Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

SIMULTANEOUS ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR NIACIN AND ATORVASTATIN TABLETS BY USING RP-HPLC TECHNIQUES

Syeda Sumaiya Quadri [a]*, Dr. Madhukar. A [b], Ghousia Fatima [a], Ayesha Begum. K [a], Farhath Unnisa [a]

[a] Shadan Women's College Of Pharmacy, Khairatabad, Hyderabad, Telangana, INDIA.

[b] Department of Pharmacy, Brilliant Group of Technical Institutions, Abdullapur, Hyathnagar, Hyderabad, Telangana, INDIA.

Received on: 04-09-2016; Revised and Accepted on: 21-09-2016

ABSTRACT

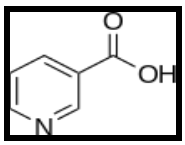
A simple, rapid, and precise reverse phase high performance liquid chromatographic (RP-HPLC) method for simultaneous analysis of Niacin and Atorvastatin in a tablet dosage form has been developed and validated. This method was performed with a Symmetry C_{18} (250 × 4.6 mm) packed with 5 µm particle column with 20:80v/v, 0.02M phosphate buffer and methanol as mobile phase at a flow rate of 1.5 ml/min. UV detection at 226 nm; Niacin and Atorvastatin were eluted with retention times of 2.33 & 6.73min, respectively. The method was continued and validated accordance with ICH guidelines. Validation revealed the method is rapid, specific, accurate, precise, reliable, and reproducible. Calibration curve plots were linear over the concentration ranges 10-450µg/mL for Niacin and 2.1-96µg/mL for Atorvastatin. Limits of detection (LOD) were 0.15 and 0.03µg/ml and limits of quantification (LOQ) were 0.5 and 0.1µg/mL for Niacin and Atorvastatin respectively. Statistical analysis was proves the method is suitable for the analysis of Niacin and Atorvastatin as a bulk, in tablet dosage form without any interference from the excipients. It was also proved study for degradation kinetics of two drugs. It may be extended for its estimation in plasma and other biological fluids.

Keywords: *RP-HPLC, Niacin and Atorvastatin, Quality control, Validation.*

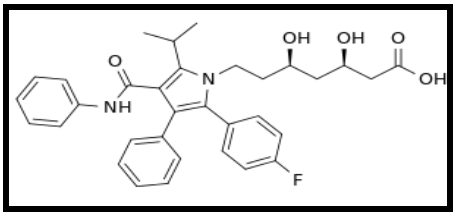
INTRODUCTION

Niacin is chemically pyridine-3-carboxylic acid [1]. Niacin is also known as vitamin B_3 and nicotinic acid, is an organic compound with the formula C6H5NO2 and, depending on the definition used, one of the 20 to 80 essential human nutrients. Niacin has not been found to be useful in decreasing the risk of cardiovascular disease in those already on a statin [2] but appears to be effective in those not taking a statin [3].

Atorvastatin is chemically (3*R*,5*R*)-7-[2-(4-Fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-propan-2-ylpyrrol-1-yl]-3,5-dihydroxyheptanoic acid [4]. Atorvastatin is marketed under the trade name Lipitor among others [5]. It is a member of the drug class known as statins, which are used primarily as a lipid-lowering agent and for prevention of events associated with cardiovascular disease. Like all statins, atorvastatin works by inhibiting HMG-CoA reductase, an enzyme found in liver tissue that plays a key role in production of cholesterol in the body.



A



B

Fig. 1: Chemical structures of Niacin (A) and Atorvastatin (B)

***Corresponding author:**
Syeda Sumaiya Quadri
Shadan Women's College of Pharmacy,
Khairatabad, Hyderabad, Telangana, INDIA.
**E-Mail: uzmi_2006@yahoo.com*

In the scientific literature, analysis of Niacin and Atorvastatin has been reported as individual ingredients and in combination with other compounds. Analytical methods have included estimation of Niacin and Atorvastatin have been analyzed in combination and with other drugs individually have also been reported by RP-HPLC [6-11], UV-Visible Spectroscopy [12].
The method described is rapid, economical, precise, and accurate and can be used for routine analysis of tablets. It was validated as per ICH guidelines [13-15].

MATERIALS AND METHODS

Experimental:

Apparatus:

The analysis was performed by using the analytical balance G285 (Mettler Toledo), the HPLC used is of Water 2695 with PDA detector. Column used in HPLC Symmetry C_{18} (250 × 4.6 mm) packed with 5 µm particle column with a flow rate of 1.5 ml/min. The mobile phase consists of 20:80v/v, 0.02M phosphate buffer and methanol which is degassed in a sonicator for about 10 minutes the injection volume is 20µl and the ultra violet detection was at 226 nm.

Reagents and solutions:

Pure sample of Niacin, Atorvastatin and other ingredients such as Methanol and water used were of HPLC and milli-q grade. Optimized chromatographic conditions are listed in Table No.1.

Preparation of stock solution:

Accurately weighed 10 mg of Niacin and Atorvastatin working standard and separately transferred into a 10ml clean dry volumetric flasks, add about 7ml of diluent to each volumetric flask and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Calibration standards at five levels were prepared by appropriately mixed and further diluted stock standard solutions in the concentration ranges from 10-450µg/mL for Niacin and 2.1-96µg/mL for Atorvastatin respectively. Samples in triple injections were made for each prepared concentration. Peak areas were plotted against the corresponding concentration to obtain the Linearity graphs.

Sample Preparation:

For the analysis of a tablet dosage form, 20 tablets were weighed individually and their average mass was determined. Then, the tablets were crushed to a fine powder. The powder equivalent to 375µg/ml of Niacin and 80µg/ml of Atorvastatin were transferred to a 10 mL volumetric flask and dissolved in 10mL of diluent, sonication was done for 15 min with swirling. After sonication, the solution was filtered through a membrane filter paper (#0.45µ). From the above stock solution 3.75mL was transferred in to 10mL volumetric flask and made volume upto the mark with diluent, the final concentrations were 375µg/ml and 80µg/ml of Niacin and Atorvastatin respectively, then injected into the chromatographic system, and analyzed quantitatively. The analysis was repeated six times and the possibility of excipient interference with the analysis was examined.

Optimization of HPLC Method:

The HPLC method was optimized and developed with a simultaneous assay method for Niacin and Atorvastatin respectively. The mixed standard stock solution (375µg/mL of Niacin, 80µg/mL of Atorvastatin) injected in HPLC. Different ratios of methanol and potassium dihydrogen orthophosphate buffer at different pH and molarities were tried (Table No. 1).

Method validation:

The method validation was done according to the ICH guidelines. The following validation characteristic parameters are accuracy, precision, linearity, and specificity, LOD, LOQ and robustness.

Linearity and range:

Linearity of the method was studied by the injecting the mixed standard solutions with the concentration ranges from 10-450µg/mL for Niacin and 2.1-96µg/ml for Atorvastatin levels of target concentrations were prepared and injected six times into the HPLC system keeping the constant injection volume. The peak areas were plotted against the concentrations to obtain the linearity graphs (Table No. 2 & Fig. 3).

Precision:

The precision of the optimized method was evaluated by carrying out six independent assays of test sample. %RSD of six assay values was calculated. Intermediate precision was carried out the samples by using another instrument and with different analyst.

Limit of Detection and Quantification:

The LOD and LOQ procedures were performed on samples contain very lower concentrations of analytes under the ICH guidelines. By applying the visual evaluation method, LOD was expressed by establishing the lowest concentration at which the analyte can be detected. LOQ was considered as the lowest concentration of analytes that can be detected and quantified, with acceptable accuracy and precision.

Robustness:

Robustness was studied by evaluating the effect of small variations in the chromatographic conditions. The conditions studied were flow rate altered by ±0.1ml/min, mobile phase composition with methanol ±5ml. These chromatographic variations are evaluated for resolution between Niacin and Atorvastatin.

System suitability:

The system suitability parameters with respect of tailing factor, theoretical plates, repeatability and resolution between Niacin and Atorvastatin peaks were defined (Table No. 3).

Specificity:

The specificity of the analytical method is the ability of the method to estimate the analyte response in the presence of additional components such as impurities, degradation products and matrix [16]. The peak purity of Niacin and Atorvastatin were assessed by comparing the Retention time of standard Niacin and Atorvastatin good correlation was obtained between the Retention time of standard and sample of Niacin and Atorvastatin.

The specificity method was also evaluated to ensure that there were no interference products resulting from forced degradation studies.

1. Forced degradation study:

Forced degradation or Stress testing of a drug substance will help to identify the degradation products, which can help to establish the intrinsic stability of the molecule.

All stress decomposition studies were performed at an initial drug concentration 375µg/ml of Niacin and 80µg/mL of TLM.

The Stability indicating study of Niacin and Atorvastatin were undergoes acid, alkali and oxidation degradation, photolysis and heat condition.

Placebo Interference: The placebo (in the present of excipients in tablet) sample were prepared as per the test method and analyzed in the HPLC. It expressed there is no additional peaks at the retention time of Niacin and Atorvastatin in the chromatograph it indicates that there is no placebo interference.

Acid Degradation: Sample was treated with 3ml of 1N hydrochloric acid and kept for 10hrs. After 10hrs the solution was neutralized with 3ml of 1N sodium hydroxide, made the volume upto the mark with diluent and analyzed using HPLC.

Alkali Degradation: Sample was treated with 3ml of 1N sodium hydroxide and kept for 10hr. After 10hr the solution was neutralized with 3ml of 1N hydrochloric acid, made the volume upto the mark with diluent and analyzed using HPLC.

Oxidative Degradation: Niacin and Atorvastatin solutions of 375µg/ml and 80µg/ml were mixed with 3mL of 30%v/v aqueous hydrogen peroxide solution and kept for 10hrs. After 10hrs made the volume upto the mark with diluent and analyzed using HPLC.

Photolytic Degradation: The **s**amples were kept under UV light for different time intervals (15mins – 7days) and made the volume upto the mark with diluent media and analyzed using HPLC.

Thermal Degradation: Samples were heated at 80^0 C for 15mins - 60mins and 220^0 C for 2-5mins and analyzed.

Accuracy:

Accuracy was carried out by applying the method to drug sample (Niacin and Atorvastatin combination of tablets) to which known amounts of Niacin and Atorvastatin standard powder corresponding to 80, 100 and 120% of label claim was added, mixed and the powder was extracted and determined by the system in optimized mobile phase. The experiment was performed in triplicate and percentage recovery, % RSD was calculated.

Analysis of marketed formulation:

The marketed formulation was assayed by above description. The peak areas were monitored at 226nm, and determination of sample concentrations were using by multilevel calibration developed on the same HPLC system under the same conditions using linear regression analyzed for Niacin and Atorvastatin in the same way as described above.

RESULTS

The simultaneous estimation of Niacin and Atorvastatin standards having concentration 375µg/ml and 80µg/ml were scanned in UV- region between 200-400 nm. λ max of Niacin and Atorvastatin was found to be at 226 nm.

The Niacin and Atorvastatin peak in the sample was identified by comparing with the Niacin and Atorvastatin standards and the Retention time was found to be around 2.33 & 6.73 mints respectively.

The estimation of Niacin and Atorvastatin tablets were carried out by RP-HPLC using Mobile phase having a composition of 200 volumes of Phosphate Buffer and 800 volumes of Methanol. Then finally filtered using 0.45µ nylon membrane filter and degassed in sonicator for 10 minutes. The column used was Symmetry C_{18} (250 × 4.6 mm, packed with 5 µm). Flow rate of Mobile phase was 1.5 ml/min, System suitability parameters such as RSD for six replicate injections were found to be less than 2%, theoretical plates - 3754.8 & 5617.7, and tailing factor - 1.66 & 1.29 for Niacin and Atorvastatin respectively.

The acceptance criteria of Method Repeatability is RSD should be not more than 2.0% and the method show Method Repeatability 0.025% and 0.062% for Niacin and Atorvastatin respectively, which shows that the method is precise.

The validation of developed method shows that the drug stability is well within the limits. The linearity of the detector response was found to be linear from 10-450µg/mL and 2.1-96µg/ml of target concentration for Niacin and Atorvastatin standards with a correlation coefficient value is greater than 0.999. The correlation coefficient of (R^2) = 0.9997 & 0.9993 for Niacin and Atorvastatin respectively, which shows that the method is capable of producing good response in PDA detector.

The Accuracy limit is the % recovery should be in the range of 98.69 - 100.81% & 98.73 - 100.37% for Niacin and Atorvastatin respectively. The validation of developed Method shows that the accuracy is well within the limit, which shows that the method is capable of showing good accuracy.

Table No. 1: Optimized chromatographic conditions for Niacin and Atorvastatin

Parameters	Method
Stationary phase (column)	Symmetry C_{18} (250 × 4.6 mm, packed with 5 µm)
Mobile Phase	20:80v/v, (0.02M phosphate buffer: methanol)
Ph	7.2 ± 0.02
Flow rate (ml/min)	1.5
Run time (minutes)	8.0
Column temperature (°C)	Ambient
Volume of injection loop (µl)	20
Detection wavelength (nm)	226
Drugs RT (min)	2.33 & 6.73

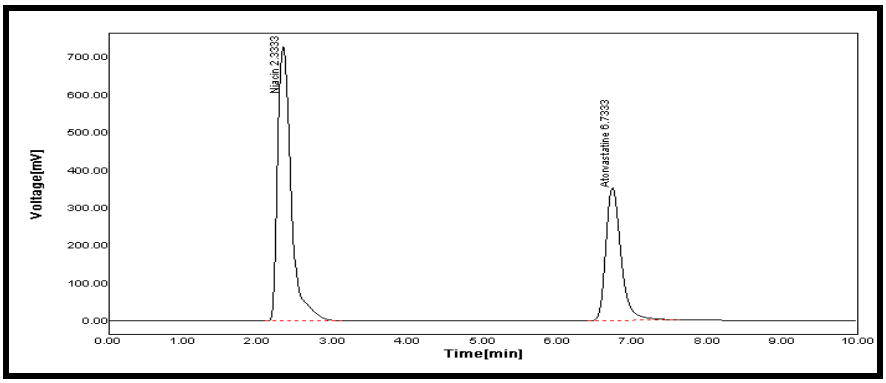


Fig. 2: Chromatogram of Niacin and Atorvastatin at 226nm

Table No. 2: Linearity Data for Niacin and Atorvastatin

Analyte	Concentration range (µg/mL)	Correlation Coefficient (R^2)	Slope	Intercept
Niacin	10-450	0.9997	21411x	34392
Atorvastatin	2.1-96	0.9993	57395x	49442

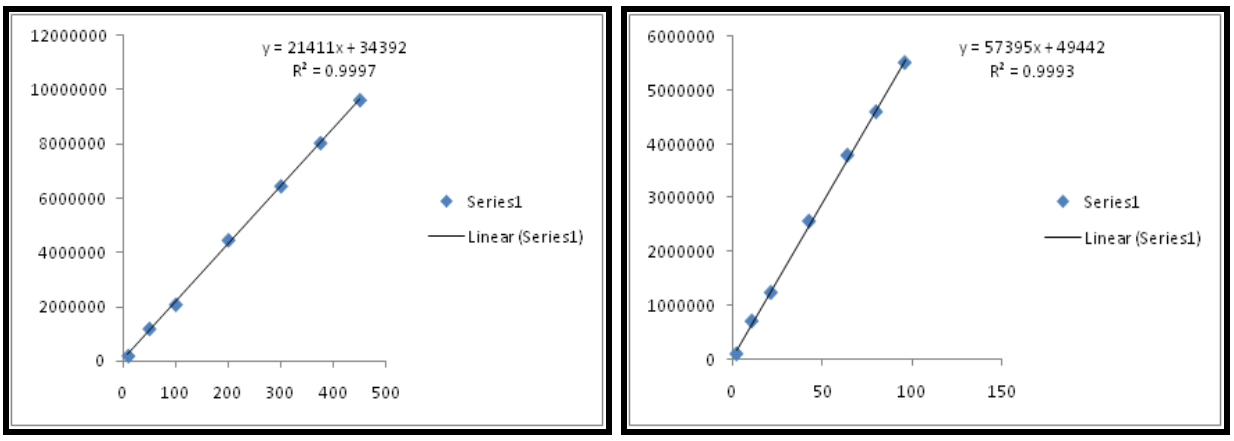


Fig. 3: Linearity Curve of Standard Niacin and Atorvastatin

Table No. 3: System suitability parameters for Niacin and Atorvastatin

System suitability parameters	Niacin	Atorvastatin
Retention time (min)	2.33	6.73
% R.S.D (n=5)	0.025	0.062
Resolution (Rs)	-	11.9815
Tailing factor (asymmetric factor)	1.66	1.29
USP plate count	3754.8	5617.7
LOD (µg/mL)	0.15	0.03
LOQ (µg/mL)	0.5	0.1

CONCLUSION

A new RP-HPLC method described in this manuscript provides a simple, convenient and reproducible approach for the simultaneous estimation and quantification of Niacin and Atorvastatin in routine quality control analysis.

REFERENCE:

1. https://en.wikipedia.org/wiki/Niacin.
2. Keene D, Price C, Shun-Shin MJ, Francis DP. Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117, 411 patients. BMJ (Clinical research ed.), **2014**; 349: g4379. doi:10.1136/bmj.g4379.
3. Bruckert Eric, Labreuche Julien, Amarenco Pierre. Meta-analysis of the effect of nicotinic acid alone or in combination on cardiovascular events and atherosclerosis, Atherosclerosis, **2010**; 210(2): 353-361.
4. https://en.wikipedia.org/wiki/Atorvastatin.
5. Pfizer product promotion page (Lipitor). Retrieved 2011-12-05.
6. Suresh Kumar GV, Rajendraprasad Y, Chandrashekar SM. Development and validation of reversed-phase HPLC method for Simultaneous Estimation of Atorvastatin calcium and Telmisartan in Tablet dosage form, Int. J. PharmTech. Res., **2010**; 2(1): 463-470.
7. Jaiprakash N. Sangshetti, Mohammed Aqeel, Zahid Zaheer, Rana Z. Ahmed, M.H.G. Dehghan, Indrajeet Gonjari. Development and validation of RP-HPLC method for determination of Atorvastatin calcium and Nicotinic acid in combined tablet dosage form, Journal of Saudi Chemical Society, 2013; 1-6. http://dx.doi.org/10.1016/j.jscs.2012.12.005**.**
8. Saroj Kumar Raul, Atna Bhaskar Aravelli, Durgasi Jhansi. RP-HPLC Method Development and Validation for the Simultaneous Estimation of Atorvastatin and Ezetimibe in Pharmaceutical dosage form, Asian J. Pharm. Clin. Res., **2015**; 8(2): 178-181.
9. A.P. Dewani, D.S. Mohale, S. Hiware, R.L. Bakal, A.V. Chandewar, Mohd. Salimuddin Farooqui. Development and Validation of RP-HPLC method for Simultaneous Estimation of Niacin and Simvastatin in tablet dosage, Ind. J. Pharm. Pharmacol., **2015**; 2(1): 21-26.
10. D.A. Shah, K.K. Bhatt, R.S. Mehta, M.B. Shankar, S.L. Baldania and T.R. Gandhi. Development and validation of a RP-HPLC method for determination of atorvastatin calcium and aspirin in a capsule dosage form, Ind. J. Pharm. Sci., **2007**; 69 (4): 546-549.
11. Pravish Kumar Tiwari, Padmakar Sathe. Development and validation of HPTLC method for niacin and simvastatin in binary combination, Advances in Bioscience and Biotechnology, **2010**; 1: 131-135. doi:10.4236/abb.2010.12018.
12. M.K. Ranganath and Raja Ram Chowdary. Simultaneous Estimation and Validation of Niacin and Atorvastatin Calcium by UV-Spectroscopy in Pure and Tablet Dosage Form Using Methanol: Water Mixture as Solvent, RGUHS J. Pharm. Sci., 2014; 4(2):70-77.
13. Code Q2(R1)-Text on Validation of Analytical Procedure Step-3 Consensus Guideline. ICH Harmonised Tripartite Guideline, **2005**.
14. Code Q2A-Text on Validation of Analytical Procedure Step-3 Consensus Guideline. ICH Harmonised Tripartite Guideline, **1994**.
15. Code Q2B-Validation of Analytical Procedure Methodology Step-4 Consensus Guideline. ICH Harmonised Tripartite Guideline, **1994**.
16. International Conference on Harmonization. Photo stability testing of new drug substance and products Q1B. International Conference on Harmonization, IFPMA, Geneva, **1996**.

How to cite this article:

Syeda Sumaiya Quadri et al., SIMULTANEOUS ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR NIACIN AND ATORVASTATIN TABLETS BY USING RP-HPLC TECHNIQUES, J. Pharm. Res., 2016; 5(9): 213-216.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil