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Research article *Pharmaceutical Analysis*

Simultaneous estimation of niacin and lovastatin by using rp-hplc in api and marketed formulations

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ABSTRACT

estimated using Phenomenex Gemini C18 (4.6mm×150mm, 5µm) particle size column. A mobile phase composed of tri ethylamine buffer and methanol in proportion of 32:68 v/v, at a flow rate of 1.0 ml/min was used for the separation. Detection was carried out at 248nm. The linearity range obtained was 30-70µg/ml for Niacin and 10-50µg/ml for Lovastatin with retention times (Rt) of 3.297min and 5.405min for Niacinand Lovastatin respectively. The correlation coefficient values were found to be 0.999 & 0.999. Precession studies showed % RSD values less than 2 % for both the drugs in all the selected concentrations. The percentage recoveries of Niacin and Lovastatinwere found to be 100.1873% for Niacin and 100.748% for Lovastatin respectively. The assay results of Niacin and Lovastatinwere found to be 99.82%. The limit of detection (LOD) and limit of quantification (LOQ) were 2.6µg/ml and 7.8µg/ml for Niacin and 3.4µg/ml 10.2µg/ml for Lovastatin respectively. The proposed method was validated as per the International Conference on Harmonization (ICH) guidelines. The proposed validated method was successfully used for the quantitative analysis of commercially available dosage form.

Keywords: Niacin and Lovastatin, RP-HPLC, ICH Guidelines, Validation.

INTRODUCTION

Analytical chemistry is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and the quantitative measurement of the substances present in bulk and pharmaceutical preparation.Measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio, and fluorescence, began to be used for quantitative analysis of variety of inorganic and biochemical analytes. Highly efficient chromatographic and electrophoretic techniques began to replace distillation, extraction and precipitation for the separation of components of complex mixtures prior to their qualitative or quantitative determination. These newer methods for separating and determining chemical species are known collectively as instrumental methods of analysis. Most of the instrumental methods fit into one of the three following categories viz spectroscopy, electrochemistry and chromatography

Advantages of instrumental methods

- Small samples can be used
- High sensitivity is obtained
- Measurements obtained are reliable
- Determination is very fast
- Even complex samples can be handled easily

Limitations of instrumental methods

- An initial or continuous calibration is required
- Sensitivity and accuracy depends on the instrument
- Cost of equipment is large
- Concentration range is limited
- Specialized training is needed
- Sizable space is required

High Performance Liquid Chromatography

HPLC is a type of liquid chromatography that employs a liquid mobile phase and a very finely divided stationary phase. In order to obtain satisfactory flow rate liquid must be pressurized to a few thousands of pounds per square inch.The rate of distribution of drugs between Stationary and mobile phase is controlled by diffusion process. If diffusion is minimized faster and effective separation can be achieved .The techniques of high performance liquid chromatography are so called because of its improved performance when compared to classical column chromatography advances in column chromatography into high speed,efficient ,accurate and highly resolved method of separation.For the recent study metformin and Sitagliptin was selected for estimation of amount of analyte present in formulation and bulk drug. The HPLC method is selected in the field of analytical chemistry, since this method is specific, robust, linear, precise and accurate and the limit of detection is low and also it offers the following advantages

- Speed many analysis can be accomplished in 20min (or) less.
- Greater sensitivity(various detectors can be employed).
- Improved resolution(wide variety of stationary phases).
- Re usable columns(expensive columns but can be used for many analysis).
- Ideal for the substances of low viscosity.
- Easy sample recovery, handling and maintenance.
- Instrumentation leads itself to automation and quantification (less time and less labour).
- Precise and reproducible.
- Integrator itself does calculations.
- Suitable for preparative liquid chromatography on a much larger scale.

Quantitative Methods in chromatography:

Internal standard method

In this technique a known quantity internal standard is chromatographed[7] and area is ascertained then a quantity of the internal standard is added to the raw sample prior to any sample pretreatment or separation operation .The peak area of the standard in sample run is compared with the peak are when the standard is run separately This ratio serves as correction factor for variation in sample size ,for losses in any preliminary operations, or for incomplete resolved adjacent sample component, must not interfere with the sample component and must never be present in sample.

$$\textbf{Area ratio} = \frac{\textbf{Area of sample}}{\textbf{Area of internal standard}}$$

$$\textbf{Sample concentration} = \frac{\textbf{Area of sample}}{\textbf{Area of internal standard}} \textbf{ x concentration of standard}$$

This technique is often used for the samples having components. It is used to evaluate the absolute purity of sample .the procedure is to total up the areas under all peaks and then calculate the percentage of total area that is contributed by compound of interest. For this method the entire sample must be eluted all components must be separated and peak must be completely resolved.

Standard addition method

Standard addition method is used in many techniques in analytical chemistry. It is of limited use in chromatography because of the difficulty of injecting accurately known amounts of sample. A sample mixture is analysed for the analyte of interest by adding a specified amount of this analyte to the sample, thus increasing its concentration. The analysis is then repeated and the resulting increase in peak area due to addition of the standard amount is noted. Hence, the concentration of the analyte in the original sample may be calculated. If the peak area for the first analysis is A1 and with the standard addition of x mg is A2, then the peak area corresponding to x mg (or x mg/litre) is (A2 - A1). Thus, the original amount of the analyte x in the sample corresponding to A1, is given by

Amount x = (x A1) / (A2 - A1) mg/litre.

An allowance for dilution due to addition of the standard amount has to be made. The main difficulty with this method concerns the reproducibility of the sample injection. A precision of better than 1 % should be achieved if valid quantitative results are to be obtained.

MATERIALS AND METHODS

Niacin from Sura labs, Lovastatin from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK).Acetonitrile for HPLC from Merck, Phosphate buffer from Sura labs.

HPLC METHOD DEVELOPMENT TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Niacin and Lovastatin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 2.25ml of the above Niacin and 0.45ml of the Lovastatin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water, Acetonitrile: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA buffer pH 4.8 in proportion 32:68 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, X- bridge column, Xterra. Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Column : Phenomenex Gemini C18 (4.6mm×150 mm, 5.0 µm) particle size
Column temperature: 38˚C
pH : 4.8
Mobile phase : Methanol: TEA buffer pH 4.8 (32:68v/v)
Flow rate : 1ml/min
Wavelength : 248nm
Injection volume : 20µl
Run time : 7 min

METHOD VALIDATION

PREPARATION OF MOBILE PHASE

Preparation of mobile phase

Accurately measured 320ml (32%) of HPLC Methanol and 680ml of TEA buffer (68%) were mixed and degassed in a digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Column : Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size
Column temperature : 38˚C
Wavelength : 248nm
Mobile phase ratio : Methanol: TEA buffer pH 4.8 (32:68v/v)
Flow rate : 1ml/min
Injection volume : 20µl
Run time 7minutes

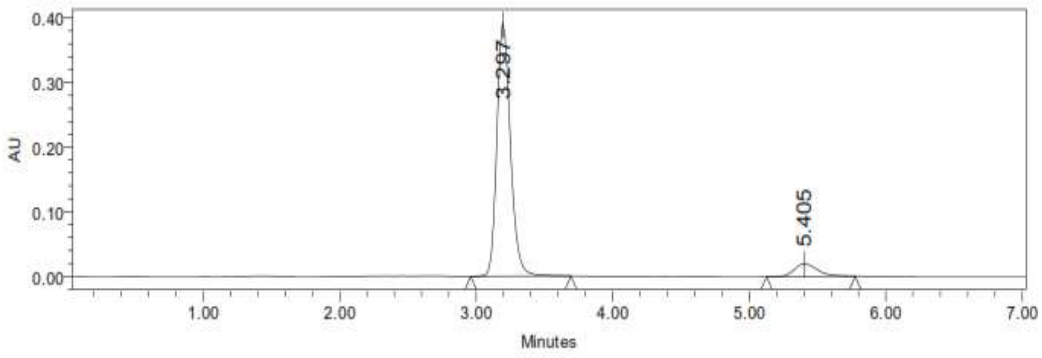

Fig 1: Optimized Chromatogram (Standard)

Table 1: Optimized Chromatogram (Standard)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP
1	Niacin	3.297	859856	42569	1.24	7896	
2	Lovastatin	5.405	5698	3652	1.36	6582	6.8

Optimized Chromatogram (Sample)

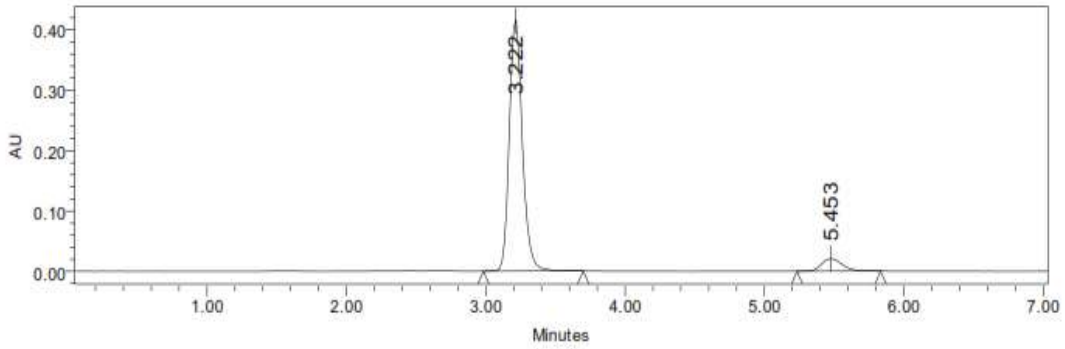

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	USP Resolution
1	Niacin	3.222	865898	43659	1.26	7985	
2	Lovastatin	5.453	5789	3785	1.38	6659	7.0

- *Resolution between two drugs must be not less than 2.*
- *Theoretical plates must be not less than 2000.*
- *Tailing factor must be not less than 0.9 and not more than 2.*
- *It was found from above data that all the system suitability parameters for developed method were within the limit.*

Table 3: Peak Results for Assay sample of Niacin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Niacin	3.297	865985	43659	1.26	7985
2	Niacin	3.294	865798	43875	1.26	7925
3	Niacin	3.295	865456	43659	1.27	7946

Table 4: Peak Results for Assay sample of Lovastatin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Resolution
1	Lovastatin	5.435	5789	3659	1.37	6659	6.9
2	Lovastatin	5.417	5798	3684	1.38	6689	7.0
3	Lovastatin	5.434	5749	3695	1.38	6648	6.9

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

The % purity of Niacin and Lovastatin in pharmaceutical dosage form was found to be 99.82%.

Linearity

Table 5: Niacin

Concentration µg/ml	Average Peak Area
30	545894
40	725985
50	897856
60	1068594
70	1245698

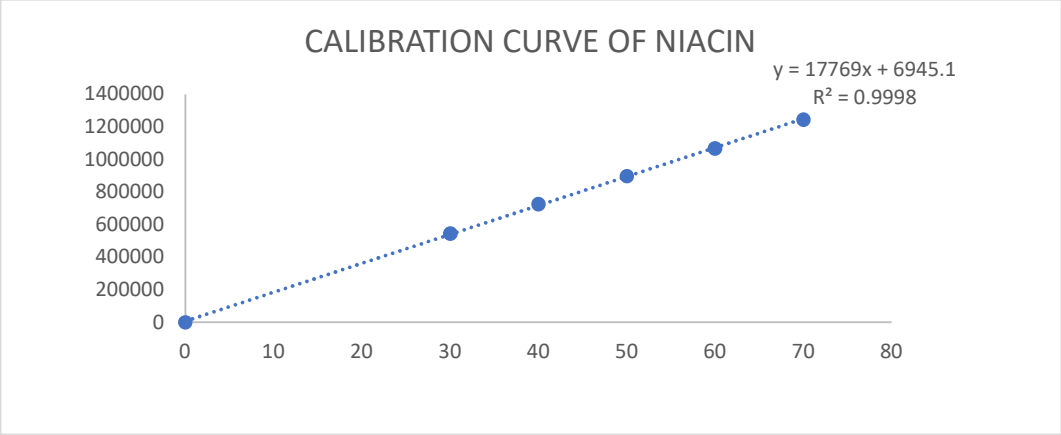

Fig 3: Calibration Curve of Niacin

Table 6: Lovastatin

Concentration µg/ml	Average Peak Area
10	2038
20	3859
30	5698
40	7489
50	9218

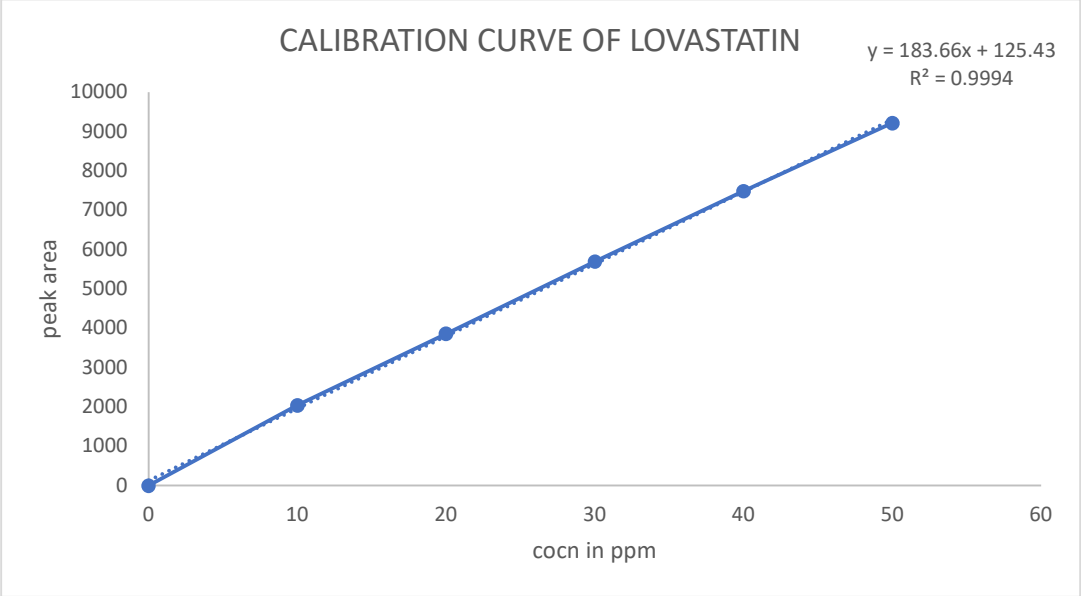

Fig 4: Calibration Curve of Lovastatin

Repeatability

Table 7: Results of Repeatability for Niacin

S. No.	Peak name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Niacin	3.213	859856	42659	7859	1.24
2	Niacin	3.253	857985	42598	7869	1.24

3	Niacin	3.297	856984	42587	7846	1.25
4	Niacin	3.215	856987	42569	7819	1.25
5	Niacin	3.254	859878	42894	7856	1.24
Mean			858338			
Std.dev			1454.222			
%RSD			0.169423			

%RSD for sample should be NMT 2
The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table 8: Results of repeatability for Lovastatin

S. No.	Peak Name	Retention time	Area(µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Lovastatin	5.441	5697	3659	6592	1.36
2	Lovastatin	5.442	5689	3648	6539	1.36
3	Lovastatin	5.409	5698	3692	6584	1.37
4	Lovastatin	5.520	5639	3648	6579	1.36
5	Lovastatin	5.424	5688	3689	6549	1.36
Mean			5682.2			
Std.dev			24.57031			
%RSD			0.432408			

Intermediate precision

Table 9: Results of Intermediate precision for Niacin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Niacin	3.211	868956	43659	7985	1.26
2	Niacin	3.211	869857	43985	7954	1.27
3	Niacin	3.210	865983	43879	7946	1.26
4	Niacin	3.212	866587	43865	7963	1.27
5	Niacin	3.211	864256	43875	7964	1.26
6	Niacin	3.297	868974	43562	7942	1.26
Mean			867435.5			
Std. Dev.			2167.095			
% RSD			0.249828			

- *%RSD of six different sample solutions should not more than 2.*

Table 10: Results of Intermediate precision for Lovastatin

S.No.			Area	Height (µV)		
1	Lovastatin	5.411	5785	3789	6659	1.37
2	Lovastatin	5.410	5798	3758	6625	1.38
3	Lovastatin	5.420	5766	3746	6649	1.38
4	Lovastatin	5.423	5746	3795	6675	1.37
5	Lovastatin	5.419	5782	3761	6653	1.38
6	Lovastatin	5.409	5786	3752	6627	1.37
Mean			5777.167			
Std. Dev.			18.40018			
% RSD			0.318498			

- *%RSD of six different sample solutions should not more than 2.*

Table 11: Results of Intermediate precision Day 2 for Niacin

S.No.			Area	Height (µV)		
1	Niacin	3.211	845985	44585	8025	1.27
2	Niacin	3.233	847895	44895	8069	1.28
3	Niacin	3.244	848985	44758	8046	1.27
4	Niacin	3.297	847859	44548	8094	1.28
5	Niacin	3.297	845984	44865	8042	1.28
6	Niacin	3.202	847898	44254	8076	1.27
Mean			847434.3			
Std. Dev.			1201.345			
% RSD			0.141763			

- *%RSD of six different sample solutions should not more than 2.*

Table 12: Results of Intermediate precision Day 2 for Lovastatin

S.No.			Area	Height		
1	Lovastatin	5.411	5898	3986	6852	1.39
2	Lovastatin	5.410	5884	3955	6864	1.39
3	Lovastatin	5.420	5863	3956	6829	1.40
4	Lovastatin	5.405	5845	3945	6874	1.39
5	Lovastatin	5.409	5896	3925	6829	1.39
6	Lovastatin	5.463	5874	3962	6825	1.40
Mean			5876.667			
Std. Dev.			20.39281			
% RSD			0.347013			

- *%RSD of six different sample solutions should not more than 2.*

Accuracy

Table 13: The accuracy results for Niacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	451144.3	25	24.998	99.992%	
100%	897248.3	50	50.104	100.208%	100.1873%
150%	1344562	75	75.278	100.362%	

The percentage recovery was found to be within the limit (98-102%).
The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table 14: The accuracy Results for Lovastatin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2895	15	15.084	100.560%	
100%	5685.333	30	30.282	100.940%	100.748%
150%	8449	45	45.335	100.744%	

- *The percentage recovery was found to be within the limit (98-102%).*
  The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 15: Results for Robustness

Niacin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.0mL/min	859856	3.297	7896	1.24
Less Flow rate of 0.9mL/min	915847	3.639	7251	1.20
More Flow rate of 1.1mL/min	842564	2.859	7415	1.21
Less organic phase	825498	3.460	7365	1.23
More organic phase	814578	3.022	7258	1.22

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Table 16: Results for Robustness

Lovastatin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical	Tailing factor
Actual Flow rate of 1.1mL/min	5698	5.405	6582	1.36
Less Flow rate of 0.9mL/min	6452	6.250	6785	1.32
More Flow rate of 0.8mL/min	5254	4.863	6365	1.34
Less organic phase	5487	6.196	6254	1.38
More organic phase	5369	5.010	6298	1.33

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Niacin and Lovastatin was done by RP-HPLC.

The TEA buffer was p^H 4.8 and the mobile phase was optimized with consists of Methanol: TEA buffer mixed in the ratio of 32:68 % v/v.

A Phenomenex Gemini C18 (4.6mm×150mm, 5.0 µm) particle size or equivalent chemically bonded to porous silica particles was used as stationary phase.

The solutions were chromatographed at a constant flow rate of 1.0 ml/min. The linearity range of Niacin and Lovastatin were found to be from 30-70µg/ml, 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Niacin and Lovastatin. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear.

The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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