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A Novel Stability Indicating Liquid Chromatographic Method for the Estimation of Rimonabant Hydrochloride in Tablet Dosage Form.

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Abstract : A novel stability indicating HPLC assay method has been developed and validated for the estimation of rimonabant hydrochloride in tablet dosage form. A RP-HPLC separation was achieved on C18 column (250X4.6 mm i.d., 5 µm) utilizing a mobile phase comprising of Buffer: ACN in ratio of 35:65 mixed and the eluents from the column were monitored using a variable wavelength detector at 220 nm. The stress testing of rimonabant hydrochloride was carried out under acidic, alkaline hydrolysis, oxidation and thermal degradation (dry heat) conditions and rimonabant hydrochloride was well resolved from its degradation products with good resolution. The proposed method has permitted the quantification of rimonabant hydrochloride in the linearity range of 2-15 µg/ml and the flow rate was maintained at 1 ml/min. The retention times of rimonabant hydrochloride observed was 4.150 min. The method was validated as per ICH (International Conference on Harmonization) guidelines. From study, it was found that drug is susceptible for degradation to hydrolytic condition and oxidation but not affected by dry heat, UV light, thermal condition.
Keywords : Rimonabant, RP-HPLC, Stability-indicating assay, Forced degradation, Validation, ICH guidelines.

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INTRODUCTION

Rimonabant N-piperino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (Figure 1) is an Antiobesity class.

Rimonabant is available as bulk material, tablet[1, 2]. No official method has been established for determination of Rimonabant. Different analytical methods for determination of Rimonabant in biological fluids have been reported and include liquid chromatography/mass spectrometry (LC/MS) [3- 12].

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Rimonabant is available as bulk material, tablet[1, 2]. No official method has been established for determination of Rimonabant. Different analytical methods for determination of Rimonabant in biological fluids have been reported and include liquid chromatography/mass spectrometry (LC/MS) [3- 12]. Rimonabant is a selective antagonist of cannabinoid type 1 (CB1) receptor. Rimonabant is the first member of a new class of compounds that target a novel physiological system, the endocannabinoid system [13] (ECS).

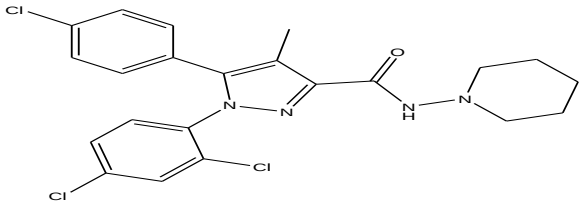


Figure 1: Chemical structure of Rimonabant hydrochloride

Literature review reveals that few analytical methods were evoked for the estimation of RMT in various matrices such as human plasma and hair [14-17], in rat and monkey plasma [18, 19], in fat cells [20], bio analytical application with RMT [21],estimation of RMT with orlistat [22] and with four major species in slimy foods[23], estimation synthetic drugs in herbal slimming formula [24], pharmacokinetic studies of RMT in rats [25], stability indicating assay of RMT by HPLC [26, 27], HPTLC [28] and HPLC estimation [29], spectrophotometric estimation [30] of RMT were reported.

The stability of a drug substance or drug product is defined as its capacity to remain within established specifications, i.e. to maintain its identity, strength, quality, and purity until the retest or expiry date [31]. Stability testing of an active substance or finished product provides evidence of how the quality of a drug substance or drug product varies with time under a variety of environmental conditions, for example temperature, humidity, and light. Knowledge from stability studies is used in the development of manufacturing processes, selection of proper packaging and storage conditions, and determination of product shelf-life [32,33].In this report a simple, rapid, accurate and economic RP-HPLC stability indicating assay for the estimation of Rimonabant in tablet.

MATERIALS & METHODS:

Materials:

Rimonabant used as reference substance (assigned purity 99.85%) was obtained from Zydus Cadila Pharmaceuticals Ltd., Changodar, (Ahmedabad, India). Tablet formulation of Rimonabant contains a single component (100mg). Acetonitrile and methanol (both LC grade) used for work were from E. Merck Ltd., (Mumbai, India). The water for RP-HPLC was of HPLC grade by Milli Q. Triflouroacetic acid used in buffer preparation for mobile phase was of AR Grade by Spectrochem. Pvt. Ltd, (Mumbai, India).

Methods

RP-HPLC Method

Instrumentation

The RP-HPLC system consisted of a Shimadzu (LC-2010C Perkin Elmer) equipped with PDA detector.

Chromatographic separation condition

A Phenomenex C18 (250mm x 4.6mm i.d., 5µm particle size) column was used at ambient temperature. The mobile phase was Acetonitrile: buffer (65:35 v/v, 1.0 ml of Triflouroacetic acid was dissolved in 2000 ml with Milli Q water, mixed and filtered through 0.45 µm Millipore HVLP filter). The mobile phase was filtered through nylon 0.45 µm - 47 mm membrane filter and was degassed daily before use. The flow rate was 1.0 ml/min, and the sample injection volume was 10 µl. The detector was set at a wavelength of 220 nm.

PREPARATION OF SAMPLE AND STANDARD SOLUTIONS

Standard preparation: Accurately weighted rimonabant reference substance (40 mg) was transferred to 100 ml volumetric flask and dissolved in diluting solution. From this solution further 5 ml was transferred in 200 ml and diluted to the mark with diluting solution with final concentration of 10µg/ml.

Sample solution preparation :As described in RP-HPLC Method, the sample solutions of rimonabant were prepared to give final concentration of 10µg/ml.

FORCED DEGRADATION OF ACTIVE PHARMACEUTICAL INGREDIENT AND TABLETS OF RIMONABANT

As such condition: The solutions were prepared without any treatment on standard and sample. In this 40 mg API, and 2 intake tablets of samples were taken in 50 ml volumetric flask, 20 ml of Methanol was added, sonicated for 10 min, and diluted to mark with diluent. Then the solutions were filtered through 0.45 µm filter and used for experiment(Figure 2,3).

Figure 2: Chromatogram of Standard solution.

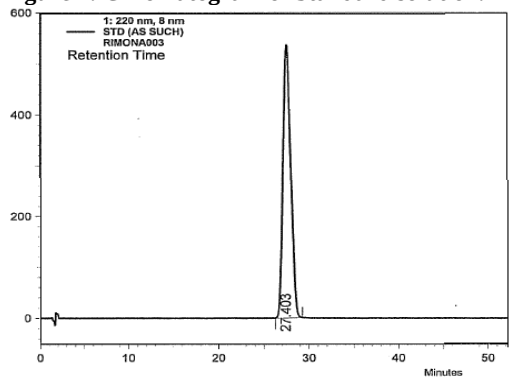
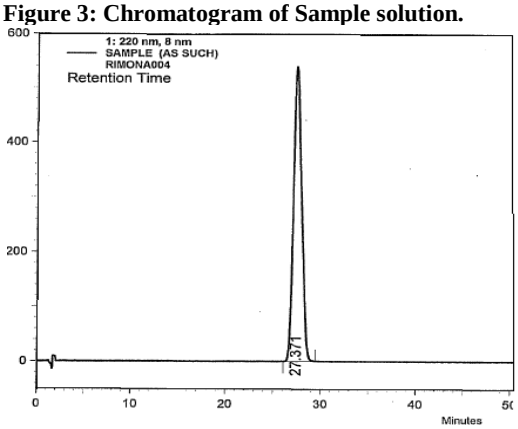


Figure 3: Chromatogram of Sample solution.



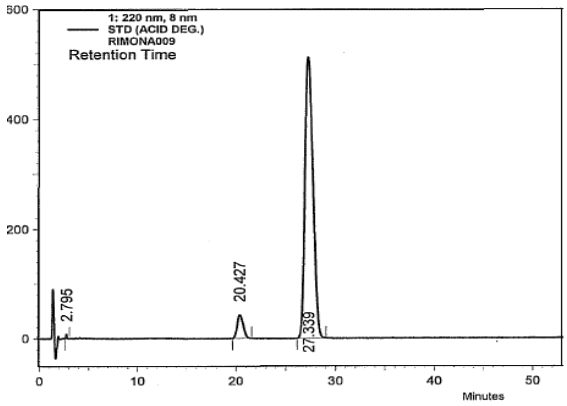


Figure 4: Chromatogram of API solution in Acidic Condition.

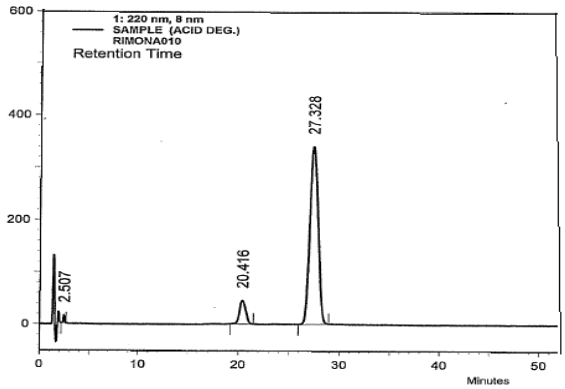


Figure 5: Chromatogram of Sample solution in Acidic Condition.

Alkali Degradation: Drug was subjected to alkaline condition to achieve degradation from 10 – 30 %. 40 mg API, and 2 intake Tablets were dissolved with 5 ml of methanol as diluent and 5 ml of 5N NaOH was added, then these solutions were subjected for heating for 2 hrs. Then after heating solutions are made cool, then all solutions were Neutralize with 5 N hydrochloric acid solution. After that mixed well and diluted to mark with Methanol as diluent. After that solutions were filtered with 0.45 µm Millipore HVLP filter and injection was made in HPLC system (Figure 6,7).

Figure 6: Chromatogram of API solution Exposed to Alkaline Condition.

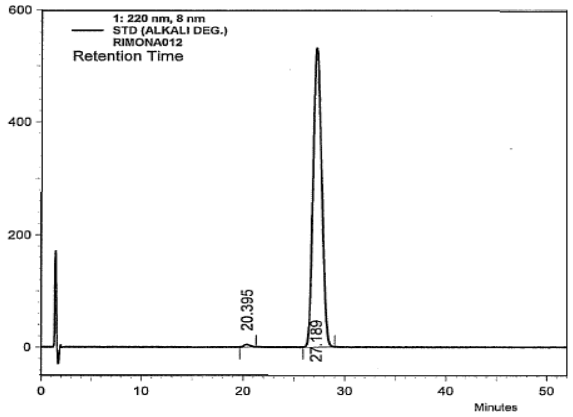


Figure 6: Chromatogram of API solution Exposed to Alkaline Condition.

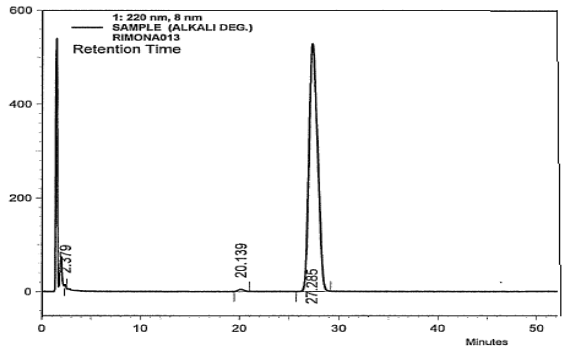


Figure 7: Chromatogram of Sample solution Exposed to Alkaline Condition.

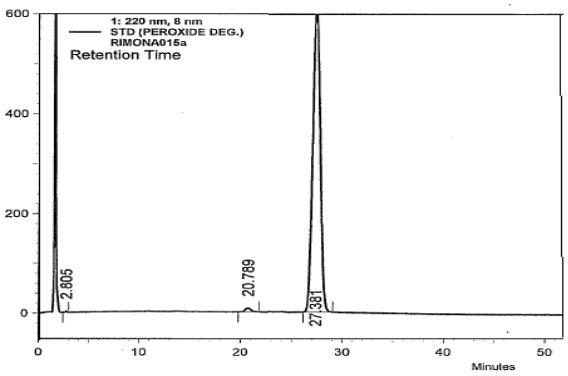


Figure 8: Chromatogram of API solution in Oxidation Condition.

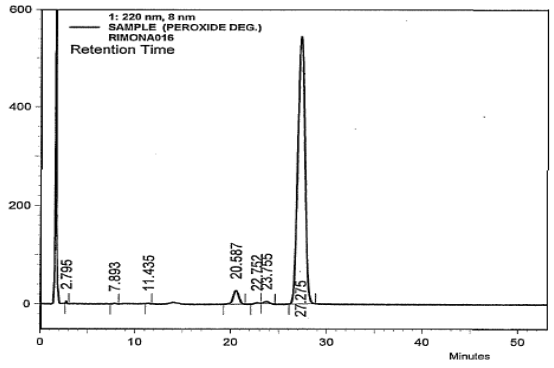


Figure 9: Chromatogram of Sample solution in Oxidation Condition.

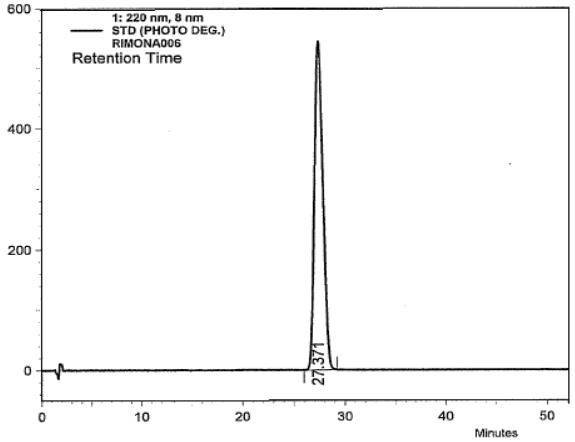


Figure 10: Chromatogram of API Exposed to UV light solution

Oxidative Degradation: Drug was exposed to oxidizing medium throw hydrogen peroxide solution. Attempt was taken to achieve degradation between 10.0 – 30.0 %. 40 mg API and 2 intake Tablets were dissolved with 5 ml of methanol as diluent and 5 ml 5 % hydrogen peroxide was added then these solutions were subjected for heating for 2 hrs. Then after heating solutions were made cooled, mixed well and diluted to mark with Methanol as diluent. After that solutions were filtered with 0.45µm Millipore HVLP filter and injection was made in HPLC system (Figure 8,9).

Figure 8: Chromatogram of API solution in Oxidation Condition.

Figure 9: Chromatogram of Sample solution in Oxidation Oxidation

Photodegradation: API, Placebo, and tablets were exposed to U.V radiation for 24 hrs. Then 40 mg API and 2 intake Tablets were dissolved with 5 ml of methanol, sonicated for 15 min. then made volume up to mark with diluent. Then solutions were filtered with 0.45 µm Millipore HVLP filter and injection was made in HPLC system (Figure10,11).

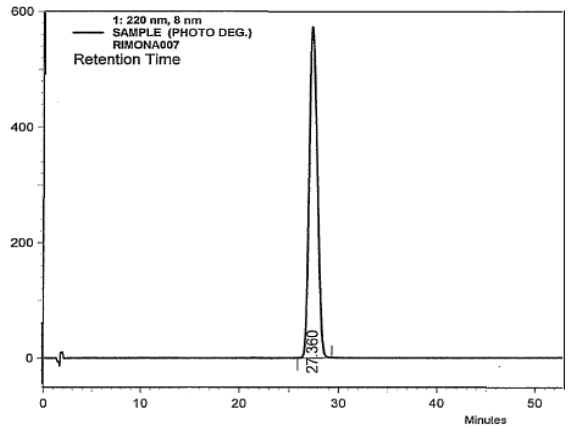


Figure 11: Chromatogram of Sample solution Exposed to UV light.

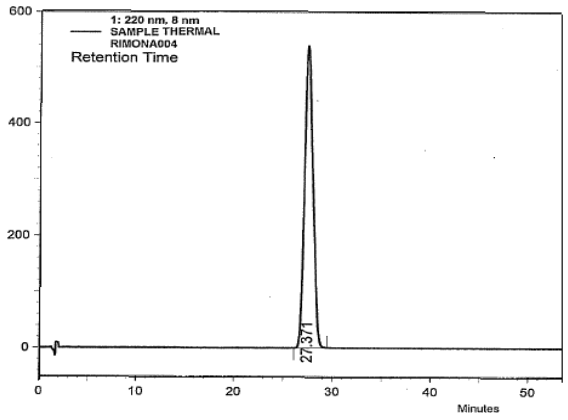


Figure 12: Chromatogram of API solution Exposed to Thermal condition.

***Thermal Degradation*:** API, Placebo, and tablets were exposed to dry heat for 5 days. Then 40 mg API and 2 intake Tablets were dissolved with 5 ml of methanol, sonicated for 15 min. then made volume up to mark with diluent. Then solutions were filtered with 0.45 µm Millipore HVLP filter and injection was made in HPLC system(Figure 12,13).

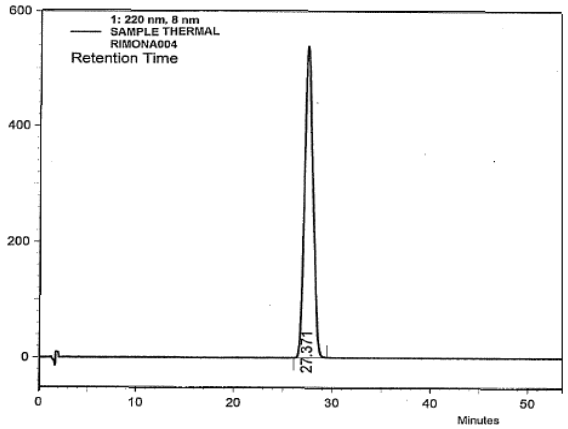


Figure 13: Chromatogram of Sample solution Exposed to Thermal condition.

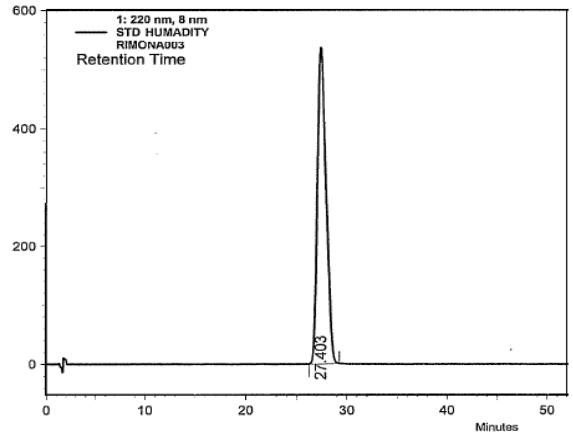


Figure 14: Chromatogram of API solution Exposed to Exposed to Humidity condition

Humidity Degradation: API and tablets were exposed to 40 °c and 75 Relative Humidity for 5 days. Then 40 mg API, 460 mg Placebo and 2 intake Tablets were dissolved with 5ml of methanol, sonicated for 15 min. then made volume up to mark with diluent. Then solutions were filtered with 0.45 µm Millipore HVLP filter and injection was made in HPLC system(Figure 14,15).

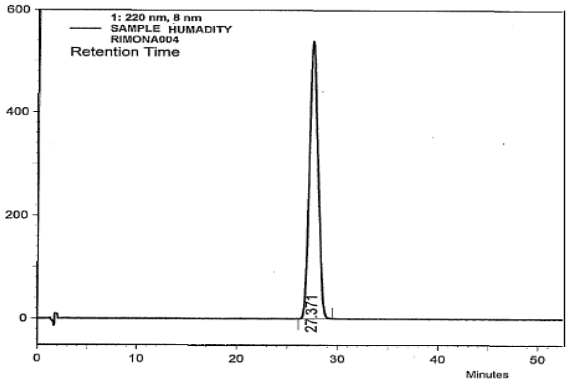


Figure 15: Chromatogram of Sample solution Exposed to Humidity condition.

RESULTS AND DISCUSSION

A simple stability-indicating RP-HPLC method has been developed for determination of RMT in the presence of its degradation products. The method was optimized to provide a good separation of the components.Mobile phase and flow rate selection were based on peak parameters (symmetry, tailing), run time, ease of preparation, and cost. A typical chromatogram for rimonabant using the C18 column with a mobile phase composition of Acetonitrile: buffer (65:35 v/v) at a flow rate of 1.0 ml/min. Rimonabant produced a sharp and symmetric peak when chromatographed with these conditions.The use of mobile phase without buffers, which shortens column life, is the main advantage of the proposed RP-HPLC method. The retention time observed allowed rapid determination of the drug, which is important for routine analysis. No interferences from the diluents, impurities, or excipients present in pharmaceutical formulations were observed at the detection wavelength. The analyte chromatographic peak was not attributable to more than one component (diode array detector peak purity test= 99999) [14].

Method Validation

The method was validated as per the ICH guidelines for different validation parameters. The method was validated for its specificity, linearity, accuracy, precision, selectivity, Limit of detection (LOD) and Limit of quantification (LOQ) (Table 1).

Table 1: Summary of the validation parameter

Parameters	**Observations**	
Specificity	No interference was found	
Linearity Correlation coefficient (r) Std. Dev. Slop Intercept	2 to 50 µg/ml 0.9998 3421.37750 63277.63902 1602.82439	
Accuracy Recovery from placebo 50% 100% 150%	% Recovery 99.9 100.0 100.1	% RSD 1.5 0.6 1.3
Precision Intermediate Precision	% Assay 99.7	% RSD 1.3
Robustness System suitability Temperature Organic phase ratio Flow rate	% RSD 0.1 0.1 0.1 0.1	
Solution stability	Stable for 24 hrs.	
LOD	0.2 µg/ml	
LOQ	0.5 µg/ml	

Stability Indicating Method:

Stability Indicating Method was developed for Rimonabant in Pharmaceutical Dosage form under hydrolytic stress condition (5N HCL, 5N NaOH); Oxidation condition (5% H_2O_2) and dry heat condition, UV light, Thermal condition. From study, it was found that drug is susceptible for degradation to hydrolytic condition and oxidation but not affected by dry heat, UV light, thermal condition (Table 2).

Table 2: Summary of data of Stability Indicating Method by HPLC

Sr. No.	*Sample*	*Condition*	*% Area*	*% Degradation*	*Peak Purity*
1	API	As Such	100	-	0.99999
2		Acidic	89.38	10.62	0.99999
3		Alkaline	99.36	0.64	0.99999
4		Peroxide	98.93	1.07	0.99998
5		UV light	100	0	0.99999
6		Thermal	100	0	0.99911
7		Humidity	100	0	0.99939
8	Tablet	As Such	100	-	0.99999
9		Acidic	85.96	14.04	0.99999
10		Alkaline	99.27	0.73	0.99997
11		Peroxide	95.04	4.73	0.99999
12		UV light	100	0	0.99995
13		Thermal	100	0	0.99966
14		Humidity	100	0	0.99922

CONCLUSION

A validated stability indicating HPLC method has been developed for the determination of RMT in API and dosage forms. The results of stress testing undertaken according to the International Conference on Harmonization (ICH) guidelines reveal that the method is selective, specific and stability-indicating. The proposed method is simple, accurate, precise, specific, and has the ability to separate the drug from degradation products and excipients found in the tablet dosage forms. The method is suitable for use for the routine analysis of RMT in either bulk API powder or in pharmaceutical dosage forms. The simplicity of the method allows for application in laboratories that lack sophisticated analytical instruments such as LC–MS and or GC–MS. These methods are complicated, costly and time consuming rather than a simple HPLC method. In addition, the HPLC procedure can be applied to the analysis of samples obtained during accelerated stability experiments to predict expiration dates of pharmaceuticals. This approach has demonstrated its strength in terms of sensitivity, rapidity, cost effectiveness when compared with other methods cited in the literature.

ACKNOWLEDGEMENTS:

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REFERENCES:

1. The Merck Index, An Encyclopedia of Chemicals, Drugs, and Biologicals Merck & Co., Inc.Whitehouse Station, NJ, 1213.
2. Sanofi-Aventis received from the FDA an approvable letter for rimonabant for weight management and a non approvable letter for smoking cessation [press release]. Paris, France: Sanofi-Aventis, 2006; pp 225.
3. McCulloch M, Zhou X, Xu Y, et al. Spear L.Department of Chemistry, Cleveland State University, 2121 Euclid Avenue, Cleveland, OH 44115-2214, USA, 2000; pp 1233.
4. Béquet F, Uzabiaga F, Desbazeille M, et al. Discovery Analytics, sanofi-aventis R&D, 2000; pp 3110.
5. Munson J, High Performance Liquid Chromatography: Theory, Instrumentation, and Pharmaceutical Applications. In, Munson, J.W., editor. Pharmaceutical Analysis-Modern Methods, 2nd Ed., Part-B, Mumbai: IMBD Publication, 2001; pp 25.
6. Beckett AH, & Stenlake JB, Practice Pharmaceutical Chemistry, CBS Publishers, New Delhi, 1997; pp 285.
7. International Conference on Harmonization of technical Requirements for registration of

Pharmaceuticals for Human Use (ICH) Guideline on Validation of Analytical Procedure-Methodology, Geneva, Switzerland,1996.

8. Lorenz LJ, High Performance Liquid Chromatography. In, R.E. Schirmer, editor. Modern Methods of Pharmaceutical Analysis, 2nd Ed., Florida:CRC Press,2000; pp 241.
9. Gelfand EV, Cannon CP, Rimonabant: a selective blocker of the cannabinoid CB1 receptors for management of obesity, smoking cessation and cardiometabolic risk factors. Expert Opin Investig Drugs, 2006; pp 307– 315.
10. Stout TH, Dorsey JG, High Performance Liquid Chromatography. In, Ohannesian, L. Streeter, A.J. editors. Handbook of Pharmaceutical Analysis, NY: Marcel Dekker Inc., 2002; pp 127.
11. Delyle SG, Abe EF, Devillier PE, and Alvarez JC
12. , Laboratory of Pharmacology – Toxicology, Universitary Hospital Raymond Poincaré, AP-HP, 104

Boulevard Raymond Poincaré, 92380 Garches, France.
13. McCulloch MC, Zhou X, Xu Y, Department of Chemistry, Clevel and State University, 2121 Euclid Avenue, Cleveland, USA 44115-2214.
14. Ducobu J, Sternon J. Rimonabant (Acomplia), specific inhibitor of the endocannabinoid system. Journal de pharmacie de Belgique 2005; 60(3):89-91.
15. Melissa M, Xiang Z, Yan X, Steve B, Linda S. Determination of endocannabinoid receptor
antagonist SR141716 (rimonabant) in plasma by liquid chromatography tandem mass spectrometry. J. Chrom. 2008;863(2):258-265.
16. Fouad CH, Joel S, Patrick CH. Liquid chromatographic method with ultraviolet absorbance detection for measurement of rimonabant in human plasma. Anal. Lett. 2009; 42(6):833-841.
17. Stanislas Grassin D, Emuri A, Philippe D and Jean Claude A. Determination of rimonabant in human plasma and hair by liquid chromatography–mass spectrometry. J. Chrom. B. 2008; 872(1-2):115-120.
18. Rajesh T, Lakshmi KS, Sharma S. Quantification of rimonabant in rat plasma by high performance liquid chromatography and its application to pharmacokinetic studies. Der Pharma Chemica 2009; (1):12-29.
19. Javors M, Sanchez JJ, Mahon MC, Lance R. Quantification of rimonabant (SR 141716A) in monkey plasma using HPLC with UV detection. J. Chromatogr. Sci. 2010; 48(6):491-495.
20. Marie Paule G, Laurence H, Franck F, et al. Identification of endocannabinoids and related compounds in human fat cells. Obesity 2007; (15)837–845.
21. Bhaumik U, Ghosh A, Chatterjee B, et al. Development and validation of a high-performance liquid chromatographic method for bio analytical application with rimonabant. J. Pharmaceutic. Biomed. Anal. 2009;49(4):1009-1013.
22. Wei MA, Qiang MA, Li FU, et al. Simultaneous determination of rimonabant and orlistat illegally added in the weight-loss functional foods by high performance liquid chromatography-tandem mass spectrometry. Chin. J. Chrom. 2010;28 (1):43-48.
23. Yu Z, Wei Q, Fan Q, Wan C. A simple and convenient method for simultaneous determination of four major species of illegal additives in slimming health food. J. Liq. Chrom. Relet. Tech. 2010;33(4):452–461.
24. Kanan SM, Abu Yousef IA, Gunasekar C, Abdo NM, Narasimhan S. Detection and quantification of synthetic drugs in herbal slimming formula. Eur. J. Sci. Res. 2009;34(3):348-357.
25. Thornton Jones ZD, Kennett GA, Benwell KR, et al. 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. Behav. 2006;(84) 353–359.
26. Hurtado FK, Ravenello A, Arend MZ, et al. Validated stability-indicating RP-HPLC method for the determination of rimonabant in a pharmaceutical dosage form. J. AOAC Int. 2010;93(3):869-875.
27. Raju TS, Ravikumar M, kumar VK, et al. A stability indicating LC method for rimonabant. Chromatographia. 2009;(69)97-102.
28. Rajesh T, Lakshmi KS, Sharma S, et al. Use of a validated stability-indicating HPTLC method to study the degradation of rimonabant. Journal of Planar Chromatography 2010; 23(2):148–155.
29. Sreekanth N, Babu Rao CH, Mukkanti K. RP-HPLC Method development and validation of rimonabant hydrochloride in bulk and pharmaceutical dosage forms. Int. J. Pharm. Pharm. Sci. 2009;(1)199-205.
30. Chakraborthy M, Gupta B, Debnath R, et al. A new spectrophotometric method development for determination of rimonabant in bulk and tablets. Int. J. Pharm. Pharm. Sci. 2009;(1)162164.
31. FDA, Guidance for Industry: Impurities in drug product draft guidance. Center for Drug Evaluation and Research (CDER), 1998.
32. International Conference on Harmonization (ICH), Guidance for Industry, Q1A (R2): Stability Testing of New Drug Substances and Products, IFPMA, Geneva, 2003.
33. Grimm W. In: Carstensen JT, Rhodes CT, editors. Drug stability, principles and practices. New York: Marcel Dekker; 2000.
34. International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2 (R1), November 2005