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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ATORVASTATIN AND NIACIN IN BULK AND COMBINED TABLET DOSAGE FORM BY USING UV–VISIBLE SPECTROSCOPY

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ABSTRACT

A simple, precise, rapid and sensitive UV spectrophotometric method was developed and validated for estimation of Niacin (NCN) and Atorvastatin (ATR) in pure and tablet dosage form using methanol: water (10:90 v/v) mixture as solvent by simultaneous equation method. The absorption maxima were found to be 257nm and 242 nm for Niacin and Atorvastatin calcium respectively. Beer's law range was in the concentration range of 10-60 µg/ml for Niacin and 5-30 µg/ml for Atorvastatin calcium with correlation coefficient within the range of 0.997 – 0.998 for both the drugs. Recovery studies were performed to assess the accuracy of the methods, the results were found to be between 99.8% to100.1% for Atorvastatin and 98.75% to 100.6% for Niacin. LOD and LOQ were found to be 0.366 µg/ml and 1.1 µg/ml for Atorvastatin and 1.65 µg/ml and 5.0µg/ml for Niacin. Robustness was done by slightly changing the solvent composition and λ_{max}. The above method has been validated according to ICH guidelines. Hence the above methods can be used for routine analysis of Niacin and Atorvastatin in pharmaceutical industries.

Keywords – *Niacin, Atorvastatin, Simultaneous equation, LOD, LOQ*

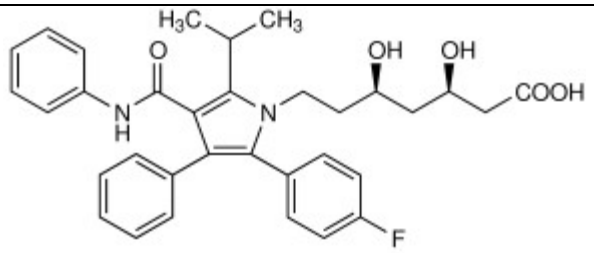
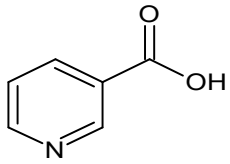
1. INTRODUCTION

Spectrophotometric analysis continues to be one of the most widely used analytical technique. The greatest use of UV-Vis absorption spectroscopy lies in its application to quantitative measurements. The reasons for this stem from the ease with which most spectrophotometric measurements can be made, their sensitivity and precision, and the relatively low cost of instrument purchase and operation.

Estimation of Niacin and Atorvastatin in combine dosage form was already reported by HPLC and UV methods in literature review. But a simple method with good sensitivity and linearity by using cost effective solvent was not reported. The present aim of the study was to develop a simple, precise, rapid and sensitive UV spectrophotometric method for estimation of Niacin (c) and Atorvastatin (ATR) in pure

and tablet dosage form and to validate according to ICH guidelines. Attempts will be made to use for routine analysis of Niacin and Atorvastatin in pharmaceutical industries [1-11].

Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic LDL receptors on the cell-surf ace to enhance uptake and catabolism of LDL. Atorvastatin also reduces LDL production and the number of LDL particles[12]. Niacin is used in treatment of pellagra, and used in addition to other lipid-lowering medication[13-14].

 **(a) Atorvastatin**	Molecular Formula: $C_{33}H_{35}FN_2O_5$ IUPAC Name: 2-(p-fluorophenyl)-β, δ-dihydroxy-5-isopropyl-3-phenyl-4-(phenylcarbamoyl) pyrrole-1-heptanoic acid (1:2) trihydrate
 **(b) Niacin**	Molecular Formula: $C_6H_5O_2N$ IUPAC Name: pyridine-3-carboxylic acid
Chemical Structure of (a) Atorvastatin (b) Niacin	

2. MATERIALS AND METHOD

2.1 Materials

Atorvastatin (Purity-99.99%) and Niacin (99.98%) samples were provided by CosmePharma, Goa. Tablets containing Atorvastatin(10mg) and Niacin (375mg) were procured from local Pharmacy. Distilled water was used in the present study was made by double distillation procedure in institutional lab. UV-Visible spectrophotometer (Shimadzu (UV-1700)) with spectral band width of 0.1 nm and wavelength accuracy of + 0.5 nm with automatic wavelength correction was used to determine the absorbance.

Instrument/ equipment	Specification
Detector	Photo multiplier tube
Software	Carywin
Uv-spectrophotometer	Shimadzu (UV-1700)
pH meter	Digital pH meter (Lab India PICO$^+$)
Analytical balance	Mettler Toledo (XS105)
Ultra-sonicator	Ultrasonic Bath Sonicator (Bandelin Sonorex)

All the glass wares used were made of Borosilicate glass and the solvents and prepared solutions were filtered through Nylon (0.45µ) filters**.**

2.2 Preparation of standard solutions

Take 1g of Atorvastatin and 1g Niacin powder and make up to 1000ml to get the concentration about 1000µg per ml for Atorvastatin and Niacin. From this take 0.5 ml ,1ml, 2ml, 3ml, 4ml, 5ml, 6ml and make up the100ml volume to get the concentrations about 5 to 60 µg/m solutions of Niacin and atorvastatin.

2.3 Preparation of test solution

Weigh accurately 20 tablets and calculate the average weight, then transfer the powder equivalent to 10mg of Atorvastatin and Niacin in to 100ml volumetric flask, add sufficient amount of solvent to dissolve and make the volume up to 100ml with solvent from this take 20ml and make up to100ml to get 20 µg/ml solution.

2.4 Method development

The solvents for the experimental study were selected based on the solubility of the drug. The solubility was performed using common solvents like methanol, water, acetonitrile. The method of analysis was carried out on the basis of absorption of drugs (Atorvastatin and Niacin) at the wavelength maximum of the each other and adsorption maximum were found to be 242 nm and 257 nm for Atorvastatin and Niacin respectively. The absorptivity values E (1%, 1cm) were determined for two drugs at all selected wavelengths. The concentration of two drugs in mixture was calculated by using simultaneous equations method[4,5].

The absorptivities of Atorvastatin at λ1(242) and λ2(257) is aX1 and aX2 ·

The absorptivities of Niacin at λ1 and λ2, aY1 and aY2 ·

The absorbances of the diluted sample(mixture) at λ1 and λ2, A1 and A2

Let Cx and Cy be the concentration of Atorvastatin and Niacin respectively in the sample. The absorbance of the mixture is the sum of the individual absorbances of X andY

At λ1 A1 =a X1 b Cx + aY1b Cy ---------- (1)

At λ2 A2 =a X2 b Cx + aY2 b Cy ---------- (2)

For measurements in 1 cm cells b=1

Rearrange eq. (2) Cy = A2 - a X2 b Cx/ aY2

Substituting for Cy in eq. (1) and rearranging

Cx = A2 aY1 - A1 aY2/ a X2 aY1 - a X1aY2 ----------(3)

Cy = A1 a X2 - A2 a X1/ a X2 aY1 - a X1aY2 --------- (4)

2.5 Method validation

The proposed method was validated as per the recommendations of ICH guidelines for the parameters like accuracy, linearity, precision, detection limit and quantitation limit robustness[6].

2.5.1 Accuracy

Accuracy of the method was determined by performing recovery studies recovery studies were carried out by standard addition method at three different levels (80%, 100% and 120%). A known amount of Atorvastatin and Niacin were added separately to pre-analyzed powder and percent recoveries were calculated at each level.

2.5.2 Linearity

The linearity of the drug was established by constructing the calibration curve with concentration on x-axis and absorbance on y-axis. From the calibration curve, it was over concentration range of 5µg/ml-60µg/ml for Niacin and Atorvastatin. The correlation coefficient (r^2) was calculated.

2.5.3 Precision

Precision determined by preparing the working standard solutions for Atorvastatin (5-25 µg/ml) and Niacin(10-62µg/ml). Absorbance's were checked at each concentration level thrice a day for three days to satisfy the interday and intraday precision. The % RSD of Atorvastatin and Niacin were Calculated for intraday and inter day precision respectively.

2.5.4 Sensitivity

The Limit of Detection (LOD) and Limit of Quantitation (LOD) of the developed method was calculated by the signal to noise ratio (S/N) using the following equations suggested by ICH guidelines[9,10].

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where, σ = standard deviation of the intercept of calibration curve and S = slope of the calibration curve.

2.5.5 Robustness

The robustness of the method was established by slightly changing the parameters like wave length and solvent composition ratio and calculate the % RSD[9].

3. RESULTS AND DISCUSSION

Initially, the solubility of Atorvastatin and Niacin was checked in various solvents. The drug was found to be soluble in methanol and water. From the overlain spectrum, it was observed that maximum absorbance of Atorvastatin was shown at 242 nm and Niacin was shown at 257nm. Hence, this value has been selected as detection wave length for the analysis. After several trials, a method using solvent composition of water and methanol in the ratio of 90:10.

The proposed method was validated according to Q2 specifications of the ICH guidelines. The mean recovery of pure drug from the analyzed solution was found to be in the range of 98.6 – 100.5 % for both drugs indicating the accuracy of proposed analytical method was with in the acceptance criteria. %RSD were found to be less than 2. % RSD of absorbance's values of Niacin working standard solutions were found to be in the range of 0.14- 1.94% and 0.22- 1.3%, for intra-day and inter-day precision respectively and 0.25-1.94% ,0.0- 2.0% for Atorvastatin. The low values of these statistical parameters validated the method. LOD and LOQ were found to be 0.366µg/ml and 1.1µg/ml for Atorvastatin and 1.65µg/ml and 5µg/ml for Niacin respectively which indicate the method has good sensitivity. The % RSD values for the Atorvastatin and Niacin were found to be below 2.0% by changing the parameters like wave length and solvent composition ratio, r^2 value of linearity curve was 0.998 and 0.997 for NCN and ATR, hence the method was said to be robust. Assay of the combined dosage form was performed by simultaneous equation method and the percentage purity of the Atorvastatin and Niacin was found to be 100% and 99.7% respectively. Thus, the proposed analytical method can be successfully applied for the routine analysis of Atorvastatin and Niacin in bulk and tablet dosage forms.

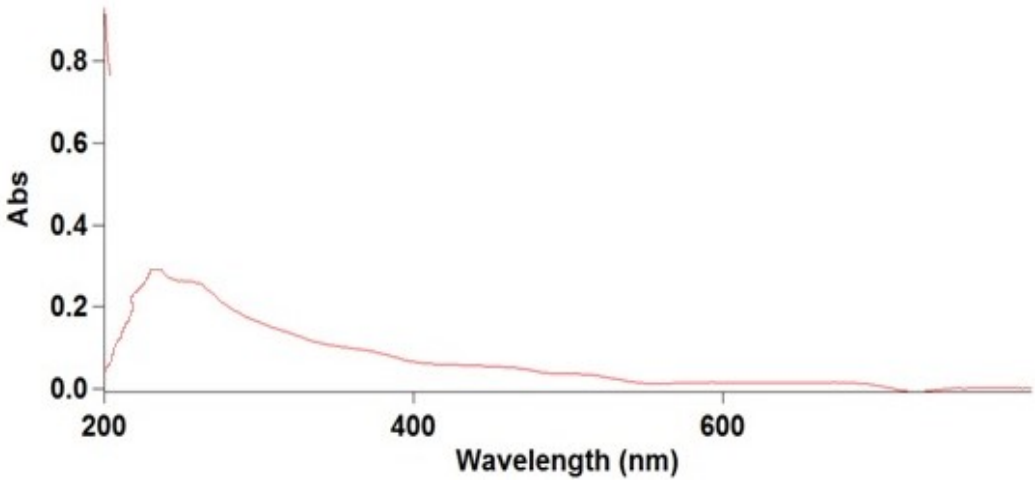


Fig.1: UV Spectra of Niacin

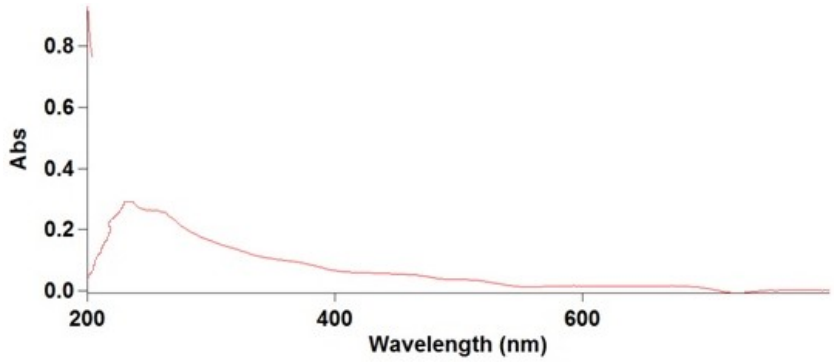


Fig.2: UV Spectra of Atorvastatin

Table 2: Absorbance's of Linearity standard solution of Niacin and Atorvastatin

Level	Concentration of Niacin(µg/ml)	Absorbance	Concentration of atorvastatin(µg/ml)	Absorbance
Level-1	10	0.101	5	0.043
Level-2	20	0.181	10	0.1
Level-3	30	0.301	15	0.138
Level-4	40	0.402	20	0.183
Level-5	50	0.492	25	0.238
Level-6	60	0.594	30	0.285

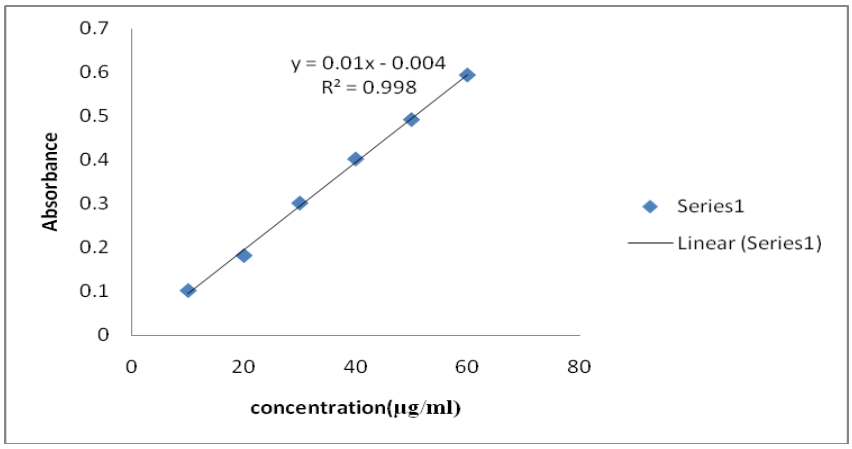


Fig.3: Calibration curve of Niacin

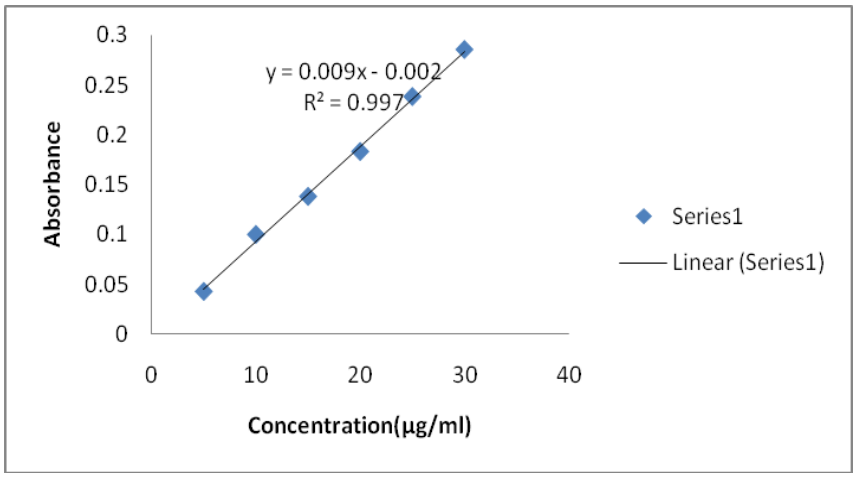


Fig.4: Calibration curve of Atorvastatin

Table 3: % Recovery data (Accuracy)

Name of the drug	% Level	Amount added (µg/ml)	Standard solution ABS	Standard +Spiked ABS	Amount recovered (µg/ml)	% Recovery
Atorvastatin	80%	16	0.162	0.341	35.93	99.8
	100%	20	0.181	0.361	40.05	100.1
	120%	24	0.215	0.396	47.20	98.3
Niacin	80%	16	0.168	0.353	36.23	100.6
	100%	20	0.185	0.371	39.5	98.75
	120%	24	0.231	0.416	47.90	99.7

Table 4: Precision data of Niacin

CONC (µg/ml)	DAY-1 ABS	DAY-2 ABS	DAY-3 ABS	AVG	STDEV	% RSD
10	0.104	0.104667	0.103	0.103889	0.000839025	0.807617
20	0.182667	0.18	0.181667	0.181445	0.001347329	0.742557
30	0.305667	0.307	0.306667	0.306445	0.000693755	0.226388
40	0.409	0.407	0.405667	0.407222	0.001677586	0.411958
50	0.492667	0.491667	0.487667	0.490667	0.002645751	0.539215
60	0.573333	0.588333	0.576667	0.579444	0.007876242	1.359275

Table 5: Precision data of Atorvastatin

CONC (µg/ml)	DAY-1	DAY-2	DAY-3	AVG	STDEV	% RSD
5	0.040633	0.0415	0.042333	0.041489	0.000850057	2.048889
10	0.099667	0.099333	0.099333	0.099444	0.000192835	0.193912
15	0.137	0.137	0.137	0.137	0	0
20	0.180333	0.181333	0.181	0.180889	0.000509211	0.281505
25	0.226	0.226333	0.227333	0.226555	0.000693755	0.306219

Table 6: Sensitivity data of Atorvastatin and Niacin

Parameter	Formula	Niacin	Atorvastatin
LOD	= 3.3 σ/S	1.65 µg/ml	0.366 µg/ml
LOQ	LO =10 σ/S	5.0µg/ml	1.1 µg/ml

Table 7: Robustness data of Atorvastatin and Niacin

Variation of Parameter		Parameters					
		Abs1	Abs-2	Abs-3	Average	STDEV	% RSD
λ_{Max} Atorvastatin	240	0.179	0.18	0.182	0.180333	0.003606	1.992017
	242	0.180333	0.181333	0.181	0.180889	0.0005092	0.281505
	244	0.179	0.18	0.182	0.180333	0.001528	0.847057
λ_{Max} Niacin	255	0.181	0.18	0.179	0.18	0.001	0.555556
	257	0.182	0.18	0.186	0.182667	0.0030550	1.672473
	259	0.182667	0.18	0.18167	0.181445	0.0013472	0.742557
Solvent atorvastatin	89:11	0.179	0.18	0.182	0.180333	0.001528	0.847057
	90:10	0.180333	0.181333	0.181	0.180889	0.0005092	0.281505
	91:9	0.179	0.18	0.182	0.180333	0.003606	1.992017
Solvent Niacin	89:11	0.182	0.18	0.186	0.182667	0.0030555	1.672473
	90:10	0.179	0.18	0.182	0.180333	0.001528	0.847057
	91:9	0.181	0.18	0.179	0.18	0.001	0.555556

Table 8: Absorbance and Absorptivity data of Atorvastatin and Niacin for simultaneous equation

Name of drug	Concentration (µg/ml)	Absorbance		Absorptivity	
		at λ_{max} 257	at λ_{max} 242	at λ_{max} 257	at λ_{max} 242
Niacin	20	0.181	0.12	0.00905	0.006
Atorvastatin	20	0.189	0.16	0.00945	0.008

Table 9: Assay data of Atorvastatin and Niacin

Name of the drug	Label claim	Amount found	% Purity
Atorvastatin	10mg	10mg	100%
Niacin	375mg	374mg	99.7%

4. CONCLUSION

It can be concluded that a simple, efficient and reliable UV method was developed and optimized for the estimation of Atorvastatin and Niacin in combined dosage form. The method developed had an improved linearity range and sensitivity. The developed method has been validated as per ICH guidelines and was found to be specific, accurate, precise, sensitive and robust. Hence, the method can be successfully applied for the routine analysis of bulk and in pharmaceutical formulation (tablet).

REFERENCES

1. Kamboj PC. Pharmaceutical Analysis.Vol1.2nded, Vallabh Publications, NewDelhi, 2007,p.1-2.
2. Indian Pharmacopoeia. Government of India Ministry of Health and Family Welfare. Indian Pharmacopoeia Commission. Ghaziabad, 2007, p.1674
3. Beckett AH, Stenlake JB. Practical Pharmaceutical chemistry. IV-Edition .part two, CBS Publishers and distributors, 2004 .p.326.
4. Sethi PD. Quantitative analysis of drugs in pharmaceutical formulations. 3rd ed, CBS Publisher, New Delhi, 1997, p.22-43.
5. Willard HH, Merritt LL, Dean JJ, Frank AS. Instrumental method of analysis. 7th ed, CBS Publishers and Distributors, New Delhi, 1986, p.2-5.
6. ICH. Q2B, Analytical validation-methodology. 1996.
7. ICH. Q2A, Text on validation of analytical procedure. 1994.
8. ICH. Q2 (R1), Validation of analytical procedures, text and methodology. 2005.
9. Hokanson GC. A life cycle approach to the Validation of analytical methods during Pharmaceutical product development. Part I, The initial validation process, Pharm Tech, 1994, p.118-30
10. Hokanson GC. A life cycle approach to the validation of analytical methods during Pharmaceutical product development. Part II, Changes and the need for additional validation, Pharm Tech, 1994, p.92-100.
11. Green JM. A practical guide to analytical method validation. Anal Chem, 1996, p.305.
12. Bernard S, Mathew M, et al. Simultaneous Estimation of Atorvastatin Calcium and Amlodipine besylate by UV Spectrophotometric method using hydrotropic solubilization. Hygeia.J.D.Med April 2013.vol.5 (1) : 105-112.
13. Ranganath MK, Raja Ram C. 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| Apr–June 2014. Vol. 4 : 70-77.
14. Ramesh S, Sheetal D, et al. Spectrophotometric method for Simultaneous Estimation of Atorvastatin and Niacin in tablet dosage. IRJP 2012.vol.3 (5): 364-367.