DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR ANALYSING ANTIHYPERLIPIDEMIC AGENTS BY QBD APPROACH

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ABSTRACT: A simple, specific, and sensitive reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the simultaneous estimation of niacin and lovastatin in bulk and tablet formulations. Chromatographic separation was achieved using a Solar C18 column (150 mm × 4.6 mm, 5 µm) with a mobile phase consisting of Methanol: Acetonitrile: 20 mM Ammonium Acetate Buffer (pH 5.5, adjusted with glacial acetic acid) in the ratio of 50:30:20 (v/v/v) at a flow rate of 1.2 mL/min. The retention times for niacin and lovastatin were found to be 2.2 min and 9.4 min, respectively. A Quality by Design (QbD) approach using a 2^3 full factorial design was applied to optimize the mobile phase, with the optimized ratio being Methanol: Acetonitrile: Buffer at 45:40:15 (v/v/v). Detection was carried out at 225 nm using a UV detector. Calibration curves showed linearity in the range of 250-750 µg/mL for niacin and 10-30 µg/mL for lovastatin. The method was validated according to ICH and USP guidelines, showing acceptable results for accuracy, precision, linearity, and robustness. This method is suitable for routine analysis of niacin and lovastatin, both individually and in combination, in pharmaceutical tablet dosage forms.

Keywords: Reverse phase - High performance liquid chromatography, Quality by Design, atherosclerotic disease, coronary heart disease, High density lipoprotein, Low density lipoprotein, very low density lipoprotein.

INTRODUCTION

Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood and it is also identified as dyslipidemia. Hyperlipidemia is an increase in one or more of the plasma lipids, including triglycerides, cholesterol, cholesterol esters and phospholipids and or plasma lipoproteins including very low-density lipoprotein and low-density lipoprotein, and reduced high-density lipoprotein levels. Hyperlipidemia is the presence of raised or abnormal levels of lipids and/or lipoproteins in the blood. It is also called hyperlipoproteinemia because these fatty substances travel in the blood attached to proteins and this is the only way that these fatty substances can remain dissolved while in circulation. It is also called as dyslipidemia. Hyperlipidaemia is the most prevalent episode of dyslipidaemia (which includes any hypo and hyper lipid levels). Hyperlipidemia is an unhealthy body condition that increases the risk of

atherosclerotic disease (ASHD) and coronary heart disease (CHD) in human. It also increases the risk of hypertension, Alzheimer's disease, pancreatitis and hepatitis. [5] Dyslipidemic persons have high risk for atherosclerosis. Atherosclerotic lesions form a localized plaque in intima and narrow the arterial lumen. Accumulations of lipids in heart induce oxidative stress and inflammatory cardiac fibrosis leads to cardiac dysfunction. Hyperlipidemia can be treated by lifestyle changes, dietary modification, reducing the other risk factors of atherosclerosis and finally with effective and safe use of drug therapy. Drug therapy start with statins monotherapy to reduce LDL-cholesterol. But in order to reach LDL and triglycerides and HDL target in high-risk cardiovascular patients. It can be genetic (primary) or caused by other health issues (secondary). HDL, produced by the liver, is beneficial as it removes excess cholesterol. Though triglycerides provide energy, VLDL particles connected to them may contribute to artery plaque buildup, increasing cardiovascular risk. High levels of LDL cholesterol (the so called "bad cholesterol") greatly increase the risk for atherosclerosis because LDL particles contribute to the formation of atherosclerotic plaques. Low HDL levels ("good cholesterol") are an independent risk factor, because reverse cholesterol transport works to prevent plaque formation, or even cause regression of plaques once they have formed.

2. MATERIALS AND METHODS

2.1. Instruments

1. UV-Visible Spectrophotometer (Shimadzu Model), Model number: UV-1780.
2. HPLC: model Shimadzu P-Series HPLC System with PDA Detector, column Solar C18 (150mm×4.6mm, i.e.5µm), It was equipped with a PDA detector, a solvent delivery pump, a sample injector, and a column thermostat. Data collection and processing were performed using Labsolution software.
3. Ultra sonic Bath: The Athena ATS-2-LED Ultrasonic Bath operates at a frequency of 40 kHz with a power output of 50W.
4. Digital pH meter: The pH meter used in the study was a Systonic S-902 model, known for its accuracy and reliability in pH measurements.
5. Analytical weighing balance: The analytical weighing balance used in the study was the Contech CAS-234 model.
6. QbD Software: STATEASE Design Expert, version 13.

2.2. Chemicals and Reagents

Methanol, Acetonitrile and water were of HPLC grade and Ammonium acetate buffer (AR grade), glacial acetic acid were obtained from Dange Trending company, (Amravati,

Maharashtra). Niacin and Lovastatin reference standards obtained as gift samples from yarrow chem. Pharma, Mumbai. Tablet dosage form containing 20 mg of lovastatin and 500 mg of Niacin (Advicor) was procured from the local market.

2.3. HPLC Condition

The mobile phase consisted of (PH 5.5) 20mM Ammonium acetate buffer: Methanol: Acetonitrile (50:30:20 v/v/v) and QbD optimize mobile phase consisted of PH 5.5 (45:40:15 v/v/v). The mobile phase was prepared freshly, and it was sonicated for 5 min before use. C18 (150mm×4.6mm, i.e.5µm). The column was equilibrated for at least 30 min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature. Flow rate at 1.2ml/min. Detection at 261 nm. Volume of injection 20µl.

2.4. Preparation of Mobile phase

The Mobile phase was prepared by mixing 20mM of ammonium acetate buffer (Ph-5.5) and Methanol: Acetonitrile in the ration of (45:40:15 v/v/v). The solution was then filtered through 0.45 microns membrane filter and degassed.

2.5. Preparation of 20mM Ammonium acetate buffer

0.308g Ammonium Acetate was weighed into 200ml beaker dissolved and diluted to 150ml with HPLC grade water the flask was shaken until the particles get dissolved. The pH was adjusted to 5.5 with Glacial acetic acid. The final volume up to 200ml and check the PH.

2.6. Preparation of Standard solution

Weigh accurate 500mg of Niacin and 20 mg of lovastatin were transferred to 100ml of volumetric flask.100ml of Methanol was added to dissolve the contents completely. Further,1ml Niacin and Lovastatin, standard stock solution was diluted to 10ml. Volumetric flask with methanol and mixed. (Concentration: 100µg/ml)

2.7. Determination of λ max

The standard solution of Niacin and Lovastatin were scanned separately in the wavelength range 200-400nm and the λ max was found to be 261 nm and 245nm for Niacin and Lovastatin respectively. And it was found that both drugs show appreciable absorbance at 225 nm, so it is used for the further study. The overlay absorption spectrum of Niacin and Lovastatin is shown in the figure 1.

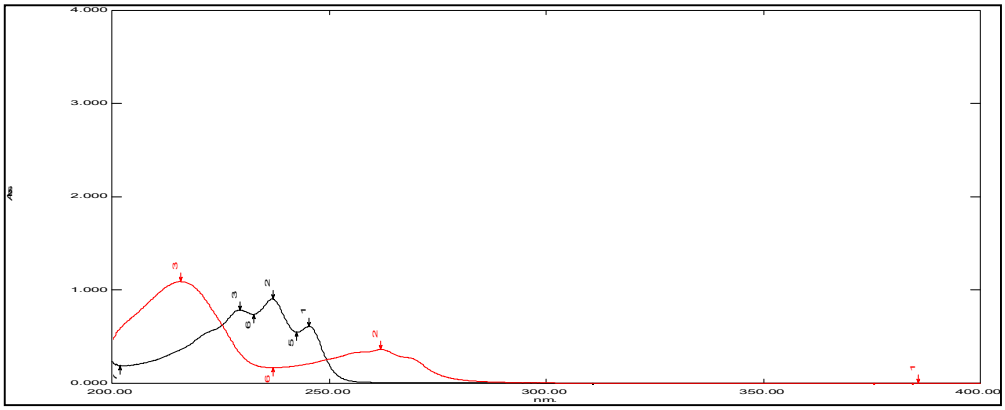


Figure No. 1: Overlayed UV spectra of Niacin and Lovastatin

Optimized chromatographic conditions:

1. Analytes: Niacin and Lovastatin

2. Column: C18(150mm×4.6mm, i.d.5µm)

3. Mobile Phase: 50:30:20 (Methanol: ACN: Ammonium acetate buffer pH 5.5)

4. Flow rate: 1.2 ml/min

5. Elution mode: Isocratic

6. Wavelength selected: 200-400 nm

7. Temperature: Room temperature (28°C)

8. Run time: 15 minutes

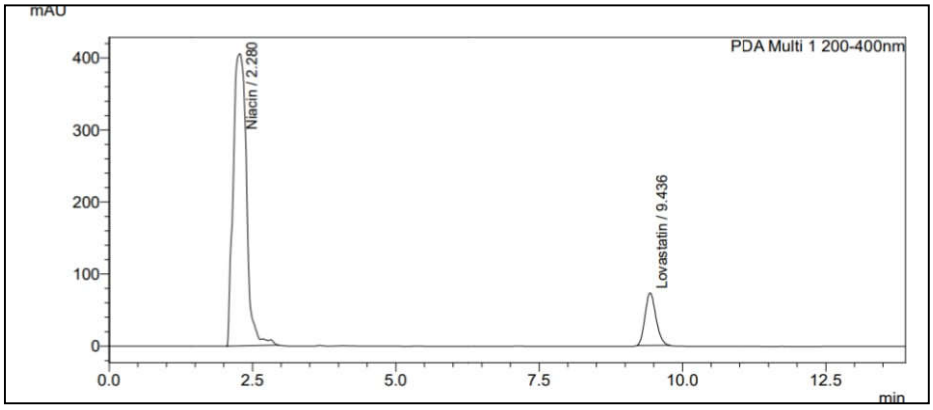


Figure 2: Standard chromatograms of Niacin and Lovastatin

Quality by Design (QbD) Based Method Optimization

Table No. 1: Statistical data for QbD plots of Niacin

		Factor 1	Factor 2	Response 1	Response 2	Response 3

Std	Run	A:Met:Acn:Buffer	B:Flow Rate	Retention Time	Tailing Factor	Plate Count
		%	ml	Min	TF	USP
2	1	45:40:15	0.8	3.4	1.17	2198
1	2	40:50:10	0.8	3.4	1.28	2178
6	3	50:30:20	1	2.6	1.44	2067
9	4	50:30:20	1.2	2.2	1.20	2056
3	5	50:30:20	0.8	3.3	1.15	2045
7	6	40:50:10	1.2	2.3	1.27	2189
8	7	45:40:15	1.2	2.2	1.45	2202
5	8	45:40:15	1	2.7	1.22	2196
4	9	40:50:10	1	2.8	1.31	2192

Table No. 2: Statistical data for QbD plots of Lovastatin

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	Run	A:Met:Acn:Buffer	B:Flow Rate	Retention Time	Tailing Factor	Plate Count
		%	ml	Min	TF	USP
2	1	45:40:15	0.8	9.2	1.22	11555
1	2	40:50:10	0.8	6.7	1.23	10121
6	3	50:30:20	1	11.2	1.17	11801
9	4	50:30:20	1.2	9.4	1.17	10905
3	5	50:30:20	0.8	13.9	1.15	13301
7	6	40:50:10	1.2	4.5	1.25	7783
8	7	45:40:15	1.2	6.2	1.23	9106
5	8	45:40:15	1	7.4	1.22	10103
4	9	40:50:10	1	5.4	1.24	8907

2.8 QbD optimize Batch

Table No. 3: Data of Chromatogram of QbD optimize Batch.

Parameter	Chromatographic Method
Preparation of Mobile Phase	Methanol: Acetonitrile: Ammonium Acetate Buffer (PH-5.5) 45:40:15
Flow Rate (mL/min)	1.2

Detection Wavelength (nm)	200-400
Injection Volume (µL)	20
Column Temperature (°C)	28
Run Mode	Isocratic

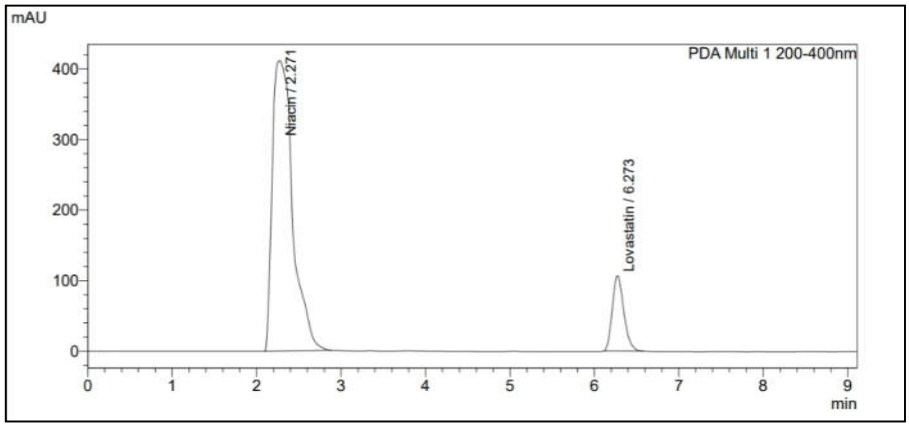


Figure No. 3: Chromatogram of QbD optimize Batch

2.9. METHOD VALIDATION PROCEDURE

The developed method was validated for the parameters listed in ICH guidelines.

2.9.1 Linearity

The method was linear in the range of 250-750µg/ml and 10-30µg/ml for both Niacin and Lovastatin. The linear correlation coefficient niacin and lovastatin were found to be 0.999 and 0.995 respectively, Calibration curve of niacin and lovastatin was obtained by plotting the peak area ratio versus the respective concentrations. The regression equation of calibration curve was Y = 68191x+17868 for Niacin and Y = 10266x+27186 for Lovastatin respectively.

Table No. 4: Linearity of Niacin

Description	Concentration	Area
50%	250	3583843
75%	375	5352784
100%	500	6885767

125%	625	8764926
150%	750	10401669
Regression equation		Y=68191x+17868
Co-relation coefficient		$R^2 = 0.999$

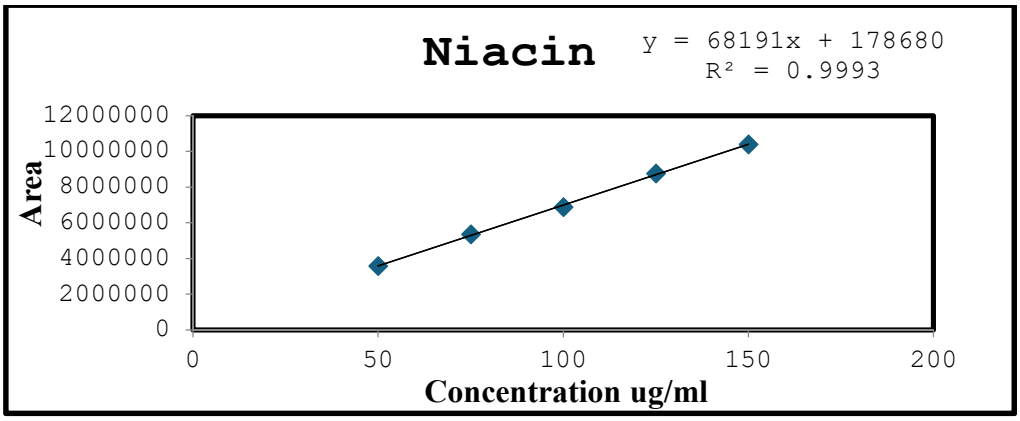
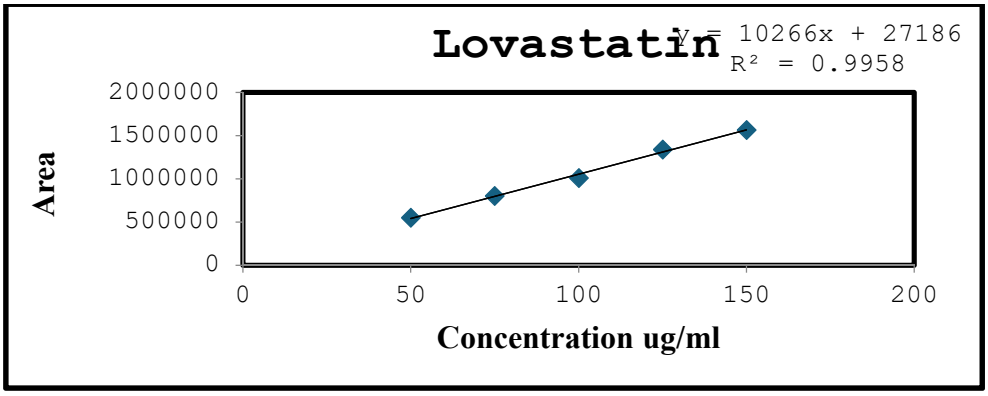


Table No. 5: Linearity of Lovastatin

Description	Concentration	Area
50%	10	550537
75%	15	802632
100%	20	1011016
125%	25	1339087
150%	30	1565527
Regression equation		Y=10266x+27186
Co-relation coefficient		$R^2 = 0.995$



2.9.2 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 Niacin and Lovastatin was found to be 130.48 µg/mL and 130.71 µg/mL respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ was 395.42 µg/mL and 396.10µg/mL for NI and LS. Results are recorded.

2.9.3 Accuracy

The accuracy of the developed RP-HPLC method was evaluated using the standard addition method at 50%, 100%, and 150% of the target concentration. Pre-analyzed samples of Niacin and Lovastatin were spiked with known standard amounts and analyzed in triplicate. Percent recovery and %RSD were calculated to assess accuracy. Niacin showed recoveries between 99.84% and 100.1%, and Lovastatin between 99.91% and 101.7%, with %RSD values below 2%. These results confirm the method's accuracy, reliability, and lack of interference from excipients.

Table No. 6: Accuracy (Recovery) Study Data for Niacin and Lovastatin

Drug	Level of % Recovery	Amount of standard		% Recovery	% Mean	SD	%RSD
		Added µg/mL	Recovered µg/mL				
Niacin	50	250	749.96	99.98	99.97333	0.1301	0.130
	100	500	1001.19	100.1			
	150	750	1248.12	99.84			
	50	5	14.99	99.91			

Lovastatin	100	10	20.34	101.7	100.9367	0.9235	0.923
	150	15	25.3	101.2			

2.9.4 Precision:

The precision of the method was evaluated through intra-day and inter-day studies for Niacin and Lovastatin at 100 ppm. Niacin showed %RSD values of 1.264% (intra-day) and 0.749% (inter-day), while Lovastatin showed 0.533% and 1.158%, respectively. These low %RSD values confirm the method's reliability, reproducibility, and minimal variability.

Table No. 7: Precision Study of Niacin

Niacin				
Conc.500ppm	**Area**	**Mean**	**SD**	**%RSD**
Intra-day	6885767	6875187	86928.7	1.264
	6783453			
	6956342			
Inter-Day		6946547	52076.3	0.749
Day 1	6886457			
Day 2	6978543			
Day 3	6978543			

Table No. 8: Precision Study for Lovastatin

Lovastatin				
Conc.20ppm	**Area**	**Mean**	**SD**	**%RSD**
Intra-day	1011016	1015563	5420.729	0.533
	1014112			
	1021562			
Inter-Day		1022487	11842.03	1.158
Day 1	1011134			
Day 2	1021564			
Day 3	1034764			

2.9.5 Repeatability:

Repeatability of the method was assessed by analyzing six replicates of 100 ppm standard solutions of Niacin and Lovastatin under identical conditions. %RSD of peak areas was calculated, showing low values for both drugs. This confirms the method's repeatability and consistency.

Table No. 9: Repeatability of Niacin and Lovastatin

Sr. No.	Peak Area	
	Niacin	Lovastatin
1	6885767	1011016
2	6887523	1012015
3	6785684	1014121
4	6846746	1011113
5	6965437	1011231
6	6875435	1010112
Mean	6874432	1011601
SD	58694.97	1375.223
%RSD	0.853	0.135

2.9.6 Robustness for the chromatographic method

Robustness of the RP-HPLC method was tested by making small deliberate changes in chromatographic conditions, such as varying the flow rate from 1.1 mL/min to 1.2 and 1.3 mL/min. The impact on resolution, capacity factor, peak height, peak width, and tailing factor was evaluated to ensure the method’s reliability under normal usage.

Table No. 10: Robustness of Niacin and Lovastatin

Parameter	Drug	Variable	Retention Time (min)	Theoretical Plates	Tailing Factor

Flow Rate	**Niacin**	1.1 ml/min	2.385	2196	1.45
		1.2 ml/min	2.388	2202	1.43
		1.3 ml/min	2.384	2192	1.42
		Mean	2.385667	2196.667	1.433333
		SD	0.002082	5.033223	0.015275
		%RSD	0.087	0.229	1.065
	Lovastatin	1.1 ml/min	6.606	9227	1.21
		1.2 ml/min	6.595	9221	1.21
		1.3 ml/min	6.578	9392	1.23
		Mean	6.593	9280	1.216667
		SD	0.014107	97.04123	0.011547
		%RSD	0.213	1.045	0.949

Parameter	**Drug**	**Variable**	**Retention Time (min)**	**Theoretical Plates**	**Tailing Factor**
Injection Volume	**Niacin**	18ug/ml	2.384	2024	1.45
		20ug/ml	2.385	2028	1.48
		22ug/ml	2.388	2032	1.47
		Mean	2.385666667	2028	1.466667
		SD	0.002081666	4	0.015275
		%RSD	0.087	0.197	1.041
		18ug/ml	6.578	9392	1.23
		20ug/ml	6.606	9227	1.21
		22ug/ml	6.579	9277	1.21

	Lovastatin	**Mean**	6.587667	9298.667	1.216667
		SD	0.015885	84.60693	0.011547
		%RSD	0.241	0.909	0.949

2.9.7 Ruggedness

Ruggedness assesses the reproducibility of results under varying conditions, such as different operators and temperatures. Niacin (500 µg/ml) and Lovastatin (20 µg/ml) at 100 ppm were analyzed under these conditions to confirm consistency across different environments.

Table No. 11: Ruggedness of Niacin and Lovastatin

Parameter	**Variable**	**Area**	
		Niacin	**Lovastatin**
Different Analyst	1	6885767	1011016
	2	7032678	1012014
	3	7045342	1014121
	SD	88701.18	1585.164
	%RSD	1.269	0.156
Room Temperature	20°C	7523229	1109852
	25°C	7527226	1107789
	30°C	7539221	1112134
	SD	8322.661	2173.42
	%RSD	0.110	0.195

2.9.8 System Suitability

The system suitability test is essential in validating analytical techniques and confirming the resolution amongst numerous peaks of interest. All critical parameters (theoretical plates, retention time, and tailing factor) in the study met complete acceptance every time. The % RSD

for the Retention time, Theoretical plate and Tailing factor of six replicates was found to be 0.365, 0.220 and 1.287 for Niacin. The % RSD for retention time, Theoretical plate and Tailing factor was found to be 0.839, 0.907 and 0.841 for Lovastatin.

Table No. 12: System suitability of Niacin

Sample	Niacin		
	Retention Time	Theoretical Plates	Tailing Factor
1	2.271	2202	1.45
2	2.265	2209	1.45
3	2.262	2210	1.46
4	2.255	2211	1.43
5	2.276	2212	1.47
6	2.256	2217	1.42
Mean	2.264166667	2210.167	1.446667
±SD	0.008280499	4.875107	0.018619
%RSD	0.365	0.220	1.287

2.9.9Application of Method to Marketed Preparation

The validated RP-HPLC method was successfully applied to the estimation of Niacin and Lovastatin in commercially available pharmaceutical formulations (Advicor). Accurately weighed quantities of the marketed formulation equivalent to 500 µg/mL of Niacin and 20 µg/mL of Lovastatin were prepared in the mobile phase and filtered through a 0.45 µm membrane filter. These test solutions were injected in triplicate under the optimized chromatographic conditions.

Table No. 13: Data of marketed preparation of Niacin and Lovastatin

Drug	Standard Conc. (µg/mL)	Peak Area	Amount Found (µg/mL)	Tailing Factor	% Assay
Niacin	500	2187148	49.98	1.491	99.96
Lovastatin	20	856634	19.87	1.451	99.35

SUMMARY

A robust, precise, and efficient RP-HPLC method was successfully developed and validated for the simultaneous estimation of Niacin and Lovastatin in bulk and pharmaceutical dosage forms. Method validation was performed in accordance with ICH Q2 (R1) guidelines, assessing key parameters such as linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantitation (LOQ). Both analytes showed excellent linearity within the selected concentration ranges, with correlation coefficients (R^2) of 0.999 for Niacin and 0.995 for Lovastatin. Accuracy studies at 50%, 100%, and 150% levels yielded recoveries within the acceptable range of 99–101%, and %RSD values were consistently below 2%, confirming the reliability of the method. Precision studies demonstrated minimal intra-day and inter-day variability. The regression equations derived from calibration curves were effectively used for back calculation of unknown sample concentrations. The method showed excellent applicability when tested on a marketed formulation, yielding % assay values of 99.96% for Niacin and 99.35% for Lovastatin, with well-resolved peaks, acceptable tailing factors, and high theoretical plate counts. As part of method optimization, a Quality by Design (QbD) approach was incorporated using a 2^3 Full factorial design. The influence of critical method parameters mobile phase composition and flow rate on critical quality attributes such as retention time, tailing factor, and plate count was evaluated. This systematic approach enhanced method understanding and robustness while identifying optimal chromatographic conditions through 3D response surface plots.

CONCLUSION

The validated RP-HPLC method is simple, rapid, accurate, and reproducible for the simultaneous estimation of Niacin and Lovastatin in pharmaceutical dosage forms. It satisfies all the required validation parameters and demonstrates strong suitability for routine quality control analysis. The integration of the QbD approach provided additional confidence in the method's robustness and ensured reliable performance under varied analytical conditions. Its

successful application to marketed formulations further affirms its utility in industrial and regulatory environments. The systematic method of QbD tools enabled identification and control of critical method variables, ensuring method reliability throughout its lifecycle. This approach not only improved method robustness but also aligned with regulatory expectations, promoting a science-based strategy in method development.

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