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Research Article

SIMULTANEOUS ESTIMATION AND VALIDATION OF ATORVASTATIN CALCIUM AND NICOTINIC ACID IN COMBINED TABLET DOSAGE FORM BY RP HPLC METHOD

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ABSTRACT

A new simple, specific, precise and accurate revere phase liquid chromatography method has been developed for estimation of atorvastatin calcium (AST) and nicotinic acid (NA) simultaneously in a combined tablet dosage forms. The chromatographic separation was achieved on a 5 – micron C 18 column (250x 4.6mm) using a mobile phase consisting of a mixture of Acetonitrile: Ammonium Acetate buffer 0.02M (68:32) P^H 4.5. The flow rate was maintained at 0.8 ml / min. The detection of the constituents was done using UV detector at 245 nm for AST and NA. The retention time of AST and NA were found be 4.803 ± 0.0013min and 3.220 ±0.0008 min respectively. The developed method was validated for accuracy, linearity, precision, limit of detection (LOD) and limit of quantification (LOQ) and robustness as per the ICH guidelines.

Keywords: Simultaneous estimation, Atorvastatin calcium, Nicotinic acid, Tablet form and RP HPLC

INTRODUCTION

Atorvastatin calcium is chemically a calcium salt of (β R, 8 R)-2-(4 – fluoro-phenyl) – α, δ di hydroxyl 5(1 methyl ethyl) 1, 3, phenyl, 4 (phenyl amino) carbonyl) -1 H pyrrole- heptanoic acid tri hydrate used as antihyperlipidaemic. It is official in Indian pharmacopoeia [1] Nicotinic acid is chemically pyridine-3-carboxylic acid, used for pellagra and hyperlipoproteinaemia. It is official both in I.P and B.P. [2]

Detailed survey of literature for atorvastatin calcium **(AST)** revealed several methods based on different techniques like extractive Spectrophotometry [3], HPLC [4-8], HPLC [9-11] for its determination in human serum, capillary electrophoresis [12], HPTLC for its determination in pharmaceutical, [13] derivative spectrophotometric methods [14] RP HPLC method with other combination [15]

Similarly literature survey for Nicotinic acid **(NA)** revealed several methods based on Spectrophotometry, [16] RP- HPLC [17-19], tandem mass spectrophotmetry[20]. Methods are reported for estimation of atorvastatin calcium and nicotinic acid in combination by RP- HPLC [21] and stability indicating RP HPLC method [22] in tablet dosage form. This paper describes a simple, precise and accurate RP HPLC method for the estimation of AST and NA combination in a tablet dosage form.

MATERIAL AND METHODS

Chemical and reagents: AST was the generous gifts from Biocon Limited Bangalore, and nicotinic acid was procured from Qualigens Fine Chemicals (Glaxo Ltd). Combination of these drugs was purchased from the local market (TONACT PLUS containing Atorvastatin calcium 10 mg and nicotinic acid 375 mg as per the label claim, marketed by Lupin Pharmaceuticals, India). HPLC grade Acetonitrile, ammonia, glacial acetic acid was procured from Merck.

RP HPLC instrumentation and chromatographic conditions

The following chromatographic conditions were established for the separation of drug and maintained the same parameter throughout the method.

System High performance liquid chromatograph10AT SHIMADZU- SPD10A detector

Column	SS Grace- C18, 250X 4.6 mm, 5 µm
Detector	UV detector
Mobile phase	Acetonitrile: Ammonium acetate 0.02M (68:32) P^H 4.5.
Detection wavelength	245 nm
Mode	Isocratic
Sample Size	20 µl
Temperature	Room temperature

Preparation of standard stock solution

a) Preparation of standard stock solution of AST: An accurately weighed quantity equivalent to 10 mg AST was dissolved in 25 ml of methanol and dissolved then the volume was made up to 50 ml with same solvent.

b) Preparation of standard stock solution of NA: An accurately weighed quantity equivalent to 375 mg NA was dissolved in 25 ml of methanol and dissolved then the volume was made up to 50 ml with same solvent.

Validation of the method [23-24]

The method was validated as per ICH guide line. The parameters checked were linearity, accuracy, precision, limit of detection, limit of quantification, robustness and specificity.

System suitability testing A standard solution was prepared using AST and NA working standard as per the test method and was injected nine times into the HPLC system. The parameters like theoretical plates, tailing factor and resolution for the standard solution were calculated.

Calibration curve

From the working standard solution of AST (200 µg/ml) and NA (7500 µg/ ml), appropriate aliquots of AST and NA stock solution were diluted in methanol to obtain final stock solution of 2, 4, 6, 8 10 µg/ml of AST and 75, 150, 225, 300, 375 µg/ml of NA.The solutions were injected using a 20 µl fixed loop system and chromatogram were recorded. Calibration curve were constructed and regression equation were computed for AST and NA.

Specificity

A blank solution (mobile phase) was injected and the chromatogram showed no inferring peaks at retention time of the two drugs. The chromatogram of AST and NA extracted from the tablet dosage form were compared with those acquired from AST and NA standards, correlation was good (in terms of retention time and area) indicates specificity of method.

Precision: (Reproducibility)

The precision of the method was verified by performing the intraday and interday precision. The intraday and interday precision of the proposed method was determined by estimating the corresponding response three times on the same day and on three different days.

Accuracy (% Recovery)

For accuracy of method, recovery studies were carried out by applying a known amount of standard AST and NA at a level of 80,100,120 % to the sample solution (standard addition method). Three determinations were performed at each level, using same chromatographic condition as describe above.

Limit of Detection (LOD) and Limit of quantification (LOQ)

The LOD and LOQ were calculated using following equations as per International conference on Harmonization guide line

$$LOD = 3.3 \times \sigma / S \qquad LOQ = 10 \times \sigma / S$$

Where σ is standard deviation of the response and S is the standard deviation of y intercept of regression lines.

Robustness

Robustness was checked by making a slight deliberate change in the experimental procedure like slight change in the temperature, flow rate and PH and the data are expressed in terms of relative standard deviation.

Analysis of the marketed products

To find the content of the marketed formulation, (TONACT PLUS, Label Claim, 10 mg of AST and 375 mg of NA), twenty tablets were weighed and average weight was determined, powdered, from this equivalent weight of 10 mg for AST and 375 mg of NA was transferred into a 50 ml volumetric flask, containing 15 ml of methanol and sonicated for 30 minutes, filtered through Whatmann filter paper No.41and then volume was made up to 50 ml with methanol.

RESULTS AND DISCUSSION

Validation

To optimize the HPLC parameters, several mobile phases were tried and satisfactory results were obtained by using the mobile phase consisting of Acetonitrile: ammonium acetate 0.02M (68:32) at of PH of 4.5, with a flow rate of 0.8ml / min.UV detection was carried out at 245nm and run time was 15min.The selection of the mobile phase was made on the basis of resolution, asymmetric factor, and theoretical plates. The retention time of AST and NA was found to 4.803 ± 0.0013min and 3.220 ±0.0008 min respectively. A typical chromatogram of the test is shown in fig.1.

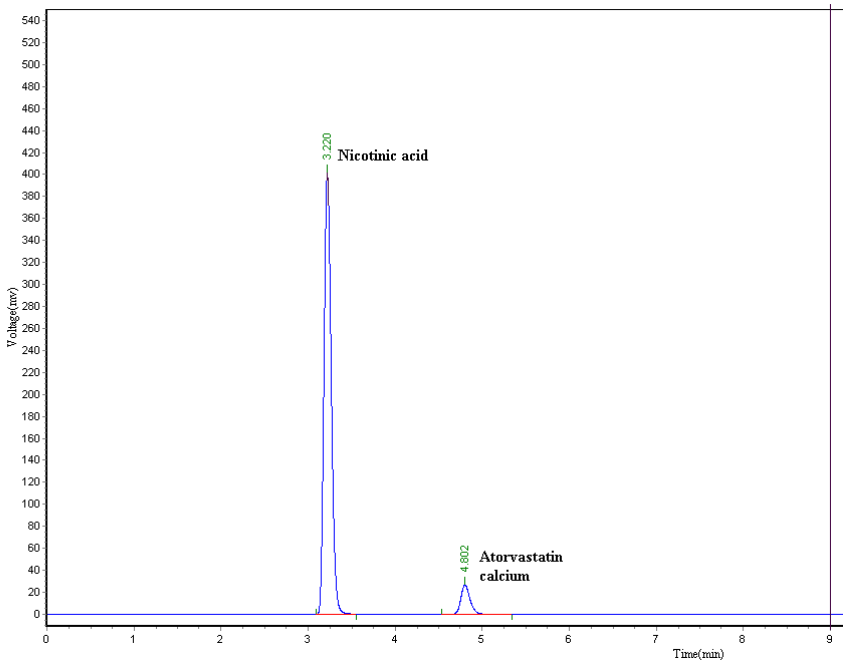

Fig. 1A: Representative Chromatogram of Atorvastatin Calcium and Nicotinic acid at 245 nm

System suitability parameters

System suitability parameters proved that the proposed method suits for the simultaneous estimation of AST and NA. After various trials performed, chromatogram for AST and NA was found satisfactory on, using mobile phase composition of Acetonitrile: Ammonium acetate 0.02M (68:32) PH 4.5.Drug peak was found to be symmetrical as observed from asymmetry factor of 1.4177 for AST and 1.1902 for NA. Resolution of the proposed method was found to be satisfactory and the results are given in table 1.

Table 1: Study of System Suitability Parameters

S. No.	Parameters	AST	NA
1	AUC(Area under the curve) *	188549.4964	2293720.083
	±S.D	1474.3166	618.7871
	% RSD	0.007819	0.30164
2	Resolution	2.8086	
3	Theoretical levels	10508.5698	7020.8085
4	Tailing factor	1.2463	1.1902
5	Asymmetry	1.4177	1.1902

* Mean of nine trials

Linearity

The linear regression data revealed a good linear relationship over the concentration range of 2-10 µg/ ml for AST and 75-375 µg/ ml for NA with correlation coefficient (r^2 = 0.9998)for AST and correlation coefficient (r^2 =0.9993)for NA respectively. The results are showed in table 2 and fig 2 and fig 3

Specificity

The method was found to be specific since no interferences chromatograms were seen when carried out in presence of additives, when only mobile phase was injected.

Precision

The proposed method was found to be precise as indicated by percent RSD not more than 2% as per ICH guidelines for interday and intra day determination. The results are shown in the table 1

Accuracy

The proposed method when used for the estimation of AST and NA from pharmaceutical dosage form after spiking with the standard, afforded recovery of 99.3% (80%), 99.51%(100%), 99.9%(120%) at different levels were found for AST and 99.41%(80%), 100.1% (100%), 100.03%(120%) for NA respectively, as shown in table 3 and table 4

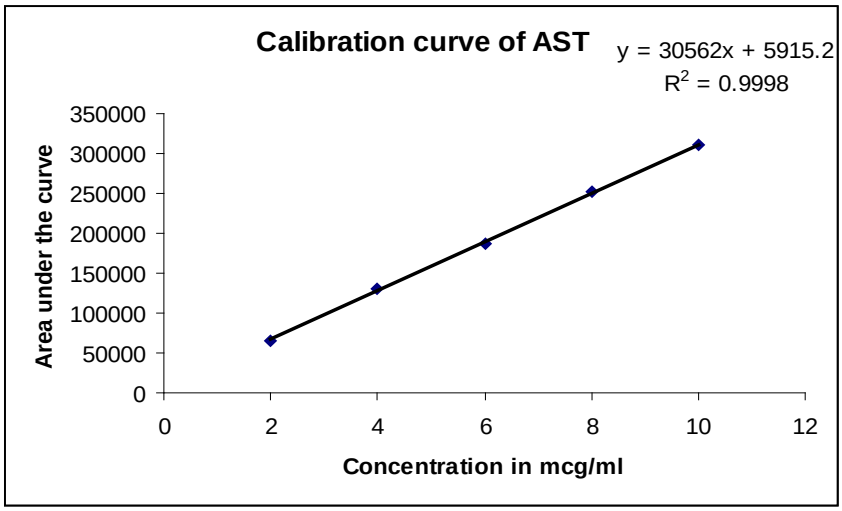

Fig. 2: Linearity graph of Atorvastatin Calcium (Mean of six trials**)**

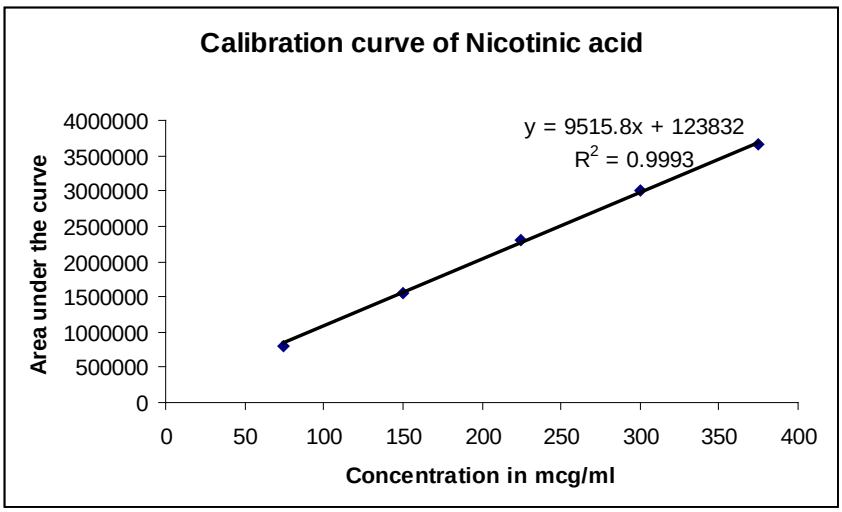

Fig. 3: Linearity graph of Nicotinic acid (Mean of six trials**)**

Table 2: Linear regression data of AST and NA

S. No	Parameters	AST	NA
1	Detection Wavelength	254 nm	254 nm
2	Linearity Range µg/ ml	2-10	75-375
3	Correlation Coefficient(r^2)	0.9998	0.9993
4	Linearity Regression equation (y=mx+c)	Y=30562x+5915.2	Y=9518.8x+123832
5.	LOD	0.08ng/ml	0.31ng/ml
6	LOQ	0.24ng/ml	0.95ng/ml
7.	Precision(%RSD)		
	Intraday	0.8187	0.3437
	Interday	0.8296	0.2483

Table 3: Recovery studies of Atorvastatin Calcium

	Excepted concentration		
	Level of recovery		
	80%	**100%**	**120%**
	8µg/ ml	**10 µg/ ml**	**12µg/ ml**
Peak Area*	151020.145	189180.536	227921.974
%RSD	0.1098	0.9515	0.3087
Mean % recovery	99.3	99.51	99.9

* Mean of three trials

Table 4: Recovery studies of Nicotinic Acid

	Excepted concentration		
	Level of recovery		
	80%	**100%**	**120%**
	300 µg/ ml	**375 µg/ ml**	**450 µg/ ml**
Peak Area*	1816356.75	2286236.166	2741634.416
%RSD	0.1540	0.1796	0.2243
Mean % recovery	99.41	100.1	100.03

*Mean of three trials

Limit of Detection (LOD) and Limit of quantification (LOQ)

The limit of detection was found to be 0.08 ng/ml and 0.31 ng/ml and limit of quantification were found to be 0.24 ng/ml and 0.95 ng/ml for AST and NA respectively. The results are shown in table 1

Robustness

Robustness was checked by making a slight deliberate change in the experimental procedure by slight change in the temperature, flow rate and P^H and no significant change in area and retention time was found in all the parameters and all the values were found to be with in 2% relative standard deviation. The results of robustness are given in the table 5, 6, 7.

Analysis of the marketed products

The proposed method was applied successfully to determine the content of AST and NA in pharmaceutical product. The results are expressed in terms of percentage in Table 8

Table 5: Robustness for flow rate studies (temperature)

Temperature (oC)	**Area (AST) ***	**Area (NA) ***
30	189975.437	2281845.56
%RSD	0.5172	0.0252
25	189641.3783	2303419.1
%RSD	0.2823	0.0063
35	190944.7916	2319482.99
%RSD	0.0557	0.0427

* mean of three readings.

Table 6: Robustness for P^H studies

P^H	**Area (AST) ***	**Area (NA) ***
4.5	186207.7657	2321064.75
%RSD	1.7364	1.6636
4.4	184836.6036	2323249.34
%RSD	0.04420	0.0129
4.6	1839605.55	2358738.3
%RSD	0.0027	0.0040

* mean of three readings.

Table 7: Robustness for Flow rate Studies

P^H	**Area (AST) ***	**Area (NA) ***
0.8	192991.3647	2313118.667
%RSD	1.5175	1.2715
0.798	195246.6576	2339216.59
%RSD	0.2210	0.01761
0.802	192971.653	2318706.51
%RSD	0.3006	0.0208

* mean of three readings.

Table 8: Analysis of tablet formulation

S.No.	**Parameters**	**AST**	**NA**
1	Label claim(mg/tab)	10 mg	375mg
2	Drug content %	99.51±0.9474	100.1±0.1779
3	% RSD	0.9520	0.1776

CONCLUSION

The proposed HPLC method is simple and the total run time for the two components is less than 7 min. The quantitation of each component was not affected by any of the possible interfering substances included during tablet manufacturing. The method is accurate and precise as indicated from the recovery study. It can be concluded that the proposed HPLC method has great promise for the simultaneous determination of two components in pharmaceutical formulations.

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REFERENCES

1. Indian Pharmacopoeia Health ministry of India, 2010; Vol.II 849
2. Indian Pharmacopoeia Health ministry of India, 2010, Vol.II 1776, British Pharmacopoeia, the stationery office on behalf of the medicines and health care products regulatory agency, 2007;Vol.II 1466-1467.
3. Erk.N Extractive spectrophotometric determination of atorvastatin in bulk and pharmaceutical formulation Analytical Letters 2003; 36 (12): 2699-2711
4. Jemal M, Ouyang Z, Chen B C, Teitz D Quantitation of atorvastatin and its bio-transformation products in human serum by HPLC with electro spray tandem mass spectrometry. Rapid Commun Mass Spectrom, 1999; 13: 1003-1015
5. Erturk S, Sevinc A E, Ersoy L, Ficicioglu S HPLC method for the determination of atorvastatin and its impurities in bulk drug and tablets. J Pharm Biomed Anal 2003;33: 1017-1023
6. Puratchikody A, Valarmathy R, Shiju P J Rukumani K. RP-HPLC determination of atorvastatin calcium in solid dosage forms. Pharm Rev 2003; 1:79-80
7. Sankar D G, Raju M S M, Sumanth K, Latha P V M HPLC method for estimation of Atorvastatin in pure and pharmaceutical dosage form. Asian J Chem 2005;17: 2571-2574
8. Stanisz B, Kania L Validation of HPLC method for determination of atorvastatin in tablets and for monitoring in solid phase. Acta Pol Pharm 2006; 63: 471-476
9. Bahrami G, Mohammadi B, Mirzaeei S, Kiani A Determination of atorvastatin in human serum by reverse phase high performance liquid chromatography with UVdetection. J Chromatogr B: Analyt Technol Biomed Life Sci 2005; 826: 41-45.
10. Hermann M, Christensen H, Reubsaet J L Determination of atorvastatin and metabolites in human plasma with solid phase extraction followed by LC-tandem MS. Anal Bioanal Chem 2005; 382: 1242-1249
11. Petkovska R, Comett C, Dimitrovska A, Development and Validation of Rapid Resolution RP-HPLC method for simultaneous determination of Atorvastatin and related compounds by use of chemometrics, Analytical letters, April 2008; 41(6):992-1009.
12. Guiphen E, Sisk G D, Scully N M, Glennon J D Rapid analysis of atorvastatin calcium using capillary electrophoresis and microchip electrophoresis. Electrophoresis 2006; 27: 2338-47
13. Dhaneshwar S R, Yadav S, Mhaske A, Kadam S HPTLC method for determination of content uniformity of atorvastatin calcium tablets. Indian J Pharm Sci 2005; 67: 182-186
14. Smita t. Kumbhar, Swapnil d. Jadhav, Neela m. Bhatia and Manish s. Bhatia Development and validation of derivative spectrophotometric method for estimation of atorvastatin calcium and amlodipine besylate in tablet dosage form, Int J Pharmacy and Pharm Sci 2011; 3, (Suppl 4): 195-197
15. Saravanamuthukumar M, Palanivelu M, Anandarajagopal K, Sridharan D Simultaneous Estimation and Validation of Atorvastatin Calcium And Ubidecarenone (Coenzyme Q10) in Combined Tablet Dosage Form by RP-HPLC Method. Int J Pharmacy and Pharm Sci 2010;2(2):36-38.
16. Illarionova E A, Syrovatskii I P, Abramova L V Spectrophotometric determination of nicotinic acid. Zavodskaya Laboratoriya 2002; 68: 9-12.
17. Tsuruta Y, Kohashi K, Ishida S, Ohkura Y Determination of nicotinic acid in serum by high performance liquid chromatography with fluorescence detection. J Chromatogr 1984; 30: 309-15
18. Tokunaga H, Okada S, Kimura T (1989) Determination of nicotinic acid in injections by high performance liquid chromatography. Eisei Shikenjo Hokoku 107: 108-112
19. Zarzycki P K, Kawalski P, Nowakowska J, Lamparczyk H High performance liquid chromatographic and capillary electrophoretic determination of free nicotinic acid in human plasma and separation of its metabolites by capillary electrophoresis. J Chomatogr A 1995; 709: 203-208
20. Hsieh Y, Chen J Simultaneous determination of nicotinic acid and its metabolites using hydrophilic interaction chromatography with tandem mass spectrometry. Rapid Commun Mass Spectrom 2005; 19: 3031-3036
21. Shah DA, Bhatt KK, Mehta RS, Shankar MB, RP-HPLC method for the determination of Atorvastatin Calcium and Nicotinic acid in combined tablet dosage form, Indian Journal of Pharm. Sci., 2007; 69(5):700-703.
22. Krishna R.Gupta, Sonali S. Askarkar and Sudhir G.Wadodkar Stability Indicating RP-HPLC Method for Simultaneous Determination of Atorvastatin and Nicotinic Acid from Their Combined Dosage Form Eurasian J. Anal. Chem. 2009; 4(3): 294-303,
23. ICH, Q2B Validation of Analytical Procedure: Methodology, in: Proceeding of the International Conference on Harmonization, Geneva, March 1996.
24. ICH, Guidance on Analytical Method Validation, in: Proceedings of International Convention on Quality for the Pharmaceutical Industry, Toronto, Canada, September 2002.