Journal of Saudi Chemical Society (2016) **20**, S328–S333

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Development and validation of RP-HPLC method for determination of Atorvastatin calcium and Nicotinic acid in combined tablet dosage form

Jaiprakash N. Sangshetti [a,*], Mohammed Aqeel [a], Zahid Zaheer [a], Rana Z. Ahmed [a], M.H.G. Dehghan [a], Indrajeet Gonjari [b]

[a] *Y.B Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Rauza Bagh, Aurangabad 431001, (M.S), India*
[b] *Government College of Pharmacy, Karad 415110 (M.S), India*

Received 21 April 2012; accepted 1 December 2012
Available online 20 December 2012

KEYWORDS

Atorvastatin;
Nicotinic acid;
Validation;
RP-HPLC;
Simultaneous estimation;
Tablet dosage form

Abstract A simple, specific and accurate reverse phase liquid chromatographic method was developed for the simultaneous determination of Atorvastatin calcium and Nicotinic acid in tablet dosage forms. The analysis has been performed by using Agilent ZORBAX SB-C18 (150 × 4.6 mm, 3.5 u) and mobile phase containing acetonitrile: distilled water (85:15) at pH 4.5 (adjusted with phosphoric acid). The detection was carried out at 261 nm with a flow rate of 1.0 ml/min. The retention times of Atorvastatin calcium and Nicotinic acid were 6.092 and 3.125 min, respectively. The method was validated according to ICH guidelines. The method was validated for specificity, precision, linearity, accuracy and robustness. The linearity for Atorvastatin calcium and Nicotinic acid were in the range of 2–12 and 10–80 μg/ml respectively. The recoveries of Atorvastatin calcium and Nicotinic acid were found to be in the range of 99.031% and 99.744% respectively. The proposed method was validated and successfully applied to the estimation of Atorvastatin calcium and Nicotinic acid in combined tablet formulation.

1. Introduction

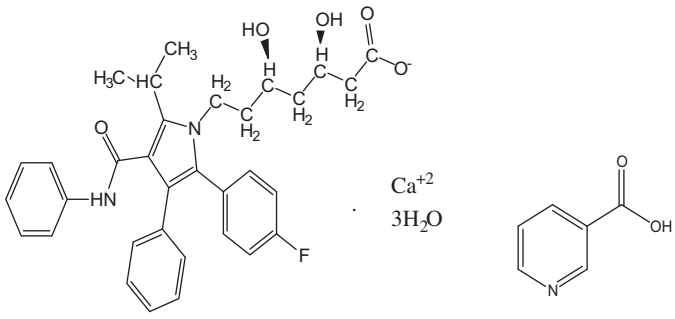
Atorvastatin is chemically (3R,5R)-7-[2-(4-fluorophenyl) -3-phenyl-4-(phenylcarbamoyl)-5-(propan-2yl)1H-pyrrol-1-yl-3, 5-dihydroxyheptanoic acid calcium salt trihydrate Fig. 1 (Gennaro, 2000). Atorvastatin selectively and competitively inhibits the hepatic enzyme HMG-CoA reductase. Nicotinic acid is chemically pyridine-3-carboxylic acid Fig. 1 (Gennaro, 2000). Nicotinic acid lowers plasma triglyceride and cholesterol concentration.

Several studies for the estimation of the drug using various techniques have been carried out for Atorvastatin and Nicotinic acid, some of them being: extractive spectrophotometric determination of Atorvastatin in bulk and pharmaceutical formulations (Erk, 2003), HPLC assay and pharmaco-kinetic

* Corresponding author. Tel.: +91 240 2381129.
E-mail address: jnsangshetti@rediffmail.com (J.N. Sangshetti).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2012.12.005



Atorvastatin calcium Nicotinic acid

Figure 1 Chemical structure of Atorvastatin calcium and Nicotinic acid.

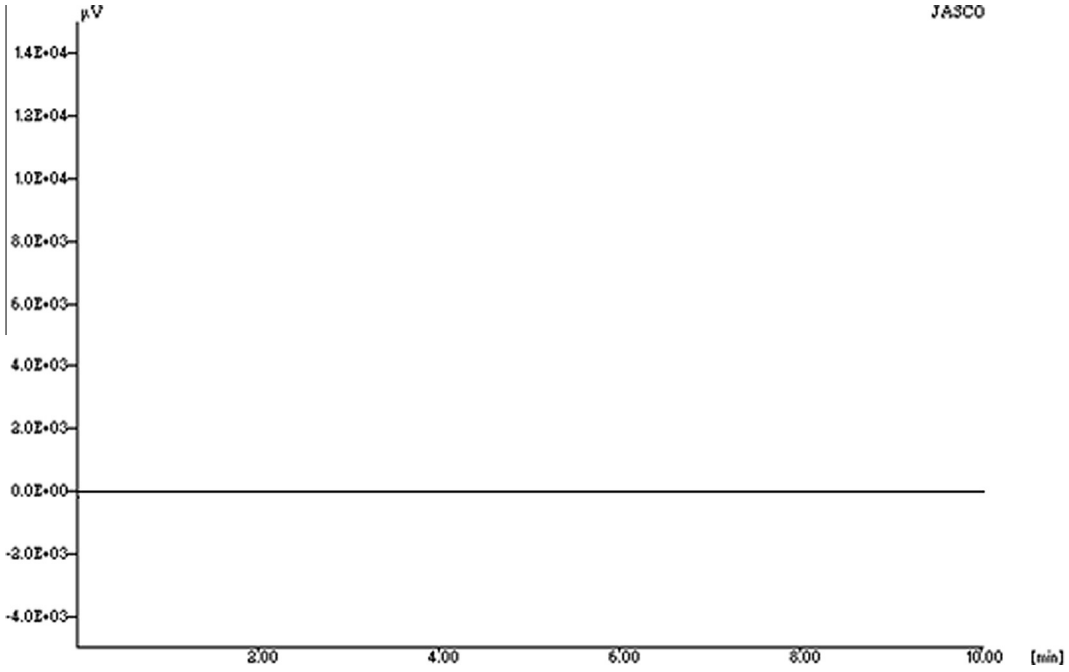


Figure 2 Chromatogram of the placebo.

study of Atorvastatin in beagle dog after oral administration of Atorvastatin self-micro emulsifying drug delivery system (Shen et al., 2006), liquid chromatographic determination of Atorvastatin in bulk drug, tablets and human plasma (Altuntas et al., 2004), HPLC method for the determination of Atorvastatin and its impurities in bulk drugs and tablets (Erturk et al., 2003), Chromatographic and capillary electrophoretic method for the analysis of Nicotinic acid and its metabolites (Iwaki et al., 2000).Though a number of methods are reported for estimation of individual drug Atorvastatin and Nicotinic acid or in combination with other drugs to our knowledge no HPLC method is available for simultaneous estimation of Atorvastatin and Nicotinic acid in combined dosage form. Considering this we have decided to develop a RP-HPLC method for simultaneous estimation of Atorvastatin and Nicotinic acid in combined dosage form. Validation of the developed method has gained significance nowadays (Bouabidi et al., 2010,Rozet et al.; 2011). The objective of validation of an analytical method is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. The developed method is validated as per ICH guidelines (ICH, 2005).

2. Experimental

2.1. Chemicals and reagents

Atorvastatin and Nicotinic acid working standards were generous gifts from Lupin Laboratory Limited Aurangabad and Aurobindo labs hyderabad, India respectively. Combination drug products of Atorvastatin and Nicotinic acid (Label claim: Atorvastatin calcium equivalent to Atorvastatin 10 mg, and Nicotinic acid 375 mg), TONACT plus (Lupin Ltd., Mumbai, India), were purchased from a local pharmacy. Acetonitrile, methanol and water used were of HPLC grade. *Ortho*phosphoric acid used was of analytical reagent grade.

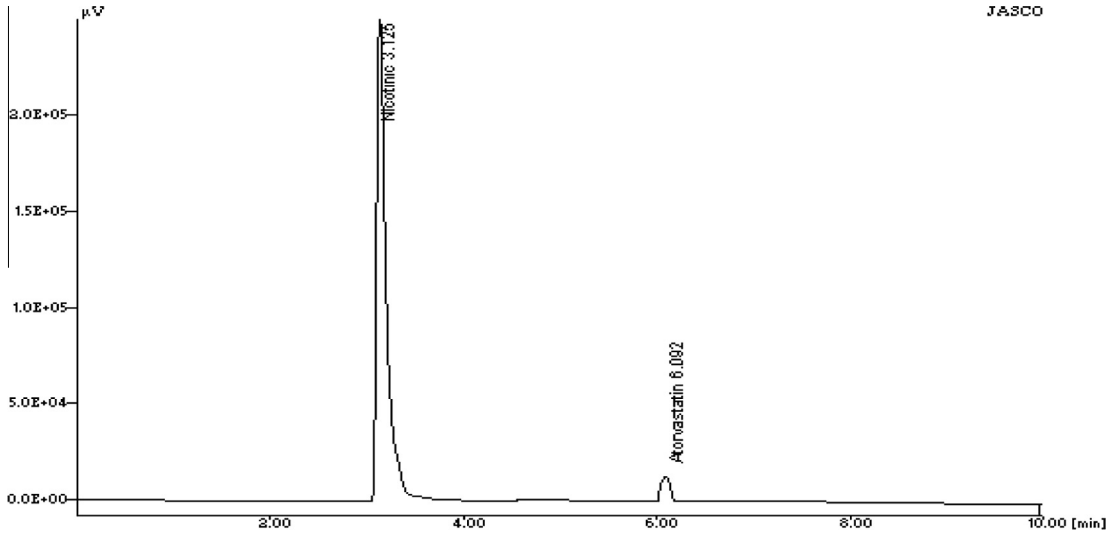


Figure 3 Chromatogram of standard preparation of Nicotinic acid (75 µg/ml) and Atorvastatin (2 µg/ml).

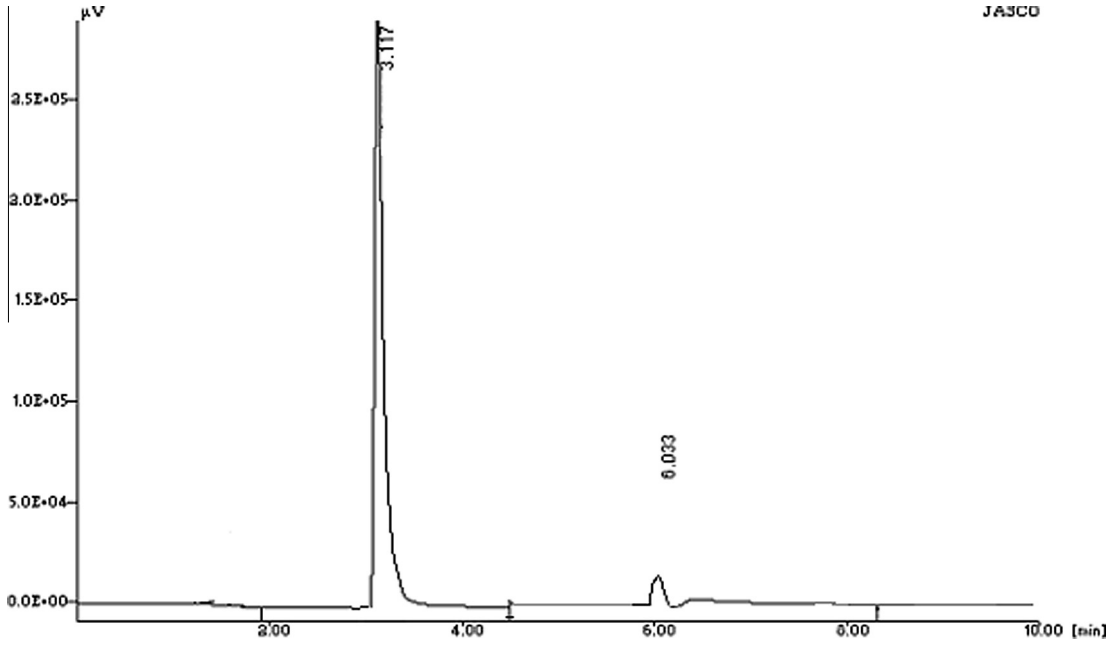


Figure 4 Chromatogram of sample preparation of Nicotinic acid (75 µg/ml) and Atorvastatin (2 µg/ml).

2.2. Instruments

JASCO HPLC LC-2000 Plus series, Pump PU-2080, detector UV-2075 plus, Browin software with Agilent ZORBAX SB-C18 (150 × 4.6 mm, 3.5 u) column were used. All weights were taken on electronic balance (Denver, Germany).

3. Method

3.1. Chromatographic conditions

Chromatographic separation was achieved at 18 °C and the detection was carried at 261 nm at a flow rate of 1 mL/min. Run time was kept at 10 min. Prior to the injection of drug solution, column was equilibrated for 60 min with the mobile phase flowing through the system. The injection volume was 20 µL for assay level. The analysis has been performed by using Agilent ZORBAX SB-C18 (150 × 4.6 mm, 3.5 u) and the mobile phase containing acetonitrile: distilled water (85:15) at pH 4.5 (adjusted with phosphoric acid).

3.2. Standard preparation

The standard stock solutions 100 ug/ml of Atorvastatin and 100 µg/ml of Nicotinic acid were prepared separately by dissolving working standards in methanol and diluting with the same solvent. Standard calibration solutions of Atorvastatin

Table 1 Summary of validation parameters for the proposed method.

Parameters	Atorvastatin	Nicotinic acid
Retention time (min)	6.092	3.125
Theoretical plates	2777.77	4123.607
Tailing factor	0.9	0.8
Linearity range (μg mL^{-1})	2–12	10–80
Correlation coefficient (*r*)	0.999	0.999
Accuracy (%)	98.93–101.16%	98.83–101.20%

and Nicotinic acid having concentrations in the range of 2–12 and 10–80 ug/ml respectively were prepared by diluting stock solutions with methanol. A representative chromatogram of standard preparation is shown in (Fig. 3).

3.3. Sample solution

Twenty tablets were weighed and finely powdered. Powder equivalent to Atorvastatin 10 mg and 375 mg Nicotinic acid was accurately weighed into a 100 ml volumetric flask, 30 ml of diluents was added and sonicated for 15 min with intermittent shaking, made up to the volume with diluents and mixed. Filter the solution through 0.45 nylon membrane filter. Dilute 2 ml of the above solution to 100 ml with diluents. A representative chromatogram of sample preparation is shown in (Fig. 4).

4. Analytical method validation

4.1. Specificity

To assess the method specificity, tablet powder without Atorvastatin and Nicotinic acid was prepared with the same excipients as those in the commercial formulation. For RP-HPLC, the solution was prepared using the same procedure as for the analytical sample. Placebo solution was injected into the HPLC system following test conditions, the chromatogram was recorded and the responses of the peaks if any measured. Chromatogram of the placebo has not shown any interference at the retention time of both Atorvastatin and Nicotinic acid (Fig. 2).

4.2. Precision

Precision was measured in terms of repeatability of application and measurement. Repeatability of standard application (System precision), inter day precision study of Atorvastatin and Nicotinic acid were carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for 3 different concentrations of Atorvastatin (2, 4, 6 μg/ml) and Nicotinic acid (10, 20, 30 μg/ml). Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. The repeatability studies were carried out by estimating the response of three different concentrations of Atorvastatin (2, 4, 6 μg/ml) and Nicotinic acid (10, 20, 30 μg/ml) for triplicate and results are reported in terms of relative standard deviation (RSD). The precision of the method is satisfactory as % RSD is not more than 2% (Table 2).

4.3. Calibration and linearity

The linearity of method is obtained by preparation of the calibration curve. The calibration curve for Atorvastatin was obtained by plotting the peak area of Atorvastatin versus concentration of Atorvastatin over the range of 2–12 μg/ml, and it was found to be linear with $r = 0.999$. Similarly, the calibration curve for Nicotinic acid was obtained over the range of 10–80 μg/ml and was found to be linear with $r = 0.999$. The slope and intercept value for calibration curve was found $y = 84879x + 38623$ for Nicotinic acid and $y = 28352x + 4738$ for Atorvastatin.

4.4. Accuracy

The recovery experiments were performed by adding a known quantity of pure standard drug into the solution of the tablet powder. The sample was then spiked with standard at levels 80%, 100% and 120% of test concentration. The resulting spiked sample solutions were assayed in triplicate and the results were compared with the expected results and expressed as percentage The recoveries of Atorvastatin and Nicotinic acid were found to be in the range of 98.93–101.16% and 98.83–101.20%, respectively (Table 1).

4.5. Robustness

Robustness of the method was determined by analyzing standard solutions at normal operating conditions and by changing some operating analytical conditions such as flow rate, pH, and detection wavelength. The conditions with the variation and the results are presented in Table 3.(see Table 4)

4.6. LOD & LOQ

LOD and LOQ were calculated by using the formula 3.3S.D/S and 10S.D/S where S.D is the standard deviation of *Y*-intercept and *S* is the slope of the calibration curve.

5. Results and discussion

For HPLC method optimization different ratios of methanol and water were tried but it was found that the peaks got

Table 2 Result of precision study.

Concentration (μg/ml)	Interday (RSD,%)	Repeatability (RSD,%)	Concentration (μg/ml)	Interday (RSD,%)	Repeatability (RSD,%)
2	1.18	0.15	10	0.18	0.10
4	0.97	0.40	20	0.19	0.08
6	0.50	0.80	30	0.12	0.10

Table 3 Robustness.

Robustness study of Atorvastatin				Robustness study of Nicotinic acid			
Chromatographic changes				Chromatographic changes			
Factor	Level	Assay	%Deviation	Factor	Level	Assay	%Deviation
Flow rate(ml/min)				Flow rate(ml/min)			
0.8	−0.2	99.06	0.03	0.8	−0.2	99.8	0.06
1	0	99.03	0	1	0	99.74	0
1.2	0.2	99.01	0.02	1.2	0.2	99.69	0.05
% Deviation at flow rate 0.8 ml is 0.03 and at 1.2 ml is 0.02% respectively				% Deviation at flow rate 0.8 ml is 0.06 and at 1.2 ml is 0.05% respectively			
pH				pH			
4.3	−0.2	98.99	0.04	4.3	−0.2	99.6	0.14
4.5	0	99.03	0	4.5	0	99.74	0
4.7	0.2	99.05	0.02	4.7	0.2	99.84	0.1
%Deviation at pH 4.3 is 0.04 and at 4.7 is 0.02% respectively				%Deviation at pH 4.3 is 0.14 and at 4.7 is 0.10% respectively			
Wavelength(nm)				Wavelength(nm)			
256	−5	99.08	0.05	256	−5	99.82	0.08
261	0	99.03	0	261	0	99.74	0
266	5	98.97	0.06	266	5	99.55	0.19
% Deviation at Wavelength 256 is 0.05 and at 266 is 0.06% respectively				% Deviation at Wavelength 256 is 0.08 and at 266 is 0.19% respectively			

Table 4 Assay results of combined dosage using proposed method (mean values of three determinations).

Formulation	Labeled amount (mg)		Amount obtained (mg)		% Recovery	
	Atorvastatin	Nicotinic acid	Atorvastatin	Nicotinic acid	Atorvastatin	Nicotinic acid
TONACT plus	10 mg	375 mg	9.90 mg ± 0.08	374.04 mg ± 0.9	99.031% ± 0.8%	99.744% ± 0.24%

distorted when methanol was used. Hence, methanol was replaced with acetonitrile. Different ratios of acetonitrile and water were tried. It was found that acetonitrile: water in the ratio 85:15 v/v at pH 4.5 adjusted with phosphoric acid at a flow rate 1.0 mL/min gave good peaks. Hence a different combination of mobile phases and chromatographic conditions was tried acetonitrile: distilled water (85:15) at pH 4.5 adjusted with phosphoric acid as the mobile phase, Agilent ZORBAX SB-C18 (150 × 4.6 mm, 3.5 u) column, 1 mL/min flow rate, 20 µL injection volume, 18 °C column oven temperature, 261 nm wavelength was found to be suitable for combination. These chromatographic conditions gave a retention time of 6.092 and 3.125 min for Atorvastatin and Nicotinic acid respectively.

Specificity of the method was checked by injecting the placebo solution, no peaks were found at the retention time of Atorvastatin and Nicotinic acid. The stability of the sample solution was evaluated by preparing a sample solution as per the proposed method and analyzed initially and at 1 h time intervals up to 24 h by keeping the sample solution at room temperature. A good linear relationship as indicated by correlation coefficient value 0.999 was observed for Atorvastatin over the range of 2–12 µg/ml and over the range of 10–80 µg/ml for Nicotinic acid correlation coefficient of 0.999. Intermediate precision was done by changing the analyst, column, chemical make with the same chromatographic conditions and the obtained results were within the limits. The robustness of the method was evaluated by deliberately varying the chromatographic conditions of the method such as, flow rate, pH and wavelength. The parameters like tailing factor and retention times showed adherence to the limits. The accuracy of the method was determined by recovery studies and the percentage recovery was calculated. The recoveries of Atorvastatin and Nicotinic acid were found to be in the range of 98.93–101.16% and 98.83–101.20%, respectively. The proposed liquid chromatographic method was applied to the determination of Atorvastatin and Nicotinic acid in their combined dosage forms (Tablet Tonact plus). The results for Atorvastatin and Nicotinic acid were comparable with the corresponding labeled amounts (Table 6).

6. Conclusion

The HPLC method developed for the analysis of mixture of Atorvastatin and Nicotinic acid in their pharmaceutical preparations is precise, accurate and with a short run time. The method was fully validated showing satisfactory data for all the method validation parameters tested. The developed method can be conveniently used by the quality control department to determine the assay of pharmaceutical preparations.

Acknowledgements

The authors are thankful to Mrs. Fatima Rafiq Zakaria Chairman Maulana Azad Educational Trust and Dr. M.H.G. Dehghan, Principal,Y.B. Chavan College of Pharmacy, Dr. Rafiq Zakaria Campus, Aurangabad 431 001 (M.S.), India for providing the laboratory facility.

References

Altuntas, T.G. et al, 2004. Liquid chromatographic determination of Atorvastatin in bulk drug, tablets and human plasma. J. Liq. Chromatogr. Relat. Technol. 27 (1), 83–93.

Bouabidi, A. et al, 2010. Critical analysis of several analytical method validation strategies in the framework of the fit for purpose concept. J. Chromatogr. A 1217 (19), 3180–3192.

Erk, N., 2003. Extractive spectrophotometric determination of Atorvastatin in bulk and pharmaceutical formulations. Anal. Lett. 36 (12), 2699–2711.

Erturk, S. et al, 2003. An HPLC method for the determination of Atorvastatin and its impurities in bulk drugs and tablets. J Pharm. Biomed. Anal. 33 (5), 101–123.

Gennaro, A.E., 2000, 21 Ed.. In: Remington's The Science and Practice of Pharmacy, vol. II Mack Publishing Co, Easton, PA, 1294, 1368, 1705.

ICH, Q2 (R1), 2005.Guideline on validation of analytical procedures: text and methodology. In: Proceedings of the International Conference on Harmonization, Geneva.

Iwaki, M. et al, 2000. Chromatographic and capillary electrophoretic method for analysis of Nicotinic acid and its metabolites. J.Chromatogr., B: Biomed. sci. Appl. 745 (1), 149–157.

Rozet, E. et al, 2011. Advances in validation, risk and uncertainty assessment of bioanalytical methods. J. Pharm. Biomed. Anal. 55 (4), 848–858.

Shen, H.R. et al, 2006. HPLC assay and pharmaco-kinetic study of Atorvastatin in beagle dog after oral administration of Atorvastatin self-micro emulsifying drug delivery system. Pharmazie 61 (1), 18–20.