RJC

RASĀYAN *J. Chem.*
Vol. 8 | No.4 |404 - 410 | October - December | 2015
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

GRADIENT RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF METOPROLOL, RAMIPRIL AND ATORVASTATIN IN TABLET DOSAGE FORM

Chandrabatla Varaprasad and K. Ramakrishna*
Department of Chemistry, Institute of Science, Gitam University,
Visakhapatnam-530045, Andhra Pradesh, India
*E-mail: karipeddirk@gmail.com

ABSTRACT

A cost-effective RP-HPLC method using a PDA detector at 210nm wavelength for simultaneous estimation of Metoprolol, Ramipril and Atorvastatin in pharmaceutical dosage forms has been developed. The method was validated as per ICH guidelines over a range of 62.5-625 µg/mL, 6.25-62.5 µg/mL and 12.5-125µg/mL for Metoprolol, Ramipril and Atorvastatin respectively. Analytical column Xbridge C18, 4.6 x 150mm, 5µ was used at a temperature of 30°C ± 0.5°C. OPA Buffer and Acetonitrile composition were used as mobile phase in a gradient flow at a flow rate of 1.0 mL/min. Retention times of 2.4 ± 0.5, 6.1± 0.5 min and 7.8 ± 0.5 min were obtained for Metoprolol, Ramipril and Atorvastatin are respectively. The percentage recoveries of Metoprolol, Ramipril and Atorvastatin, are 100.82%, 100.05% and 100.7% respectively.

Keywords: RP-HPLC, Metoprolol, Ramipril, Atorvastatin, Simultaneous estimation.

INTRODUCTION

Metoprolol [Fig.1A] (*RS*) -1-(Isopropylamino)-3-[4-(2-methoxyethyl) phenoxy] propan-2-ol [1].It is used to treat angina (chest pain), hypertension (high blood pressure) and to prevent heart attack.

Ramipril [Fig. 1B]' (2*S*, 3aS, 6aS)-1-[(2*S*)-2-{[(2*S*)-1-ethoxy-1-oxo-4-phenylbutan-2-l] amino} propanoyl]-octahydrocyclopenta[*b*]pyrrole-2-carboxylic acid belongs to a group of ACE inhibitors used to treat hypertension. It works by relaxing blood vessels, causing them to widen thereby lowering high blood pressure helps prevent heart attack [2-4].

Atorvastatin [Fig.1C] [5], (3*R*,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid is a synthetic HMG-CoA reductase inhibitor that lowers plasma cholesterol levels [6].

In the literature, few methods were reported for the determination these drugs either individually or in combination in pharmaceutical dosage forms. These methods include: spectrophotometric methods [7-16], HPLC methods [17-23], LC/MS/MS methods [24-26] were reported. Only stability indicating methods [27-28] with the combination of these drugs is available, and no official method for the simultaneous estimation of Metoprolol, Ramipril and Atorvastatin is available. In the view of the importance, the author developed and validated a cost effective RP-HPLC assay method for Metoprolol, Ramipril and Atorvastatin in fixed dosage formulation as per ICH and other relevant guidelines. [29-33]

EXPERIMENTAL

Reagents and Chemicals

Working standards of Metoprolol (MPL), Ramipril (RPL) and Atorvastatin (ATN) were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples. HPLC grade Water, Methanol and Acetonitrile were procured from Merck chemical division, Mumbai and tablets of METPURE-AR5 containing Atorvastatin 10mg, Metoprolol 25 mg, Ramipril 5 mg were obtained from the pharmacy.

Instrumentation and Chromatography Conditions

2996 series of Waters Photodiode array detector attached to 2995 series of Waters HPLC, which is having Hamilton syringe and auto sampler opted for chromatography. A degasser to remove the dissolved air and column oven to maintain the desired temperature is also available in the system. OPA buffer: Acetonitrile as mobile phase, at a flow rate of 1.0 mL/min in gradient flow (Table-1), and Xbridge, C18, 4.6 x150mm, 5µ as a stationary phase with an injection volume of 10 µL were fixed as chromatography conditions. Detector wavelength was set at 210 nm.

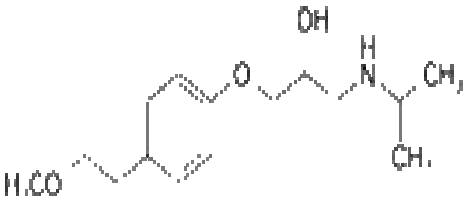

Fig.-1A: Structure of Metoprolol

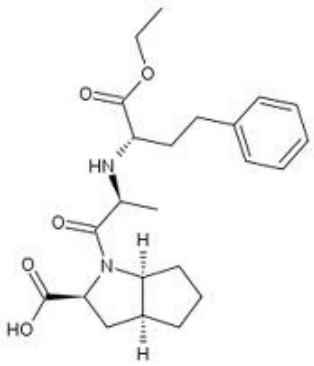

Fig.-1B: Structure of Ramipril

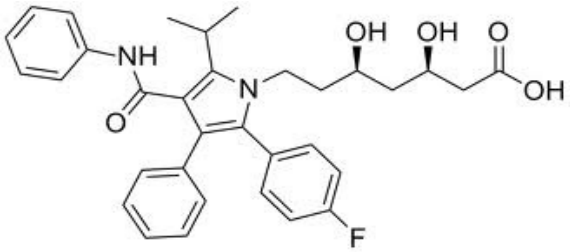

Fig.-1C: Structure of Atorvastatin

Table 1: Gradient Program of mobile phase

Elution	Time	Flow	%A	%C
	0.01	1	75	25
	2.5	1	75	25
	6	1	25	75
Gradient	7.1	1	75	25
	11	1	75	25

Working Standard Stock Solution Preparation

250 mg of Metoprolol, 25 mg of Ramipril and 50 mg of Atorvastatin of working standard were accurately weighed, and these samples were transferred to a 100ml volumetric flask, containing diluent. The mixture was sonicated for 5 minutes to aid dissolution and finally made up to the volume with the same diluent.

Preparation of Calibration Curve Standards

Aliquot of 0.25mL, 0.5mL, 1.0mL, 1.25mL and 1.5mL and 2.5mL were pipette out from stock-A into 10 ml volumetric flask separately and volume was made up to 10mL with diluent. This gives the solutions of 62.5, 125.0, 250.0, 312.5, 375.0 and 625.0 µg/mL respectively for Metoprolol, 6.25, 12.5, 25.0, 31.25, 37.5 and 62.5 µg/mL respectively for Ramipril and 12.5, 25.0, 50.0, 62.5, 75.0 and 125 µg/mL respectively for Atorvastatin.

Tablet Solution Preparation

Twenty tablets of METPURE-AR5 containing 10 mg of Atorvastatin 25 mg of Metoprolol and 5 mg of Ramipril were weighed and ground into fine powder. Powder equivalent to the weight of five tablets was accurately weighed and transferred to a 100ml volumetric flask containing a few mL of diluent. After thorough mixing and sonication, this solution is filtered using 0.45 micron filter paper 2.0 ml of this solution was transferred to 10 ml volumetric flask and the solution was made up to the volume with diluent.

RESULTS AND DISCUSSION

Method Development

After the selection of the drug combination, both the drugs were dissolved in suitable diluent to get a clear solution. The mobile phase was optimized by modifying different combinations of buffers and organic solvents. The pka value of both the drugs was also considered for optimization of pH of the buffer. 210 nm is identified as the isoabsorptive point for all the three drugs and hence it is used as detection wavelength in the present assay.

The resolution and the peak shape of both the drugs found significant with the mobile phase composition of Orthophosphoric acid buffer and Acetonitrile at a flow rate of 1.0 ml/min were used, excellent elution of the three drugs with low retention and run times was observed. Different columns like Xterra, Inertsil, Inspire columns were tried and finally X-bridge C18, 4.6 x150mm, 5µ column with a flow rate of 1.0 ml/min. Mobile Phase of OPA buffer and acetonitrile in the ratio of 30:70 v/v, column temperature at 30°C had resulted in excellent elution of the three drugs with low retention and run times. The retention of for Metoprolol, Ramipril and Atorvastatin were found to be 2.4 ± 0.5, 6.1 ± 0.5 min and 7.8 ± 0.5 min, respectively. A typical chromatogram was shown below in figure-2.

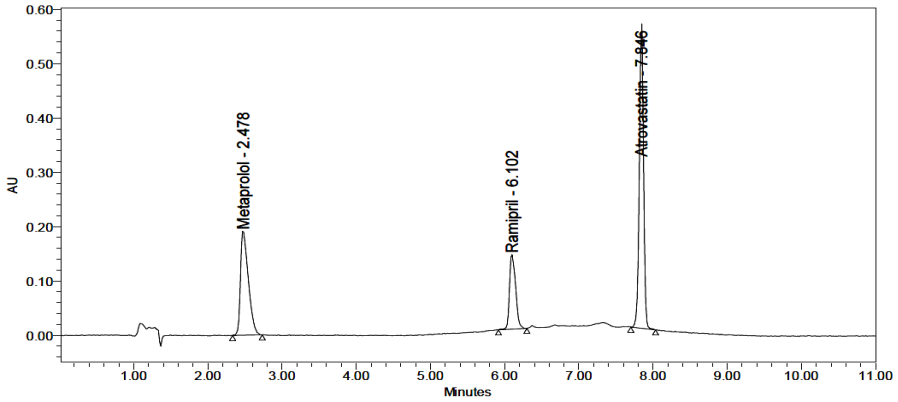

Fig.-2: Typical Chromatogram of MPL, RPL and ATN

METHOD VALIDATION

System Suitability

System suitability test was performed by injecting six replicate injections 100% target solution of Metoprolol (MPL), Ramipril (RPL) and Atorvastatin (ATN). The parameters such as the number of theoretical plates, area and peak tailing were determined and were observed that all the parameters were within the limits. Results were shown in Table-2.

Table-2: System suitability of MPL, RPL and ATN

PARAMETERS	MPL	RPL	ATN
Tailing Factor	1.58	1.21	0.95
No of theoretical plates	2492	24368	91113

Specificity

Specificity experiment was performed by injecting samples of the mobile phase, placebo, sample solution, unspiked and spiked sample. The results showed no interference at the retention time of Metoprolol,

Ramipril and Atorvastatin.The representative chromatogram of Placebo is shown in Figure-3.

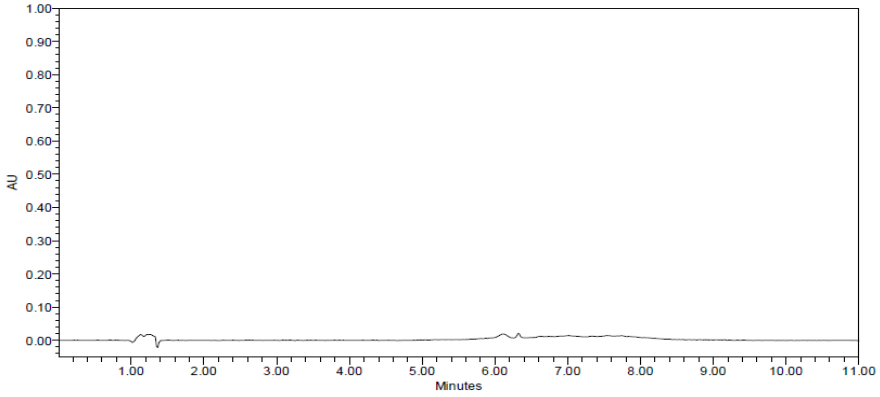

Fig.-3: Specificity chromatogram

Linearity

Standard solutions of Metoprolol (62.5-625.0µg/mL), Ramipril (6.25-62.5µg/mL) and Atorvastatin (12.5µg/mL-125µg/mL) respectively were prepared and injected under the chromatographic conditions described above. Calibration curves were drawn the concentration of drug versus corresponding peak areas obtained at 210nm. The results showed a significant correlation between detector response and concentration level of each drug within the concentration range. All the three drugs showed a linear response as shown in Figure (4-6). The equation Y= (mx+c) was used to represent the linearity as follows-

Y (MPL) =5636.x +29570, Y (RPL) = 29436.x+20510 and Y (ATN) = 44950.x +35874

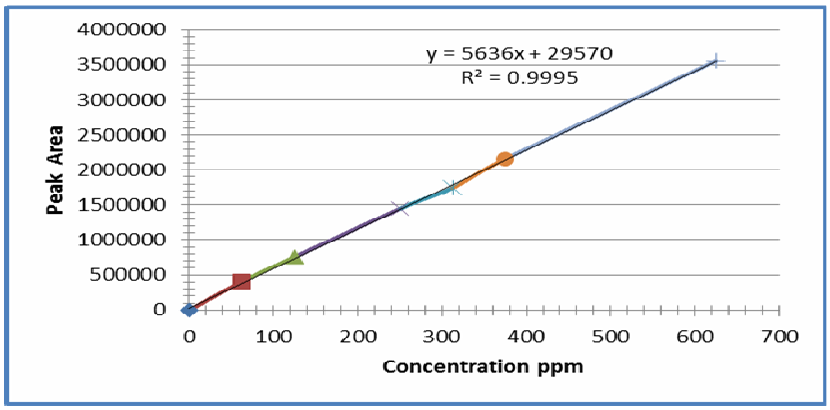

Fig.-3: Calibration Curve for Metoprolol

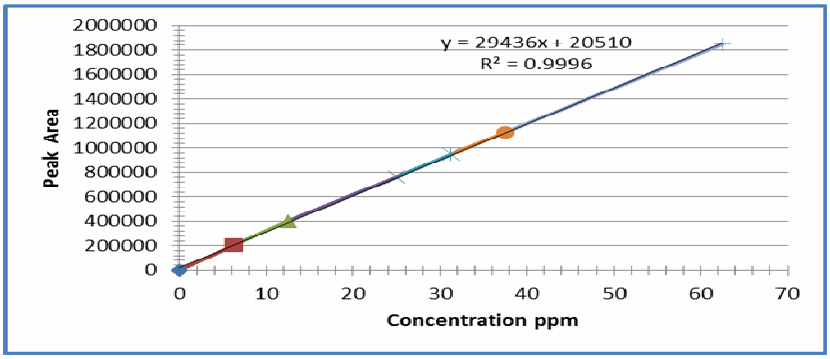

Fig.-4: Calibration Curve for Ramipril

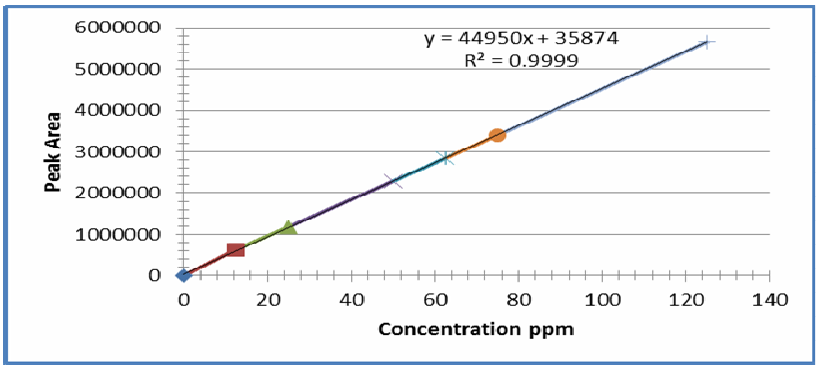

Fig.-5: Calibration Curve for Atorvastatin

Accuracy

Known amounts of reference solution for all the three drugs Metoprolol, Ramipril and Atorvastatin equivalent to 50%, 100% and 150% of the label claim were added to the tablet solutions of Metoprolol, Ramipril and Atorvastatin. These results are summarized in Table- 3. The percent mean recovery for Metoprolol, Ramipril and Atorvastatin are 100.82, 100.05 and 100.70 respectively, indicating that the method was accurate.

Table-3: Recovery Experiments of MPL, RPL and ATN

Amount Spiked			Standard Amount added			% Recovered		
MPL	RPL	ATN	MPL	RPL	ATN	MPL	RPL	ATN
125	12.5	25	250	25	50	102.29	99.30	101.31
125	12.5	25	250	25	50	102.19	99.61	101.74
125	12.5	25	250	25	50	102.28	100.91	98.50
250	25	50	250	25	50	99.89	99.88	99.87
250	25	50	250	25	50	100.43	101.12	100.37
250	25	50	250	25	50	100.21	98.43	101.33
375	37.5	75	250	25	50	99.87	100.44	101.01
375	37.5	75	250	25	50	99.99	100.74	100.89
375	37.5	75	250	25	50	100.25	100.02	101.31
					AVG	100.82	100.05	100.70
					SD	1.09	0.86	1.00
					%RSD	1.08	0.86	0.99

Repeatability

The precision of the developed method was assessed for intraday (Precision) and interday (by varying the analyst and HPLC column called as intermediate precision). The obtained % RSD for Metoprolol, Ramipril and Atorvastatin were 0.73, 1.57 and 1.19 for Intraday and 0.79, 1.47 and 1.21 respectively.

Robustness

Robustness is performed by making slight variations in the Flow rate, column temperature and concentration of the mobile phase. The changes and the results were tabulated in Table 4. No significant effect was observed with the above changes indicating the robustness of the method.

Stability of Sample Solution

The stability studies were carried out in mobile phase after 24hrs at ambient temperature using the mentioned chromatographic conditions. From these studies, it was revealed that Metoprolol, Ramipril and Atorvastatin were stable in mobile phase for at least for 24hrs indicating the reliability of analysis in the proposed procedure. Results are shown in Table-5.

Table-4: Robustness table of Metoprolol, Ramipril and Atorvastatin

	Changed value	Retention time			Tailing factor			% assay		
		MPL	RPL	ATN	MPL	RPL	ATN	MPL	RPL	ATN
Column Temperature	25	2.314	6.054	7.475	1.27	0.93	0.92	101.8	101.5	101
	35	2.415	6.115	7.870	1.37	0.83	0.72	100.7	100.9	101.3
Flow Rate (mL)	0.9	2.116	6.044	7.435	1.51	1.13	0.96	98.5	99	98.9
	1.1	2.006	5.945	7.335	1.51	1.13	0.96	100.1	99.2	99.2
Mobile-Phase	-5	2.125	5.995	7.415	1.51	1.13	0.96	98.8	99	99.4
	+5	2.015	5.884	7.315	1.12	1.88	0.97	101.2	101.9	101.6
Average								100.18	100.25	100.20
Std Dev								1.32	1.34	1.19
%RSD								1.31	1.33	1.19

Table-5: Stability data of MPL, RPL and ATN

	% Assay	
Drug	0 hr	24hr
MPL	98.79	100.70
RPL	98.65	100.02
ATN	98.57	99.64

LOD and **LOQ**

In the present chapter LOD and LOQ of MPL, RPL and ATN were determined by linearity curve method. LOD and LOQ were calculated by using the equations. LOD = 3.3 σ/S and LOQ= 10 σ/S, where "σ "is the standard deviation of the response, and "S" is the slope of the linearity curve.

The LOD values were 2.29µg/mL, 0.05µg/mL and 1.08µL for MPL, RPL and ATN respectively. The LOQ values were 6.95µg/ml, 0.15µg/mL, and 3.26µg/ml for MPL, RPL and ATN respectively.

Analysis of Formulations

The proposed method was applied for the estimation of Metoprolol, Ramipril and Atorvastatin in tablet dosage form and the results are reported in Table 6. The high recovery with low RSD value confirmed the appropriateness of the proposed method.

Table-6: HPLC data for Analysis of Tablets

DRUG	MPL	RPL	ATN
%Assay	98.79	98.65	98.57
%RSD	0.78	0.91	0.37

*Mean of six (n=6) determinations

CONCLUSION

A new RP-HPLC method was developed and validated for simultaneous estimation of Metoprolol, Ramipril and Atorvastatin in tablet dosage form. The calibration curve was found to be linear over a concentration range of 62.5-625 µg/mL, 6.25-62.5 µg/mL and 12.5-125µg/mL for Metoprolol, Ramipril and Atorvastatin respectively. A linear equation was established to provide the best fit for the concentration vs. detector response. The goodness of fit was consistently found to be 0.99 during the validation. No interference or overlapping of the peaks either due to excipients or diluents was observed during the Selectivity experiment at the retention time of Metoprolol, Ramipril and Atorvastatin. The obtained % RSD value of < 2 confirms that proposed method is effectively precise. Further, the separation of the analytes was completed in 11 minutes only, making the proposed RP-HPLC method conveniently adopted for the routine quality control analysis of other combination formulations containing these drugs.

REFERENCES

1. G. Stephen Ball, S. Ian. Robertson, *The American Journal of Cardiology*, **59 (10)**, D23(1987)**.**
2. http://livertox.nih.gov/Ramipril.htm#overview
3. S. Yusuf, P. Sleight, J. Pogue, J. Bosch, R. Davies,G. Dagenais, *N. Engl. J. Med*., **342(3)**,145 (2000)**.**
4. G.R. Dagenais, S. Yusuf, M.G. Bourassa, Q. Yi, J. Bosch, E.M. Lonn, S. Kouz, J. Grover, *Circulation*. (2001).
5. "Atorvastatin Calcium", drugs.com. Retrieved 3 April (2011)
6. Lea, Andrew, Mc Tavish, Donna, *Drugs*, **53 (5)**, 828(1997).
7. Manisha B. Jadhav, Sachin S. Suryawanshi, Sachin R. Tajane, K.N.Tarkase, *Int. J. Pharma. Pharm. Sci.***, 4 (3)** 387(2012).
8. A.S.Jadhav, K.N.Tarkase, Deshpande, *Der Pharmacia Lettre* **4(3)**, 763(2012).
9. O.E. Afieroho, O. Okorie, T.J.N. Okonkwo, *Tropical J. Pharm Res.*, **11(2)**, 275(2012).
10. Erdal Dinc, Dumitru Baleanu, *Asian J. Chem*., **23(6)**, 2377 (2011).
11. Permender Rathee, Hema Chaudhary, Sushila Rathee, Dharmender Rathee, *Indian Pharmacist,* **9(91)**, 84 (2010).
12. A. Shravya, R.S. Chandan, B. M. Gurupadayya, M. Sireesha, *Int. J. Pharm. Tech.*, **2(4)**, 1046 (2010).
13. Sagar B Wankhede, Nitin R Dixit, Sohan S Chitlange, *Der Pharma Chemica*., **2(1)**, 134 (2010).
14. Vandana Patel, Rajesh Baldha, Deepa Patel, *Asian J. Chem*., **22(4),** 2511 (2010).
15. R Vijayalakshmi, A.R.Magesh, M.D.Dhanaraju, *Oriental J. Chem.,* **26(1)**, 255 (2010).
16. B Stanisz, W Rafa. *Chemia Analityczna,* **53(3)**, 417 (2008).
17. Boyka G Tsvetkova, P Lily, *J Chem Pharm Res*., **5(1)**, 168 (2013).
18. Olabemiwo, Ojeyemi Mathew, Mahesh, Pottabattula Rao, A.B.N.Nageshwar rao, V.Jayathirtha, *Asian J Chem*., **24(6)**, 2762 (2012).
19. B.Brahmaiah, K. Sujana ,A. Prameela Rani, Asian *J. Pharm Clin. Res*., **6(1)**, 23 (2013).
20. Shradhanjali Singh, Kirti S. Topagi, Mrinalini C. Damle, *Res. J. Pharm. Tech*., **2(4)**, 749 (2009).
21. Bilal Yilmaz, *Int. J. Pharm. Sci. Review* Res., **1(1)**, 39 (2010).
22. Lakka Narasimha Swamy, Nishant Goswami, Penugonda Balakrishna, Vaddi Sailaja, *Int J Res Pharm Sci*., **2(4)**, 608 (2011).
23. A Patel, C Macwana, V Parmar, S Patel, *J. of AOAC Int*., **95(2)**, 419 (2012).
24. Sha Li , Xingli Wang, Kelong Peng , Zhiguo Ma , Xiaoqi Zhang , Shaolian Fu, Xiaofei Li, Linlin Li, Aihua Hong and Jie Jiang , *Molecules,* **17**, 2663 (2012).
25. Syed N.Alvi, Saleh Al-Dgither, Muhammad M.Hammami, *Analy Chem: An Indian J*., **7(7)**,540 (2008).
26. Yu, Qi, Hu, Zhe-Yi, Zhu, Fan-Yuan, Zhu, Jin-Hui, Wan, Li-Li, Li, Yan, Guo, Cheng, *Chromatographia*, **73(3-4)**, 257 (2011).
27. Shaik Harun Rasheed, M Ramakotaiah, P Sandhya Vani, Mulla Arief, Gajavalli, Silpa Rani, *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **1(4)**, 816(2010).
28. Seshadri Raja Kumar, Desai Makarand Madhukar, Thummala Veera Raghavaraju, Deepa Krishnan, Dama Venugopala Rao, Ivon Elisha Chakravarthy, *Scientia Pharmaceutica* ,**78(4)**, 821(2010).
29. ICH Harmonised Tripartite Guideline. Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline. Geneva, Switzerland: (1994).
30. ICH Harmonised Tripartite Guideline, Code Q2B- Validation of Analytical Procedure Methodology Step-4 Consensus Guideline. Geneva, Switzerland: (1994).
31. A.H. Beckett, J.B. Stenlake, (2002), *Practical Pharmaceutical Chemistry. 4th ed., New Delhi;* CBS Publishers and Distributors, pp. 157-66.
32. Indian Pharmacopoeia, (2007), Ghaziabad, Indian Pharmacopoeia Commission, pp. 558-59, 1005-6.
33. British Pharmacopoeia, (2009), London, British Pharmacopoeia Commission, pp. 2861, 5094.

[RJC-1333/2015]