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Article

Simple Titrimetric Analysis for Determination of Pitavastatin Calcium in Bulk and Formulation Dosage

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Abstract: A simple, convenient, economic and accurate method is reported for the determination of pitavastatin calcium in pure and dosage form. This method is an indirect determination of pitavastatin by determining the amount of calcium present in the drug. Pitavastatin belongs to the group of anticholesterol agents used in the treatment of hypercholesterolemia. Analytical methods used so for to determine the concentration of this active pharmaceutical ingredient in dosage forms in the quality control are based mainly on the instrumental methods. The present work is based on the complexometric titration of Ca^{2+} with EDTA. The titration was carried out both by direct titration with EDTA and back titration with Zn^{2+}. Eriochrome black T was used as the indicator at pH = 10 in both the titrations. The optimum reaction conditions and other analytical parameters were evaluated.

Keywords: pitavastatin; calcium; EDTA; eriochrome black T; complexometric titration.

1. Introduction

The statin drugs inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and, thereby, suppress cholesterol biosynthesis. In the 1970s, Endo et al. [1, 2] studied how certain fungi protected themselves against others. In 1978, they reported the discovery of mevastatin, the first statin drug. Eventually, through the laboratory of Goldstein and Brown [3, 4], statin drugs emerged as the most effective means of reducing elevated levels of plasma cholesterol. There are currently seven statins available in pharmaceutical industries, that is, lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, rosuvastatin, and pitavastatin [5, 6]. First-generation statins, such as lovastatin and mevastatin, were isolated from fungi. However, second- and third- generation statins have been developed by either modification of first-generation statins or through chemical synthesis in the laboratory. In general, all the statins share similar chemical characteristics, with second- and third-generation statins having several aromatic rings and an aliphatic fatty acid side chain, and first-generation statins which have a decalin ring and an aliphatic side chain. Pitavastatin calcium (PVT) [7], mono calcium bis {(3R, 5S, 6E)-7-[2-cyclopropyl-4-(4-flurophenyl)-3-quinolyl]-3-5-dihydroxy-6-heptenoate} (Fig 1.), is a lipid-lowering agent [8], used in hyperlipidemia.

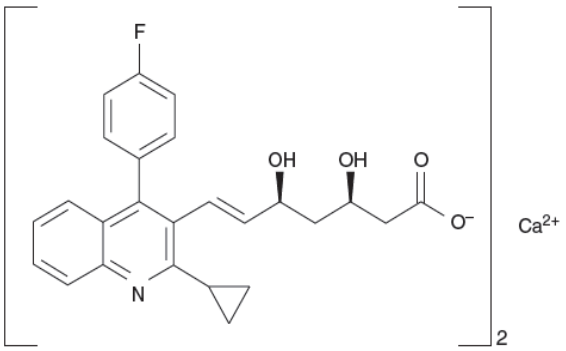


Figure 1. Chemical structure of PVT

Several analytical techniques have been used for the determination of PVT, such as, Deng et al. [9] and Hualv et al. [10] estimated pitavastatin by LC-MS method employing C18 column with different lengths (50 mm and 150 mm) and mobile phases (acetonitrile: 0.1 % formic acid (90:10, v/v) and acetonitrile: 0.025 % formic acid (70:30, v/v), respectively) in clinical samples; Bakyalakshmi et al. [11] estimated PVT by HPTLC using silica gel 60F254 (10-20) cm plate and ethyl acetate: methanol: NH_3: 1- drop formic acid (7:2:0.8, v/v/v) at room temperature; Krishna et al. [12] estimated PVT spectrophotometrically employing three methodologies such as oxidation, complex formation and Prussian blue formation and followed the reactions at 510 nm, 530 nm and 755 nm respectively.

Gomes et al. [13] validated the stability of PVT under different conditions employing BEH C18 (100 × 2.1 mm, 1.7 µm) as stationary phase and 0.03% orthophosphoric acid (0.3 mL/L) and acetonitrile under the gradient elution mode and detected at 245 nm. Sathishkumar et al. [14] estimated PVT by HPLC method using 0.5% AcOH: acetonitrile (35:65) as mobile phase and C18 as the stationary phase in the room temperature with a flow rate of 1 mL/ min, and Panchal et al. [15] employed the same C18 column as the stationary phase and 0.1% orthophosphoric acid: acetonitrile: trimethylamine (19.8:80:0.2, v/v/v) with pH = 3±0.05 at room temperature with a flow rate 1.4 mL/min. Both the methods were executed with isocratic elution mode and detected by diode array detector.

Virupaxappa et al. [16] estimated PVT spectrophotometrically and estimated indirectly by following the decrease in the intensity of $KMnO_4$ after oxidation of PVT at 550 nm. Ergin et al. [17] estimated all statin drugs except lovastatin and paravastatin by spectrophotometric means and based on the charge transfer reaction of drugs as n-electron donor, a 7,7,8,8-tetracyanoquinodimethane (TCNQ) as π-acceptors to give highly colored complex species. The interaction of statins with TCNQ in acetonitrile yielded a bluish-green colored chromogen, which absorbs strongly at 843 nm.

Up to this stage there was no report concerning the estimation of PVT by simple titration method. The present method is a simple and low cost titration analysis which is desirable for the determination of PVT in pharmaceuticals. PVT contains two pitavastatin molecules (one fluoride substituted aromatic ring, one quinoline ring to which one cyclopropyl group and an aliphatic fatty acid side chain with two hydroxyl groups bonded to the ring in the adjacent position) complexed with a calcium ion. EDTA is the most commonly used chelating agents as it can form complexes with a wide range of metals. Metal ions (Lewis acids) react with electron pair donors (Lewis bases) to form coordination compounds or complexes. A chelate is produced when a metal ion coordinates with two or more donor groups from the ligand. EDTA has six sites which can donate an electron pair to Ca^{2+} and hence Ca-EDTA complex is more stable than Ca-indicator complex. The most common indicator is eriochrome black T [EBT]. EBT binds to metal ions to give a red color. Upon release of the metal to EDTA, it becomes blue. The proposed method is simple, accurate and easy to apply to routine analysis**.**

2. Materials and Methods

2.1. Apparatus

A standard borosil burrets, pipetts, standard flasks, measuring cylinders and conical flasks are calibrated as per International Conference on Harmonization (ICH) guidelines [18].

2.2. Materials and Method

All solutions were prepared with doubly distilled water. All the chemicals (analytical reagent grade) were used in this work. The procedure for preparation of all reagents had been referred from the textbook [19].

2.2.1. Disodium ethylene diaminetetraacetate (EDTA)

The 0.01 M EDTA was prepared by dissolving 3.72 g of disodium EDTA dehydrate salt in double distilled water in a 1 L volumetric flask and standardized with 0.01 M $ZnSO_4$ a primary standard solution.

2.2.2. Zinc sulphate heptahydrate

The 0.01M $ZnSO_4$ solution was prepared by dissolving 1.61 g of zinc sulphate heptahydrate in 1 L volumetric flask, dissolved with 500 mL of water and made to volume with double distilled water.

2.2.3. NH_3-NH_4Cl buffer (pH = 10)

The 142 mL ammonia (NH_3) was taken in 400 mL beaker and 17.5 g ammonium chloride (NH_4Cl) salt was weighed and transferred to that beaker and made to 250 mL with water.

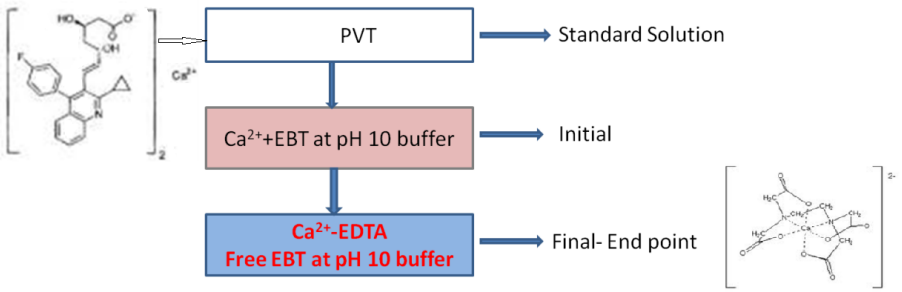
2.2.4. Pitavastatin calcium

A 0.01M standard pitavastatin calcium drug solution was first prepared by dissolving 881.1 mg in 5 mL 2 M H_2SO_4 and diluting to the mark with distilled water in 100 mL calibrated flask. The stock solution was diluted approximately to get the working concentration.

2.2.5. Recommended Procedure

2.2.5.1 Direct titration

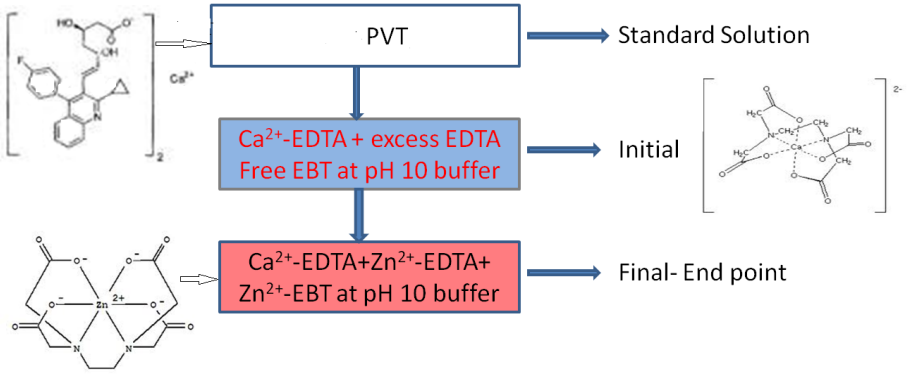
The 5 mL of 0.01 M PVT solution was taken into 250 mL conical flask and 100 mL water added to it. The 5 mL buffer solution was added and pinch of EBT indictor was added, the initial color was wine red. The solution was titrated against 0.01 M EDTA solution. The end point was wine red to blue color and has been explained in Scheme 1.



Scheme 1. Direct titration between 0.01 M PVT and 0.01 M EDTA

2.2.5.2 Back titration

To 5 mL of 0.01 M PVT excess (~10 mL) of 0.01 M EDTA solutions was added and 100 mL water was added followed by 5 mL of buffer (pH 10) solution and a pinch of EBT indicator. The initial color was blue. The solution was titrated against standard 0.01 M $ZnSO_4$ solution. The end point was blue to wine red color and the procedure was explained in the Scheme 2.



Scheme 2. Titration between 0.01 M EDTA and 0.01 M $ZnSO_4$

2.2.5.3 Analysis of formulations

Pivasta 4, 2 & 1 (Zydus cadila healthcare) tablets were purchased from local pharmacy. The 40 tablets were ground well, initially average weight for 40 tablets were noted. The required amount of PVT to make 0.005 M PVT was taken into 250 mL beaker dissolved with 50 mL water and 10 mL 2 M H_2SO_4 filtered through Whatman filter paper 3 or 4 times washed the residue with water and made up to 100 mL with water. The coating material TiO_2 doesn't react with EDTA and also with the indicator (EBT), and was confirmed with pure TiO_2. Both the direct and back titrations were performed by following the above procedure. The blank correction was done with other excipients like starch.

3. Results and Discussion

The titration results for pure drug by both direct and back titrations are presented in the Table 1. The results are in very good agreement with the expected theoretical values, which are comparable with each other for direct and back titration. The values are highly precise and can be visualized from the RSD values.

The titration was performed with formulation tablets and results are presented in Table 2.

The titrations are extended for different PVT contents of tablets of same brand Pivasta from Cadila Health care Pvt., and results are presented in Table 3. The results obtained in titration are plotted against labeled tablet value with correlation coefficient $R^2 = 0.9999$ and slope 0.95 (Fig. 2).

Table 1. Titration results of PVT in form of bulk drug

S. No.	Direct Titration		Back Titration		Theoretical Value (mg)	Assay (%)
	Ca content (mg)	PVT content (mg)	Ca content (mg)	PVT content (mg)		
1	1.96	43.08	2.00	43.96	44.05	99.80
2	1.98	43.52	1.98	43.52	44.05	98.80
3	1.96	43.08	2.00	43.96	44.05	99.80
4	1.98	43.52	2.00	43.96	44.05	99.80
5	1.98	43.52	2.00	43.96	44.05	99.80
6	1.98	43.52	2.00	43.96	44.05	99.80
7	1.98	43.52	2.00	43.96	44.05	99.80
8	1.98	43.52	1.98	43.52	44.05	98.80
Average	1.98	43.41	2.00	43.85	44.05	99.55
SD	0.0093	0.2035	0.0093	0.2035		0.97
RSD (%)	0.47	0.47	0.46	0.46		1.00

Table 2. Titration results of PVT in form of tablet dosage

S. No.	Direct Titration		Back Titration		PVT Label value (mg)
	EDTA Volume (mL)	PVT Observed value (mg)	EDTA Volume (mL)	PVT Observed value (mg)	
1	4.8	1.92	4.8	1.92	2.00
2	4.8	1.92	4.8	1.92	2.00
3	4.7	1.88	4.8	1.92	2.00
4	4.7	1.88	4.8	1.92	2.00
5	4.8	1.92	4.7	1.88	2.00
6	4.8	1.92	4.7	1.88	2.00
Average	4.77	1.91	4.77	1.91	2.00

Table 3. Titration results of different concentrations of Pivasta tablets

Tablet Brand	PVT Labeled Value (mg)	PVT Observed Value (mg)	
Pivasta 1	1	0.96 0.95 0.96	0.96
Pivasta 2	2	1.92 1.92 1.92	1.92
Pivasta 4	4	3.84 3.80 3.82	3.82

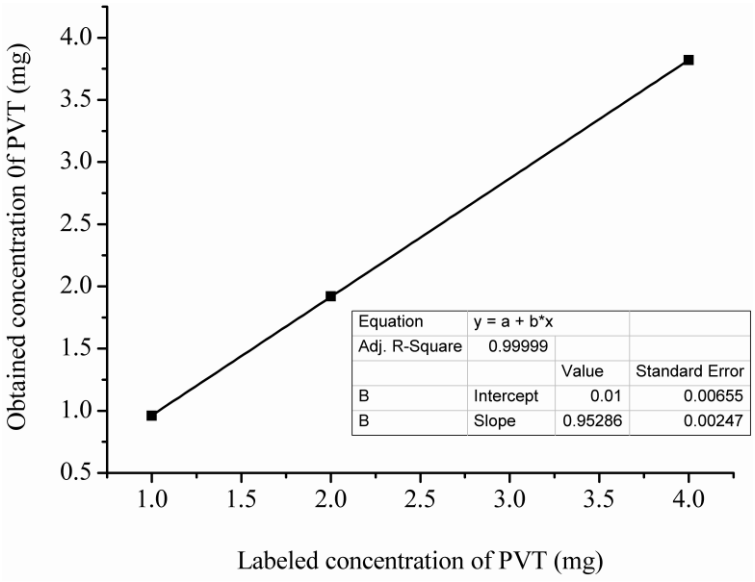


Figure 2. Plot of labeled tablet concentration vs obtained concentration in titration (R^2 = 0.9999).

The present method was checked for its validity at different laboratory with different persons. The results obtained from them are presented in Table 4. The results such as precision, variance t-test and F-test values are highly encouraging, precise and comparable; hence the method can be extended to any analytical laboratory for quality control purposes. The comparisons of precisions between various methods have also been made and were presented in Table 5. From the above results presented so for and comparisons made it can be inferred that titration method to determine PVT is a very good and simple method to perform in any simple pharmaceutical industrial labs.

Table 4. Ruggedness reports of titration method

Methods	t-Test	F-Test	RSD (%)
Titrimetric	0.47	7.58	0.47
UV-Visible	1.13	1.19	0.42
HPLC	-	-	0.22
HPTLC	-	-	0.35
UPLC	-	-	1.04

Table 5. Comparison of statistical results of various methods

Ruggedness	Direct Titration		Back Titration	
	Inter Analyst	Inter lab	Inter Analyst	Inter lab
Precision	0.32	0.31	0.42	0.39
Variance	0.47	0.48	0.32	0.33
Student test (t-test)	1.69	1.84	8.58	7.86
F-test	0.32	0.31	0.42	0.39

4. Conclusions

An effective method for the determination of PVT using EDTA as a reagent has been developed. The method is simple and easy to perform and cost effective compared with many other methods and do not entail any stringent experimental variables which affect the reliability of results. The proposed method can be used for the determination of PVT for bulk and formulation dosages in pharmaceutical industries.

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