acetonitrile was selected for extraction as it shows highest extraction recovery with minimum dilution factor. The low, medium and high quality control samples of rimonabant were found to be 68.20, 68.59 and 68.57% (Table 3).

Table 3. Extraction recovery in human plasma (*n*=6).

Concentration (ng mL^{-1})	% Mean recovery ± SD	% RSD[a]
LQC[b] (50)	68.20 ± 0.07	0.10
MQC[c] (400)	68.59 ± 0.49	0.71
HQC[d] (1000)	68.57 ± 0.31	0.45

[a] % Relative standard deviation; [b] Low quality control; [c] medium quality control; [d] high quality control

3.8. Stability

Twenty-four hours room temperature storage and freeze-thaw cycles for low, medium and high quality control samples indicated that rimonabant was stable in human plasma under experimental condition (Table 4).

Table 4. Stability data for developed method

Stability Condition	LQC[a]		MQC[b]		HQC[c]	
	% NC[c]	% C[d]	% NC[c]	% C[d]	% NC[c]	% C[d]
Stock solution stability	98.21	-1.79	98.89	-1.11	98.76	-1.24
Bench top stability	97.63	-2.37	97.22	-2.78	98.47	-1.54
Post processing stability	96.23	-3.77	98.82	-1.18	99.79	-0.21
Freeze & thaw stability	98.75	-1.25	97.47	-2.53	98.35	-1.65
Long term stability	97.46	-2.54	97.79	-2.21	99.64	-0.36

[a] Low quality control; [b] Medium quality control; [c] High quality control;
[c] % Nominal concentration; [d] % Change

4. Conclusion

The HPLC method for determination of rimonabant in human plasma has been developed. Method validation has been demonstrated by a variety of tests for specificity, sensitivity, linearity, precision, accuracy, recovery and stability. As the limit of quantitation of method is far less than C_{max} of drug, the described assay method can be applied without any interference to quantitate rimonabant in human plasma samples obtained from a volunteer following the administration of single 20 mg dose of drug. The result of analysis suggests the applicability, reproducibility and utility of the method for direct estimation of rimonabant in plasma sample.

References

1. Carai MAM, Colombo G and Gessa G L (2005) Rimonabant: The first therapeutically relevant cannabinoid antagonist. Life Sci 77: 2339.
2. Patel PN and Pathak RA (2007) Rimonabant: a novel selective cannabinoid-1 receptor antagonist for treatment of obesity. J Health-Syst Pharm 64(5): 481.
3. Rinaldi MC, Barth F, Heauime M, Shire D, Calandra B, Congy C, Aartinez S, Maruani J, Neliat G, Caput D, Ferra P, Soubrie P, Breliere JC and Le G (1994), SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350(2-3): 240.
4. Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J and Rosenstock J (2006) Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients. JAMA 295: 761.
5. Pertwee RG (1997) Pharmacology of cannabinoid CB1 and CB2 receptors. Pharmacol Ther 74: 129.
6. McAllister SD, Griffin G, Satin LS and Abood ME (1999) Cannabinoid receptors can activate and inhibit G protein-coupled inwardly rectifying potassium channels in a xenopus oocyte expression system. J Pharmacol Exp Ther 291(2), 618.
7. Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma AL and Mitchell RL (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 48(3): 443.
8. Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi C M, Le Fur G and Casellas P (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. Biochem J 312(2): 637.

9. Rafael M and Fernando RF (2002) Cannabinoid Addiction: Behavioral Models and Neural Correlates. J Neurosci 21(9): 3326.
10. Vries TD, Shaham Y, Homberg J, Crombag H, Schuurman K, Dieben J, Vanderschuren L and Schoffelmeer A (2001) A cannabinoid mechanism in relapse to cocaine seeking. Nat Med 7(10): 1151.
11. Nirogi R, Kandikere V, Mudigonda K and Ajjala D (2008) Liquid chromatography tandem mass spectrometry method for the quantification of rimonabant, a CB1 receptor antagonist, in human plasma. Biomed Chromatogr 22(5): 469.
12. Satyanarayana RT, Kumar RM, Kumar KV, Raghavachary KSV, Varma MS, Rao CSNM and Swamy P Y (2009) A stability-indicating LC method for rimonabant. 69(1-2): 97.
13. Hsieh Y, Duncan CJG and Brisson JM (2007) Fused-core silica column high-performance liquid chromatography/tandem mass spectrometric determination of rimonabant in mouse plasma. Anal Chem 79: 5668.
14. Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM), 2001.