ISSN No:-2456-2165

Development and Validation Method for the Determination of Atorvastatin Calcium Tablets Drugs by Using High Performance Liquid Chromatography (HPLC) in Pharmaceutical Formulation

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Abstract:- The objective of this research is to describe the optimization, validation, and application of chromatographic techniques for determination of Atorvastatin Calcium in their pharmaceutical formulation. In this work a simple, rapid, accurate and sensitive analytical methods have been developed and validated. This method is a direct spectro chromate graphic analytical method depend on the chromatographic separation of Atorvastatin calcium compound. This method was developed. By using C18 column with a mobile phase consisting of buffer solution and acetonitrile. The flow rate was adjusted at 1.5 ml/min, injection volume 20 µL, with UV-Detector the maximum absorption peak (λmax) at 238 nm. Column oven at ambient temperature, and retention time was found to be 4.405 min. Under the Optimized condition, beer's law correlating the absorbance (Y) with concentration (X) was obeyed in the range of 2.0 to 35 µg/ml methods.

I. INTRODUCTION

Atorvastatin calcium, chemically (3R,5R)-7-[2-(4-Fluorophenyl)-5-isopropyl-3-phenyl-4-(phenylcarbamoyl)-1H-pyrrol-1-yl]-3,5-dihydroxyheptanoic acid calcium salt [1], is an inhibitor of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reeducates this enzyme catalyzes the conversion of HMG-CoA to mevalonate, an early and rate-limiting step in cholesterol biosynthesis[2]. Atorvastatin is administered as the calcium salt of the active hydroxyl acid and between 10 and 80 mg per day is used 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].

➢ *System Suitability Testing Study*

System suitability testing is to see if the operating system is performing properly or not. As in case of HPLC, an acceptable approach is to prepare a solution containing the analyte and a suitable test compound. If the method being used is to control the level of impurities, the minimum resolution between the active component and the most difficult to resolve impurities should be given [56]. To reduce the raised lipid levels in patients with primary hyperlipidemia [3].

To the best of our knowledge, no single rapid stability-indicating UV-Spectrophotometric method was reported for the determination of Atorvastatin calcium. And their related substances were used to validate the method [4].

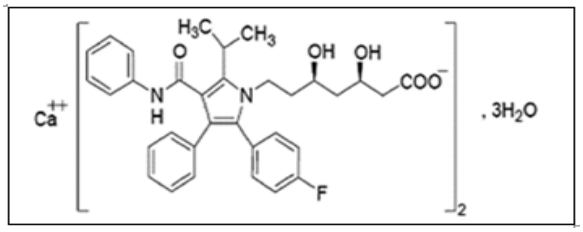

Fig 1:- chemical structure of atorvastatin calcium

➢ *Validation of Analytical Methods*

Validation is defined as finding or testing the truth of something. The objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose [6].

Method validation provides documented evidence, and high degree of assurance that an analytical method employed for a specific 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.

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II. MATERIALS AND METHODS

A. *Reagents and Solutions*
- ✓ Atorvastatin working standard,
- ✓ whatmann filter paper No. 41.

B. Instrumentation

The following instruments and equipment's or apparatus were used during the course of this work:
- ✓ FTIR (Fourier Transform Infra-Red spectrophotometer), (SHIMADZU, KYOTO, JAPAN), Model FTIR-8400s.

➢ ***Preparation of solutions***

- *Solubility Studies of Atorvastatin by UV-Spectrophotometer Analysis*

Solubility of ATV was determined at (28±2) °C. An excess amount of the drug was taken into 25 ml volumetric flasks each containing combinations of methanol and deionized water in ratio (5: 95,10 : 90, 30:70, 40:60, 50:50) , by adding methanol firstly to the drug .

C. Determination of maximum absorption

➢ *Determination of the wavelength of maximum absorption (λ max) of Atorvastatin Calcium*

20 mg of Atorvastatin Calcium working standard was accurately weighed and transferred to 100-ml volumetric flask, 30 ml of methanol was added to and dissolved by sonication for 1 minute for dissolution, The solution was cooled at room temperature and completed to the mark with deionized water to give 200 µg/ml stock solution which was diluted suitably to produce. (5.0, 10.0, 25.0, 50,100) µg /ml of ATV. This solution was scanned in the spectrum mode from 200-800 nm. From the spectrum of the drug obtained λ_{max} of ATV was determined at 291 nm (Figure 2.7.3).

➢ *Determination of the wavelength of maximum absorption (λ max) of placebo of Atorvastatin Calcium*

From the total weight of tablet placebo PLC composition 164.3 mg accurately weighed 20.0 mg of PLC Placebo of Atorvastatin tablets compositions (Table 2.2) and transferred to 100 ml volumetric flask, 30 ml of methanol was added to the weight of PLC and dissolved by sonication for 1.0 minute for dissolution. The solution was cooled at room temperature and completed to the mark with deionized water to give 200 µg /ml stock solution which was diluted suitably to produce (5.0, 10.0, 25.0, 50, and 100) µg/ml of PLC. This solution was scanned in the spectrum mode from 200-800 nm. From the spectrum of the PLC composition there is no any spectrum obtained in the λmax of ATV drug at 203 nm (Figure 3.7.2).

➢ *Determination of the wavelength of maximum absorption (λ max) of 30 % Me OH (Atorvastatin Calcium Solvent)*

30 ml of methanol (spectroscopic solvent) was transferred to 100 ml volumetric flask 50 ml of deionized water was added to the solvent, cool, and complete the volume to the mark with deionized water and scanned in the UV-Spectrophotometer in the range of (200 to 800) nm, The maximum absorption wavelength of solution was found to be 202 nm (Figure 3.7.1).

D. Preparations of standard solutions

➢ *Preparation of standard stock solution of Atorvastatin Calcium standard solution for UV-Spectrophotometer*

20 mg of Atorvastatin calcium working standard (100.20% purity) was accurately weighed, dissolved in 30 ml methanol, transferred quantitatively into 100 ml volumetric flask, completed the volume to the mark with deionized water and mixed well, Sonication for 60 seconds for dissolution. From these stock solutions, working standard solutions having different concentrations 5-80 µg/mL each were prepared by appropriate dilutions.

E. Preparations of sample solutions

➢ *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 100 ml volumetric flask and dissolved in 10 ml of methanol, Sonication for 60 second then added addition of 40 ml of methanol was added and mixed, completed to the mark with deionized water and filtered through Whatmann filter paper No. 41. From the above solution 1.0 ml was taken and transferred to 10 ml volumetric flask and diluted to the mark with methanol : water (30:70) to get a 20 µg /ml solution and measured at 291 nm.

F. Physical analysis and characterization for tablets and raw materials under study

➢ *Analytical test methods*

- *Description*

View sample of the product and recorded the appearance with respect to color and shape to the provided sample.

- *Weight variation*

By using analytical sensitive balance Conducted on twenty (20) tablets were selected randomly, and weighed individually, record average, minimum and maximum weight of tablets and relative standard deviation percentage was recorded.

- *Uniformity of content by weight variation*

By using analytical sensitive balance Thirty (30) tablets were selected randomly, and weighted individually ten (10) tablets were weighted and recorded.

- *Dimension*

By using the hardness tester and for ten (10) tablets, width and length were determined for the tablets of drugs

ISSN No:-2456-2165

and maximum and minimum and average for dimension results was calculated.

- *Thickness*

By using the hardness tester and for same ten (10) tablets in diameter test, determined the minimum and maximum and average for thickness results.

- *Disintegration*

By using disintegration tester, randomly selected six tablets of the sample and introduce to disintegration tester on tablet in each tube.

- *Melting point for tablets and raw material*

2.0 mg from the sample accurately weighed, transferred to aluminum pans and sealed. All samples were run at a heating rate of 20 ºC/min over a temperature range 40-430 ºC using Shimadzu DSC-60 Thermal Analyzer.

➢ *Characterization of active ingredient and tablets drugs:*

- *FTIR Spectroscopy*

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

III. RESULTS AND DISCUSSIONS

A. Method Development

At the beginning, we tried many methods to develop a method for the two drugs with different mobile phase composition, buffer, pH, column, absorbance and flow rate. All methods had been applied showing asymmetrical peaks, overlapping, and unusual chromatograms for the drugs separately and in mixture in solution.

In this research project, two instrument HPLC and UV-Spectrophotometer, two drugs: Sildenafil citrate tablets & Atorvastatin tablets, and four new methods (A,B,C and D) were developed and validated as assay methods for the quantitative determination of Sildenafil citrate and atorvastatin calcium in their pharmaceutical formulations (tablets): method (A) for quantitative determination of sildenafil citrate tablets by using HPLC. And method (B) for quantitative determination of the second drug: atorvastatin tablets by using HPLC, method (C) for quantitative determination sildenafil citrate tablet by using UV-Spectrophotometer. Method (D) for quantitative determination of atorvastatin in pharmaceutical formulation (tablets), High Performance Liquid Chromatograph and UV-Spectrophotometer were used as mainly instrument in this research project with the aid of some other instrument.

B. Identification of active pharmaceutical ingredient (API) and tablets for drugs under study by using FTIR

➢ *Identification of Atorvastatin calcium (Active Ingredient)*

To assist in the identification of the Atorvastatin and Sildenafil Citrate FTIR spectrum of Atorvastatin and Sildenafil Citrate sildenafil positive (by HPLC and UV-Spectrophotometer) products were analyzed. FT-IR spectroscopy is concerned with the interaction between a molecule and radiation from the IR region of the EM spectrum (4000 – 400 cm-1). FTIR spectrum can be divided into two approximate regions:

- Functional group region (4000-1500 cm-1), valuable information are obtained from this region to interpret any IR spectrum.
- Fingerprint region (< 1500 cm-1), usually consists of a very complicated series of absorptions that are characteristic for a particular compound [60] The Fingerprint Region of an Infra-Red Spectrum, Chemguide, available from

FTIR is a very fast technique considering that no further sample preparation is needed and spectrum acquisition requires only a few seconds. By an accurate comparison between the IR spectrum of any sample with that of the original drug it is possible to determine whether they have the same composition or not, thus it permits to ascertain definitively if the investigated sample is falsified or not.

In this study, a Shimadzu 8400S Fourier Transformation Infra-Red (FTIR) spectrophotometer was employed for qualitative analysis of sildenafil Citrate and Atorvastatin Calcium in the identified (by HPLC and UV-Spectrophotometer) market samples [61]. Spectra were recorded with 25 scans and a resolution of 2cm-1.The approach was:

Firstly, the IR bands distinct for the reference substance due to its functional groups were identified. Each substance has a unique IR spectrum.

Secondly, presence of these distinct bands was searched in the IR spectrum of the samples.

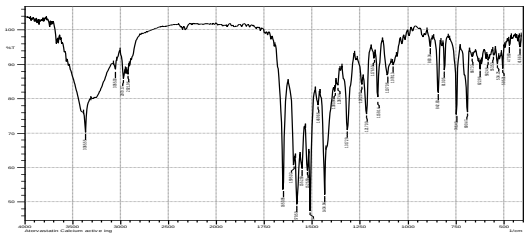

Fig 2:- FTIR spectra of Atorvastatin STD (reference)

The IR spectrum showing percentage transmission (%T) versus wave number (cm-1) of Atorvastatin calcium (ATV) is shown in Figure (2) with characteristic peaks of aromatic N-H stretching and C=O stretching at 3364.21 cm-1 and 1649.81 cm-1, respectively. However formulation exhibited similar peaks but with a negligible shift for aromatic N-H stretching and C=O stretching at 3363.17 cm-1 and 1647.67 cm-1. It is evident from the figure that ATV in nanoparticles doesn't undergo any chemical reaction with any of the excipients used in the preparation.

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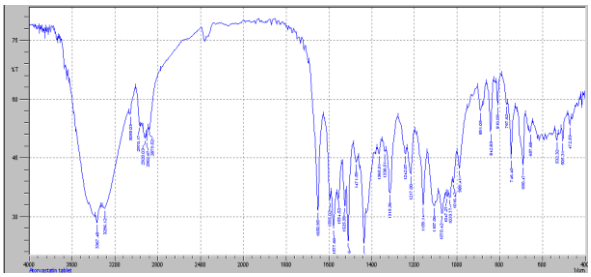

Fig 3:- FTIR spectra of Atorvastatin tablets (sample)

The FT-IR absorption spectra of atorvastatin was obtained using KBr pellet technique and the spectra was found to exhibit characteristics absorption bands at 3240, 1627, 1620 1180,1100,3600, 828 cm-1, showing N-H, C=O, C=C, C-O, C-N, O-H and aromatic substitution bands respectively of ATV Calcium. The chemical structure of ATV Calcium was shown in the 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 500 cm-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 in the light path and the spectrum was obtained show figure (3).

C. Physical analysis of Active Ingredient and excipient (formulation)

➢ *Identification of Atorvastatin calcium and excipient (Tablets 40 mg) Tablets product specification*

ATV Calcium that was received as a gift sample from AZAL Pharmaceutical industries Co.Ltd was characterized for various physical properties like color, average weight, diameter, melting point, thickness, hardness, disintegration.

Test	Specification	Results	Comment
Description	White,round,biconvex,film coated Tablet with break line on one side	Complies	Complies
Average weight	Nominal tablet weight: 185.0mg; limits:171.0mg to 189.0 mg.	186.5 mg	Complies
Diameter	8.5 mm to 8.9 mm	8.6 mm	Complies
Thickness	2.9 mm to 3.9 mm	3.1 mm	Complies
Hardness	NLT 3.0 Kp	1.9 Kp	Complies
Disintegration	NMT 30 mm	6.50 min	Complies
Solubility	Freely soluble in methanol & soluble in DMSO & DMF, very slightly soluble in water.	Complies	Complies
Melting point	159.0-160 ^{O}C	160 ^{O}C	complies

Table 1:- Identification of Atorvastatin calcium and excipient (Tablets 40 mg) Tablets product specification (physical tests)

D. Methodology

➢ *Specivity and selectivity*

Study Conducted Through Chromatograph Standard, Test, placebo and Blank Solutions; Also, Standard and Sample under stress condition chromatographed to prove that there is no peak Interference, and test method able to separate Atorvastatin Clearly" calculated as base

➢ *Calculations:*

Actual Atorvastatin content on dried basis "mg":
(Actual wt. X P X (100-WC) X MWB)/ (100 X100 XMWC)
Calculated Content as Atorvastatin "mg":
(AT X STD1wt X P X (100-WC) X MWB X100) / (AS X 100 X 100XMWC)

Specivity and Selectivity					
Conditions	Response of mean beak	Resolution	Tailing Factor	No.Of theoretical Plates	Area%
Blank	_ve	_ve	_ve	_ve	_ve
Placebo1 solution	_ve	_ve	_ve	_ve	_ve
Placebo 2 solution	_ve	_ve	_ve	_ve	_ve
Standrad1 solution	1511568.5	3.34	1.09	14394.918	97.592
Standrad2 solution	1496094.5	3.34	1.1	14269.973	96.776
Sample1 solution	1510581.5	3.49	1.11	14263.808	96.927
Sample2 solution	1493849	3.35	1.11	14223.636	96.189

Table 2:- specefity and selectivity values of condition of ATV under study

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Atorvastatin Peak appears clearly in Standard and Test Solutions at Retention Time: 4.0 minute. With good resolution and tailing factor, doesn't appear in Blank Solutions and placebo solutions, "There is No. Interference with blank or placebo.

System suitability Parameters for solutions under stress conditions					
Conditions	Response of mean beak	Resolution	Tailing Factor	No.Of theoretical Plates	Area%
Standard with 0.1N HCl	1356039.5	3.36	1.11	14192.966	89.664
Test with 0.1N HCl	1409494.5	3.5	1.12	14197.345	87.809
Standard with 0.1N NaOH	1494705.5	3.33	1.11	14155.752	98.171
Test with 0.1N NaOH	1490855.6	3.37	1.12	14183.663	96.574
Standard with 30% H2O2	1482369	3.23	1.12	14083.411	35.037
Test with 30% H2O2	1451210	3.42	1.11	14220.173	33.809
Standard heated at 80°C	1466116.5	3.36	1.12	14300.42	95.933
Test heated at 80°C	1341482.5	6.671	1.11	14280.108	93.456

Table 3:- System suitability Parameters for solutions under stress conditions

Sample under stress conditions show same behavior as standard, also degradation due to excipient doesn't interface with main peak and calculated Content doesn't differ than expected by not more than 2.25 mg and that approve degradation due to excipient practically doesn't interface with main peak Standard under Stress Conditions show degradation peaks doesn't interface with Atorvastatin peak with 0.1N HCL & 0.1N NaOH and 80°C, and calculated content doesn't differ than expected by not more than 2.16 mg, in hydrogen peroxide the area % decreased due to increase in secondary peaks area. Which approve that degradation products due to acid, base hydrolysis and oxidation practically doesn't interface with main peak.

IV. CONCLUSION

Specify of ATV approved by absence of main peak in placebo, blank, and presence of it in standard and sample solution, there is no interference between degradation products and main peak. ATV show good resolution between main peak and degradation peaks, using standard and product under stress conditions.

❖ Linearity, LOD, LOQ and Range

Through serial dilution from working standard to conduct linear calibration curve, and calculate correlation coefficient " R" coefficient of determination "R2" , slope, Y-intercept and relationship equation. LOD, LOQ and Range calculated. Procedure and calculations: atorvastatin concentration. Calculated by equation:
(STD wt. X preparation Dilution X P X (100 - WC) XMWB) / (100 X 100 XMWC)

1.Preparation dilution: dilution at which standard weight dissolved, i.e.: for level1; preparation dilution = 1/50 X 1/100= 0.0002
2. Actual weight "mg" calculated by equation atorvastatin conc. / 0.002 where 0.002: dilution factor for sample and standard in the test method actual content in "mg": is a Collected data to use mainly in accuracy calculation." mentioned here to explain data in table below.

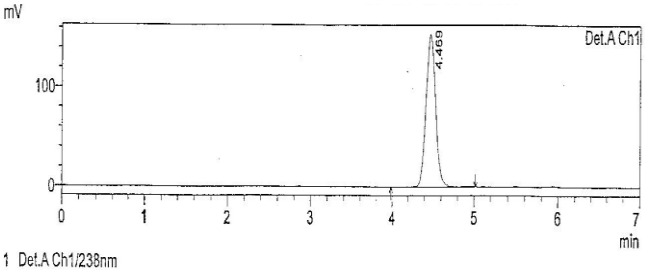

Fig 4:- Chromatogram of Atorvastatin calcium standard

Peak #	Name	Ret.Time	Area	Height	Area %
1	Atorvastatin	4.469	1222502	154800	100.00
Total	-	4.469	1222502	154800	100.00

Table 4:- chromatogram values of atorvastatin calcium standard

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Peak #	Name	Theoretical plate	K[1]	Tailing factor	Resolution
1	Atorvastatin	6850.372	7.939	1.130	00.00
Total	-	6850.372	7.939	1.130	00.00

Table 5:- atorvastatin calcium standard parameters

3. Each concentration injected 5 consecutive times, average, RDS%, and relative response calculated
4. Blank injected and major response recorded and its standard deviation is calculated
5. Relative response calculated by dividing average of response by concentration
6. Calibration curve plotted between average of response and concentration, linear relationship generated.
7. On basis of blank standard deviation, LOD (=3.3xδ/S) and LOQ (=10xδ/S) calculated." δ blank STDV and S: calibration curve slope

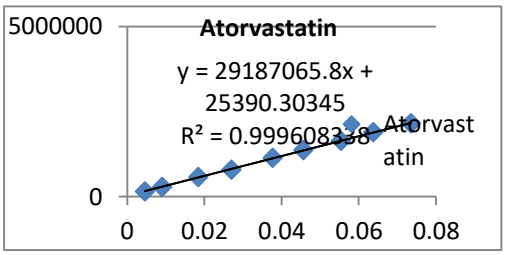

Fig 5:- Calibration curve proposed method

➢ *Linearity*

Linearity of ATV approved by calibration curve; linearity study start from concentration of 0.00460046 mg/ml to 073534982mg/ml as atorvastatin calcium, correlation coefficient of determination "R²" = 0.9996, equation X= (Y-25390)/29187065

From Calibration Curve:
1. Calibration curve found linear due to correlation of determination " R²" = 0.9996, slope = 29187065.8

2. Y- intercept = 25390 and relationship equation = 29187065 X + 25390.3 , " concentration" = Y-25390.3/29187065 3. LOD = 0.001µg/ ml and LOQ = 0.003 µg/ ml atorvastatin base approved by solution prepared with 0.001µg/ ml, and 0.003µg/ml

➢ *LOD*

0.0011 µg/ml calculated with reference to blank standard deviation and slope.

➢ *LOQ*

0.0034 µg/ml calculated with reference to blank standard deviation and slope

➢ *Range*

Range of ATV established through linearity measurement to be from 12.4% to 177.2% assay.
Range: from 2 to 35 mg (per mg) and from: 12.0 to 177.0 % as percentage

Range of ATV established through linearity measurement to be from 12.4% to 177.2%assay

❖ *Accuracy*

By Data Collected from Linearity Measurements: Contents of solutions Calculated "Found contents calculated using Average of Instrument response" and Compared with Actual content of prepared solutions. By Data Collected from Precision Repeatability measurements: between prepared concentrations and result found

➢ *Preparations and Calculations:*

1.Standard Preparations Used in Linearity with concentrations of 0.0046, 0.0091, 0.0184, 0.0270, 0.0377, 0.0456, 0.0554, 0.0637, and 0.0735. Calculated as Atorvastatin on dried basis and with reference to Assay of standard.
A. Thus Concentration divided by Sample dilution factor " 0.002'' to get actual content of this concentration.
B. by using instrument response of thus solutions against standard, content can be calculated form equation:
Found Content: (Response of solution X Standard Wt.) / Response of Standard Note:
Level #5 with concentration 0.0378 is method standard
2. Placebo spiked with Standard Preparations "as Product" used in Repeatability with concentrations of 50%, 100% and150% Assay.
A. Content Calculated for each one "as product" from equation Found Content (AT X STD wt. X DFS X P X (100-WC)) / (AS X DF. X100 X 100)
3. For both kind of solutions; %Recovery calculated by equation: % Recovery = Found Content X 100 /Actual Content.
4. Difference between found content and actual content calculated.
* From linearity Results From Linearity and within 9 different concentration from 11 % to 170%; Recovery (%) found to be 100.11% with %RSD 3.86%.

RECOMMENDATION

Method found accurate with % of Recovery =99.09 %; with +/- 1.47

❖ *Precision*

Precision of analytical method developed through repeatability and intermediate precision" ruggedness". Repeatability approved through 9 preparations from 3 different concentrations, while intermediate precision approved through Day to Day and Analyst to analyst analysis.

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Preparations and calculation:

➢ *Repeatability*

1. From product labeled claim, 3 concentration prepared" 50%, 100%, 150%" from standard and placebo combination.
2. Actual atorvastatin weight calculated with reference to standard assay. Water content and molecular ratio, through following equation: Actual Atorvastatin weight: Standard Wt. x (STD assay/100) x ((100-WC)/100) x (MWB/MWC) . Actual Atorvastatin assay: Actual Atorvastatin weightx100/ labeled Claim
3. Each preparation inject 3 times, individual and average of response, %RSD, Calculated
4. Individual and average of retention time and %RSD calculated.

B. Intermediate Precision / Ruggedness:

1. from the same batch prepared test and standard solution and calculate assay as day to day Precision.
2. From same batch prepared test and standard solution and injected it, and the same day and the same batch prepared test solution and injected it against his own standard, "as analyst to analyst precision"
3. Day to day and analyst to analyst Average assay and %RSD Calculated ,Over all 3 assays average and %RSD calculated.

➢ *Intermediate precision and Ruggedness*

Method found Repeatable with RSD % doesn't exceed 0.5% when testing instrument response and retention time Method found precise with RSD % doesn't exceed 2.0% when testing intraday and intra analyst precision

➢ *Robustness*

Robustness tested through changing in Flow rate and mobile phase Composition, and record its effect on retention time, peak shape and system suitability parameters.

1. Decrease flow rate to 1.0 ml/min. increase peak retention time to 17.9 min and peak area increased.
2. Changed the mobile phase to decrease the peak retention time to 13.5 min and the peak area decrease.

➢ *Application of method B*

The proposed method was applied to the pharmaceutical formulation containing atorvastatin calcium. The result is shown in table 3.5.21 indicate that the high accuracy of the proposed method for the determination of the drug studied. The proposed method has the advantages of being virtually free from interference by excipient. The percentage was Validity of the analytical test method under study is approved and method can be used in routine work.

Brand name and dosage form	Labeled claim (mg/tablet)	Amount found (mg/tablet)	(% found ± RSD)
atorvastatin tablets	50	49.545	99.09± 0.44

Table 6:- determination of Atorvastatin tablets by the proposed method

*RSD, relative standard deviation.
*Mean value of three determinations

❖ 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.

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