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DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE ESTIMATION OF ATORVASTATIN CALCIUM HYDRATE RESIDUE BY USING UV SPECTROPHOTOMETER

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Keywords:

Atorvastatin Calcium Hydrate, Residue method development, Swab sticks and swabbing

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ABSTRACT: A new, simple and sensitive spectroscopic method has been developed for the estimation of Atorvastatin calcium hydrate residue by using swabbing technique. In the proposed method the absorbance was measured 245.0nm corresponding to the absorbance maxima of Atorvastatin calcium in the ratio methanol and water as 90:10 as a solvent. Shimadzu-2450 UV-Visible spectrophotometer with UV Prove 2.31 software, capable of multi-component analysis, was used for quantization. Linearity range was observed in the concentration range of 1-10µg/ml for Atorvastatin. The % recovery of Atorvastatin was found out to be 100.2%. The method was validated statistically and recovery study was performed to confirm the accuracy of the method. The method was found to be rapid, simple, accurate and precise.

INTRODUCTION: Atorvastatin calcium hydrate chemically is **[**R-(R', R')]-2-(4-fluorophenyl)-beta, delta-dihydroxy-5-(1-methylethyl)-3-phenyl-4[(phenyl amino) carbonyl]- lH-pyrrole-1-heptanoic acid, calcium salt (2:1) [1, 2]. Atorvastatin calcium is a white to off-white crystalline powder[1] used for Antilipemic agents (synthetic cholesterol-lowering agent), Anticholesteremic agents.

Atorvastatin, a selective, competitive HMG-CoA reductase inhibitor, is used to lower cholesterol and triglycerides in patients with hypercholesterolemia and mixed dyslipidemia and in the treatment of homozygous familial hypercholesterolemia.

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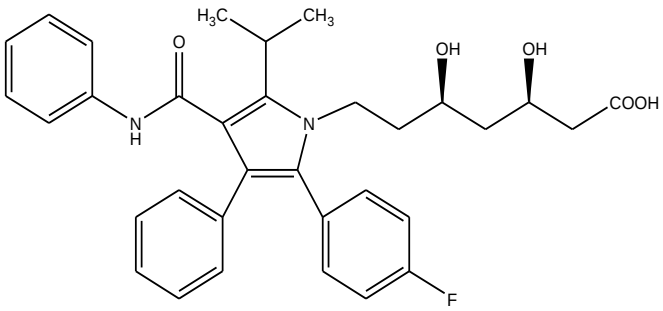
Atorvastatin has a unique structure, long half-life, and hepatic selectivity, explaining its greater LDL-lowering potency compared to other HMG-CoA reductase inhibitors [3, 4].

Many analytical methods like Spectroscopic methods [5-10], HPLC [11-19], UPLC [20], HPTLC [21], has been reported for determination of Atorvastatin (structure of Atorvastatin has shown in **Fig. 1**).



Atorvastatin

FIG. 1 STRUCTURE OF ATORVASTATIN

However, no spectroscopic method has been reported till date by using swabbing technique. The aim of this work is to develop a spectrophotometric procedure for the determination of atorvastatin calcium hydrate residue by swabbing technique. However, if it is not possible, a solvent in which solubility of API at least 10 times the acceptance unit is selected.

Selection of detection method: The method generally used for Residue estimation is UV detection method.

Selection of Wavelength: The wavelength selection is generally done based on the UV Spectrophotometric and assay method. A wavelength where there is less interference and more response or absorption occurs by the drug substance can be selected for this method.

Estimation of non-interference from Swab blank: The solvent selection for residue analysis should not show any peak at the retention time of analyte peak. To establish the non-interference of blank, prepare the swab blank accordingly.

Establishment of Recovery Factor: The acceptance criteria for the recovery in residue analysis shall not less than 80% to 120%. 10ml of diluent in a cleaned test tube was transferred. A clean swab was placed into the test tube and standard stock solution of the drug substance in the selected solvent was prepared.

1ml on to a 5*5 SS Plate was spread very slowly and simultaneously careful drying was performed so that no drop of solution went out of the plate.

The volume was adjusted in such a manner that the final concentration of the test solution was same as that of the standard stock solution. The plate was allowed to dry and performed swabbing. The swabbing was done as per the direction given in **Fig. 2a and Fig. 2b**.

The total swabbing was collected into the test tube sonicated, filtered and measured the absorbance at specified wavelength by UV Spectrophotometer.

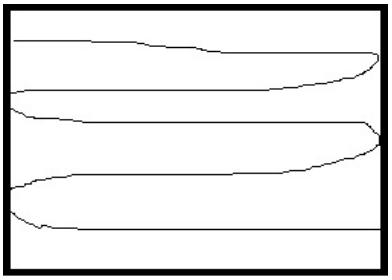


FIG: 2A SWABBING SURFACE IN HORIZONTAL

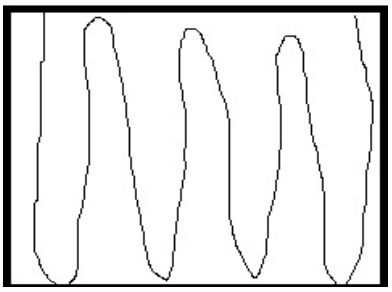


FIG: 2B SWABBING SURFACE IN VERTICAL

Limit of detection and Limit of quantification: Establishment of limit of detection (LOD) and limit of quantification (LOQ) for analyte peak is based on signal noise ratio (S/N). The method was developed in such a way that the limit of quantification was less than or equal to the acceptable limit.

$$LOD = 3.3\ \sigma / S \text{ and } LOQ = 10\ \sigma / S$$

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

Selection of Standard concentration: In general the standard concentration shall be equal to that of the acceptance limit. However, if the response of the analyte is very low, then slightly higher standard concentration than the acceptance limit can be selected; provided the linearity up to that concentration.

EXPERIMENTAL METHOD: A simple UV spectrophotometric method was developed for the determination of Atorvastatin. The drug Atorvastatin was exhibiting maximum absorbance at 245nm in mixture of Methanol and water in the ratio of 90: 10. The proposed method was statistically validated.

Sample: The Atorvastatin Calcium reference standard batch no. GS/1O11/ATC/C/5 assigned purity 99.6% was supplied by Jubilant Organosys Limited.

Reagents and Chemicals: Methanol (HPLC Grade) and other chemicals were of analytical grade. The water used as HPLC Grade Purified Water.

Instrumentation: Spectral and absorbance measurements were made on a Shimadzu-2450 UV-Vis Spectrophotometer with UV Probe 2.31 software by using 1 cm quartz cells. Mettler Toledo electronic balance was used for weighing the standard.

Scanning and determination of maximum Wavelength (λ_{max}): In order to ascertain the wavelength of maximum absorption (λ_{max}) of the drug, different concentrations (1-10 µg /ml) of the drug in Diluent (Methanol: Water: 90:10) were scanned using UV- spectrophotometer within the wavelength region of 200 – 400 nm against Diluent as blank.

Method:

1. **Selection of Swabbing Solvent:** The selection of solvent for residue estimation of API was based on solubility of the drug Atorvastatin Calcium Hydrate.

2. **Preparation of Diluent:** A mixture of Methanol (HPLC Grade) and Water (HPLC Grade) in the ratio of 90:10 was prepared.

3. **Preparation of Standard Stock Solutions:** Standard stock solution was prepared by dissolving 54mg of Atorvastatin Calcium Hydrate Working Standard equivalent to about 50mg of Atorvastatin with diluents, in a 10 ml of volumetric flask to get a concentration of 1µg/ml (5000 µg/ml) solution.

4. **Preparation of Standard solution:** From the prepared stock 1ml was taken in a 50ml volumetric flask and diluted to 50ml with diluent to get working standard solution of 100 µg/ml.

5. **Pre-treatment of Swab (pt swab):** Desired swab sticks were taken and dipped into sufficient amount of methanol in 50ml volumetric flasks and sonicated for 30mins. Then removed and rinsed with fresh methanol.

6. **Preparation of Swab solution:** Three pre-treated swab sticks were taken in three individual clean test tubes and 10ml of diluent was added and sonicated for 10mins. The solution was filtered with 0.45 µm nylon membrane filter.

- **Procedure:** Blank, Standard solution and PT Swab blank were scanned. Rinsed solvent, diluent, filtered swab blank (filtered through 0.45 µm nylon membrane filter) are also scanned in a wavelength range between 200-400nm.

7. **Preparation of Accuracy 100% stock solution:** 10ml of stock solution was taken in a 50ml of volumetric flask and diluted up to 50ml with diluent.

- **Procedure for swabbing:** Accurately 1ml of stock solution was slowly dropped on a clean 5*5cm SS Plate and the solution was evaporated by using a dryer. The surface was swabbed in horizontal and vertical manner. Then the swab was transferred in a test tube with 10ml diluent and sonicated for 10mins.

8. **Preparation of Accuracy 100% level sample:** 1ml of accuracy 100% stock solution was transferred on a cleaned 5*5cm SS Plate and proceeds as per swabbing procedure. The absorbance of accuracy 100% sample at 245nm by using diluent as blank with three replicates measurement of standard solution.

9. **Preparation of Linearity level:** Previously prepared standard solution used as linearity stock. From the stock solution 0.1ml, 1ml, 2ml, 4ml, 6ml, 8ml were taken. The absorbance of each linearity level was measured with taking diluent as blank and LOD & LOQ value were measured. The quantifications were carried out by keeping these values to the straight line equation of calibration curve.

Residue validation of Atorvastatin calcium hydrate:

Specificity:

1. **Preparation of Diluent:** A mixture of Methanol (HPLC Grade) and Water in the ratio of 90:10 was prepared.

2. **Preparation of Standard Stock Solutions:** Standard stock solution was prepared by dissolving 54mg of Atorvastatin Calcium Hydrate Working Standard equivalent to about 50mg of Atorvastatin with diluent to a 100ml of volumetric flask to get concentration of 1mg/ml (5000 µg/ml) solution.

3. **Preparation of Working Standard Solutions:** From the prepared stock 1ml was taken in a 50ml volumetric flask and diluted 30ml with diluent and sonicated to dissolve and make up the volume with diluent to get working standard solutions of 100 µg/ml. The absorbance was measured maximum at 245 nm, against diluent as blank.

4. **Pre-treatment of Swab (PT Swab):** Desired text wipe swab sticks were taken and dipped into sufficient amount of methanol in 50ml volumetric flasks and sonicated for 30mins. Then the swabs were removed and rinsed with fresh methanol and then the swabs were dried before use for validation.

5. **Pre-treatment of Swab Solution (Swab Blank):** Three pre-treated swab sticks were taken in three individual clean test tubes and 10ml of diluent was added and sonicated for 10mins. The solution was filtered with 0.45 µm nylon membrane filter.

6. **Swabbing Procedure:** Standards of atorvastatin (6 replicates), Rinse solvent (water), Swab solvent (Diluent) and PT Swab blank-1, 2, 3 were analysed as per the method and the interference was checked at 245nm.

Accuracy:

1. **Preparation of Accuracy 50% Level Stock Solution:** 10ml of standard stock solution was taken and diluted to 100ml of volumetric flask with diluent.

2. **Preparation of 100% Level Stock Solution:** 10ml of standard stock solution was taken and diluted to 50ml with diluent.

3. **Preparation of Accuracy 200% level stock solution:** 10ml of standard stock solution was taken and diluted to 25ml with diluent.

4. **Preparation of swabbing for 50%, 100%, 200% Accuracy:** 1ml of accuracy 50% level stock solution was slowly dropped on a clean 5*5cm SS Plate. The solution was evaporated by using a dryer and swabbed the surface in horizontal and vertical manner. The swab in a test tube with 10ml diluent was transferred and sonicated for 10mins.

   3 replicates sample of 50% accuracy were prepared. Similarly 3 replicates sample were prepared each for both accuracy 100% and 200% stock solution. Then absorbance of each sample at 245nm by using diluent as blank was measured and percentage of recovery was calculated. The accuracy of standards was determined.

Precision: The precision of the proposed method was ascertained by actual determination of six replicates of fixed concentration of the drug within the Beer's range and the absorbance was found by the proposed method. From these absorbances, Mean, Standard deviation and % RSD were calculated.

- **Method:** Prepared diluent, reagents, PT swab, swab blank and standard in specificity test were used here. Total 6 sample preparations were required for method precision. Out of 6 samples, 3 samples were prepared in accuracy 100%.

Limit of Detection (LOD) & Limit of Quantitation (LOQ): The absorbance of predicted values of LOD & LOQ levels for 6 times was measured.

RESULTS AND DISCUSSION:

Scanning result and maximum Wavelength (λ_{max}): The resulting spectra were shown in **fig. 3a, fig. 3b** and the absorption curve showed characteristic absorption maxima at 245nm for Atorvastatin.

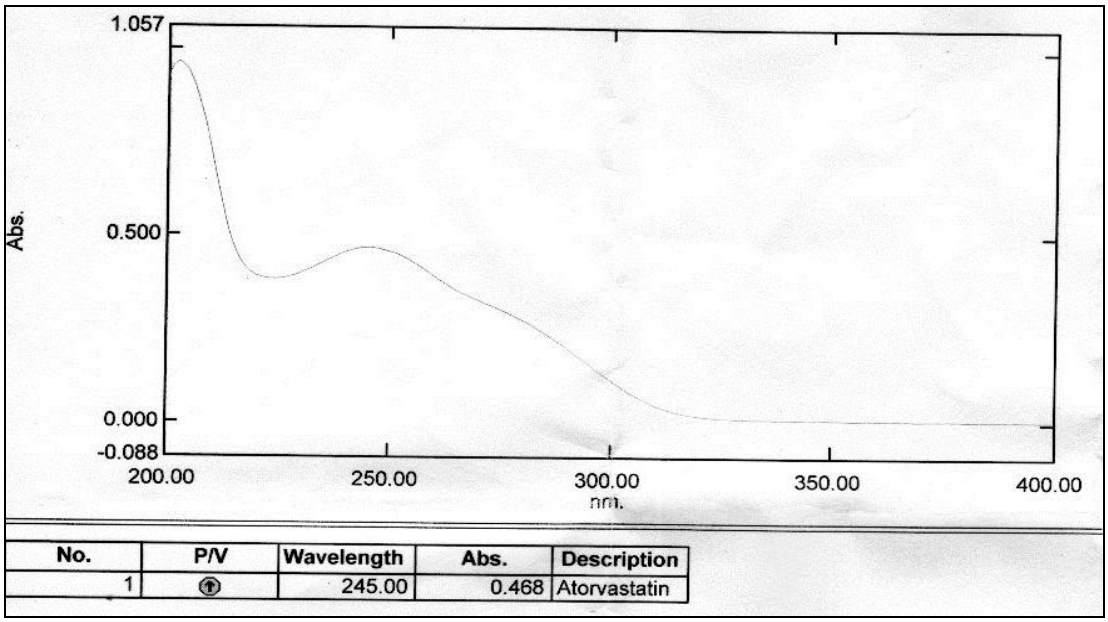


FIG: 3A UV SPECTRUM OF ATORVASTATIN IN DILUENT [Sample: Atorvastatin (10 µg/ml); Reference: Diluent]

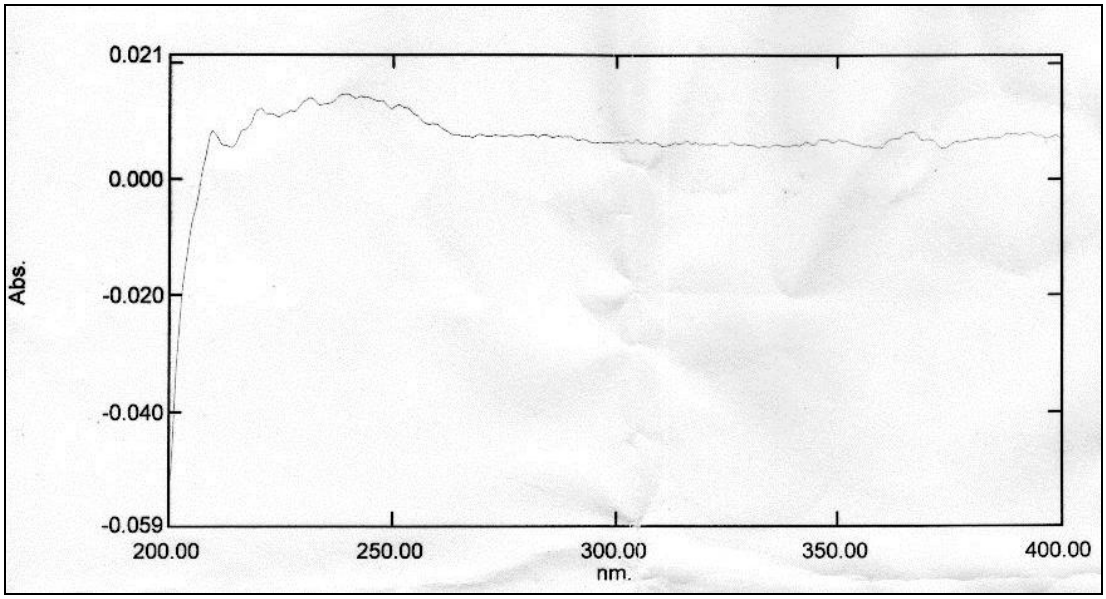


FIG: 3B UV SPECTRUM OF SWAB SOLVENT IN DILUENT

TABLE 1: SOLUBILITY STUDY RESULT OF ATORVASTATIN

Solvent	Solubility
Aqueous solutions of pH 4 and below. (Water)	Insoluble
Distilled water, pH 7.4 phosphate buffer, and acetonitrile	Very Slightly Soluble
Ethanol	Slightly soluble
Methanol	Freely Soluble
Methanol: Water::90:10	Soluble

The maximum absorption of the drug atorvastatin calcium hydrate standard was found at wavelength 245nm and also the interference of Blank, Standard (3times), rinsed solvent water, PT Swab blank-1, 2, and 3 were determined. The % of interference found within the limit not more than 2% (**Table 2**).

TABLE 2: % OF INTERFERENCE DETERMINATION

Sample	Absorbance	% Interference at 245nm	Mean of Standard
Blank (Diluent)	0	0	
Standard-1	0.4342		
Standard-2	0.4344		0.4343
Standard-3	0.4343		
Rinse Solvent Water	-0.0241	-5.5491	
Swab Solvent Diluent	-0.0068	-1.5657	
PT-Swab Blank-1	0.0001	0.023	
PT-Swab Blank-2	-0.0026	-0.5986	
PT-Swab Blank-3	-0.0008	-0.1842	

Accuracy: The accuracy of the method was determined by calculating recoveries of Atorvastatin by method of standard additions and the % of recovery were 100.2% and shown in **Table 3 and Table 4**.

TABLE 3: STANDARD ABSORBANCE AT 245nm

Standard	Absorbance
1	0.4632
2	0.4632
3	0.4654
Mean	0.4639
SD	0.0013
% RSD	0.28

TABLE 4: CALCULATION OF ACCURACY OF ATORVASTATIN RESIDUE CLEANING METHOD DEVELOPMENT

Recovery Level	Observed Area Counts (µv*sec)	Amount Recovered (µg)	Recovery Stock Solution added (ml)	Actual amount added (µg)	% Recovery
1/1	0.4650	100.353	1	100.116	100.2

% of Recovery = 100.2

Linearity: LOD & LOQ value as shown in **Table 5** and the calibration curve (**Figure 4**) was found to be linear over the range of 1-10 µg/ml for Atorvastatin.

TABLE 5: LOD & LOQ DETERMINATION

Stock Solution Taken (ml)	Dilute with diluent	Conc. (ppm)	Absorbance
0.1	10	0.1	0.0099
1	10	1.001	0.048
2	10	2.002	0.0989
4	10	4.005	0.1909
6	10	6.007	0.2849
8	10	8.009	0.3761
Standard Solution (10)	NA	10.012	0.4681

σ =0.001682543, (σ = Residual Standard Deviation or Regression Line), Slope = 0.046455278, LOD = 0.120 & LOQ =0.362

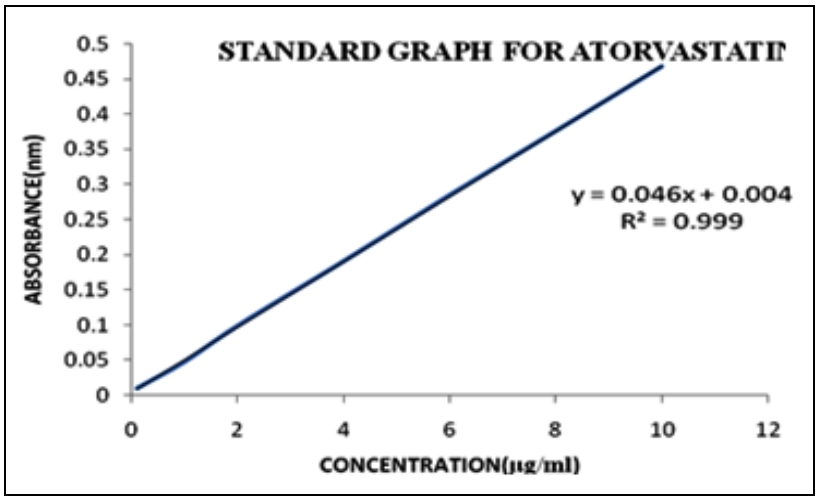


FIG. 4: STANDARD GRAPH OF ATORVASTATIN

Method Validation Results:

Specificity:

TABLE 6: SPECIFICITY TEST OF ATORVASTATIN CALCIUM HYDRATE RESIDUE

Sample	Absorbance	% Interference at 245nm
Blank (Diluent)	0	0
Rinse Solvent Water	-0.0296	-6.961
Swab Solvent Diluent	-0.0026	-0.611
PT-Swab Blank-1	0.0005	0.118
PT-Swab Blank-2	0.0012	0.282
PT-Swab Blank-3	0.0001	0.024

Accuracy:

TABLE 7: ACCURACY READINGS

Sample ID	Absorbance	Amount Recovered	Actual Amount Added	%Recovery of Pure drug	Statistical	Analysis
A_1 : 50%	0.2210	52.036	50.058	104.0	Mean	102.4%
A_2 : 50 %	0.2228	52.460	50.058	104.8	SD	3.5445
A_3 : 50 %	0.2089	49.187	50.058	98.3	% RSD	3.46
A_4 : 100 %	0.4170	98.185	100.116	98.1	Mean	99.4%
A_5 : 100 %	0.4171	98.209	100.116	98.1	SD	2.1939
A_6 : 100 %	0.4333	102.023	100.116	101.0	% RSD	2.21
A_7 : 200 %	0.8417	198.183	200.232	99.0	Mean	102.5%
A_8 : 200 %	0.8700	222.132	200.232	110.9	SD	7.112
A_9 : 200 %	0.8351	196.629	200.232	98.2	% RSD	6.93

TABLE 8: RECOVERY LEVEL

Accuracy	Recovery Level	% Recovery	Statistical Analysis
50% Accuracy Level	Recovery Level-I-1	104	Over all Mean = 101.5
	Recovery Level-I-2	104.8	
	Recovery Level-I-3	98.3	
100% Accuracy Level	Recovery Level-II-1	98.1	Over all SD = 4.4181
	Recovery Level-II-2	98.1	
	Recovery Level-II-3	101.9	Over all %RSD = 4.35
200% Accuracy Level	Recovery Level-III-1	99	% Recovery Factor = 0.981
	Recovery Level-III-2	110.9	
	Recovery Level-III-3	98.2	

TABLE 9: ACCURACY STANDARDS

Standard No.	Absorbance	Statistical Analysis
1	0.4296	
2	0.4253	Mean = 0.4252
3	0.4246	SD = 0.000
4	0.4252	%RSD = 0.07
5	0.4252	
6	0.4254	

Precision:

TABLE 10: PRECISION READINGS

Sample No.	Absorbance	Residue in µg/ml	Statistical Analysis
1	0.417	0.597	
2	0.4171	0.597	Mean = 98.958
3	0.4333	0.598	SD = 1.8760
4	0.4254	0.599	%RSD = 1.90
5	0.4185	0.601	Min. = 96.631
6	0.4108	0.598	Max. = 102.623

TABLE 11: LOD & LOQ DETERMINATION

Sample ID	Absorbance of LOD Solution	Absorbance of LOQ Solution
1	0.004	0.0153
2	0.0037	0.0158
3	0.0038	0.0163
4	0.005	0.0165
5	0.0051	0.0141
6	0.0039	0.0153

DISCUSSION: The present work provides an accurate, rapid, sensitive method for the estimation of atorvastatin calcium hydrate residue. From the optical characteristics of the proposed method, it was found that atorvastatin calcium hydrate obeys linearity within the concentration range of 1-10µg/ml.

The drug atorvastatin calcium hydrate standard was found to be maximum absorption in a wavelength at 245nm from figure 3a and the % of interference found within the limit not more than 2% from the Table 2.

From Table 5 and Table 6 residual standard deviation or regression line (**σ)** was found to be **0.001682543** and slope = **0.046455278, LOD= 0.120 & LOQ = 0.362.**

From Table 6, the scanning indicates that no interference from rinse solvent, swab solvent and swab blank indicating specificity of the method and the % of interference observed from rinse solvent water, Swab solvent diluent, and swab blank at 245nm were within defined limit (2%) and no interference was observed from the pharmaceutical adjuvants.

From the results shown in Table 9, it was found that the % RSD is less than 2, which indicates that the method has good reproducibility.

From the results shown in accuracy Table 4 and Table 8, it was found that the percentage recovery values of atorvastatin residue cleaning method is 100.2, which was validated and getting % recovery 101.5, indicates that the proposed method is accurate.

Hence, the proposed method was evaluated statistically and was validated in terms of precision, linearity and accuracy.

CONCLUSION: The proposed method is simple, sensitive and reliable with good precision and accuracy. The method is also specific while estimating the residue method development for cleaning. Hence, the method can be used for the determination of atorvastatin calcium hydrate in routine analysis.

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