Research Article **CODEN: AJPAD7** **ISSN: 2321-0923**

Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry

Journal home page: www.ajpamc.com

ANALYATICAL METHOD DEVELOPMENT AND VALIDATION OF LOVASTATIN AND NIACIN BY USING RP-HPLC METHOD

R. Vasanthi[1]*, J. Prasad[1], M. Alagar Raja[1], V. Prashanthi[1], V. Shrisha[1], David Banji[1], D. Selva Kumar[2]

[1*]Department of Pharmaceutical Analysis and Quality Assurance, Nalanda College of Pharmacy, Nalgonda, Telangana State, India.

[2]School of Pharmacy, Taylors University, Malaysia.

ABSTRACT

A new simple specific, sensitive, precise reverse phase high performance liquid chromatography method has been developed for simultaneous estimation of lovastatin and niacin. The determination was carried out by using Symmetry C8 (4.6 x 250mm, 5µm) column with the mobile phase containing acetonitrile: phosphate buffer (Ph4.±0.5) in the ratio of 65:35 v/v. The optimized flow rate was 0.7ml/min and the UV detection was carried out at 240 nm. The retention time of lovastatin and niacin were found to be 3.093 min and 6.196 min respectively. The method was found to be linear in the concentration range 2.0-10µg/ml for lovastatin and niacin respectively. The method was validated as per ICH guidelines. The proposed method was successfully applied for the estimation of lovastatin and niacin in pharmaceutical dosage forms.

KEYWORDS

Lovastatin, Niacin, UV, HPLC and ICH guidelines.

Author for Correspondence:

R. Vasanthi,

Department of Pharmaceutical Analysis and Quality Assurance, Nalanda College of Pharmacy, Nalgonda, Telangana State, India.

Email: vasanthi.rangapuram@gmail.com

INTRODUCTION

Lovastatin (Figure No.1) is an inhibitor of 3-hydroxy-3 methylglutaryl-coenzyme. A Reductase (HMG-CoA Reductase), an enzyme that catalyzes the conversion of HMG-CoA to mevalonate. Mevalonate is a required building block for cholesterol biosynthesis and lovastatin interferes with its production by acting as a reversible competitive inhibitor for HMG-CoA, which binds to the HMG-CoA Reductase. Niacin (Figure No.2) (also known as vitamin B_3, nicotinic acid, or less commonly vitamin PP; archaic terms include pellagra-preventive and anti-dermatitis factor) is an organic compound. Niacin is involved in both

DNA repair, and the production of steroid hormones in the adrenal gland. Niacin treatment also decreases the serum levels of apolipoprotein B-100 (Apo B), the major protein component of the VLDL (very low-density lipoprotein) and LDL fractions[1].The combination of lovastatin with niacin is an attractive therapeutic option because both have well-recognised record of utility in lipoprotein metabolic disorders. This combination is also used in the treatment of cardiovascular diseases such as heart attacks, stroke which are caused by fat clogging the blood vessels.

Literature survey reveals some analytical methods for quantitative determination of lovastatin and niacin individually and in combination with other drugs and some with niacin like spectrophotometric method[2-3]capillary electrophoresis method[4-5] and chromatographic methods [6-10].

In theses present study a successful attempt has been made to develop a rapid, precise, accurate and comparatively economical RP-HPLC method for quantitative estimation of Isotretinoin in. The developed method validated and recovery studies were conducted and studied by using various statistical parameters according to ICH guidelines [11].

MATERIALS AND METHODS

Chemicals and Reagents

Acetonitrile and water HPLC grade, orthophosphoric acid, potassium dihydrogen phosphate of AR grade were obtained from Merck, Mumbai, India. Pharmaceutical grade lovastatin and niacin were obtained as a gift samples from Novo Nordisk Ltd, Hyderabad, India.

Instruments

The HPLC system (WATERS model 2487) consisting of dual λ absorbance detector containing 515 HPLC pump, Rheodyne injector (7725i) with 20µl fixed loop. The output signal was monitored and integrated using Empower 2 software. A Symmetry C8 (4.6 x 250mm, 5 µm, Make Waters) or equivalent column was used for separations.

Preparation of 0.01M potassium dihydrogen ortho phosphate

Dissolve 1.36gm of potassium dihydrogen phosphate in sufficient water to make up to 1000ml.

Mobile phase preparation

The mobile phase was prepared by mixing 0.01M potassium dihydrogen phosphate adjust to 4 P^H with ortho phosphoric acid and acetonitrile in the ratio of (35:65% v/v).

Preparation of standard stock solution

Initially 50mg of Lovastatin was weighed accurately and transferred to 100ml volumetric flask, about few ml of methanol was added and sonicated to dissolve. The final volume was made up to mark with methanol and 1ml of this solution transferred to 100ml volumetric flask, volume was made up to the mark with 100ml of mobile phase to obtain 50µg/ml of Lovastatin solution. Finally 4ml of this solution transferred to10ml volumetric flask, volume was made up to the mark with mobile phase to obtain final concentration of Lovastatin solution as 20µg/ml. Different aliquots 1,2,4,6,8,10 ml of standard stock solutions were transferred into 10ml volumetric flasks and volume was adjusted to mark to obtain the concentration in the ranging from 5µg/ml to 50µg/ml.

Determination of Working Wavelength (λ max)

Standard solutions of lovastatin and niacin were scanned separately in the wavelength range of 200-400nm and the λ max was found to be 238 nm and 262 nm for lovastatin and niacin respectively. From the overlaid spectra it was found that both the drugs show appreciable absorbance at 240 nm, so it is used for the further study. The overlaid absorption spectrum of lovastatin and niacin is shown in Figure No.3.

Preparation of Standard solution

About 50 mg of Lovastatin and 50 mg of Niacin were weighed into a 100 mL volumetric flask, to this few ml of mobile phase was added, sonicated and the volume was made up to mark with the mobile phase.

Preparation of Sample solution

Twenty capsules were weighed accurately. A quantity of powder equivalent to 40 mg of Lovastatin and 500mg of Niacin was taken in 100

mL volumetric flask and made up to the mark with mobile phase. From above solution 4 mL of the clear solution is pipette out in to 100 mL volumetric flask and made up to the volume with mobile phase. The resulting solution is used to record the chromatogram figure.

Assay

20 µL of the standard solution was injected five times into the chromatographic system, chromatograms were recorded and peak areas were measured. 20 µL of the sample solution was injected in five times into the chromatographic system, chromatograms were recorded and peak areas were measured.

METHODS DEVELOPMENT

The developed method was fully validated for the parameters as per ICH guidelines.

System Suitability

System suitability is done by replicate analysis of 6 injections at the concentration of 16µg /mL of Lovastatin and 200µg /mL of Niacin were injected six times and the chromatograms were recorded for the same.

Linearity

Linearity is determined by a series of three to six injections of five or more standards. Peak areas (or heights) of the calibration standards are usually plotted in the Y-axis against the nominal standard concentration, and the linearity of the plotted curve is evaluated through the value of the co-relation coefficient (r2). The methods were linear in the range of 8-24 ppm lovastatin 100-300 ppm for niacin and inject each level into the chromatographic system and measure the peak area.

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (preanalyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug.

Precision

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day variation). Repeatability was examined by analyzing six determinations of the same batch of each component at 100% of the test concentration. Which confirms that the method is sufficiently precise. Intermediate precision (inter-day variation) was studied by assaying five samples containing the nominal amount on different days by different analysts using different LC systems. Solutions corresponding to each concentration level were injected in duplicate. The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample.

Limit of detection and limit of quantification

The limit of detection and quantification were calculated using signal to noise ratio. The LOD for lovastatin and niacin were tested at specific level i.e 0.115µg/ml and 0.121µg/ml. The LOQ for lovastatin and niacin were tested at specific level i.e 0.384µg/ml and 0.036µg/ml.

Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase, p^H of buffer, flow rate. The flow rate was varied by 1 ± 0.2 mL/min. the percentage of organic modifier was varied by 65 ± 5% and column temperature was varied by 30 ± 5°C. Their effects on the retention time (TR), tailing factor (T), theoretical plate numbers (N) and repeatability of peak areas (n = 3) were studied.

RESULTS AND DISCUSSION

The goal of this study was to develop a new RP-HPLC method, several mobile phase compositions were tried for separation and quantification of lovastatin and niacin in bulk and pharmaceutical dosage forms. To develop an effective method for the analysis of the drugs preliminary tests were performed in order to select adequate and optimum conditions. Parameters such as detection wavelength, mobile phase composition and p^H, mobile phase comprising of Acetonitrile: Buffer in 65:35 at a flow rate 0.7 ml/min to get a better reproducibility and repeatability. Quantification was achieved with UV detection at 240 nm and the retention time for lovastatin and niacin were found

to be 3.093 and 6.196 mins respectively. A typical chromatograms of niacin and lovastatin is shown in Figure No.4,5. The optimized method was validated as per ICH guidelines.

System suitability

System suitability tests were carried out on freshly prepared standard solutions and the parameters are summarized in Table.No.1.

Linearity

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Lovastatin and Niacin is 0.999and 0.999 respectively. It shows that the good correlation exist between the drug and response. The results are summarized in the Table No.2.

Accuracy

The % Recovery for each level obtained for Lovastatin was 102.0 % and Niacin was 101.87% as per the ICH guidelines the results were within the limit. The results are shown in Table No.4.

Precision

The % RSD of 6 determinations of Lovastatin and Niacin for System precision intraday and inter day was found to be within the acceptance criteria of not more than 2.0%. The results are tabulated in Table No. 5, 6, 7 and 8.

Limit of Detection and Limit of Quantification

Limit of detection result for Lovastatin and Niacin was found to be 2.9 and 2.84 respectively and were within the limits. S/N ratio for Lovastatin and Niacin were found to be 9.96 and 10.3 respectively and were within the limits. Results are summarized in Table No.9 and 10.

Table No.1: System Suitability of Proposed Method

S.No	Parameters	Lovastatin	Niacin
1	Theoretical plates	3462.1	3771.7
2	Resolution	---	4.9
3	Tailing factor	1.4	1.3
4	Retention Time (min)	3.093	6.19

Table No.2: Linearity data of Lovastatin

S.No	Linearity Level	Concentration	Area
1	I	8ppm	424798
2	II	12ppm	631169
3	III	16ppm	850951
4	IV	20ppm	1052639
5	V	24ppm	1279197
Correlation Coefficient			0.999

Table No.3: Linearity Data of Niacin

S.No	Linearity Level	Concentration	Area
1	I	100pm	2157342
2	II	150ppm	3252253
3	III	200ppm	4347236
4	IV	250ppm	5379374
5	V	300ppm	6493722
Correlation Coefficient			0.999

Table No.4: Results of Accuracy

S.No	% of pure drug spiked	Pure drug		Formulation		Lovastatin		Niacin	
		Lovastatin	Niacin	lovastatin	Niacin	% recovery	Statistical analysis	% recovery	Statistical analysis
1	**50%**	8	100	16	200	98.9	**Mean** = 99.10 **SD** = 0.135 **%RSD** = 0.13	100.8	**Mean** = 98.05 **SD** = 0.058 **%RSD** = 0.05
2	**50%**	8	100	16	200	98.7	--	100.1	---
3	**50%**	8	100	16	200	98.7	--	100.5	---
4	**100%**	16	200	16	200	100.3	**Mean** = 97.58 **SD** = 0.032 **%RSD** = 0.03	100.9	**Mean** = 98.03 **SD** = 0.045 **%RSD** = 0.04
5	**100%**	16	200	16	200	100.6	---	100.8	---
6	**100%**	16	200	16	200	100.4	---	101.0	---
7	**150%**	24	300	16	200	101.1	**Mean** = 98.80 **SD** = 0.005 **%RSD** = 0.005	101.7	**Mean** = 98.03 **SD** = 0.015 **%RSD** = 0.01
8	**150%**	24	300	16	200	101.0	---	101.2	---
9	**150%**	24	300	16	200	100.9	---	101.8	---

Table No.5: Results of Method Precision Intraday of Niacin

S.No	Injection	Area
1	Injection-1	4571058
2	Injection-2	4445474
3	Injection-3	4411762
4	Injection-4	4446748
5	Injection-5	4439496
6	Injection-6	4441688
7	**Average**	4459371
8	**Standard Deviation**	56215.5
9	**% RSD**	1.26

Table No.6: Results of System Precision Inter day of Niacin

S.No	Injection	Area
1	Injection-1	4413840
2	Injection-2	4401169
3	Injection-3	4404313
4	Injection-4	4409147
5	Injection-5	4400229
6	**Average**	4405740
7	**Standard Deviation**	5712.1
8	**% RSD**	0.13

Table No.7: Results of Method Precision Inter day of Lovastatin

S.No	Injection	Area
1	Injection-1	872598
2	Injection-2	871624
3	Injection-3	873012
4	Injection-4	875293
5	Injection-5	878851
6	Injection-6	878420
7	**Average**	874966
8	**Standard Deviation**	3089.5
9	**% RSD**	0.35

Table No.8: Results of System Precision Inter day of Lovastatin

S.No	Injection	Area
1	Injection-1	852762
2	Injection-2	852155
3	Injection-3	857107
4	Injection-4	858890
5	Injection-5	858238
6	**Average**	855831
7	**Standard Deviation**	3150.7
8	**% RSD**	0.37

Table No.9: Limit of Detection Chromatogram of Lovastatin and Niacin

S.No	Name	Retention time	Area	s/n
1	Lovastatin	3.102	454798	2.9
2	Niacin	6.189	2157342	2.84

Table No.10: Limit of Quantification Chromatogram of Lovastatin and Niacin

S.No	Name	Retention time	Area	s/n
1	Lovastatin	3.112	631169	9.96
2	Niacin	6.215	3252253	10.3

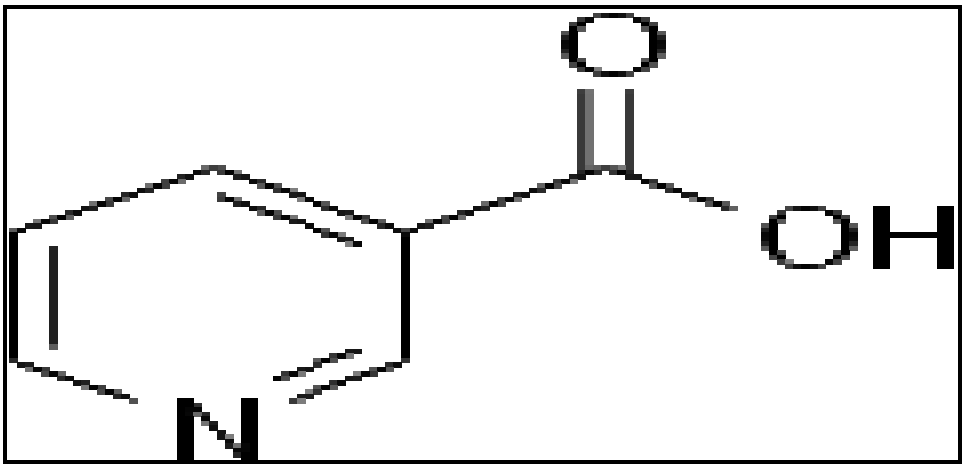

Figure No.1: Lovastatin

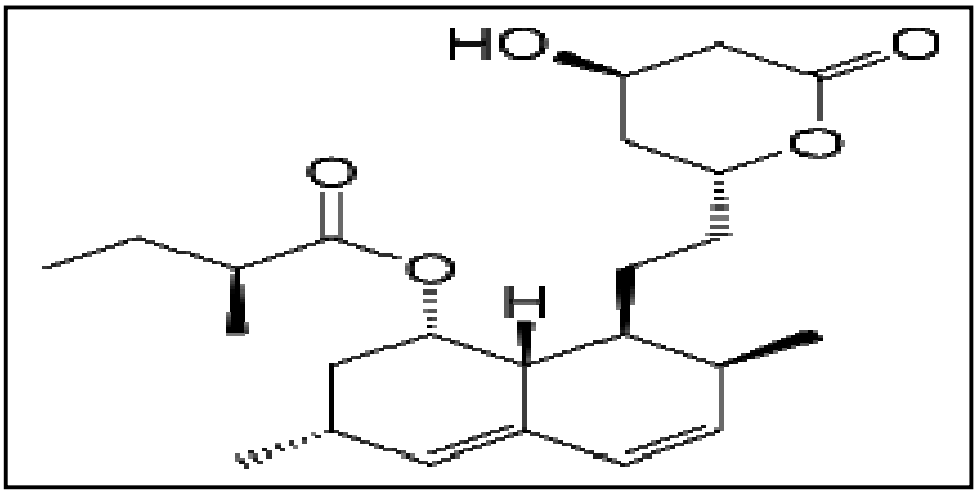

Figure No.2: Niacin

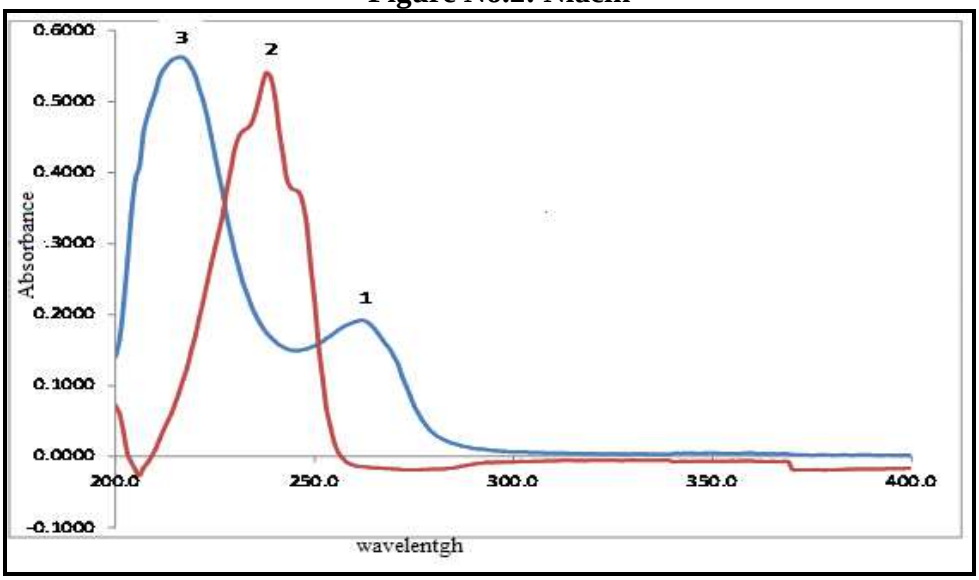

Figure No.3: Overlaid Absorption Spectrum of Lovastatin and Niacin

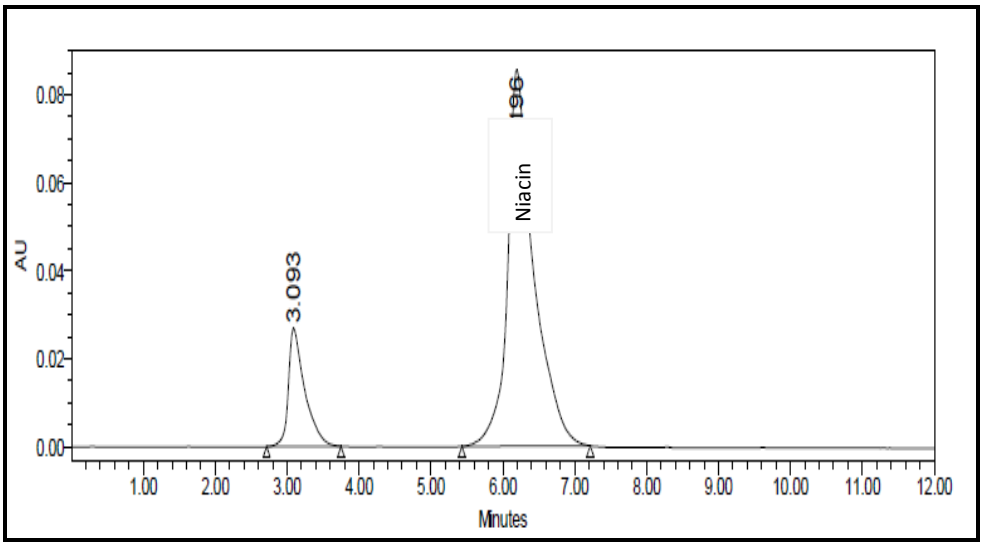

Figure No.4: Standard Chromatograms of Lovastatin and Niacin

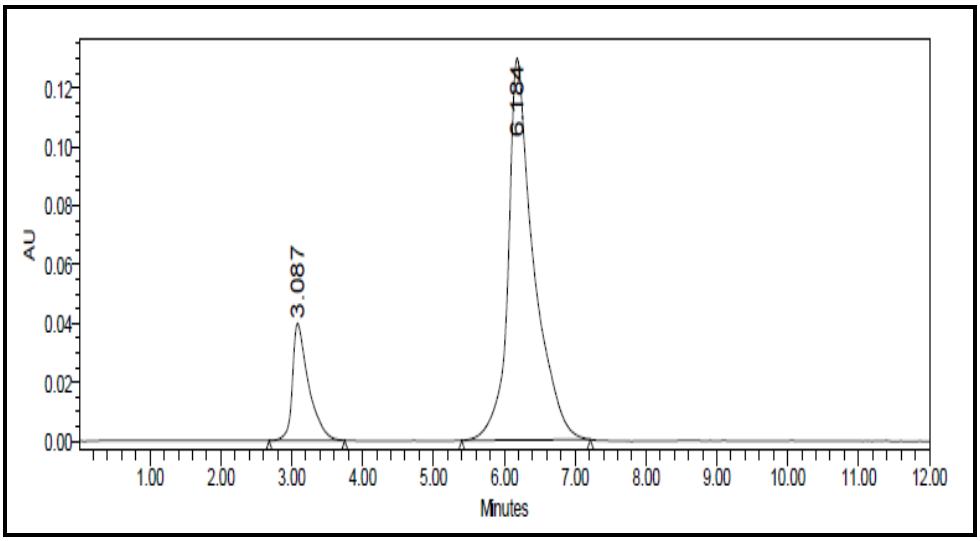

Figure No.5: Sample Chromatogram of Lovastatin and Niacin

CONCLUSION

The proposed method is found to be simple, precise, accurate and rapid for the determination of lovastatin and niacin in pure and its pharmaceutical dosage forms. Validation parameters include system suitability, specificity, linearity, accuracy, precision, robustness, and ruggedness was determined according to the ICH guidelines. The proposed method was showed good linearity, precision and accuracy for sensitive quantitative determination of lovastatin and niacin in pharmaceutical formulations without interference.

ACKNOWLEDGEMENT

I express my gratitude respectable, guide, our principal, Vice-principal, and my Parents for providing all the encouragement and I am also thankful to Novo Nordisk Ltd, Hyderabad, India for providing a gift sample of standard lovastatin and Niacin.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Tunaru S, Lattig J, Kero J, Krause G, Offermanns S. Characterization of determinants of ligand binding to the nicotinic acid receptor GPR109A (HM74A/PUMA-G), *Mol Pharmacology*, 68(5), 2005, 1271-80.
2. Sharaf El-Din M M K, Attiakam, Nassar M W I, Kaddah M M Y. Colorimetric determination of simvastatin and lovastatin in pure form and in pharmaceutical formulations, *Spectrochim Acta, AMol Biomol Spectroscopy*, 76, 2010, 423-428.
3. Kazemipour M, Ansari M, Ramezani H and Moradalizadeh M. Simultaneous determination of lovastatin and niacin in tablet by first and third derivative spectrophotometry and H-point standard addition methods, *Research in Pharmaceutical Sciences*, 7(2), 2012, 95-102.
4. Li M, Fan L Y, Zhang W, Sun J, Cao C X. Quantitative analysis of lovastatin in capsule of Chinese medicine Monascus by capillary zone electrophoresis with VIS detector, *J Pharm Biomed Anal*, 43, 2007, 387-392.
5. Windahl K L, Trenerry V C, Ward C M. The determination of niacin in selected foods by capillary electrophoresis and high performance liquid chromatography: acid extraction, *Food Chem*, 65, 1999, 263-270.
6. Sirnivasu M K, Narasa R A, Roa D S. Determination of Lovastatin and Simvastatin in pharmaceutical dosage forms by HPLC, *Indian Drugs*, 41(3), 2004, 156-159.
7. Payal Chauhan Rakesh Parmar, Nehal Sha. Stability Indicating RP- HPLC Method for the Determination of Niacin and Lovastatin in Bulk Drug and Tablet Formulation, American *Journal of pharmtech Research*, 4(2), 2014, 548-561.
8. Pfuhl P, Karcher U, Häring N, Baumeister A, Tawab M A, Schubert - Zsilavecz M. Simultaneous determination of niacin, niacinamide and nicotinuric acid in human plasma, *J Pharm Biomed Anal*, 36, 2005, 1045-1052.
9. Pillai A D, Ravishankar M N, Handral R D. High Performance Thin Layer Chromatographic method for estimation of Lovastatin Tablets, *Ind J Pharm Sci*, 5, 2011, 420-422.
10. Yuan H, Wang F, Tu J, Peng W, Li H. Determination of lovastatin in human plasma by ultra-performance liquid chromatography-electro spray ionization tandem mass spectrometry and its application in a pharmacokinetic study, *J Pharm Biomed Anal*, 46, 2008, 808-813.
11. International Conference on Hormonization validation of Analytical Procedure Methohodology Q2B, 1996.

Please cite this article in press as: R. Vasanthi *et al*. Analytical Method Development and Validation of Lovastatin and Niacin by using RP-HPLC Method, *Asian Journal of Pharmaceutical Analysis and Medicinal Chemistry*, 3(3), 2015, 128 - 136.