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Research Article

DEVELOPMENT AND VALIDATION OF A REVERSE PHASE HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF ROSUVASTATIN CALCIUM AND FENOFIBRATE IN TABLET DOSAGE FORM

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ABSTRACT

A simple, precise, accurate, and rapid HPLC method has been developed, and validated for the determination of Rosuvastatin Calcium and Fenofibrate simultaneously, in combined tablet dosage form. The mobile phase used was a mixture of Acetonitrile and water (90:10 v/v). The detection of Rosuvastatin Calcium and Fenofibrate was carried out at 240 nm with a flow rate of 1.0ml/min. The retention time (min) for Rosuvastatin calcium and Fenofibrate were 2.30, 4.92 respectively. Results of the analysis were validated statistically, and by recovery studies. The proposed method can be successfully used to determine the drug contents of marketed formulation.

Keywords: Rosuvastatin calcium (RST), Fenofibrate (FB), RP-HPLC, Simultaneous determination, Retention time.

INTRODUCTION

Rosuvastatin (RST) is chemically bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methylsulfonyl) amino] pyrimidin-5-yl](3R,5S)-3,5-dihydroxy hept- 6-enoicacid] calcium salt[1]. It is a selective and competitive inhibitor of HMG-Co A reductase, the rate limiting enzyme that converts 3-hydroxyl 3-methyl glutaryl coenzyme A to mevalonate, a precursor of cholesterol. Rosuvastatin produces its lipid modifying effects in two ways. First, it increase the number of hepatic LDL receptors on the cell surface to enhance uptake and catabolism of LDL. Second, Rosuvastatin inhibits hepatic synthesis of VLDL, which reduces the total number of VLDL and LDL particles[2].

Chemically, Fenofibrate is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester[3]. Fenofibric acid, the active metabolite of fenofibrate, produces reduction in total cholesterol, LDL cholesterol, apolipoprotien B, Total triglyceride and triglyceride rich lipoprotein (VLDL) in treated patients. In addition, treatment with fenofibrate results in increase in high density lipoprotein (HDL) and apoprotiens apo AI and apo AII[4].

Several studies have shown that therapeutic modulation of LDL size and sub class is of great benefit in reducing the risk of cardiovascular events. This seems particularly true for statins and fibrates when they are administered to higher risk patients, such as those with type 2 diabetes or vascular disease. Data reporting out comes with the use of Rosuvastatin, the latest statin molecule introduced to the market is promising[5]. The hypothesis that was tested in ACCORD Lipid was that in high-risk patients with type 2 diabetes, combination treatment with a fibrate (both to raise HDL cholesterol levels and to lower triglyceride levels) and a statin (to reduce LDL cholesterol levels) would reduce the rate of cardiovascular events, as compared with treatment with a statin alone[6].

Simultaneous determination of both drugs is highly desirable as this would allow more efficient generation of clinical data and could be more cost - effective than separate assays. The literature studies show various analytical methods reported for the estimation of Rosuvastatin calcium in pharmaceutical formulations[7-10]. Several HPLC methods[11-13] with mass spectrometry and tandem mass spectrometry are reported for the determination of Rosuvastatin calcium, in combination with fenofibrate or other drugs in plasma[14-16]. Reports are available for estimation of Fenofibrate in bulk and formulations using nuclear magnetic resonance (NMR) spectrometry and LC[17] and in human plasma by LC/tandem mass spectrometry (LC/MS/MS) with electrospray ionization[18]. Various RP HPLC methods are reported for the estimation of Fenofibrate in combination with other lipid lowering drugs[19,20]. However only one reference has been found for the simultaneous estimation of Rosuvastatin calcium and Fenofibrate in Tablet dosage form[21].

The aim of this study was to develop a simple, precise and accurate reverse phase high performance chromatographic method for simultaneous estimation of Rosuvastatin calcium and Fenofibrate in a tablet dosage form. This method was simple, rapid and provides accurate and precise results, as compared with other methods which have been reported. Criteria employed for assessing the suitability of said solvent system were cost effectiveness in terms of time required for analysis, solvent noise and preparatory steps involved in the extraction of the drug from the formulation excipients for the estimation of drug contents.

MATERIALS AND METHODS

Materials

Pharmaceutical grade Rosuvastatin calcium and Fenofibrate were supplied by Chandra labs, Hyderabad and were used without further purification. All chemicals and reagents were HPLC grade and Analytical grade. Tablets containing Rosuvastatin calcium 5mg and Fenofibrate 67mg were procured from Glennmark Pharmaceuticals limited.

Instrumentation and Chromatographic Conditions

HPLC system consisted of a pump (model LC-20 AT). Manual injector was used. Loop used was of 20µl capacity per injection. UV detector (model SPD-20AV) was used. Detection was carried out at 240nm and the software used was spinchrom data system. Hypersil C18 (250X4.6mm, 5µm) column was used. Different mobile phases were tested in order to find the best conditions for composition of mobile phase was determined to be Acetonitrile: Water (90:10, v/v) and flow rate was set to 1.0ml/min.

Method

Standard stock solution containing 50 and 670 µg/ml of Rosuvastatin calcium and Fenofibrate, respectively was prepared by dissolving 5 and 67mg of both drugs in 100ml of mobile phase. For estimation of Rosuvastatin calcium and Fenofibrate in tablets, an accurately weighed quantity of tablet powder equivalent to 5mg Rosuvastatin calcium and 67mg Fenofibrate were transferred to a 100ml volumetric flask, diluted with mobile phase, sonicated for 10 minutes and further diluted to 100ml with mobile phase. The solution was sonicated before it was used to analysis.

RESULTS AND DISCUSSION

To develop a precise, accurate and suitable RP- HPLC method for the simultaneous estimation of RST and FB, different mobile phases

were tried and the proposed chromatographic conditions were found to be appropriate for the quantitative determination. The results obtained by the assay of marketed formulation are summarized in Table.1. System suitability tests were carried out as per USP XXIV and parameters are summarized in Table.2**.**

Method Validation

The proposed HPLC method was validated as per ICH guidelines[22].

Specificity

Specificity of the methods was determined by the complete separation of Rosuvastatin calcium and Fenofibrate with parameters like retention time, asymmetry and capacity factor. The condition of the method was exactly effective and efficient.

Linearity

The plot of peak area of standard solutions versus concentration was found to be linear in the range of 2-12 µg/ml and 26.8-160.8 µg/ml for Rosuvastatin calcium and Fenofibrate and coefficient of correlation (r^2) was found to be 0.9995 and 0.9993 respectively. Typically the regression equation for the caliberation curve was found to be Y = 28.578 x +2.0721 for Rosuvastatin Calcium and Y = 14.417 x + 21.4242 for fenofibrate

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

Accuracy (Recovery studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 80%, 100% and 120%. Known amounts of standard RST and FB were added to pre-analyzed samples and were subjected to the proposed HPLC method. Results of recovery studies are shown in Table 3.

Robustness of method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, and mobile phase ratio on the retention time and tailing factor were studied. The method was found to be unaffected by small changes like ± 0.2 change in flow rate and ± 2 change in mobile phase.

Limit of Detection(LOD) and Limit of Quantitaion(LOQ)

LOD and LOQ of RST and FB were determined by Calibration Curve method, LOD and LOQ were determined by the formulae

$$LOD = \frac{3.3\ \sigma}{S}$$

$$LOQ = \frac{10\ \sigma}{S}$$

Where σ is the standard deviation of the intercept and 'S' is the slope of the calibration curve.

σ = 1.967, 13.87129 and S = 28.57, 14.41 for RST and FB respectively.

The LOD of RST and FB is found to be 0.227 µg/ml and 3.17µg/ml respectively, and The LOQ of RST and FB is found to be 0.688 µg/ml and 9.6252µg/ml respectively.

Forced Degradation Studies

Forced degradation studies were carried out in presence of acid, alkali, H_2O_2 and heat. To the sample bearing concentration 2µg/ml and 26.8µg/ml of Rosuvastatin calcium and Fenofibrate respectively, added 0.1ml of 0.1N Hcl, 0.1ml of 0.1N NaOH and 0.1ml of 5%H_2O_2 and at temperature of 50^{o}C for 6 hours individually. The results were stability indicating and are shown in Fig2, 3, 4 and 5.

Forced degradation in presence of 0.1N Hcl

Two different degraded products of Rosuvastatin were found at a RT of 2.740 and 3.220, but the peaks of RST and FB was found to be undisturbed and intact when subjected to forced degradation in 0.1N HCl.

Forced degradation in presence of 0.1N NaOH

Two different degraded products of Rosuvastatin were found at a RT of 1.953 and 2.953, but the peaks of RST and FB was found to be undisturbed and intact when subjected to forced degradation in 0.1N NaoH.

Forced degradation in presence of 5%H_2O_2

No change was observed when subjected to forced degradation in the presence of 5% H_2O_2

Forced degradation in presence of 50^{o}C temperature

No change was observed when the forced degradation was carried out at 50^{o}C temperature.

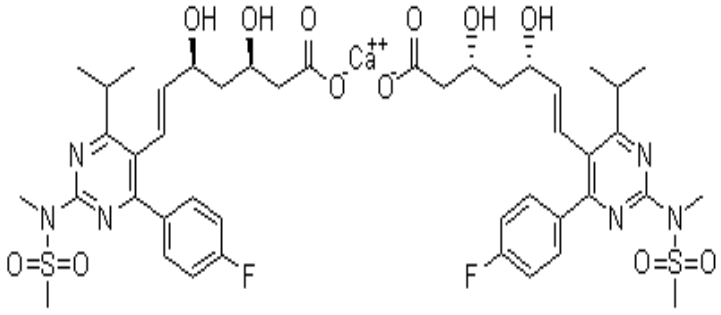

Fig. 1: Structure of Rosuvastatin Calcium

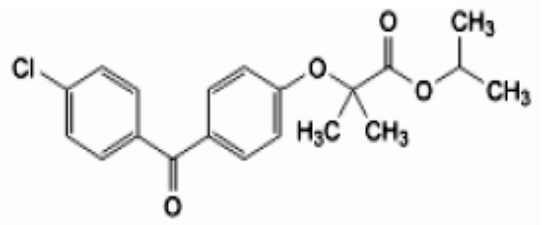

Fig. 2: Structure of Fenofibrate

Table 1: Results of RST and FB in Marketed Formulation

Marketed Formulation	Drug	% of Amount Recovered
Razel – F5	Rosuvastatin Calcium	101.1
	Fenofibrate	99.38

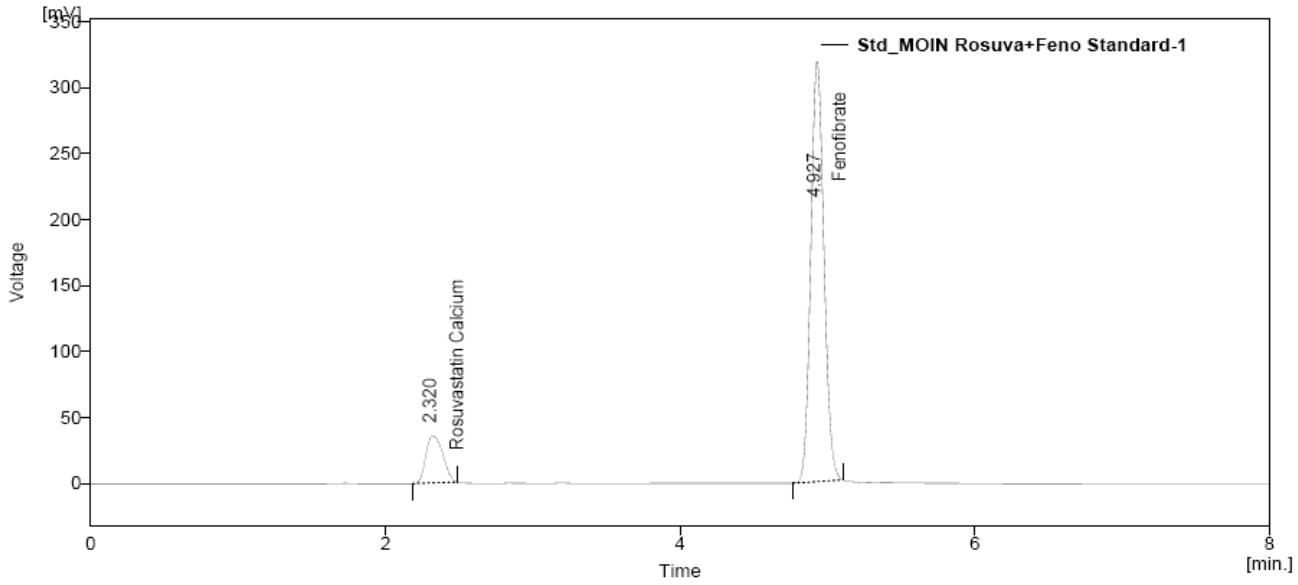

Fig. 3: HPLC chromatogram of Rosuvastatin calcium and Fenofibrate tructure of Rosuvastatin Calcium

Table2: System suitability parameters

Parameters	Rosuvastatin calcium	Fenofibrate
Retention time	2.30	4.92
Asymmetry	1.500	1.200
Theoretical plates	2177	13447
Resolution factor	-	13.1
Calibration range (µg/ml)	2-12	26.8-160.8
Correlation coefficient	0.9995	0.9993
%RSD	0.454	0.764

RSD; Relative standard deviation

Table 3: Recovery Studies

Accuracy (Recovery Studies)	Rosuvastatin Calcium (RST) (Average percentage recovery)	Fenofibrate (FB) (Average percentage recovery)	% RSD RST	% RSD FB
80%	99.15	99.79	0.00541	0.133824
100%	99.57	99.84	0.038966	0.063986
120%	99.79	100.14	0.345051	0.087715

RSD: Relative Standard Deviation

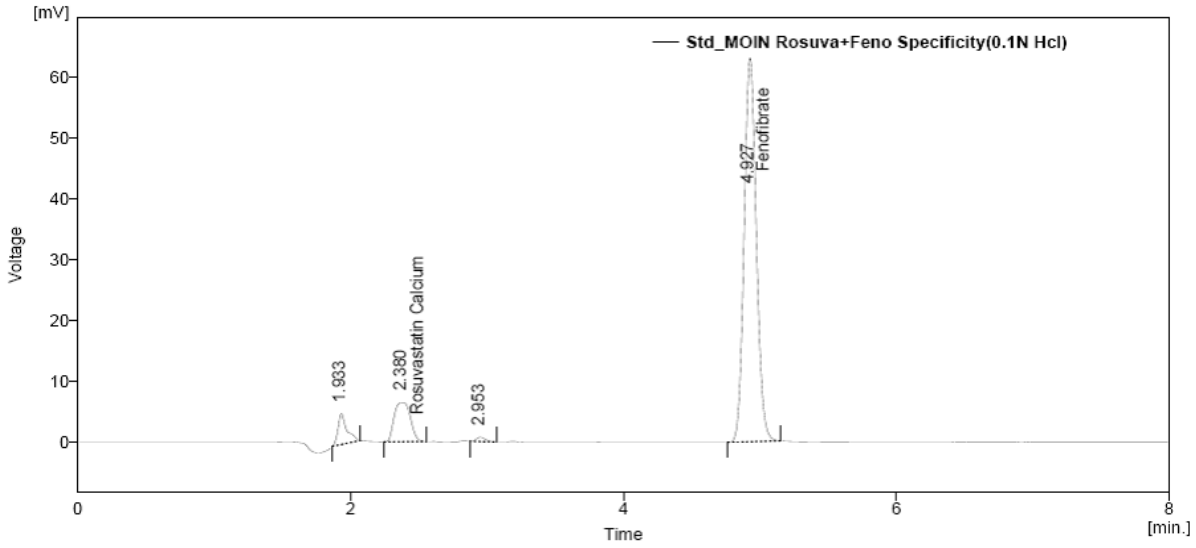

Fig. 4: Forced degradation in presence of 0.1N HCl

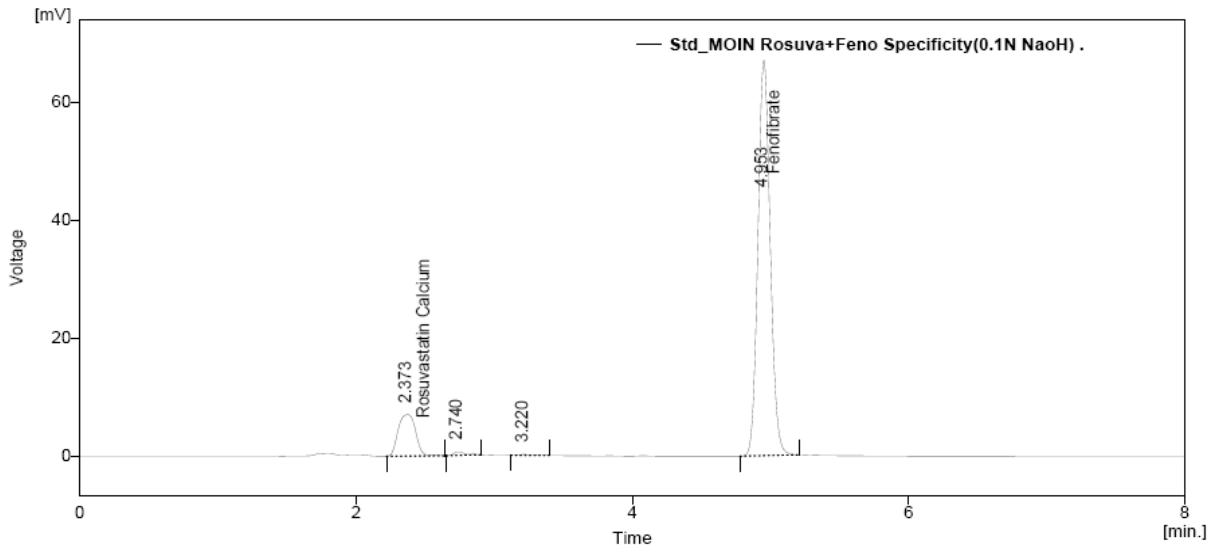

Fig. 5: Forced degradation in presence of 0.1N NaOH

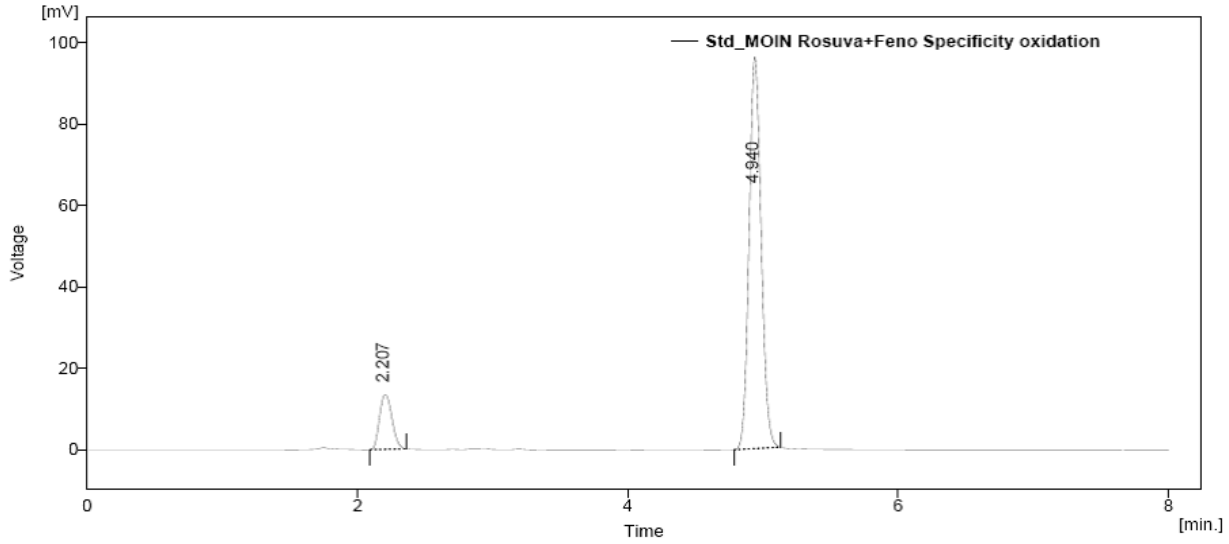

Fig. 6: Forced degradation in presence of 5%H_2O_2

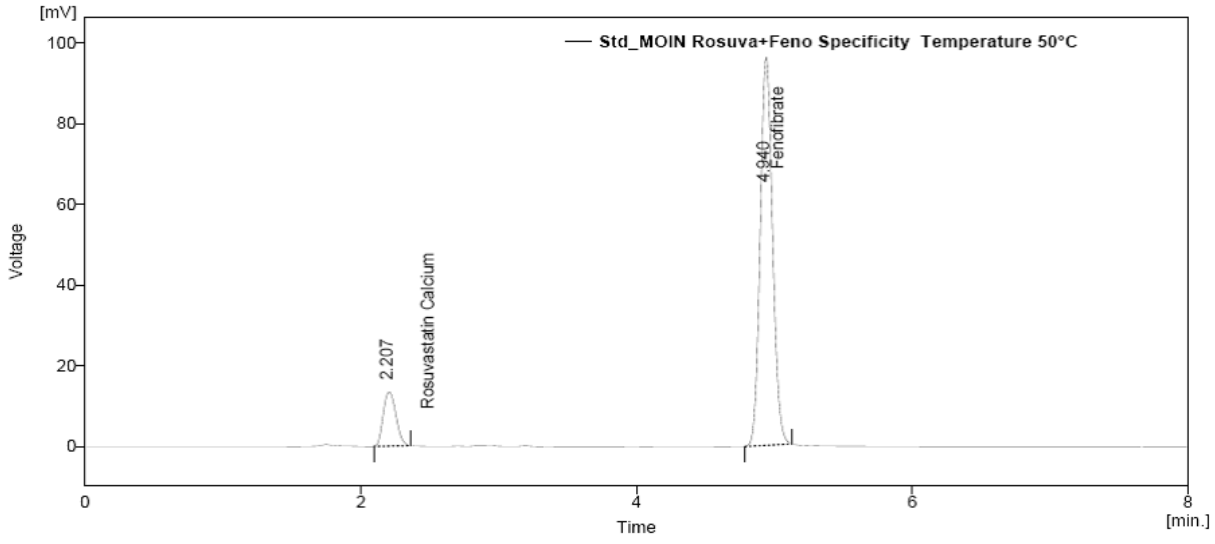

Fig. 7: Forced degradation in presence of 50^{0}C temperature

CONCLUSION

A simple, specific, linear, precise and accurate RP-HPLC method has been developed and validated for quantitative determination of Rosuvastatin Calcium and Fenofibrate in new tablet formulation. The results of stress testing of the drug, undertaken accordingly reveal that the degradation products were formed in hydrolytic (acid and base) conditions and the method being able to separate the main drug from its degraded product. The method is very simple, specific and rapid with the total run time of 5 min which makes it especially suitable for routine quality control analysis.

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REFERENCES

1. Sweetman SC. Martindale The Complete Drug Reference. 34th edition, London: Royal Pharmaceuitical Society of Great Britain. 2005; 996.
2. Fergus McTaggart. Atherosclerosis Supplements. 2003; 4(1): 9-14.
3. Maryadele JO, editor Merck index. 13th ed. NJ (USA): Merck Research Lab., 2001; 4005.
4. Jadwiga Najib. Clinical therapeutics. 2002; 24(12): 2022-2050
5. Rizzo M, Rini GB & Berneis K. Advances in therapy. 2007; 24 (3): 575-82.
6. Ginsberg HN, Elam MB, Lovato LC, Simons Morton DG & Byington RP. N Engl J Med., 2010; 362(17): 1563-1574.
7. Uyar B, Celebier M & Altinoz S. Pharmazie. 2007; 62(6): 411-413.
8. Suslu I, Celebier M & Altinoz S. Chromatographia. 2007; 66(1): 65-72.
9. Alka Gupta, Mishra P & Shah K. E-Journal of Chemistry. 2009; 6(1): 89-92.
10. Khalid Pasha Md, Syed Muzeeb, Shaik Jafar Sadik Basha, Dhanya Shashikumar, Ramesh Mullangi, Nuggehally R, Srinivas. Biomedical Chromatography. 2006; 20(3): 282- 293.
11. Mehta T.N, Patel AK, Kulkarni GM & Suubbaiah G. J AOAC International. 2005; (884): 1142-1147.
12. Vittal S, Shitut NR, Kumar TR, Vinu MC, Mullangi R & Srinivas NR. Biomed. Chromatogr. 2006; 20(11): 1252-59.
13. Kaila HO, Ambasana MA, Thakkar RS, Saravaia HT, Shah Ak. Indian Journal of Pharmceutical Sciences. 2010; 72(5): 592-598.
14. Lan K, Jiang X, Li Y, Wang L, Zhou J, Jiang Q & Ye L. J Pharm Biomed Anal. 2007; 44(2): 540-46.
15. Gao J, Zhong D, Duan X & Chen X. J Chromatogr B Analyt Technol Biomed Life Sci. **2007;** 856(1-2): 35-40.
16. Trivedi RK, Kallem RR, Mullangi R & Srinivas NR. J Phar Biomed Anal. 2005;39(3-4): 661-69.
17. Lacroix PM & Dawson BA. J. Pharm. Biomed. Anal. 1998; 18: 383–402.
18. Trivedi R, Kallem R & Mullangi R. J. Int. Med. Res. 2004; 16: 97–99.
19. Jain N, Raghuwanshi R & Jain D. Indian J Phar Sci. 2008; 70(2): 263-65.
20. Kadav AA & Vora DN. J Pharm Biomed Anal. 2008; 48(1): 120-126.
21. Suresh Kumar GV, Rajendraprasad Y. International Journal of PharmTech Research. 2010; 2(3).
22. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline on Validation of Analytical Procedure Methodology, ICH, Geneva, Switzerland. 1996.