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Development and Validation of a Complexometric Titration Method for the Determination of Rosuvastatin Calcium in Raw Material

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Authors' contributions

This work was carried out in collaboration between all authors. Author LDS managed the literature searches and wrote the protocol. Authors MB, SLB and LP managed the experimental process and performed the titration analysis. Author AIS designed the study and wrote the manuscript. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: An easy, sensitive and inexpensive volumetric method for the determination of rosuvastatin calcium in raw material has been developed.
Methodology: The titrimetric method is based on the reaction of calcium with a solution of Disodium ethylene diaminotetraacetate (EDTA) - Magnesium 0.01 M. Hydroxynaphtol blue was used as indicator. It changes from pink to blue at pH = 10 at the end point of the titration. The method was validated for linearity, precision and accuracy, following the suggestions of the International Conference on Harmonization (ICH).
Results: The linearity of the volumetric method was determined by analysis of six replicates at 80%,

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100% and 120% and three replicates at 90% and 110% of analyte concentration. The calibration curve was linear, with r = 0.9998. Assay method precision was evaluated by carrying out six independent assays of bulk drug and the intermediate precision was also verified using different analyst and different day in the same laboratory. Accuracy (mean recovery 99.0%) and precision were found to be satisfactory.
Conclusion: The proposed method can be used for quality control assay of rosuvastatin calcium in bulk drug.

Keywords: Rosuvastatin calcium; titrimetric; assay; complexometric titration; EDTA.Mg.

1. INTRODUCTION

Rosuvastatin calcium (Fig. 1) is bis [(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2- [methyl (methylsulfonyl)amino] pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt. It is a competitive inhibitor of the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), the enzyme that converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonate, precursor for cholesterol. It is a cholesterol lower agent [1]. The usual doses are 5, 10, 20 and 40 mg.

Rosuvastatin calcium alone has been determined by Spectrophotometric methods [2-4], TLC methods [5-7] and a Stability indicating HPTLC method [8].

A literature study revealed some high-performance liquid chromatographic methods for the quantitation of rosuvastatin calcium [4,9-13]. Most of the analytical techniques for rosuvastatin calcium described in the literature are based on the HPLC determination of this drug with another active drug substance [1,14-22].

Due to the need for an absolute analytical technique for rosuvastatin calcium, we have developed this volumetric technique. The present technique is concerned using a complexometric titration for the quantification of Calcium (Ca). Complexometric titrations with metal ions serve as analytical determinations of raw material at the millimole concentration. The substances that have a pair of unshared electrons (e.g. on N, O, S atoms in the molecule) are capable of satisfying the coordination number of the metal. The metal ion is a Lewis acid (electron pair acceptor) and the complexing agent is a Lewis base (electron pair donor). The complexing agent use a number of molecules which depends on the coordination number of the metal and the complexing groups of it.

A chelating agent is an organic substance that has two or more groups capable of complexing with a metal ion, forming a chelate. This titration is called a chelometric titration, which is a particular type of complexometric titration. There are six complexing groups in EDTA. The EDTA is represented by the symbol H4Y. The four hydrogens in the formula refer to the four acidic hydrogens on the four-carboxyl groups. It is a triprotic acid. The pair of unshared electrons is located on each of the two nitrogen atoms and each of the four-carboxyl groups [23].

There are not satisfactory indicators for calcium by direct titration. Eriochrome Black T cannot be used as an indicator because it forms a weak complex with calcium to give a sharp end point. Nevertheless, with a small addition of magnesium chloride to the EDTA solution,

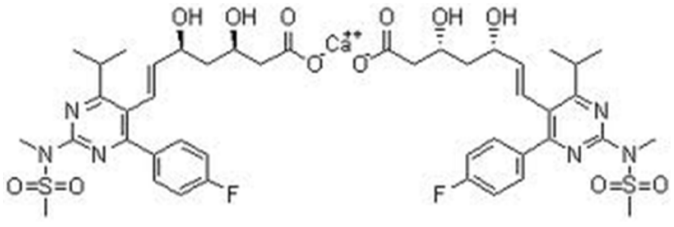

Fig. 1. Rosuvastatin calcium

calcium can be determined by direct titration. The titrant solution contains a magnesium-EDTA complex which is introduced into the titration mixture. As Ca2+ forms a more stable complex with EDTA than magnesium, the following reaction occurs:

$$MgY^{2-} + Ca^{2+} <===> CaY^{2-} + Mg^{2+}$$

Hydroxynaphthol blue is used as indicator; it is itself a chelating agent. It changes from pink to blue in the presence of calcium at pH 10.

The present manuscript describes an easy, sensitive and inexpensive volumetric method for the quantitation of rosuvastatin calcium in raw material.

The method was validated for linearity, precision and accuracy, following the suggestions of the International Conference on Harmonization (ICH) [24].

2. MATERIALS AND METHODS

2.1 Apparatus

A standard borosil burrets, pipetts, standard flasks, measuring cilindres and conical flasks are calibrated as per International Conference on Harmonization (ICH) guidelines [25].

2.2 Materials

Rosuvastatin calcium (100.0%) was obtained from Optimus drugs (Hyderabad, India). Disodium EDTA was AR Grade (Merck Química Argentina, Argentina); Magnesium chloride hexahydrate AR Grade (Alcor, Argentina) Ammonia AR Grade (Conc 20%, Reactivo Raudo Analítico, Argentina), Ammonium chloride AR Grade (J. T. Baker, Mexico), Calcium carbonate ACS grade (Mallinckrodt, USA), Hydrochloric acid ACS grade (J. T. Baker, Mexico), Hydroxy naphthol blue AR Grade (Anedra, Argentina). Purified (distilled) water was passed through a 0.45 µm membrane filter.

2.2.1 Magnesium chloride 1% (w/v)

1.07 g of magnesium chloride hexahydrate was taken in a 50 ml volumetric flask, dissolved with 30 ml of distilled water and made to volume with the same solvent.

2.2.2 Ammonia 6M

The 51 mL ammonia (NH_3) 20% was taken in 100 mL volumetric flask and made to volume with distilled water.

2.2.3 Disodium ethylene diaminotetraacetate (EDTA) - Magnesium volumetric solution

4.0 g of disodium EDTA dehydrate salt was dissolved in 500 mL of distilled water in a 1 L volumetric flask. Add 10 mL of magnesium chloride 1% and 2 mL of ammonia 6M and made to 1 L with purified water.

2.2.4 Ammonia – ammonium chloride buffer

Dissolve 6.0 g ammonium chloride in 100 mL of ammonia 6 M. Stir until total dissolution.

2.2.5 Diluted hydrochloric acid (0.1N)

Prepare by diluting 100 ml of 1N hydrochloric acid solution with sufficient water to make 1000 mL.

2.3 Standardization

The 0.01 M EDTA - magnesium solution was standardized with calcium carbonate, placing 30 mg previously dried at 110ºC to constant mass in a 250 mL conical flask, dissolve in 10 mL of distilled water and stir until dissolution. Add 10 mL of 0.1N hydrochloric acid. Add 10 mL of ammonia – ammonium chloride buffer and 30 mL of distilled water. Add 25 mg of hydroxy naphthol blue and shake during 1 min. Until the pink colour changes to blue at the end of titration, the solution was titrated with 0.01M EDTA - magnesium solution. Each mL of 0.01 M EDTA - magnesium solution is equivalent to 1.0008 mg of calcium carbonate.

2.4 General Procedure

250 mg of rosuvastatin calcium was exactly weighed, placed in a 500 ml conical flask, and dissolved in 200 mL of purified water and 25 mL of ammonia – ammonium chloride buffer. Stir during 10 min. Add 25 mg of hydroxy naphthol blue and shake during 1 min (pH= 10.0) Titrate with 0.01M EDTA - magnesium solution until the pink colour changes to blue at the end of titration.

Each mL of 0.01 M EDTA - magnesium solution is equivalent to 10.0114 mg of rosuvastatin calcium.

2.5 Method Validation

2.5.1 Linearity

Linearity was determined by analysis of six replicates at 80%, 100% and 120% and three

replicates at 90% and 110% of analyte concentration.

2.5.2 Precision

Precision of the assay method was performed by analyzing six individual samples of rosuvastatin calcium raw material. Intermediate precision was checked by analyzing six individual samples by two different analysts on different days.

2.5.3 Accuracy

Recovery studies were done to evaluate the accuracy of the method, 6 samples each at three different levels (80%, 100% and 120%). It was calculated the amount of rosuvastatin calcium recovered in relation to the added amount.

3. RESULTS AND DISCUSSION

The described method has been suitably applied to the quantitation of rosuvastatin calcium in raw material.

Linearity was determined by analysis of six replicates at 80%, 100% and 120% and three replicates at 90% and 110% of analyte concentration. The correlation coefficient ("r") value was 0.9998 and the calibration curve showed adequate linearity over the concentration range. The regression equation for the calibration curve was found to be y = 0.1035x + 0.2495. The method was lineal, the calibration graphs was fulfilled by the high value of the correlation coefficient and the intercept value that was not statistically (P = .05) different from zero (Table 1 and Fig. 2).

The precision is usually expressed as the RSD of a series of measurements. It expresses the proximity of compliance between a series of assessments obtained from multiple sampling of the aforementioned homogeneous specimen under the prescribed conditions.

Table 1. Linearity

% of nominal value	Weighed (mg) (RSD)	Vol cons (RSD)
80	200.6 (0.6)	21.0 (0.7)
90	226.4 (0.5)	23.7 (0.4)
100	251.8 (0.6)	26.3 (0.7)
110	276.2 (0.1)	28.7 (0.3)
120	301.7 (0.5)	31.5 (0.4)
Slope[(a)]	0.1035.±0.0011	
Intercept[(b)]	0.2495±0.3706	

[a]Confidence limits of the slope (p= 0.05); [b]Confidence limits of the intercept (p= 0.05)

The intermediate precision of the volumetric method was performed by analyzing the samples on two different days by two different persons. The results were presented both separately and as the mean. For precision tests the conclusion was: average values 99.5 and 100.9%, RSD 0.4% and 0.5%. "t" Test comparing two assays with 95% confidence for 10 degrees of freedom disclosed that both means were not sufficiently different *inter se* ($t_{n-2,\ \alpha:0.05}$) = 2.23 (Table 2).

The percentage recuperation range and RSD values were 98.5-99.7% and within 0.3% respectively. Accuracy was also evidenced by making a graph of the amount of rosuvastatin calcium measured against the amount present in the samples, both expressed in mg. Linear regression analysis provided slopes not sufficiently different from 1 (t test P=.05), intercepts not significantly different from zero (t test P=.05) and r = 0.9999, the RSD was 0.3. (Table 3 and Fig. 2).

Table 2. Intermediate precision

Analyst 1 sample N°	Weighed (mg)	Percentage	Analyst 2 sample N°	Weighed (mg)	Percentage
1	249.8	99.3	1	250.6	100.9
2	250.2	99.1	2	252.3	101.3
3	250.4	99.4	3	250.1	101.1
4	249.7	100.1	4	252.0	101.4
5	250.8	99.6	5	250.0	100.3
6	251.8	99.3	6	250.9	100.4
Mean	250.4	99.5		251.0	100.9
RSD	0.3	0.4		0.4	0.5

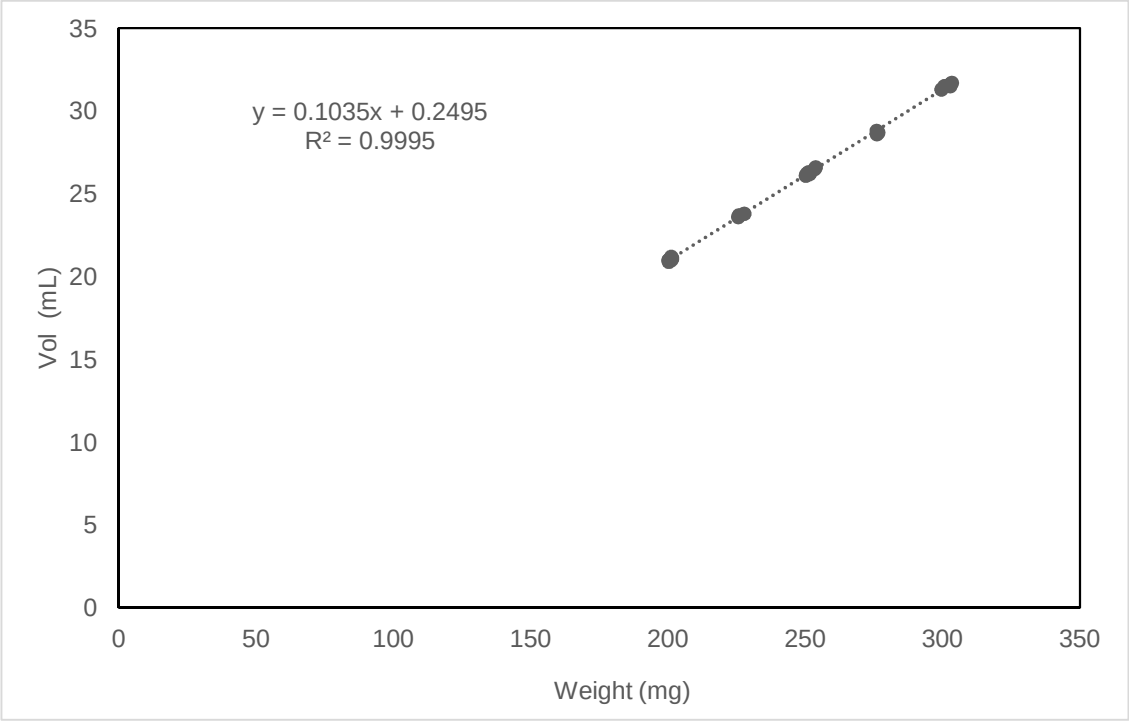

Fig. 2. Linearity

Table 3. Accuracy

% of nominal value	Added amount (mg)	Found amount (mg)	Recovery (%)	Average recovery (n=3)	RSD (%)
80	194.0	191.6	98.8	99.1	0.4
	194.2	192.5	99.1		
	193.1	190.7	98.8		
	192.2	189.8	98.7		
	193.1	191.6	99.2		
	193.0	191.6	99.3		
	194.0	193.5	99.7		
100	241.2	238.2	98.8	98.9	0.2
	242.5	239.1	98.6		
	241.5	239.1	99.0		
	242.1	240.0	99.2		
	244.3	241.8	99.0		
	244.7	242.7	99.2		
120	288.8	285.6	98.9	98.9	0.2
	292.5	289.3	98.9		
	290.3	287.5	99.0		
	289.9	287.5	99.2		
	291.7	288.4	98.9		
	291.9	287.5	98.5		
Mean (n=18)				99.0	0.3

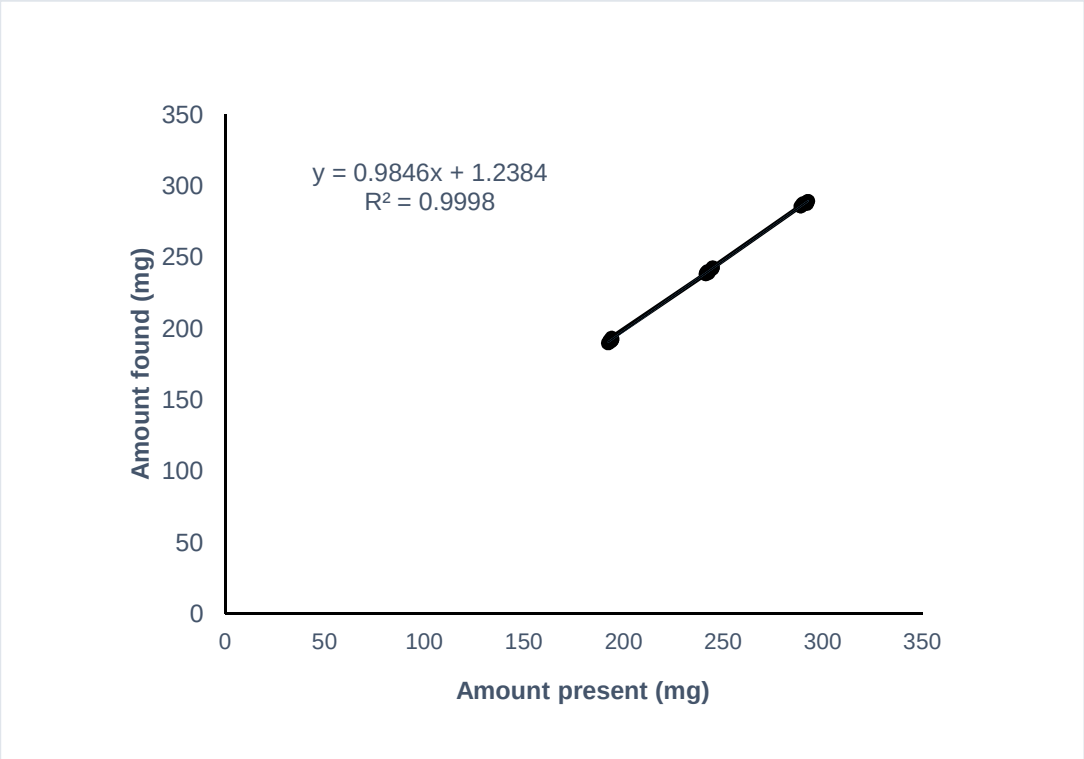

Fig. 3. Accuracy

4. CONCLUSIONS

The volumetric method proposed is easy, sensitive and inexpensive and can consequently be applied to the determination of rosuvastatin calcium in raw material. Method validation including linearity, precision and accuracy generated acceptable results.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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