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UV SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PITAVASTATIN CALCIUM AND EZETIMIBE INCOMBINED PHARMACEUTICAL FORMULATION

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ABSTRACT

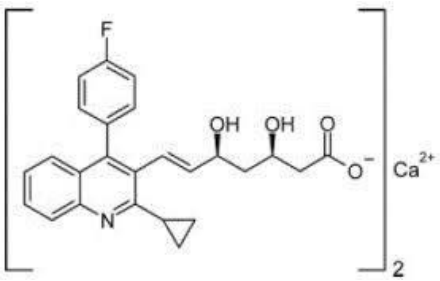
Introduction: A simple, rapid, accurate and economical UV spectrophotometric method for the simultaneous determination of Pitavastatin Calcium and Ezetimibe in combined Pharmaceutical formulation has been developed. **Aim:** The aim was to create a system that could determine both components concurrently while being selective, sensitive, and accurate. The strategy was approved in terms of Linearity, Accuracy (%recovery), Precision (Interday and Intraday), LOD, LOQ and Assay. **Materials and Method:** The approach is based on the use of methanol as a solvent and simultaneous equation for the study of both drug. Pitavastatin Calcium has absorbance maxima at 244.60nm and Ezetimibe has absorbance maxima at 234nm in methanol. **Results:** For, Pitavastatin Calcium and Ezetimibe, linearity was obtained in the concentrationranges of 1-5 µg/ml and 5-25 µg/ml, respectively. In UV r^2 was estimated 0.999 for Pitavastatin Calcium and 0.997 for Ezetimibe. Intraday Precision was figured 0.35-0.80 and 0.44-0.58 forPitavastatin calcium and 0.25-0.34 and 0.13-0.27 for Ezetimibe. Interday Precision was figured 0.27-0.78 and 0.44-0.46 for Pitavastatin calcium and 0.26-0.34 and 0.21-0.30 for Ezetimibe. Accuracy of Pitavastatin calcium was counted in the range of 98.7-101.5% and Ezetimibe was in the range of 98.3-101.3%. LOD value was deliberated 0.051 µg/ml for Pitavastatin Calcium and 0.124µg/ml for Ezetimibe. LOQ value for both drug was found to be 0.155 µg/ml and 0.375 µg/ml respectively. Assay of marketed formulation valued 98.86±0.0016 and 99.18±0.0021 of both drug.

KEYWORDS: UV Spectrophotometry, Pitavastatin Calcium, Ezetimibe, Methanol, Validation.

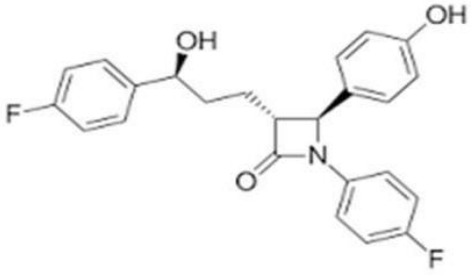
INTRODUCTION

Pitavastatin Calcium is a statin medication.[1] It's chemical name is mono calcium bis {(3R, 5S, 6E)-7-[2- cyclopropyl-4-(4-flurophenyl)-3-quinolyl]-3-5-dihydroxy-6-heptenoate}.[2] It lowers LDL (bad) cholesterol and triglycerides while raising HDL (good) cholesterol level in the blood. Pitavastatin Calcium is a brand new member of 3-hydroxy-3methylglutaryl coenzyme family. In the United States, statins (reductase inhibitors) are approved for the treatment of primary hyperlipidemia and mixed dyslipidemia. It's a synthetic lipid-lowering agent that's taken orally. Pitavastatin Calcium is lipid-lowering drug that works by inhibiting cholesterol synthesis via a competitive mechanism.[3] Ezetimibe is a dyslipidemic medication that is used to treat hyperlipidemia.[4] It received FDA approval in 2002. It's chemical name is (3R,4S)-1- (4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl) azetidin- 2-one.[5] Ezetimibe is an inhibitor of intestinal cholesterol absorption that is used to lower total cholesterol, LDL and non-high-density lipoprotein (HDL) in patients with primary hyperlipidemia, mixed hyperlipidemia, familial hyper cholesterol and homozygous sitosterolemia (phytosterolemia). Ezetimibe can be used as a monotherapy for doctors. Ezetimibe blocks cholesterol absorption at the small intestine's brush border through the Niemann-pick C1-Like-1 sterol transporter (NCP1L1).[6] The combined dosage forms of Pitavastatin calcium and Ezetimibe are available in the market for the treatment of high cholesterol. According to a review of the literature, UV[7,8], HPLC[9-13] and HPTLC[14,15] methods have been published for Pitavastatin Calcium and Ezetimibe[16-26] and also for combination HPLC[27] and HPTLC[28] method have been published. The simultaneous equations are used in this review article to plain, reliable, precise, rapid, and cost-effective spectrophotometric method for simultaneous estimation of Pitavastatin Calcium and Ezetimibe in tablet dosage form using methanol as a solvent.

Pitavastatin Calcium



Ezetimibe



MATERIALS AND METHOD

Apparatus

The absorbance of all the solution was measured using a Shimadzu double beam UV-Visible spectrophotometer with a spectral width of 2 nm, a wavelength precision of 0.5 nm, and a pair of 10 mm matched quartz cells.

Reagents and Materials

Pitavastatin calcium was gifted by Enaltec Labs Pvt. Ltd, Mumbai and Ezetimibe was gifted by Yarrow Chem Products, Mumbai. The commercial fixed dose combination Pitsafe-Ez Tablet (AAR ESS Remedies Pvt. Ltd) was procured from local market. Throughout the project, methanol and calibrated glassware were used.

Preparation of Stock Solution

Pitavastatin Calcium (20mg) and Ezetimibe(100mg) were correctly weighed and transferred to separate 50ml volumetric flask, where they were dissolved and diluted to the mark with methanol to provide a standard solution with concentration of Pitavastatin calcium 400 µg/ml and Ezetimibe 2000 µg/ml. 2.5ml of each solution was accurately measured and transferred to 50ml volumetric flask, where they were diluted to the mark with methanol to achieve a solutionwith a concentration of 20µg/ml of Pitavastatin calcium and 100µg/ml of Ezetimibe.

METHOD

Pitavastatin Calcium (20µg/ml) and Ezetimibe (100µg/ml) standard solution were scanned separately in the UV range of 200-400 nm to determine the wavelength maxima of both drugs. The λmax value of Pitavastatin calcium and Ezetimibe were found to be 244.60 nm and 234 nm, respectively. Using solution with concentration of 20µg/ml of Pitavastatin Calcium and 100 µg/ml of Ezetimibe, five solution with concentration of1, 2, 3, 4, and 5 µg/ml for Pitavastatin calcium and 5, 10, 15, 20, and 25 µg/ml for Ezetimibe were prepared in methanol. At 234 nm and 244.60 nm, the absorbance of the resulting solutions was determined, and the calibration curves were plotted at these wavelength.

VALIDATION OF THE PROPOSED METHOD

Linearity (Calibration Curve)

The calibration curves were plotted over a concentration range of 1-5 µg/ml and 5-25 µg/ml for Pitavastatin Calcium and Ezetimibe, respectively. Accurately measured standard solution of both drug (0.5, 1, 1.5, 2 and 2.5 ml) were transferred to a series of 10ml of volumetric

flaskand diluted to the mark with methanol. The absorbance of the solution were measured at 234nmand 244.60nm against methanol as blank. The calibration bands were built by plotting absorbances versus concentrations and the regression equation were calculated.

Precision

The intra-day and inter-day accuracy of the proposed method was decided by analysing the corresponding responses three times on the same day and on three different days three diverse concentration of standard arrangements of Pitavastatin Calcium and Ezetimibe.

Accuracy

The method's accuracy was calculated by using the conventional addition method to calculate Pitavastatin Calcium and Ezetimibe recovery. Known concentration of Pitavastatin Calcium and Ezetimibe standard solution were applied at 80, 100, and 120% to pre-quantified Pitavastatin Calcium and Ezetimibe sample solution (2 mg/ml for Pitavastatin Calcium and 10 mg/ml for Ezetimibe). By plugging the obtained values into the respective regression line equations, the quantities of Pitavastatin Calcium and Ezetimibe were calculated.

Limit of Detection and Limit of Quantification

Calculating the signal-to-noise ratio(S/N) using the following equations designated by International Conference on Harmonization (ICH) guidelines yielded the drug's Limit of Detection (LOD) and Limit of quantification (LOQ).

$LOD = 3.3 \times \sigma/S$ $LOQ = 10 \times \sigma/S$

Where, σ is the standard deviation of the response andS is the slope of the calibration curve.

Assay

Weigh accurately 20 tablets and powdered it. Tablet powder is equivalent to 10mg of Ezetimibe(2mg of Pitavastatin Calcium). Weigh the powder, pass it to a 50ml volumetric flask and dilute it with methanol to desired concentration [200 µg/ml Ezetimibe (40µg/ml Pitavastatin Calcium)]. Take 2.5ml of above solution and dilute it with methanol to make a 10ml solution [50 µg/ml Ezetimibe (10 µg/ml Pitavastatin Calcium)]. Furthur dilution was accomplished byplacing 1.5ml of above solution in 10ml volumetric flask and filling the rest with methanol [7.5µg/ml Ezetimibe (1.5 µg/ml Pitavastatin Calcium)].

RESULT AND DISCUSSION

Two wavelengths were used to analyse the drugs in this method. Furthermore, calibration curves for both drugs were prepared at wavelengths of 234nm (max of Ezetimibe) and 244.60 nm (max of Pitavastatin Calcium. In the concentration ranges of 1-5 µg/ml (Pitavastatin Calcium) and 5-25 µg/ml (Ezetimibe), respectively, linear correlation was found between absorbances and concentrations of Pitavastatin Calcium and Ezetimibe (Fig.1). The r^2 value of Pitavastatin Calcium was found to be 0.9991 and for Ezetimibe it was 0.9971. Pitavastatin Calcium and Ezetimibe intraday and interday precision for given method were found to be less than 2% RSD, indicating that the methods are accurate. Pitavastatin Calcium and Ezetimibe were found to have percentage recovery rates of 98.28-101.71% and 98.08-102.00%, respectively. Pitavastatin Calcium's LOD and LOQ were found to be 0.051 µg/ml and 0.124 µg/ml, respectively, while Ezetimibe's were 0.155 µg/ml and 0.375 µg/ml. Pitavastatin Calcium and Ezetimibe assay finding for given method was in the range of 98.7-101.5% and 98.3-101.3%, respectively. This is similar to a labelled claim.

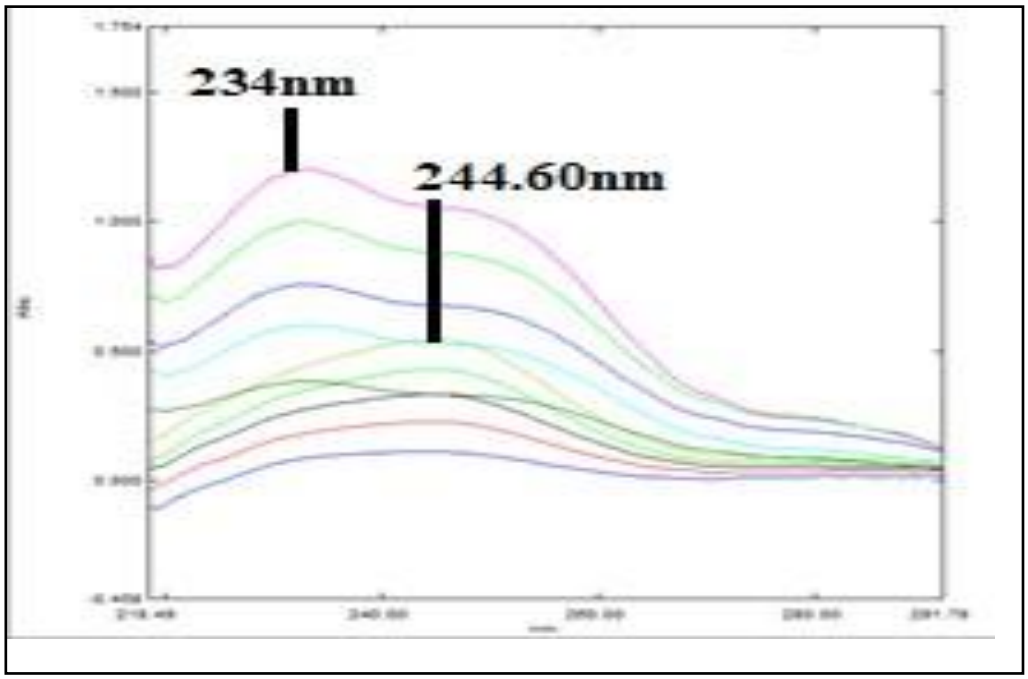


Fig. 1: Overlain Spectra of Pitavastatin Calcium (1-5 µg/ml) and Ezetimibe (5-25µg/ml), at 244.60nm and 234nm respectively.

Table 1: Summary of UV Validation Parameter.

Sr. No.	Parameter	Pitavastatin Calcium		Ezetimibe	
1.	LINEARITY RANGE	1-5 µg/ml		5-25 µg/ml	
2.	λmax	234nm	244.60nm	234nm	244.60nm
3.	CORRELATION CO-EFFICIENT	R2=0.9985	R2=0.9991	R2=0.9971	R2=0.9983
4.	REGRESSION EQUATION	y = 0.0901x + 0.0055	y = 0.1068x + 0.0082	y = 0.0405x + 0.1772	y= 0.0359x + 0.1553
5.	INTRADAY PRECISION (%RSD)	0.35-0.80	0.44-0.58	0.25-0.34	0.13-0.27
6.	INTERDAY PRECISION (%RSD)	0.27-0.78	0.44-0.46	0.26-0.34	0.21-0.28
7.	LOD	0.051 µg/ml		0.124 µg/ml	
8.	LOQ	0.155 µg/ml		0.375 µg/ml	

Table 2: Accuracy.

Level of recovery %	Amount of pure drug added (mg)		% recovery	
	PTV Ca.	EZE	PTV Ca.	EZE
80%	1.6	8	99.41	99.85
100%	2	10	100.47	99.41
120%	2.4	12	100.44	99.59
Mean	-	-	100.10	99.62

Table 3: Assay.

Formulation	Labelled amount added (mg/tab)		Amount found (mg/tab)		Assay (% estimated) Mean ± SD (n=3)	
Pitsafe-EZ	PTV Ca.	EZE	PTV Ca.	EZE	PTV Ca.	EZE
	2	10	1.98	9.92	98.86±0.001	99.18±0.002

CONCLUSION

For simultaneous determination of Pitavastatin Calcium in tablet dosage form, the proposed spectrophotometric method was found to be simple, sensitive, accurate, and precise. For Pitavastatin Calcium and Ezetimibe analysis, the method uses methanol, which is readily available and inexpensive. As a result, the approach was found to be cost effective for estimating Pitavastatin Calcium and Ezetimibe from tablets.

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