*Research Journal of Pharmaceutical Sciences*_____ __ISSN 2319 – 555X
Vol. **8(1),** 5-14, June **(2019)** *Res. J. Pharmaceutical Sci.*

Development and validation method for the determination of atorvastatin calcium tablets drugs by using UV-spectrophotometer in pharmaceutical formulation

Alrasheed A.W. Mohammed[1*], Salah Elnaiem Mohammed[2], Hanaa S. Boshra[3], Muzamil Hassaballa Abdelmula[4], Munzir Omer Mohammed[1] and Ahmed Mohammed Ali Hammad[4]

[1]Central laboratory, Shambat Campus, Khartoum University, Sudan
[2]Lecturar at Chemistry Department, Faculty of science, Khartoum University, Sudan
[3]Lecturar at Chemistry Department, Faculty of Pharmacy, Elrazi University, Sudan
[4]Excellece Center of Pesticide and Environmental Science, Department of crop protection, Faculty of Agriculture, Khartoum University, Sudan
vocard@hotmail.com

Available online at: www.isca.in, www.isca.me
Received 22nd March 2019, revised 5th June 2019, accepted 25th June 2019

Abstract

The objective of this research is to describe the optimization, validation, and application of spectrophotometric techniques for determination of Atorvastatin Calcium in their pharmaceutical formulation (tablets). In this paper simple, rapid, accurate and sensitive spectrophotometric methods have been developed and validated. This method is a direct spectrophotometric analytical method depend on dissolve of atorvastatin calcium in diluted anhydrous methanol methanol. The maximum absorption wavelength for determination of ATV drug was found to be 291 nanometer (nm), for Beer's law was obeyed in the concentration range from 5 to 35 µg/ml for UV- Spectrophotometric analysis method.

Keywords: Development, validation, determination, atorvastatin, UV-spectrophotometer, pharmaceutical formulation.

Introduction

In this study we have many properties of Atorvastatin calcium, it's a member of the drug class known as astatines, it reduce of 3-hydroxy-3-methylglutaryl-COA (HMG-CoA) reeducates this enzyme catalyzes the conversion of HMG-CoA to mevalonat[1]. Atorvastatin Calcium (ATV) is chemically known as (β*R*, 8R)-2-(4-fluorophenyl)-α, δ-dihydroxy-5-(1-methylethyl)-3-Phenyl-4-[(phenyl amino) carbonyl] 1H-pyrrole-1-heptanoic salt[2]. Atorvastatin is administered as the calcium salt of the active hydroxyl acid and between 10 and 80 mg per day is used to reduce the raised lipid levels in patients with primary hyperlipidemia [3]. In literature we have no enough validated methods for determination of ATV by using UV-Spectrophotometer in their pharmaceutical formulation and their related substances were used to validate the method[4].The empirical formula of atorvastatin calcium is $(C_{33}H_{34}FN_2O_5)_2Ca.3H_2O$ and its molecular weight is 1209.42g/mol. Its chemical structural:

Atorvastatin calcium (ATV) is a white - off to white crystalline powder that is Very slightly soluble in distilled water insoluble in aqueous solutions of pH 4 and below, neutral buffer solution, acetonitrile, and soluble in primary alcohol and weakly soluble in ethanol[5].

A few methods appeared analysis of ATV individually depend on Ultra Violet-Spectrophotometric, this methods mentions in the literature, especially chromatographic techniques are time consuming[6], costly and require expertise. The atorvastatin tablets are not yet official in any pharmacopoeia. To the best of my knowledge, no spectrophotometric methods have been described for the determination of drug in pharmaceutical formulation. There for, they were desirable to develop simple and fast procedures that could be applied in quality control laboratories for determination of drug.

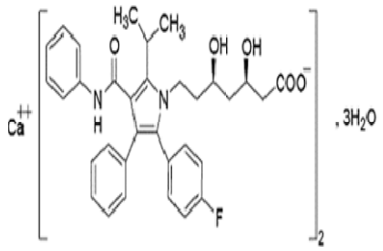

Figure-1: Chemical structure of atorvastatin calcium.

Validation of analytical methods: Validation is defined as finding or testing the truth of something. The purpose of any analytical procedure in validation is demonstrate this method has been suitable for analysis of this drug or not[7].

Method validation has been provides documented proof, and high degree of assurance that an analytical method used for a target analysis test is suitable for intended use. Become increasingly aware of the necessity of ensuring that the data submitted have been acquired for marketing authorization using

validated analytical methodology. The international conference on harmonization (ICH) has introduced guideline for analytical methods validation[8].

The most applied validation characteristics are: accuracy, precision (repeatability precision, intermediate precision and reproducibility), specificity, detection limit (LOD), quantitation limits (LOQ), linearity and range of linearity.

The parameters that required for validation and approach adopted for each particular are dependent on the type and applications of analytical method[8].

Materials and methods

Atorvastatin working standard and methanol spectroscopic (DUKSAN, pure chemical, Korea).

Instrumentation: UV-Visible Spectrophotometer model UV-1800 (SHIMADZU, KYOTO, JAPAN) and Solubility Studies of Atorvastatin by UV-Spectrophotometer Analysis Solubility of ATV was determined at room temperature. Amount of the drug was taken into volumetric flasks each containing combinations of methanol and deionized water with different concentrations by adding methanol firstly to the drug.

Determination of the wavelength of maximum absorption (λ max) of Atorvastatin Calcium: 0.200 (20mg) of ATV powdered and accurately weighed ,dissolved and transferred to a volumetric flask, anhydrous methanol was added and dissolved for dissolution and the solution was completed to the mark with deionized water to give 200µg/ml stock solution which was diluted suitably to produce. The above solution was diluted and scanned by UV-Spectrophotometer from the spectrum of the drug obtained λ_{max} of ATV was determined at 291nm.

Atorvastatin Calcium STD solution: 0.020g from Atorvastatin calcium were weighed, dissolved in anhydrous methanol then transferred quantitatively into volumetric flask, completed the volume to the mark with deionized water and mixed well. From this stock solutions, working standard solutions having different concentrations 5-80µg/mL were prepared by appropriate dilutions with the same solvent.

Preparation of sample solution of Atorvastatin for UV-Spectrophotometer: 20 tablets of ATV tablets drugs were selected randomly from provided samples were accurately weighed and powdered. A quantity of powder equivalent to 20 mg of Atorvastatin Calcium tablets drugs were accurately weighted and transferred to volumetric flask and dissolved in anhydrous methanol, and mixed, completed to the mark with deionized water. From the above solution 1.0ml was taken and transferred to volumetric flask and diluted to the mark with the same solvent to get 20µg/ml solution and measured at selected lambda.

FTIR Spectroscopy: Solid samples of tablets and standard ingredients were pressed into KBr pellets and recorded at frequencies from 4000 to 400cm^{-1}.

Results and discussion

At the beginning, we tried many methods to develop a method for the drug with different concentrations In this method UV-Spectrophotometer has been used, Atorvastatin tablets, this new method was developed and validated as assay methods for the quantitative determination of atorvastatin calcium in their pharmaceutical formulations (tablets), UV-Spectrophotometer was used.

Identification of Atorvastatin calcium (Active Ingredient): Identification of atorvastatin calcium (ATV) by using FTIR has been concern , the region of FTIR started from 400cm-1 to 4000cm-1, also it have region started from 4000cm-1 to 1500 cm-1 it called functional group region it interpret any FTIR spectrum. Second region called finger print region it less than 1500cm-1, it more complicated region in IR. It compare between standard drug spectrum and sample to attach it.

Shimadzu 8400S Fourier Transformation Infra-Red FTIR has been used to analyze of Atorvastatin Calcium for market samples using FTIR[10]. Many repetition has been repeated more than 20 times to get in Figure-2.

The IR spectrum showing percentage transmission (%T) versus wave number (cm-1) of Atorvastatin calcium (ATV) is shown in Figure-3 many characteristic of chromatogram of C=O stretching and aromatic N-H stretching at1649.81cm-1 and, 3364.21cm-1 respectively. However formulation show similar peaks but with a negligible shift for C=O stretching and aromatic N-H stretching at1647.67cm-1 and 3363.17cm-1. That prove from the figures that is ATV in nanoparticles doesn't undergo any chemical activity and reaction with any of the excipients used in this formulation.

A spectra of ATV showed above by using FTIR instrument showed exhibit many characteristics of different functional groups in different values started from 828 up to 3240 included aromatic functional groups include C-N, O-H, N-H, C=O, C=C, C-O, in ATV drug and aromatic substitution bands, structural of ATV calcium was shown in the above Figure-3.

Identification of Atorvastatin and excipient (Tablets 40 mg): Infrared (IR) spectroscopy was conducted using Thermo Nicolet Nexus 670 Spectrophotometer and the spectrum was recorded in the wavelength region of 6000 to 500cm-1. The procedure consisted of dispersing a sample (drug and excipients) in KBr and compressing into discs by applying a pressure. The pellet was placed.

Identification of Atorvastatin calcium and excipient (Tablets 40 mg) Tablets product specification: Drug under study

collected received from local market, multi tests has been done to confirm standard values of these parameters such as hardness, average weight, weight variation, friability, diameter, thickness, disintegration.

In the light path and the spectrum was obtained show Figure-3.

Determination of solubility: Atorvastatin calcium solubility was tested in different organic and aqueous solvents. It's found freely soluble in anhydrous methanol.

Method validation: This new method of analysis has been validated according to ICH guideline[11] roles such as robustness, precision, accuracy, limit of detection (LOD), ruggedness, specificity, limit of quantification (LOQ) and linearity.

Determination of maximum absorption: A standard solution of this sample has been scanned from 200nm up to 800nm for determination maximum absorbance of wavelength[12]. High absorbance has been obtained in 291nm by using anhydrous methanol. Beside this lambda many wavelength has been obtained in above range of scan. The drugs obey the Beer's law in the concentration range of 10mg/L to 100MG/l (µg/mL).

Ultraviolet absorption spectrum: An ultraviolet scan, generated using a Lambda 25 UV/VIS Spectrophotometer (SHIMADZU, KYOTO, JAPAN) at a scan speed of 480nm.min-1, is depicted in Figure-4.

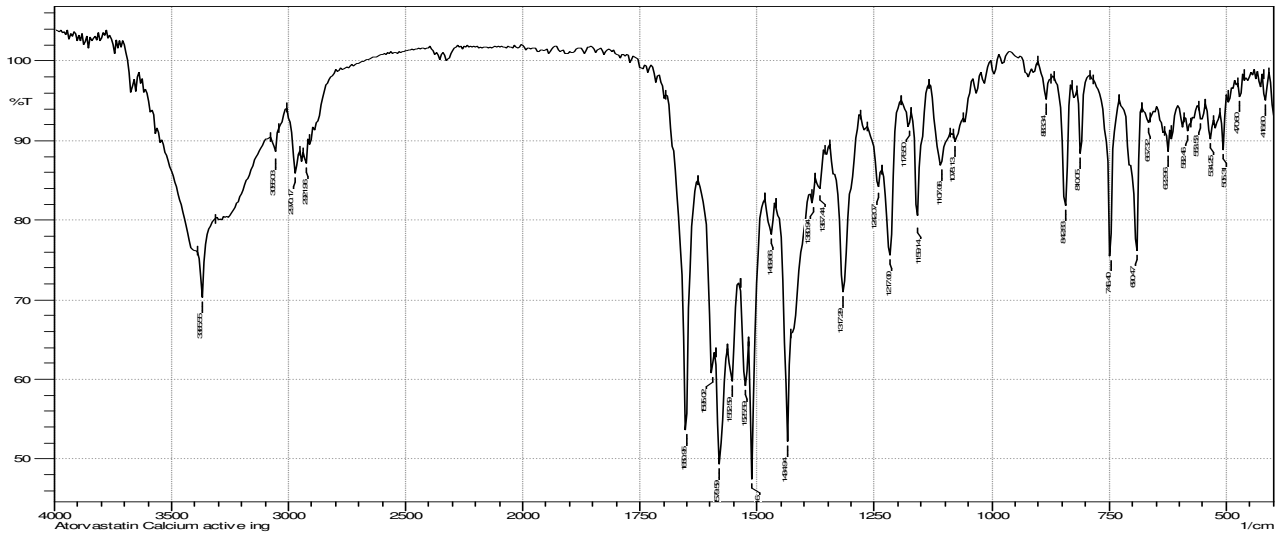

Figure-2: FTIR spectra of Atorvastatin STD.

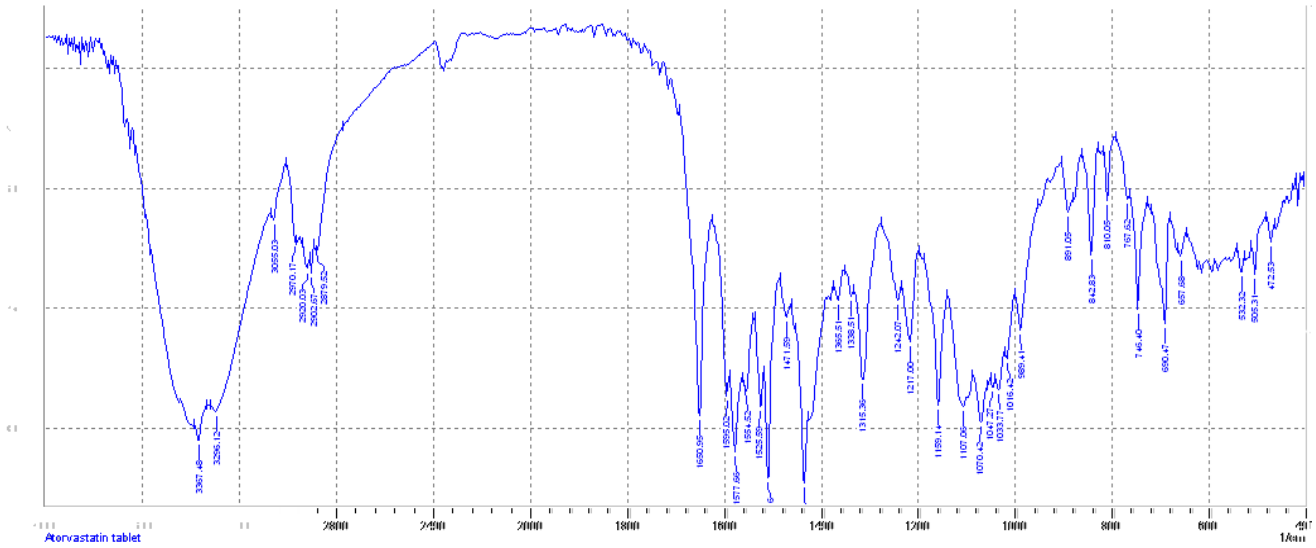

Figure-3: FTIR spectra of Atorvastatin tablets (sample).

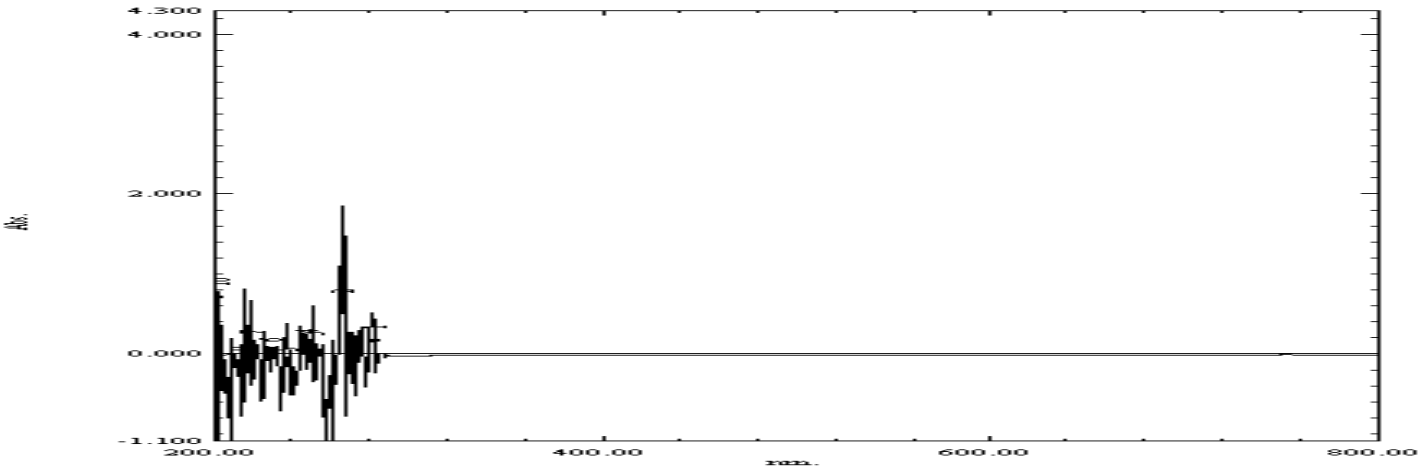

Figure-4: Absorption spectra of atorvastatin tablets drug solvent scanned spectral. λ_{max}= 202nm.

Table-1: The values of multi wavelength absorbance of drug solvent shows many different absorbance values.

No.	P/V	Wavelength nm.	Abs.
1		282.00	0.107
2		265.00	0.558
3		251.00	0.028
4		248.00	0.066
5		237.00	- 0.111
6		229 .00	- 0.042
7		219.00	0.052
8		213.00	- 0.159
9		204 .00	- 0.235
10		202.00	0.655
11		277.00	- 0.037
12		272 .00	0.010
13		258 .00	-0.617
14		241.00	-0.241
15		234 .00	-0.217
16		224 .00	-0.223
17		214 .00	-0.186
18		207 .00	-0.377
19		203 .00	- 0.473

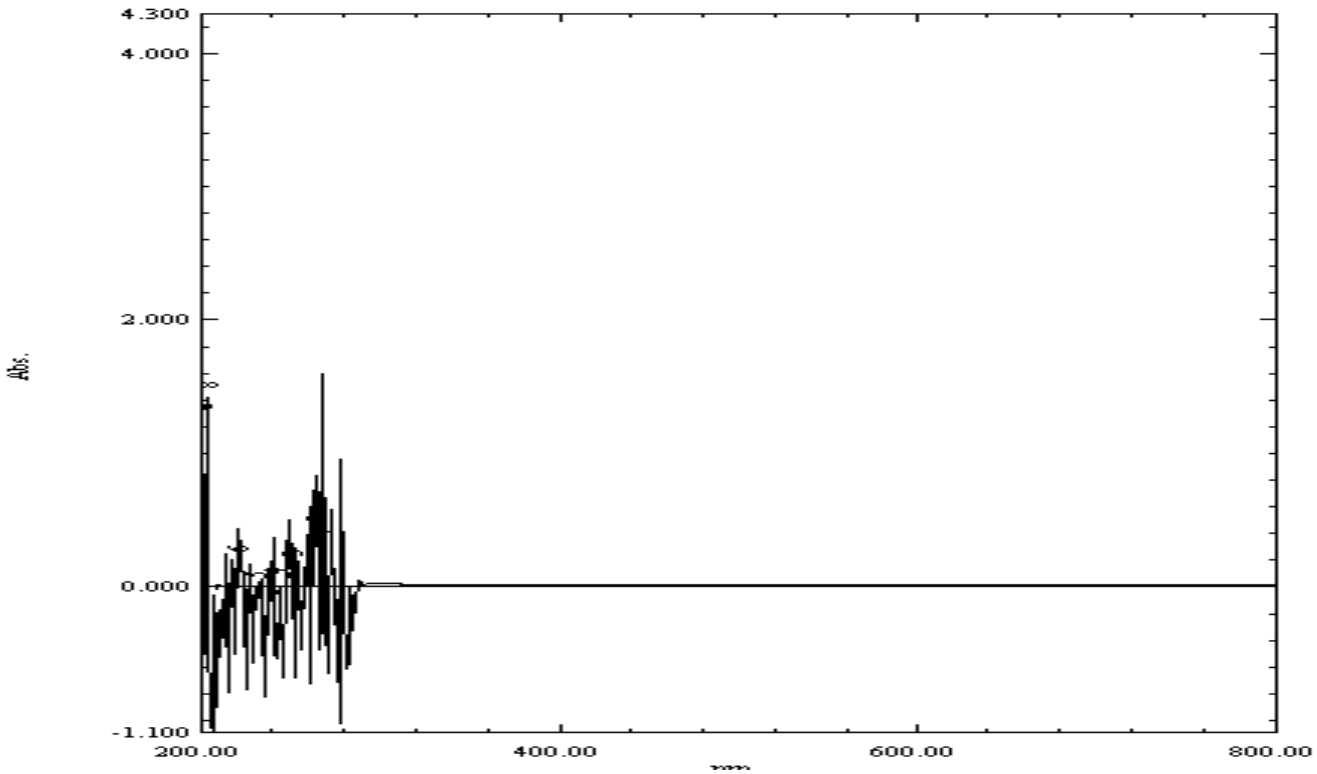

Figure-5: Absorption spectra of placebo (excipients) of atorvastatin tablets λ_{max} at = 203nm.

Table-2: Absorbance values of excipients (placebo) of ATV drug.

No.	P/V	Wavelength nm.	Abs.
1		268.00	0.183
2		264.00	0.300
3		250.00	0.030
4		240.00	-0.105
5		230.00	- 0.134
6		221.00	0.061
7		214.00	0.225
8		203.00	1.295
9		283.00	- 0.246
10		277.00	-0.182
11		270.00	0.026
12		255 .00	-0.132
13		244.00	-0.296

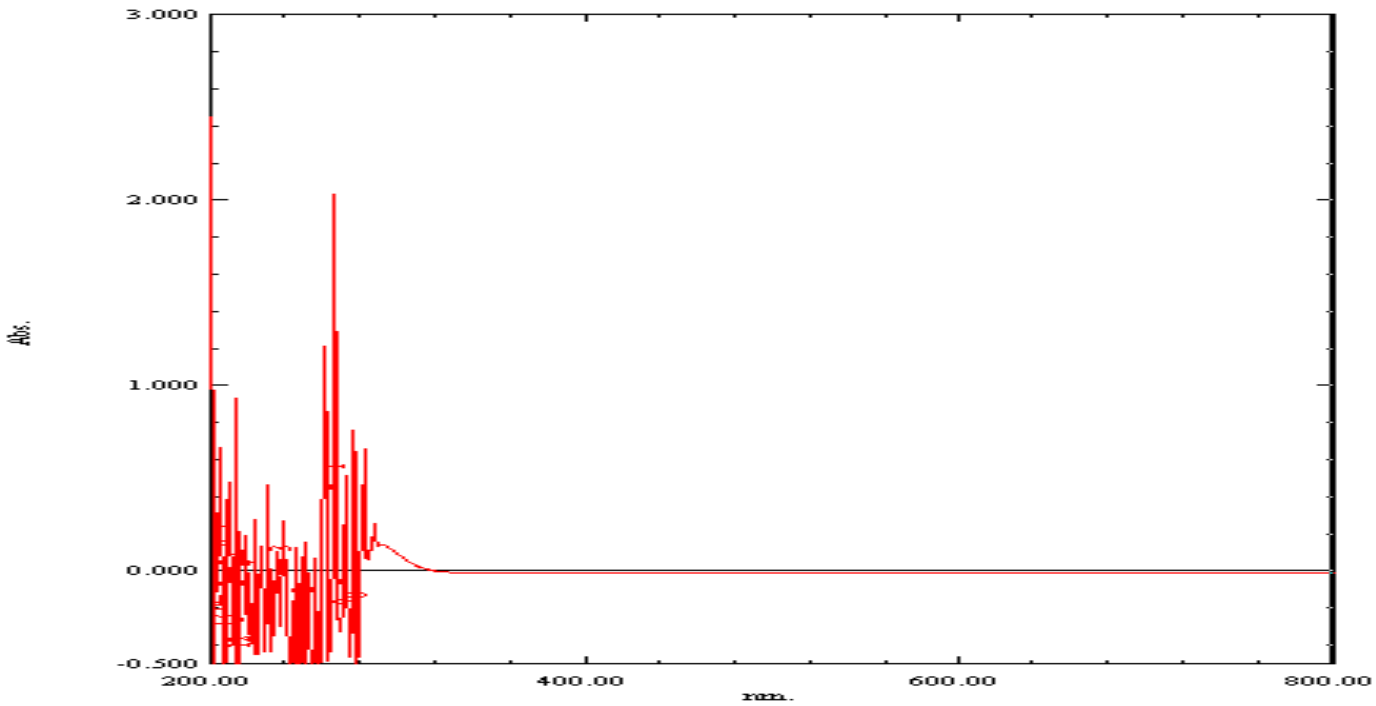

Figure-6: Absorption spectra of 10µg/ml ATV pure drug λ_{max} at 291nm.

Table-3: The values of absorbance's of pure drug of ATV λ_{max} at 291nm.

No.	Wavelength nm.	Abs.
1	326.00	-0.007
2	316.00	0. 007
3	308.00	0.038
4	304.00	0. 061
5	298.00	0.103
6	291.00	0.140
7	284.00	0.061
8	279.00	-0.464
9	269.00	- 0.330
10	257.00	-0.673
11	242.00	-1.136
12	222 .00	-0.331
13	298.00	-0.009
14	202.00	0.107

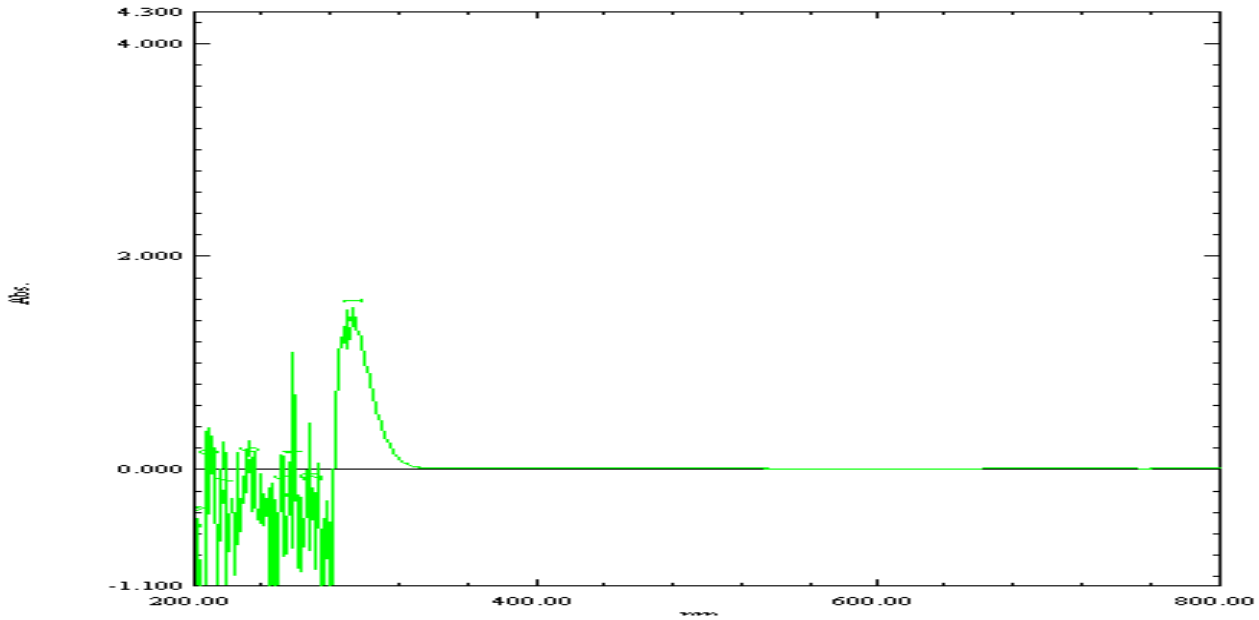

Figure-7: Absorption spectra of 100 µg/ml of ATV λ_{max}= 291nm.

Table-4: absorption values of 100100µg/ml of ATV λ_{max}= 291nm.

No.	P/V	Wavelength nm.	Abs.
1		291.00	1.356
2		269.00	-0. 303
3		267.00	-0.281
4		258.00	-0.052
5		252.00	- 0.306
6		232.00	- 0.033
7		217.00	-0.324
8		209.00	- 0. 059
9		202.00	- 0.590
10		277.00	-0.705
11		26300	- 0.459
12		253 .00	-0.326
13		244.00	-0.542
14		223.00	-0.515
15		214 .00	-0.407
16		203 .00	-1.115

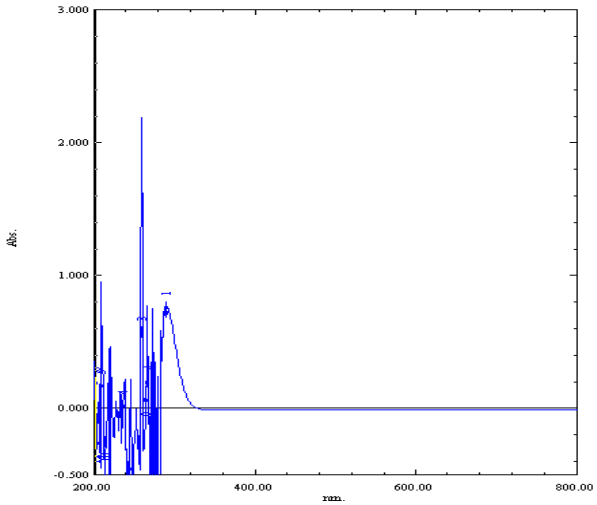

Figure-8: Absorption spectra of 50µg/ml of ATV STD λ_{max}= 291nm.

Table-5: Absorbance values of ATV 50µg/ml at 291nm.

No.	Wavelength nm.	Abs.
1	330.00	-0.004
2	318.00	0. 053
3	308.00	0.235
4	301.00	0. 467
5	296.00	0.650
6	291.00	0.710
7	284.00	0.489
8	202.00	0.154
9	276.00	- 1.766
10	271.00	-0.239
11	262.00	-0.226
12	257 .00	-0.462
13	252.00	-0.110

Specificity and selectivity: Specificity and Selectivity for method validation performed when standard and unknown has been analyzed and interference from blank[12] and placebo was checked using PDA detector and peak purity was confirmed.

Specificity: Validation guidelines have many others important parameters one of these called specificity, different concentrations has been analyzed, the analysis results prove that there is no interfere between tablet drug compositions of formulation to be that a specificity has been confirmed.

Selectivity: Study Conducted through Chromatograph Standard, Test, placebo and Blank Solutions.

Sensitivity: According to the general roles guidance of ICH Q2 R, 1 it depending on signal to noise. Limit of detection (LOD) has been detected which noise to high of signal by ratio of 3 obtained for limit of quantitation (LOQ) asignal with 10 ratio.

Linearity, LOD, LOQ and range: Linearity of ATV standard: From calibration curve: Atorvastatin calcium ATV linearity has been studded by using standard solution prepared with different concentrations values started from 5.0mg/L (0µg/ml) to 35.0 mg/L (0µg/ml), standard curve showed Figure-9.

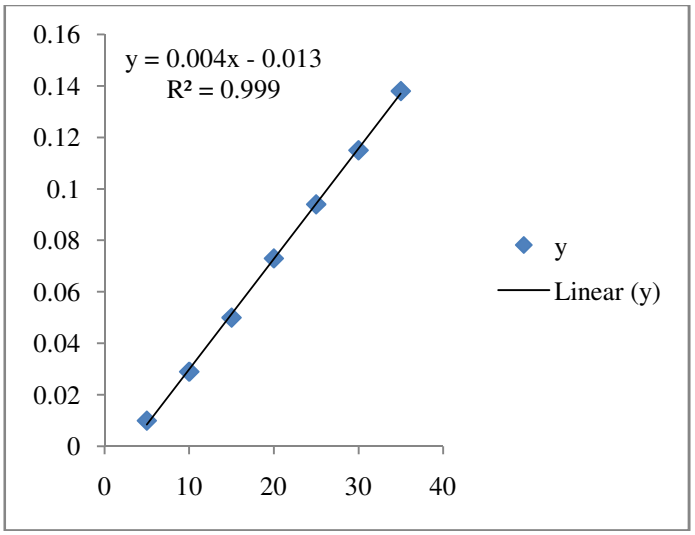

Figure-9: Linearity curve of pure drug (atorvastatin).

Table-6: Linearity study for analytical method validation of ATV tablets

Conc mg/l	Absorption of STD at 291nm	Absorption of Tablet at 291 nm
5.00	0.0100	0.0100
10.00	0.0290	0.0289
15.00	0.0500	0.0510
20.00	0.0730	0.0732
25.00	0.0940	0.0935
30.00	0.1150	0.1149
35.00	0.1380	0.1375

Limit of detection: The limit of detection (LOD) and limit of quantification (LOQ) were obtained by calculations, using the standard formula as per the ICH guidelines, Limit of detection was 0.594ppm to the ATV calcium at 291nm

$$LOD = 3.3 \frac{\sigma}{S}$$

Limit of quantification: Limit of Quantification was found to be 1.800µg/mL Atorvastatin calcium at 291nm.

$$LOQ = 10 \frac{\text{standard deviation (STD DEV)}}{\text{Slop (S)}}$$

Where STD DEV is Standard deviation of the response and S is slope of the standard curve.

Accuracy studies: One of the validation parameter is Accuracy it has been determined by recovery study of ATV with many different level of concentrations from reference material.

Recovery studies: To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120% level. From the total amount of drug found.

Precision: Precision was studied to find out intraday and inter-day (measure the samples over three respective period of days) different UV-Spectrophotometer analysis procedures of ATV at two different contents as 10mg/L (µg/ml) and 15 mg/L (µg/ml) in three repetition for any sample. Relative standard deviation and standard error were calculated at each concentration level[13].

Ruggedness: Ruggedness it one of importance parameter in validation, in this method the sample has been analyzed six repetition to compare RSD % by two analysts in same laboratory of analysis.

Inference: The linearity was calculated by least squares linear regression analysis of calibration curve[14]. The constructed calibration curve was linear over the concentration range of 5.0 ng/ml – 35.0 µg/ml (n=3).

Application of method (D): Modification and development of analytical method involves mainly method which is simple, accurate and precise and also having more advantages over existing procedure. For this target previous studes was carried out which was found to be beneficial more performance and modern a procedure. Curtsey scan disclosed there isn't reported procedure by using Ultra Violet –Visible spectrophotometric[15] up to date reported to the best of our knowledge. So for the consideration of economy rapidity, and simplicity [16], attempts were made to development fast, simple, precise, and accurate procedure for determination of ATV drug in his pharmaceutical formulation (tablets) dosage form. In a practically side using SHIMADZU. Ultra Violet-Visible Spectrophotometer model UV-1800S.

Table-7: Accuracy of the proposed method (n = 3).

Excess drug added to analyte %	Theoretical content (µg/ml)	Conc. found((µg/ml) ±SD	%Recovery	%RSD	SE
0	10	9.494 ± 0.076	99.023	0.344	0.043
50	15	15.107 ± 0.109	99.233	0.255	0.053
100	20	20.426 ± 0.074	100.123	0.413	0.062
150	25	24.454 ± 0.532	100.422	0.525	0.061

Table-8: Precession of the proposed method.

Conc. (µg/ml)	Repeatability (Intra-day precision)			Repeatability (Inter-day precision)		
	Mean abs ±SD (n=3)	SE	%RSD	Mean abs ±SD (n=3)	SE	%RSD
10	9.569 ± 0.076	0.053	0.354	9.692 ± 0.086	0.047	0.406
15	15.013± 0.109	0.062	0.256	15.102± 0.098	0.039	0.340
20	20.601 ± 0.074	0.074	0.425	20.046 ± 0.059	0.058	0.386
25	24.703 ± 0.532	0.060	0.536	24.614 ± 0.498	0.054	0.496
30	29.565± 0.080	0.065	0.469	29.611± 0.064	0.049	0.369
35	35.048± 0.0610	0.081	0.502	35.033± 0.054	0.074	0.419

Determination of ATV drug in his pharmaceutical formulation (tablet) form was carried out for all.

Finally we have Results accessing from the practically statistical tests were explicatively and it revealed that results were significant. For that fast, simple, precise and accurate procedure was developed and can be used for the determination of ATV his pharmaceutical formulation (tablet) form[17].

Conclusion

According to the (ICH) guidelines this method has been developed and validated for routine applications in quality control laboratories for analysis of atorvastatin calcium in their pharmaceutical formulations.

Table-9: Summary of the present study.

Validation Parameter	Atorvastatin calcium
Detection wave Length	293 nm
Beer's Limit	5–35µg/ml
Linearity	5–35µg/ml
R^2	0.9995
Intercept	0.013
Slope	0.0043
LOD	0.394µg/ml
LOQ	1.800µg/ml
Precision	%RSD < 2
Recovery	98-102%

References

1. Nigović B., Damić M., Injac R., Glavač N.K. and Štrukelj B. (2009). Analysis of Atorvastatin and Related Substances by MEKC. *Chromatographia,* 69, 1299-1305.

2. Erk N. (2003). Extractive Spectrophotometric Determination of Atorvastatin in Bulk and Pharmaceutical Formulations. *Anal Lett*., 36, 2699-2711.

3. Castaño G., Mas R., Fernández L., Illnait J., Mesa M., Alvarez E. and Lezcay M. (2003). Comparison of the efficacy and tolerability of policosanol with atorvastatin in elderly patients with type II hypercholesterolaemia. Drugs Aging, 20, 153-154.

4. Pfizer Ireland Pharmaceuticals (2006). Dublin, Ireland. LAB-0021-11.0, Revised June.

5. Prajapati K.P. and Bhandari A. (2011). Spectroscopic method for estimation of atorvastatin calcium in tablet dosage form. *Indo Global Journal of Pharmaceutical Sciences*, 1(4), 294-299.

6. Guideline I.H.T. (2005). Validation of analytical procedures: text and methodology Q2 (R1). In International conference on harmonization, Geneva, Switzerland, 11-12.

7. All rights are reserved by Arunadevi S. Birajdar (2015). Determination of Atorvastatin and Fenofibrate in a Fixed Dose Combination by High Pressure Liquid Chromatograph. *Human Journals Research Article*, 3(4). www.ijppr.humanjournals.com

8. Jim Clark (2000). The Fingerprint Region of an Infra-Red Spectrum. Chemguide, available from http:// www.che mguide.co.uk/a nalysis/ir/f ingerprint.html.

9. Francis A. Carey (2000). Organic Chemistry. 4th edition: The McGraw-Hill Companies.

10. Melnikov P., Corbi P.P., Cuin A., Cavicchioli M. and Guimarães W.R. (2003). Physicochemical properties of sildenafil citrate (Viagra) and sildenafil base. *Journal of pharmaceutical sciences*, 92(10), 2140-2143.

11. Lever A.B.P. (1985). Inorganic Electronic Spectroscopy. 2nd ed., Elsevier, Amsterdam, 161.

12. Guideline I.H.T. (1996). Guideline for good clinical practice E6 (R1). ICH Harmon Tripart Guidel, 4.

13. Dr. Joachim Ermer and Dr. John H. McB. Miller. (2005). Method Validation in Pharmaceutical Analysis: A Guide to Best Practice. Wiley omline library DOI:10.1002/3527604685.

14. British Pharmacopeia (BP) (2014). Atorvastatin calcium monograph. volume II.

15. Miller T.G. (1984). Goffman, social acting, and moral behavior. *Journal for the theory of social behaviour*, 14(2), 141-164.

16. García-Martínez J., Pelechano V. and Pérez-Ortín J.E. (2011). Genomic-wide methods to evaluate transcription rates in yeast. *Methods Mol Biol*, 734, 25-44.

17. United States Environmental Protection Agency, WHO, (2006). Directed Evolution of Three Biocatalysts to Produce the Key Chiral Building Block for Atorvastatin, the Active Ingredient in Lipitor. *United States Environmental Protection Agency*.