Research Article **www.ijcps.com**

International Journal of
Chemical and Pharmaceutical Sciences
2015, June., Vol. 6 (2)

ISSN: 0976-9390
IJCPS

Development and validation of simultaneous estimation of lovastatin and niacin in bulk and pharmaceutical dosage forms by RP-HPLC

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Received: 11 June 2015, Revised and Accepted: 18 June 2015

ABSTRACT

A simple, specific, sensitive, precise and reproducible a reverse phase high performance liquid chromatography new method has been developed for simultaneous estimation of lovastatin and niacin. Lovastatin and Niacin is Anti-hyperlipidemic Sustained Release drug. The determination was carried out by using symmetry C_8 (4.6×250mm, 5µ) column with (pH4) potassium di hydrogen phosphate: acetonitrile (35:65v/v) as the mobile phase and the detection wavelength at 240nm.The flow rate is 0.7 ml/ min. The Retention time of lovastatin, niacin was 3.093 min and 6.196 min respectively. Linearity for the lovastatin (LS) and niacin (NI) were found to be in the range of 8-24 µgm and 100 - 300 µgm respectively. The method was validated as per the ICH guidelines. The proposed method can be successfully applied for the estimation of lovastatin and niacin in pharmaceutical dosage forms.

Keywords: RP-HPLC, Lovastatin, Niacin, Sustained Release, Validation.

1. INTRODUCTION

Lovastatin and Niacin used in the effective treatment of hyperlipidemia .Lovastatin is an inhibitor of 3-hydroxy-3methylglutaryl-coenzymeA Reductase (HMG-CoA Reductase), an enzyme that catalyzes the conversion of HMG-CoA to mevalonate. Mevalonate is required for building block of cholesterol synthesis. Lovastatin is chemically known as(1S,3R,7S,8S,8aR)-8-{2-[(2R,4R)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl1,2,3,7,8,8a-hexahydronaphthalen-1-yl(2S)-2 methylbutanoate. Niacin is involved in both DNA repair, and the production of steroid hormones in the adrenal gland. Niacin chemically known as pyridine-3-carboxylic acid. The combination of lovastatin with niacin can been attractive option because both have excellent records for the treatment of lipoprotein metabolism disorders, and prevention & therapy of arthrosclerosis related disorders.

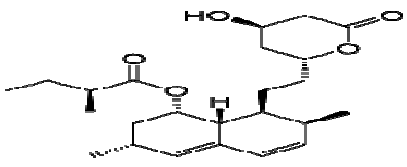

Figure - 1a: Chemical structure of Lovastatin.

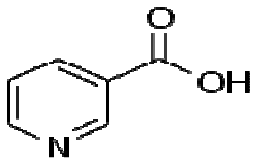

Figure - 1b: Chemical structure of Niacin.

In literature survey some analytical methods were reported for the quantitative determination of lovastatin, alone or in combination with other drugs by HPLC methods LC-MS/MS methods, micelle electro kinetic chromatographic method, volt metric technique , spectrophotometric method ,and methods reported for niacin, alone or in combination with other drugs by HPLC methods, LC-MS/MS method, UV method and HPTLC method. The aim of the study is to develop a simple, sensitive, accurate, precise and cost effective method for determination of lovastatin and niacin its related substances in bulk drugs and pharmaceutical formulations within 10 minutes. [1-4]

2. MATERIALS AND METHODS

2.1. Instruments

WATERS HPLC model 2487 Dual λ absorbance detector containing 515 HPLC pump and Rheodyne injector (7725i) with 20µl fixed loop. Chromatographic separation was performed by using symmetry C_8 column 4.6 × 250mm internal diameter and 5µparticle size. Detection wavelength is observed by using UV-3000+LABINDIA double beam with UV win 5 software UV spectrophotometer with 1cm matched quartz cells, model no.UV-2371.Citizen electronic balance was used for weighing. Global digital pH meter was used.

2.2. Chemicals and reagents

Acetonitrile, methanol and water were of HPLC grade and ortho phosphoric acid (OPA), pure potassium dihydrogen phosphate AR grade were obtained from Merck, Mumbai India. Lovastatin (LS) and Niacin (NI) reference standards obtained as gift samples from Hetero Pharmaceuticals Pvt. Ltd., Hyderabad, India. Tablet dosage form containing 40 mg of lovastatin and 500 mg of niacin (MEVACOR) was procured from the local market.

2.3. HPLC condition

The mobile phase consisted of pH4 potassium dihydrogen phosphate: acetonitrile (35:65v/v) .The mobile phase was prepared freshly and it was sonicated by using PCI Mumbai 3.5liter capacity Sonicator for 5 min before use. C_8 column (4.6×250mm,5µparticlesize). The column was equilibrated for at least 30min with the mobile phase flowing through the system. The column and the HPLCsystem were kept at ambient temperature.Flowrate at 0.7ml/min. Detection at 240nm.Volume of injection20µl.

2.4. Preparation of mobile phase

The mobile phase was prepared by mixing 0.05M potassium dihydrogen phosphate (pH- 4) and acetonitrile in the ratio of (35:65%v/v). The solution was then filtered through 0.45 microns membrane filter and degassed.

2.5. Preparation of 0.05M potassium dihydrogen phosphate

Dissolve 6.8 gm of potassium dihydrogen phosphate in sufficient water to produce 1000ml.

2.6. Preparation of standard solution

Weigh accurate 40mg of lovastatin and 500mg of niacin were transferred to100ml volumetric flask. 30 ml of mobile phase was added to dissolve the contents completely. The volume was made up to the mark with same mobile phase.

2.7. Determination of λ max

The standard solution of lovastatin and niacin were scanned separately in the wave length range of 200-400nm and the λ max was found to be 238 nm and 262 nm for lovastatin and niacin respectively. And it was found that both drugs show appreciable absorbance at 240 nm, so it is used for the further study. The overlay absorption spectrum of lovastatin and niacin is shown in the Figure 2.

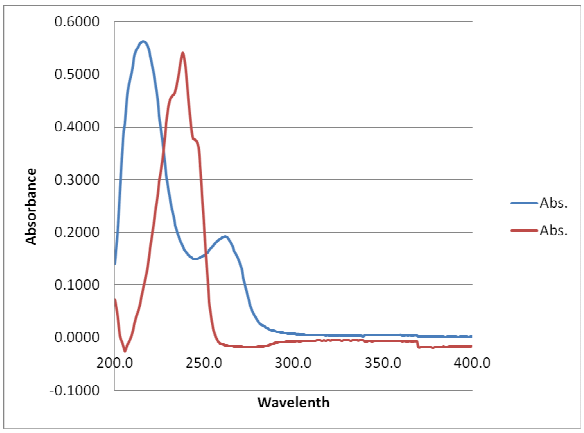

Figure – 2: Overlay absorption spectrum of lovastatin and niacin.

2.8. Analysis of tablet dosage forms

Twenty tablets containing 40mg of lovastatin and 500mg of niacin were weighed, and finely powdered. A quantity of powder sample equivalent to 20mg of lovastatin and 20mg of niacin transferred to 100ml volumetric flask. 30ml of mobile phase was added and sonicated for 5min to dissolve the contents as completely as possible and the volume was made up to the mark with the same mobile phase. Filtered and an appropriate volume of the aliquot was transferred to 10ml volumetric flask and the volume was made up to the mark. Twenty micro liters of the solution was injected into the chromatographic system and the peak areas were measured and the quantitation was carried out by keeping these values to the regression equation of corresponding calibration curve.

Optimized chromatographic conditions:

Stationary phase : symmetry C_8 column (4.6 ×250mm, 5µm).

Mobile phase : phosphate buffer (pH 4): acetonitrile (35:65)

Flow rate : 0.7 ml/min

Run time (min) : 10min

Detection : At 240 nm

Injection (volume) : 20µl

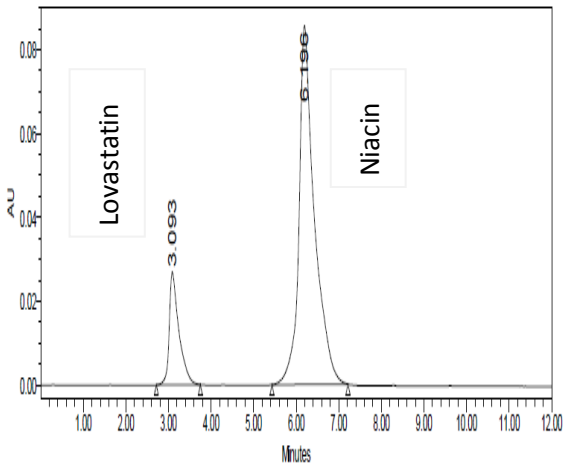

Figure – 3: Standard chromatograms of Lovastatin and Niacin.

2.9. METHOD VALIDATION PROCEDURE

The developed method was validated for the parameters listed in ICH guidelines **[5-8].**

2.9.1. Linearity

The method was linear in the range of 8-24 µgm and 100 - 300 µgm for both lovastatin and niacin. The linear correlation coefficient lovastatin and niacin were found to be 0.999 and 0.999 respectively, and are recorded in table1and 2 Calibration curve of lovastatin and niacin was obtained by plotting the peak area ratio versus the respective concentrations (Figure 4 and 5). The regression equation of calibration curve were Y=53257X for lovastatin and Y=21600X+6033for niacin respectively.

2.9.2. Accuracy

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed tablet solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For both the drugs, recovery was performed in the same way. The recovery studies were performed in triplicate. This standard addition method was performed at 50%, 100%, 150% level and the percentage recovery was calculated. Percent recovery was within the range of 98.9 to100.6 for LS and 100.8 to 101.7 for NI which indicates that the method was accurate. Results are recorded in table no 4.

2.9.3. Precision

For the precision study, repeatability study was carried out for short time interval under the same chromatographic condition. The sample was injected in six replicate. The peak area for all the six replicate was recorded. The mean and % relative standard deviation (%RSD) was calculated. The intraday %RSD for LS and NI were found to be 0.80 and 0.126 respectively. The interday %RSD for LS and NI were found to be 0.35 and 1.79 respectively. From the data obtained the developed RP-HPLC method was found to be precise. Results are recorded in table no 5.

2.9.4. Limit of detection and limit of quantification

The limit of detection and quantification were calculated using standard deviation of response and slope of the calibration curve .the LOD for lovastatin and niacin was found to be 0.115µg/ml and 0.121µg/ml respectively. The LOQ is the smallest con of the analyte, which gives response that can be accurately quantified. The LOQ was 0.384µg/ml and 0.036µg/ml for LS and NI. Results are recorded in table no 6.

2.9.5. Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like mobile phase ratio, PH of buffer ,flow rate. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed RP-HPLC method is robust. Results are shown in the table no 7.

2.9.6. Specificity studies

Commonly use excipients (starch, lactose, magnesium, and stearate) were spiked into a preweighed quantity of drug mixture. The chromatogram was taken by appropriate dilutions and the amount of each drug present in the sample mixture was determined.

Degradation studies

Specificity studies were performed by exposing the bulk drug under different stress conditions. NO degradation was observed in case of 0.1N HCL, 0.1N NaOH, 5% H2O2. Thus the method developed for the analysis of lovastatin and niacin is specific.

Table – 1: System Suitability and precision of proposed method

Parameters	Lovastatin	Niacin
Theoretical plates	3462.1	3771.7
Resolution	-	4.9
Tailing factor	1.4	1.3
Retention Time (min)	3.093	6.19
Intraday (n=3)	0.80	1.26
Inter day (n=3)	0.35	1.79

Table - 2: Linearity results of Lovastatin		
Linearity Level	**Concentration**	**Area**
I	5µg/ml	424798
II	10µg/ml	631169
III	20µg/ml	850951
IV	30µg/ml	1052639
V	40µg/ml	1279197
Correlation Coefficient		0.999

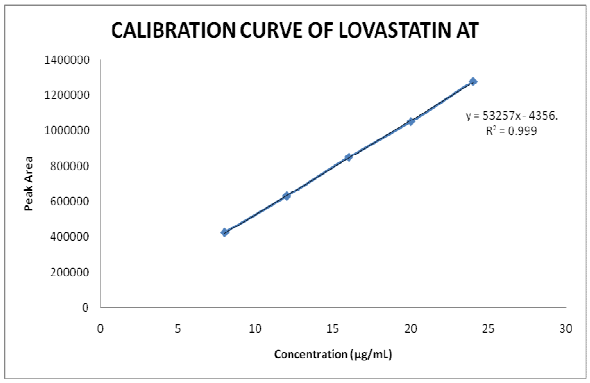

Figure – 2: Calibration curve of lovastatin.

Table – 3: Linearity results of niacin		
Linearity Level	**Concentration**	**Area**
I	5µg/ml	2157342
II	10µg/ml	3252253
III	20µg/ml	4347236
IV	30µg/ml	5379374
V	40µg/ml	6493722
Correlation Coefficient		0.999

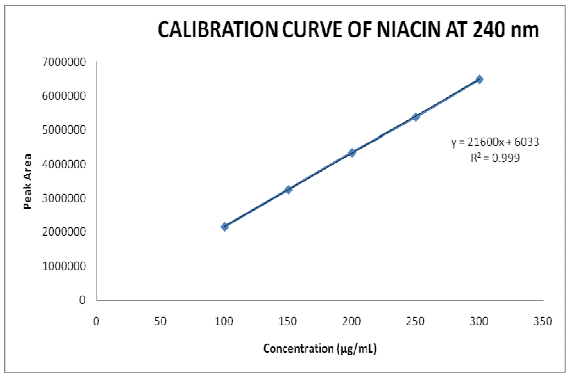

Figure – 3: Calibration curve of niacin.

Table – 4 : Results of Accuracy					
Sample	**Accuracy**	**Standard drug**	**Formulation**	**% of recovery**	**Standard deviation**
Lovastatin	50%	8	16	99.10%	*SD=0.135,%RSD=0.13
	100%	16	16	97.58%	*SD=0.032,%RSD=0.03
	150%	24	16	98.80%	*SD=0.005%,RSD=0.005
Niacin	50%	100	200	98.05%	*SD=0.058,%RSD=0.05
	100%	200	200	98.03%	*SD=0.045%RSD=0.04
	150%	300	200	98.03%	*SD=0.015,%RSD=0.01
***n=3, RSD – Relative standard deviation; *RSD: Relative standard deviation**					

Table – 5: Results of Precision		
Injection	**Area of lovastatin**	**Area of Niacin**
Injection-1	870406	4571058
Injection-2	871772	4445474
Injection-3	859987	4411762
Injection-4	869712	4446748
Injection-5	858249	4439496
Injection-6	875649	4441688
Average	867629	4459371
SD	**6927.0**	**56215.5**
% RSD	**0.80**	**1.26**

Table – 6 : Results of LOD and LOQ		
Parameter	**Lovastatin (ug/ml)**	**Niacin (ug/ml)**
LOD	0.115µg/ml	0.121µg/ml
LOQ	0.384µg/ml	0.036µg/ml

Table - 7: Results of Robustness

Condition	Variation	Average area		%RSD	
		Lovastatin	Niacin	Lovastatin	Niacin
Mobile phase: Phosphate buffer (4pH): acetonitrile: (35:65)	(pH4)potassiumdihydrogen phosphate: acetonitrile (15:85v/v)	849535	4795288	0.149	0.07
	pH 3.5.potassium dihydrogen phosphate: acetonitrile (25:75v/v)	840670	4408142	0.830	0.830
	pH 3 potassium dihydrogen phosphate: acetonitrile (45:55v/v)	857877	4364174	0.292	0.521
Flow rate: 0.6-0.8ml/min	Less flow 0.6	839838	4509668	0.016	0.464
	Actual flow 0.7	840987	4408142	0.830	0.340
	More flow 0.8	845591	4398260	0.3165	0.076

Table - 8: Results of analysis of formulation and recovery study of the proposed method

Formulation	Labeled claim (mg)	% Recovery
MEVACOR	Lovastatin-40mg	99.6
	Niacin -500mg	101.4

3. RESULTS AND DISCUSSION

To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with symmetry C_8 (4.6 x 250mm, 5 µm, Make: water) or equivalent column and mobile phase comprising of potassium dihydrogen ortho phosphate (4 pH): acetonitrile 35:65% (v/v) at a flow rate of 0.7ml/min. to get better reproducibility and repeatability. Quantification was achieved with UV detection at 240nm based on peak area. The retention time for lovastatin and niacin were found to be 3.093min and 6.196min, respectively. The optimized method was validated as per ICH guidelines. The system suitability parameters observed by using this optimized conditions were reported .A linearity range of 8-24µg/ml with correlation coefficient 0.999 was established for lovastatin and 100-300µg/ml with correlation coefficient 0.999 was established for Niacin. The results of recovery study (98.9 % lovastatin and 98.01 % for niacin) suggest that the method has good recovery. The precision of the proposed method was carried in terms of the repeatability, inter-day and intra-day time periods. The low % RSD (<2) values of inter-day (0.35%and 1.79%) and intra-day (0.80%and1.26%) variations for LS and NI, respectively, reveal that the proposed method is precise. The LOD and LOQ values for lovastatin were found to be 0.115µg/ ml, 0.384µg/ml and for niacin was 0.121 µg/ml, 0.036 µg/ml. The results of robustness in the present method showed no significant changes. The results of analysis of tablet indicated that no interference due to common tablet excipients was observed with the developed method. Therefore, the proposed method can be used for routine analysis of two drugs in their combined pharmaceutical dosage form.

4. CONCLUSION

The proposed method was found to be simple, precise, accurate and rapid for determination of Lovastatin and Niacin from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. Hence, this method can be easily and conveniently adopted for routine analysis of Lovastatin and Niacin in pure form and its dosage form and also can be used for dissolution or similar studies.

Acknowledgments

The authors are thankful to Managements and Principal of Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad India for providing needed facilities to carry out this research work. The Authors also thankful to the Hetero Pharmaceutical Pvt. Ltd., for providing gift samples of lovastatin and niacin.

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