Development & Validation of Complexiметric Titration Method for Analysis of Atorvastatin calcium In Raw Material and Tablet Dosage Form

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Abstract

An easy, sensitive and inexpensive titremitric method for determination of Atorvastatin calcium in raw material and tablet dosage form was developed. The method based on the reaction of calcium with a solution of Disodium Ethylene diamine tetra acetate (EDTA) - Magnesium 0.01M. Eriochrome Black T was used as indicator where a blue color observed at the end point at pH = 10.5. The method was validated following the suggestions of the International Conference on Harmonization (ICH) guidelines for linearity, precision, robustness and accuracy. No interference was observed in the presence of common pharmaceutical excipients. The method was found to be linear in the range (10-120) mg with R^2 equal to 0.9994. Recovery percent for standard Atorvastatin calcium (80,100,120 mg) was equal to 99.76±0.6. Accuracy was also confirmed based on three-level standard addition method (50,100,150 %) with a mean percentage recovery of 102.08±1.46. RSD % in Roubstness was found to be equal to 2.2. Further the method was precise with RSD% 0.9 for repeatability.

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Introduction

Atorvastatin calcium(ATV) chemically (bR, dR)- 2-(4-fluorophenyl)-b,d-dihydroxy-5-(1-methyl-ethyl)-3-phenyl-4-[(phenylamino)carbonyl]-lH-pyrrole-1-heptanoic acid, calcium salt (2:1), with molecular weight 1155.3 and molecular formula $C_{66}H_{62}CaF_2N_4O_{10}$[1], [2].

Atorvastatin is intended for the treatment of dyslipidemia as primary indication , as it is a competitive inhibitor of 3-hydroxy-3-methyl glutaryl-co enzyme A (HMG Co-A) reductase. Atorvastatin is available in 10, 20,40 and 80 mg tablet dosage form.

Atorvastatin is poorly soluble leading to low absolute and systemic bioavailability of about 14 and 30 % respectively[2]–[5].

One of the life threatening disease in both developed as well as in some parts of developing countries of the world is obesity; so Atorvastatin is used for treatment and prevention of the risk Atherosclerosis. Thus, there is a need to develop a more simple, rapid, accurate, precis and inexpensive methods to analyize Atorvastatin in bulk and pharmaceutical dosage forms[6].

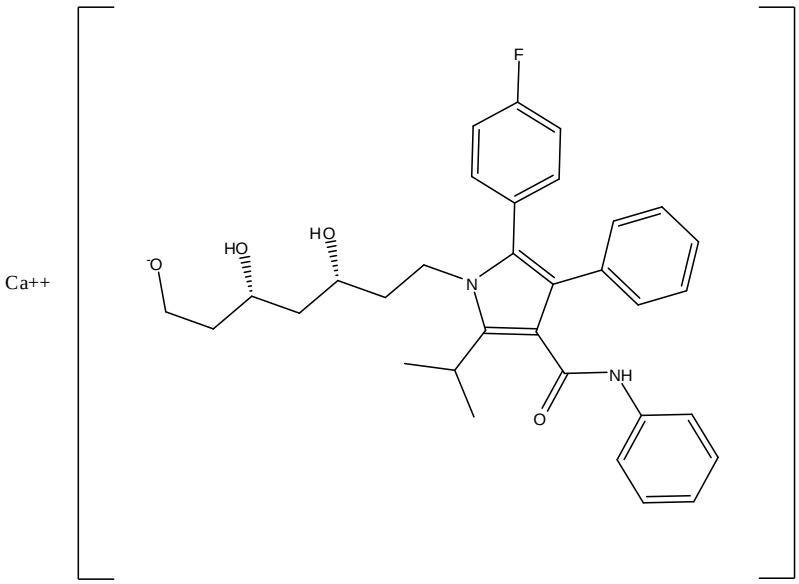

Figure 1: Structure of Atorvastatin Calcium.

In this context; determination of Atorvastatin relied on estimation of quantity of calcium ion in the analyte taking into account that each calcium ion complexes to two molecules of Atorvastatin, ultimately quantifying amount of calcium ion indicates amount of Atorvastatin present in the analyte.

Even though Eriochrome Black T cannot be used as an indicator in direct titration as it forms a weak complex with calcium at the end point ; introduction of small quantities of magnesium chloride to the EDTA solution notably enhances stability of Eriochrome Black T –calcium complex leading to stable color at the end point.

2. Materials and methods

2.1.1. Apparatus:

Pipettes (Marienfeld Germany)Tol ±0.05,Measuring cylinders (polylab) ±o.5,Beakers(ISOlabGermany),Burettes(ISOlabGermany)±o.05,Volumetricflasks(ISOlabGermany) ±0.4,Boro 3.3,Conical flasks (ISOlab Germany), Spatula.

2.1.2. Instruments:

PH meter (HANA instrument).

Electronic balance (BOECO Germany, S\N405399, made in Europe).

Oven.

2.1.3. Materials:

Atorvastatin calcium standard (Azal Company, assay99.9)

Disodium EDTA (SDFCL, india,assay 98%)

Magnesium chloride hex hydrate (Chemlab NA, assay 99%)

Methanol (Chemlab NA, assay 99.8)

Hydrochloric acid 0.1 M (Chemlab NA assay 37%)

Erochrome Black triturate (EBT) (SURchem Product LTD)

Ammonia (Labtech chemical, assay25 %)

Ammonium chloride (Labtech chemical, assay 99% by argentometry)

Purified (distilled) water

Calcium carbonate (Oxford Laboratory reagent, assay98%).

2.1.4. Preparation of reagents:

2.1.4.1-Magnesium chloride 1% (w/v):

To 50 ml volumetric flak 1.07 g of magnesium chloride hexahydrate was transferred, then dissolved with 30 ml of distilled water and made up to the volume.

2.1.4.2-Ammonia 6M:

From ammonia (NH3) 25%, 44 ml was placed in 100 mL volumetric flask and completed with distilled water.

2.1.4.3-Disodium ethylene diaminotetraacetate (EDTA) - Magnesium volumetric solution:

In l L volumetric flask 4 g of disodium EDTA dehydrated salt was dissolved with 500 mL of distilled water, then 10 mL of magnesium chloride 1% and 2 mL of ammonia 6 M were added and then completed to l L with purified water .

2.1.4.4- Ammonia – ammonium chloride buffer:

Six grams of ammonium chloride was dissolved by 100 mL ammonia 6 M, Stirred until total dissolution.

2.1.4.5-Diluted hydrochloric acid (0.1M):

Prepared by diluting 8.3 ml of 37%(w\v) hydrochloric acid solution with sufficient water to make 1000 mL .

2.1.4.6-Sample preparation:

We picked one brand of Atorvastatin calcium (Amistatin 20 mg) to be tested. Samples were weighed and crushed uniformly with the help of a mortar and pestle. A weight equivalent to 20 mg of Atorvastatin calcium was transferred into volumetric flask and dissolved by 20 ml methanol.

2.2. Standardization

The 0.01 M EDTA - magnesium solution was standardized with calcium carbonate; 30 mg of previously dried calcium carbonate was placed in a 250 mL conical flask and dissolved by 10 mL of distilled water , then stirred until complete dissolution. To titration mixture, 10 mL of 0.1 M hydrochloric acid and of ammonia – ammonium chloride buffer were added, followed by addition of 30 ml distilled water. Pinch of Erochrome black T was used as indicator; the titration was conducted until the pink color changed to blue at the end point.

The solution was titrated with 0.01 M EDTA- magnesium solutions. Each mL of 0.01 M EDTA - magnesium solution is equivalent to 1.0009 mg of calcium carbonate.

2.3. General Procedure

Fifty milligrams(50 mg) of Atorvastatin calcium was exactly weighed, placed in a 250 ml conical flask, and dissolved with 20 mL of methanol. Then 5 mL of ammonia – ammonium chloride buffer (pH= 10.5) was added. Pinch of EBT blue was incorporated and titrated against 0.01 M EDTA - magnesium solution until the pink color changed to blue at the end of titration.

Each mL of 0.01 M EDTA - magnesium solution is equivalent to 11.553 mg of Atorvastatin calcium.

2.4. Method Validation

2.4.1. Linearity:

linearity was determined by analysis of ten replicates(10,20,30,50,62.5,80,90,100,110 and 120)mg and the corresponding volume of EDTA was estimated followed by construction of standard calibration curve .

2.4.2. Precision

The precision was performed by analyzing six replicates of samples containing 50 mg Atorvastatin calcium.

2.4.3. Accuracy

Recovery studies were carried out to evaluate the accuracy of the method, using 9 samples at three different levels (80%, 100% and 120%) from standard Atorvastatin calcium.

Accuracy was also confirmed by standard addition method at three levels (50%, 100% and 150%).

2.4.4. Robustness

Robustness of the method was determined by performing the analysis using different burettes.

3. Results

3.1. Linearity

Table 1: Linearity

W.t taken (mg)	Final volume (ml)	Content %
20	1.7	98.5
30	2.5	96.8
50	4.25	99.1
62.5	5.55	103.7
80	7	102.0
90	7.75	100.3
100	8.7	100.4
110	9.5	100.6
120	10.55	102.5

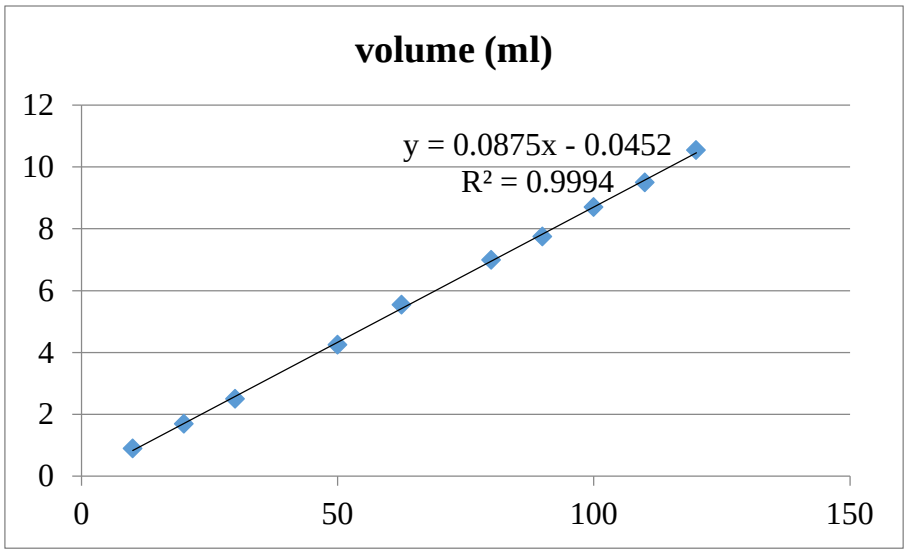

Figure 2 : Linearity curve.

3.2. Accuracy:

3.2.1. Percentage recovery

Table 2: Accuracy

Wt(mg)	volume(ml)	Actual wt %	Recovery%	RSD%
80	6.6	78.577	98.2	
80	6.7	79.76	99.7	0.876
80	6.7	79.76	99.7	
100	8.4	100	100	
100	8.4	100	100	0
100	8.4	100	100	
120	10.1	120.2	100.1	
120	10.1	120.2	100.1	0
120	10.1	120.2	100.1	
				RSD 0.6

3.2.2. Stander addition method

Table 3: Stander addition method

Level of recovery%	Initial amount of sample (mg)	Amount of standard added(mg)	Total amount present(mg)	Total amount recovered(mg)	Recovery %	Average recover %	RSD %
50%	20	10	30	30.32	101.7	101.7	0
	20	10	30	30.32	101.7		
	20	10	30	30.32	101.7		
100%	20	20	40	40.65	101.9	101.9	2.8
	20	20	40	40.06	99		
	20	20	40	41.24	104.9		
150%	20	30	50	52.16	102.5	102.5	0
	20	30	50	52.16	102.5		
	20	30	50	52.16	102.5		
						102.08	**1.46**

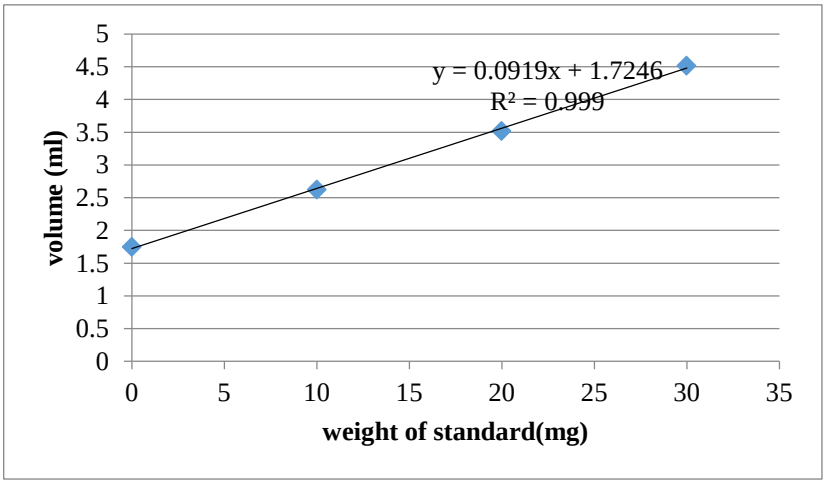

Figure 3 : standard addition curve

3.3. Precision (repeatability)

Table 4: precision (repeatability)

No. of titration	Final volume	Content %
1	4.3	102.3
2	4.2	99.9
3	4.2	99.9
4	4.2	99.9
5	4.2	99.9
6	4.2	99.9
RSD 0.9		

3.4. Robustness

Table 5: Roubstness

Wt of standerd (mg)	Final volume	Content%
50	2.5	99.4
50	2.6	103.3
50	2.6	103.3
RSD 2.2		

4. Discussion

Atorvastatin became one of most prescribed drug for treatment of dyslipidemia which currenty a major interest for many pharmaceutical companies. Many manufacturers use in-house method for analysis of Atorvastatin calcium since it lacks a known official method for analysis. Accordingly there is a massive need to develop a new, simple, economic and rapid method for quantitative analysis of Atorvastatin calcium in both raw material and final dosage forms.

A direct complexiметric titration was employed as proposed method, however a minor modification performed by addition magnesium chloride to EDTA solution.

As described by Adriana I. Segall and her co-worker, magnesium chloride notably improves stability of EDTA-calcium complex; thus getting a sharp and reliable end point.[7] Atorvastatin practically is insoluble in water and partially soluble in ethanol and water-methanol co-solvent; but it was absolutely soluble in methanol as reported by Safila Naveed[8].

The relationship between tested analyte(different weights) and response(burette reading) is expressed by Linearity; which measured by regression coefficient (R^2).Following ICH guidance it must be of value less than one. In this context, the linearity calculated in range (20-120)mg with R^2 equal to 0.9994, the method seemed to be of greater linearity when compared to R^2 of Surendra Singh Inda who calculated R^2 to be 0.998 .

Considering ICH guideline, recovery of active ingredient in the tested sample after performing the method under assessment directly relate to the accuracy of the method; the recovered amount calculated as percentage from taken amount and known as percentage recovery.

Here in, recovery was executed using three weights(80,100,120)mg with corresponding percentage of 99.7 % , 100 % and 100.1 % respectively; as a result accuracy of the method was established.

Based on literature, Accuracy is also confirmed by standard addition method. This test also use recovery percent; however in this method a known amount from standard substance is added to a previously measure amount of the sample so that percentage recovery expresses the final amount of active substance existed in the analyte.

The average recovery percent performed at three levels (50 %, 100 % and 150 %) was 102.8 % indicating Accuracy of the method.

Precision (a measure of repeatability) indicates closeness of response value (burette reading) upon repeating the titration several times taking the same weight under same conditions. Precision expressed as RSD % for all readings. In this research RSD % was 0.9 % ; consequently the method considered to be of good Precision.

The degree to which titration could withstand change in conditions such as temperature, PH and operators is indicated by Roubstness test.

Robustness results also expressed as RSD % of final volumes of burette readings; calculated RSD % was 2.2 upon changing the burettes only. Accordingly the method was Robust.

5. Conclusion

The developed method was valid; since it successfully satisfied the desired criteria of the ICH guidelines for linearity, accuracy, precision and robustness. The method is economical as compared to other reported analytical method. So this method suitable for routine analysis of commercially available formulation of Atorvastatin Calcium. This method is also suitable for determination of Atorvastatin Calcium in Tablet dosage Form without interference from common pharmaceutical excipients.

6. Acknowledgement

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7. References

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