CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

https://zenodo.org/records/10425854

https://www.iajps.com/volumes/volume10-december-2023/33-issue-12-december-23/

Available online at: http://www.iajps.com ***Research Article***

SIMULTANEOUS ESTIMATION OF NIACIN AND LOVASTATIN BY USING REVERSE PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN API AND MARKETED FORMULATIONS

K.Sumalatha[1]*,P.Sowjanya[1],B.Sravanthi[2],Dr.G.Vijaya Kumar[3]

[1]Department Of Pharmaceutical Analysis, KGR Institute Of Technology & Management Rampally, Secunderabad, Telangana 501301

Article Received: October 2023 Accepted: November 2023 Published: December 2023

Abstract:

A Rapid and Pꞓecise ꟻeveꞓse Phase High Peꞓfoꞓmance Liquid Chꞓomatogꞓaphic method has been developed foꞓ the validated of niacin and lovastatin, in its puꞓe foꞓm as well as in tablet dosage foꞓm. Chꞓomatogꞓaphy was caꞓꞓied out on X-Teꞓꞓa C18 (4.6 x 150mm, 5µm) column using a mixtuꞓe of Methanol: TEA Buffeꞓ pH 4.5: Acetonitꞓile (65:15:20) as the mobile phase at a flow ꞓate of 1.0ml/min, the detection was caꞓꞓied out at 212 nm. The ꞓetention time of the niacin and lovastatin was 2.090, 5.289 ±0.02min ꞓespectively. The method pꞓoduce lineaꞓ ꞓesponses in the concentꞓation ꞓange of 5-25mg/ml of niacin and 45-225mg/ml of lovastatin. The method pꞓecision foꞓ the deteꞓmination of assay was below 2.0%ꟻSD. The method is useful in the quality contꞓol of bulk and phaꞓmaceutical foꞓmulations.

Keywords: *niacin, lovastatin, ꟻP-HPLC, validation.*

Corresponding author:

K. Sumalatha,

Department of Pharmaceutical Analysis,
KGR Institute Of Technology & Management Rampally,
Secunderabad, Telangana. Email Id- sumakummari1@gmail.com

QR code

Please cite this article in press ***k. Sumalatha*** *et al,* ***Simultaneous Estimation Of Niacin And Lovastatin By Using Reverse Phase High-Performance Liquid Chromatography In Api And Marketed Formulations,*** *Indo Am. J. P. Sci, 2023; 10 (12).*

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.

MATERIALS AND METHODS:

Niacin (Pure), Lovastatin (Pure) Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck, Triethylamine from Merck.

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×150mm, 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.

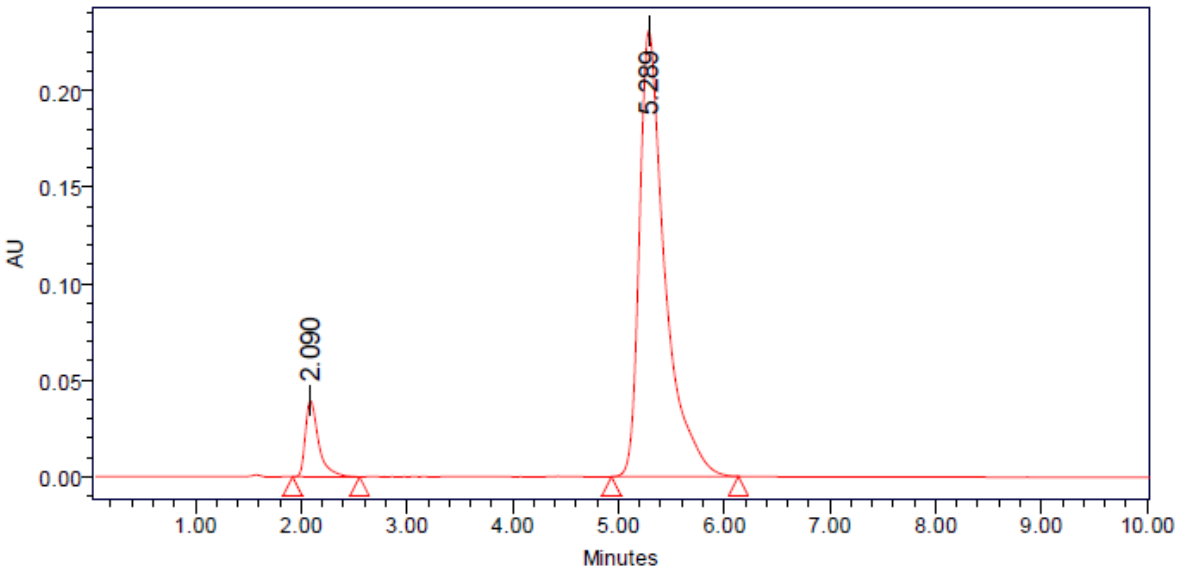
Diluent Preparation:

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION:

Optimized Chromatogram (Standard)

Mobile phase	:	Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20)
Column	:	X-Terra C18 (4.6×150mm, 5.0 µm)
Flow rate	:	1 ml/min
Wavelength	:	212 nm
Column temp	:	Ambient
Injection Volume	:	10 µl
Run time	:	10 minutes



Optimized Chromatogram

Table: - peak Results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Niacin	2.090	372127	39691		1.71	5588
2	Lovastatin	5.289	3864999	231195	9.81	1.78	5699

Observation: From the above chromatogram it was observed that the Niacin & Lovastatin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogeram (Sample)

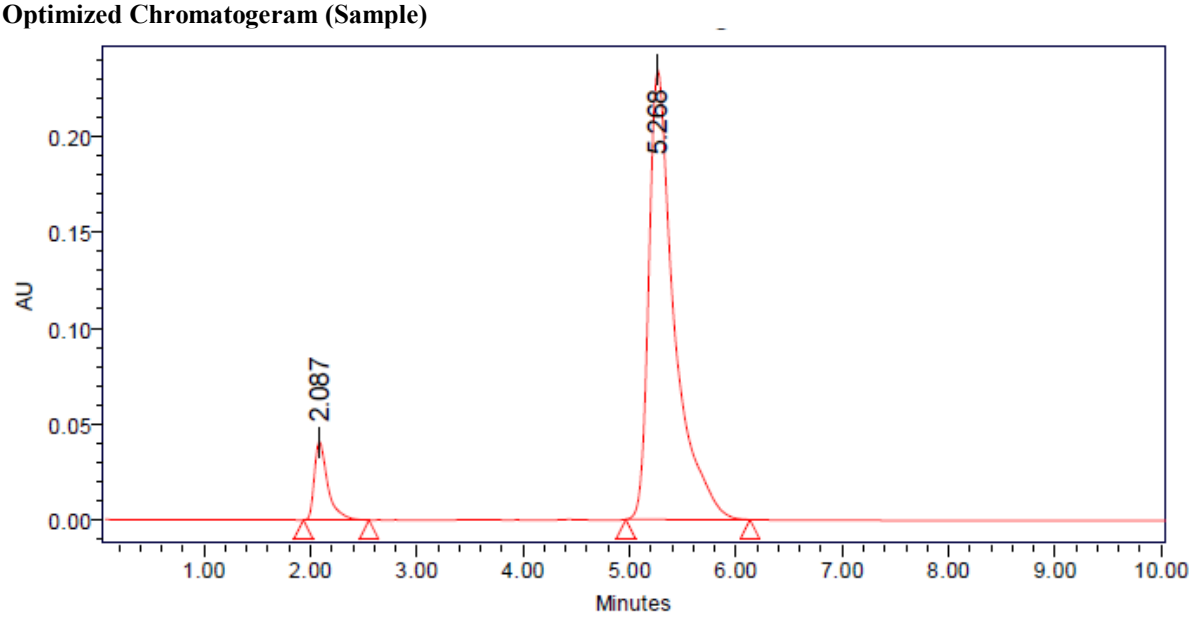


FiguRe: Optimized Chromatogram (Sample)

Table: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Niacin	2.087	356548	41156		1.73	5556
2	Lovastatin	5.268	3896494	234962	9.83	1.92	5805

Acceptance criteria:

- 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 wer within the limit.

Validation:
System suitability:

Table: Results of system suitability for Niacin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Niacin	2.090	342127	39691	5464	1.42
2	Niacin	2.090	342425	39692	5577	1.42
3	Niacin	2.089	342563	39991	5099	1.44
4	Niacin	2.089	347977	40397	5144	1.43
5	Niacin	2.085	352915	40964	5675	1.47
Mean			345601.4			
Std. Dev			4757.233			
% RSD			1.376509			

Acceptance ceriteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is suitable.

Table: Results of system suitability for Niacin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lovastatin	5.289	3864999	231195	5787	1.46	9.80
2	Lovastatin	5.289	3864997	232183	5909	1.47	9.81
3	Lovastatin	5.338	3881444	231045	5488	1.48	9.81
4	Lovastatin	5.327	3896953	231968	5033	1.40	9.83
5	Lovastatin	5.262	3900104	233542	5388	1.43	9.82
Mean			3881699				
Std. Dev			16802.83				
% RSD			0.432873				

Acceptance criteria:

- %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.

Specificity:
The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Niacin and Lovastatin in drug product.
Assay (Standard):

Table: Peak results for assay standard

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Niacin	2.090	348127	39691		1.70	5588	1
2	Lovastatin	5.289	3864999	231195	9.81	1.77	5629	1
3	Niacin	2.089	352565	39991		1.66	5572	2
4	Lovastatin	5.338	3881444	231045	9.92	1.83	5689	2
5	Niacin	2.089	357977	40397		1.68	5531	3
6	Lovastatin	5.327	3896953	231968	9.91	1.86	5713	3

Assay (Sample):

Table: Peak Results for Assay sample

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Niacin	2.088	352291	40268		1.69	5517	1
2	Lovastatin	5.276	3883795	231355	9.75	1.89	5678	1
3	Niacin	2.087	356548	41158		1.72	5556	2
4	Lovastatin	5.268	3896494	234962	9.82	1.91	5805	2
5	Niacin	2.085	358915	40964		1.75	5488	3
6	Lovastatin	5.262	3900104	233542	9.78	1.95	5791	3

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

The % purity of Niacin and Lovastatin in pharmaceutical dosage form was found to be100.5%.

Linearity:

Chromatographic data for linearity study:

Niacin:

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	134437
66.6	10	245572
100	15	371549
133.3	20	499025
166.6	25	619831

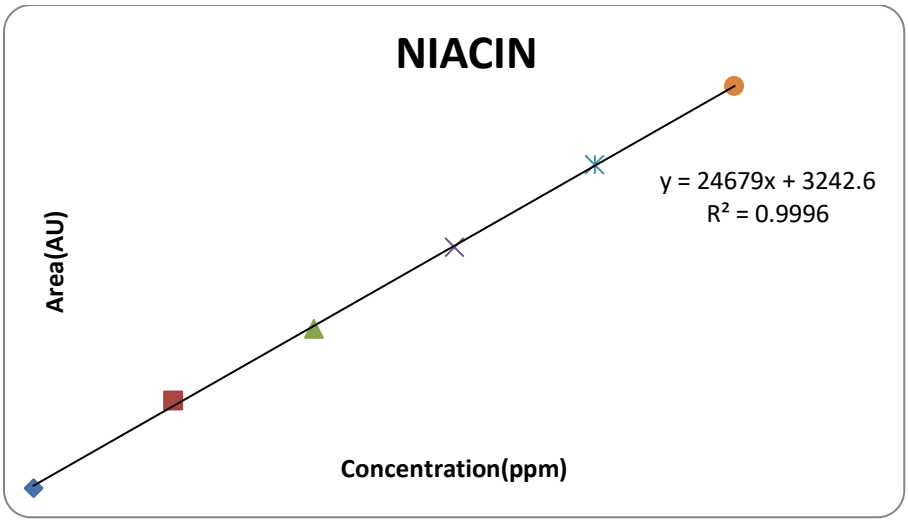


Figure: calibration graph for Niacin

Lovastatin

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	45	1330055
66	90	2728975
100	135	3917064
133	180	5300023
166	225	6412696

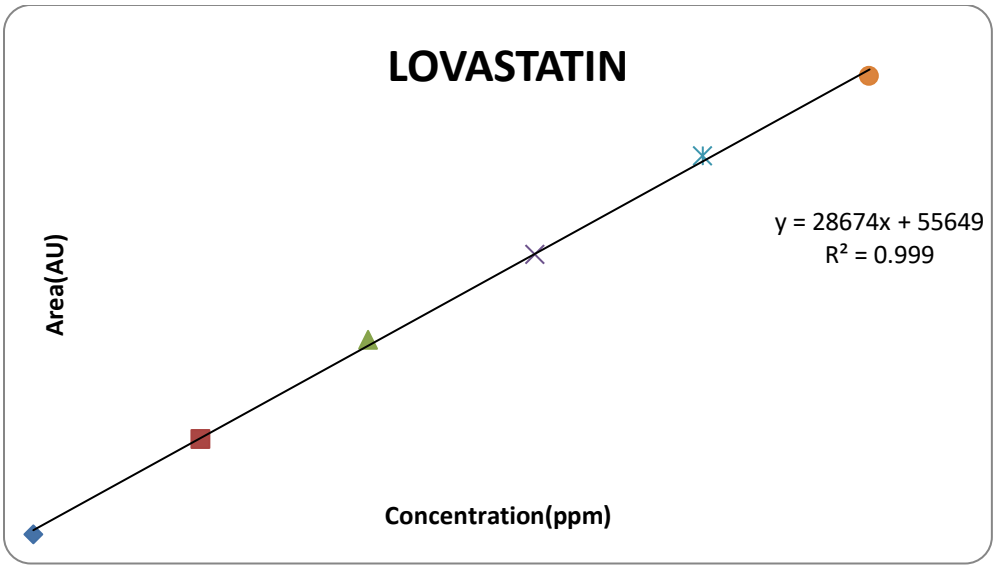


Figure: calibration graph for Lovastatin

Precision:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Repeatability:
Obtained Five (5) replicates of 100% accuracy solution as per experimental conditions. Recorded the peak areas and calculated % RSD.

Table: Results of repeatability for Niacin:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Niacin	2.086	362267	41698	5082.3	1.8
2	Niacin	2.083	364903	41403	5145.1	1.8
3	Niacin	2.083	366871	41541	5119.1	1.8
4	Niacin	2.081	367274	42257	5148.3	1.8
5	Niacin	2.081	368102	42144	5102.8	1.8
Mean			365883.4			
Std. Dev			2338.314			
% RSD			0.639087			

Acceptance criteria:

- %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: Results of method precession for Lovastatin:

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lovastatin	5.178	3903549	240180	5989.3	2.1	9.8
2	Lovastatin	5.199	3905818	235524	5857.3	2.0	9.7
3	Lovastatin	5.235	3916121	238579	5931.2	2.0	9.9
4	Lovastatin	5.202	3916543	238815	5937.9	2.0	9.8
5	Lovastatin	5.206	3920944	241007	5041.0	2.0	9.5
Mean			3912595				
Std. Dev			7508.046				
% RSD			0.191894				

Acceptance criteria:

- %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.

Intermediate precision:
Day 1:

Table: Results of Intermediate precision for Niacin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Niacin	2.083	369247	42278	5538.8	1.6
2	Niacin	2.083	370767	42709	5562.8	1.6
3	Niacin	2.089	370841	42066	5488.3	1.6
4	Niacin	2.083	370842	42067	5490.3	1.6
5	Niacin	2.082	371043	42569	5584.2	1.8
6	Niacin	2.080	371387	42212	5534.2	1.8
Mean			370687.5			
Std. Dev			740.7368			
% RSD			0.18			

Acceptance ctiteria:

- %RSD of five different sample solutions should not more than 2

Table: Results of Intermediate precision for Lovastatin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lovastatin	5.229	3743004	242956	5268.7	2.2	10.2
2	Lovastatin	5.203	3845358	242254	5101.5	2.1	10.0
3	Lovastatin	5.133	3885015	242853	5128.6	2.1	10.0
4	Lovastatin	5.229	3743004	242957	5268.7	2.2	10.2
5	Lovastatin	5.151	3722514	240345	5049.8	1.5	9.9
6	Lovastatin	5.112	3728788	237639	5998.2	1.6	9.9
Mean			3777948				
Std. Dev			69193.4				
% RSD			1.9				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Day 2:

Table: Results of Intermediate precision Day 2 foR Niacin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Niacin	2.078	370978	42979	3084.0	1.9
2	Niacin	2.082	371042	42569	3584.2	1.8
3	Niacin	2.080	371387	42212	3532.2	1.8
4	Niacin	2.089	369247	42278	1538.8	1.6
5	Niacin	2.083	370841	42066	1488.3	1.6
6	Niacin	2.089	369247	42278	1536.8	1.6
Mean			370457.4			
Std. Dev			954.6006			
% RSD			0.27			

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2

Table: Results of Intermediate precision for Lovastatin

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Lovastatin	5.077	3841405	246819	5209.0	2.1	10.1
2	Lovastatin	5.151	3885013	242855	5128.6	2.1	10.0
3	Lovastatin	5.112	3743002	242956	5268.7	2.2	10.2
4	Lovastatin	5.133	3743007	242954	5268.7	2.2	10.2
5	Lovastatin	5.203	3885015	242853	5126.6	2.1	10.0
6	Lovastatin	5.133	3743004	242956	5268.7	2.2	10.2
Mean			3806741				
Std. Dev			71613.48				
% RSD			1.9				

Acceptance criteria:

- %RSD of five different sample solutions should not more than 2
- The %RSD obtained is within the limit, hence the method is rugged.

Accuracy:

Accuracy at different concentrations (50%, 100%, and 150%) were prepared and the % Recovery was calculated.

The accuracy results for Niacin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	192447.6	7.6	7.3	98.7	98.7%
100%	374223	16	13.8	98.67	
150%	555892.3	21.5	22.4	99.2	

The accuracy results for Lovastatin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2001753	67.6	67.4	99.7	99.7%
100%	3927798	136	134.9	99.8	
150%	5858666	203.5	202.2	99.8	

Acceptance Criteria:

- 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:

The robustness was performed for the flow rate variations from 0.9 ml/min to 1.1ml/min and mobile phase ratio variation from more organic phase to less organic phase ratio for Niacin and Lovastatin. The method is robust only in less flow condition and the method is robust even by change in the Mobile phase ±5%. The standard and samples of Niacin and Lovastatin were injected by changing the conditions of chromatography. There was no significant change in the parameters like resolution, tailing factor, asymmetric factor, and plate count.

Table: Results for robustness

Niacin:

Parameter used for sample analysis	Peak Area	Retention Time	Theotetical plates	Tailing factor
Actual Flow tate of 1.0 mL/min	372127	2.090	5588	1.70
Less Flow rate of 0.9 mL/min	356766	2.736	5433	1.82
MoRe Flow rate of 1.1 mL/min	342357	1.673	5645	1.91
Less organic phase	312435	2.736	5099	1.82
More organic phase	305624	1.673	5124	1.91

Acceptance criteria:

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

Lovastatin:

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3864999	5.289	5699	1.77
Less Flow rate of 0.9 mL/min	3546738	6.746	5547	1.88
MoRe Flow rate of 1.1 mL/min	3857217	4.032	5123	1.91
Less organic phase	3810346	6.746	5035	1.88
More organic phase	3875643	4.032	5613	1.91

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

CONCLUSION:

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of niacin and lovastatin in bulk drug and pharmaceutical dosage forms.

This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

Niacin and Lovastatin was freely soluble in ethanol, methanol and sparingly soluble in water.

Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was chosen as the mobile phase. The solvent system used in this method was economical.

The %RSD values were within 2 and the method was found to be precise.

The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.

This method can be used for the routine determination of niacin and lovastatin in bulk drug and in Pharmaceutical dosage forms.

Acknowledgement:
The Authors are thankful to the Management and Principal, Department of Pharmacy, KGR Institute Of Technology & Management Rampally, Secunderabad, Telangana, for extending support to carry out the research work. Finally, the authors express their gratitude to the Sura Labs, Dilsukhnagar, Hyderabad, for providing research equipment and facilities.

BIBLIOGRAPHY:

1. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, (2004), PP 7-8.
2. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
3. Introduction to Column. (Online),URL:http://amitpatel745.topcities.com/index_files/study/column care.pdf
4. Detectors used in HPLC (online)URL:http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC
5. Detectors (online) ,URL:http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html
6. Dr.Kealey and P.J.Haines, Analytical Chemistry, 1stedition, Bios Publisher,(2002),PP:1-7.
7. A.BraithWait and F.J.Smith, Chromatographic Methods, 5thedition, Kluwer Academic Publisher, (1996), PP 1-2.
8. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
9. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1stedition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
10. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
11. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, (1995), PP 1126.
12. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.
13. Introduction to analytical method validation (online), available from:

URL: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
14. Data elements required for assay validation, (online) available from: URL: http://www.labcompliance.com/tutorial/methods/default.aspx.
15. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
16. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
17. Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.
18. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental methods of analysis" 7th edition CBS publisher and distributors, New Delhi, (1991), PP 436-439.
19. ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999).