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Application of UV-Spectrophotometry and RP-HPLC for Simultaneous Determination of Atorvastatin Calcium and Ezetimibe in Pharmaceutical Dosage Form

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Abstract:

Two methods are described for the simultaneous determination of Atorvastatin calcium and Ezetimibe in binary mixture. The first method was based on UV-spectrophotometric determination of two drugs, using simultaneous equation method. It involves absorbance measurement at 232.5 nm (λ_{max} of Ezetimibe) and 246.0 nm (λ_{max} of Atorvastatin calcium) in methanol; linearity was obtained in the range of 5 – 25 $\mu g.mL^{-1}$ for both the drugs. The second method was based on HPLC separation of the two drugs in reverse phase mode using Luna C_{18} column. Linearity was obtained in the concentration range of 8-22 $\mu g.mL^{-1}$ for both the drugs. Both these methods have been successively applied to pharmaceutical formulation and were validated according to ICH guidelines.

Keywords: Atorvastatin calcium, Ezetimibe, UV- spectrophotometry, HPLC.

1. Introduction:

Atorvastatin calcium chemically [R-(R, R*)]-2-(4-flurophenyl)-β,δ-dihydroxy-5(1-methylethyl)-3-phenyl-4- [phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate, is a synthetic HMG –CoA reductase inhibitor [1]. It has been demonstrated to be efficacious in reducing both cholesterol and triglycerides [2]. The chemical structure of Atorvastatin calcium is shown in Fig 1. The typical dose of Atorvastatin calcium is 10-80 mg per day and it reduces 40-60% LDL [3]. Literature survey reveled that various analytical methods such as HPLC [4, 5], GC-MS [6], LC-MS [7], HPLC-Electrospray tendem mass spectrometry [8] and HPTLC [9]

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have been reported for estimation of Atorvastatin calcium from its formulations and biological fluids.

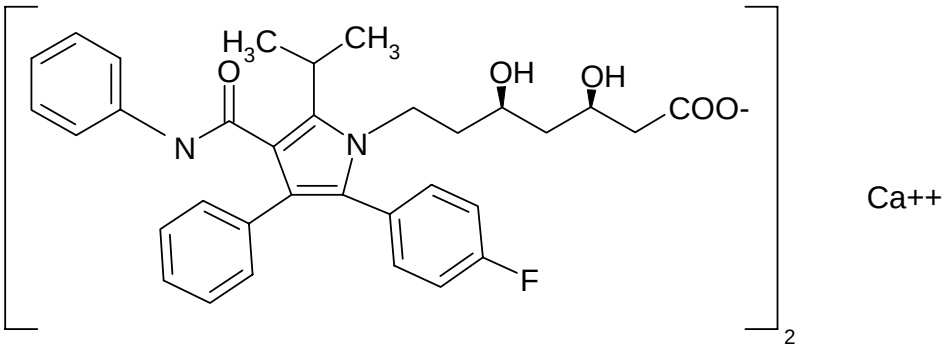

Fig 1. Structure of Atorvastatin Calcium

Ezetimibe chemically 1-(-4-flurophenyl)-(3S)-hydroxypropyl]-(4S)-(4-hydroxyphenyl)-2-azeti-dinone, is a selective cholesterol absorption inhibitor [10], which potentially inhibits the absorption of biliary and dietary cholesterol [11]. Additional studies in human indicated that ezetimibe does not affect serum fat – soluble vitamins [12]. The dose of Ezetimibe is 10 mg daily, when added on to statin therapy there is an increase in the lipid lowering effect [13]. The structure of Ezetimibe is shown in Fig 2. There are very few methods reported for estimation of Ezetimibe in pharmaceutical dosage form, which includes a validated RP –HPLC [14], spectrophotometric method using floin – Ciocalteu reagent [15].

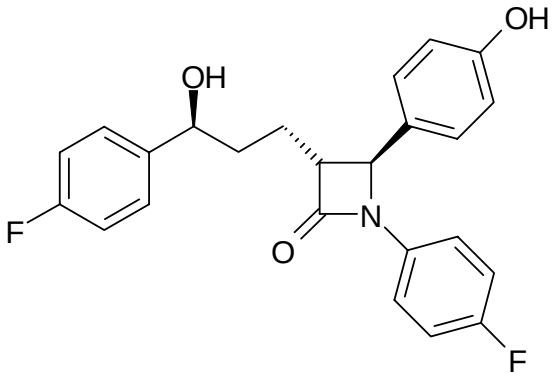

Fig 2. Structure of Ezetimibe

Both these drugs are not official in Indian Pharmacopoeia, British Pharmacopoeia, United States and European Pharmacopoeia.

At present no HPLC and UV spectrophotometric methods are reported for the simultaneous estimation of Atorvastatin calcium and Ezetimibe in tablet formulation.

Therefore, it was thought worthwhile to develop simple, precise, accurate UV-spectrophotometric and HPLC methods for simultaneous determination of Atorvastatin calcium and Ezetimibe in tablets.

2. Experimental:

2.1. Materials:

Pharmaceutical grade Atorvastatin calcium (batch no. AU 1030E04) and Ezetimibe (ET 120804) were kindly supplied as a gift sample by Blue Cross Laboratories Ltd., Nashik, (M.S.) India, used without further purification and certified to contain 99.53 % (w/w) and 99.66% (w/w), respectively on dried basis. All chemicals are of HPLC grade and were purchased from Qualigens fine Chemicals, Mumbai, India.

2.2. UV- spectrophotometry:

UV-Vis spectrophotometer 1601 (Shimadzu, Japan) with spectral bandwidth of 2 nm and 10 mm matched quartz cells was used.

Standard stock solutions of 100 $\mu g.mL^{-1}$ were prepared by dissolving 10 mg of each in 100 mL of methanol. From these stock solutions, working standard solutions having concentration 15 $\mu g.mL^{-1}$each were prepared by appropriate dilutions. They were scanned in the wavelength range of 400–200 nm and the overlain spectrum was obtained (Fig 3). Two wavelengths 232.5 nm (λ_{max} of Ezetimibe) and 246.0 nm (λ_{max} of Atorvastatin calcium) were selected for the formation of simultaneous equation. The calibration curves were found to be linear in the concentration range of 5– 25 $\mu g.mL^{-1}$, for each drug. The absorptivity coefficients of each drug at both wavelengths were determined. The concentration of two drugs in the mixture were calculated using equations [16],

$$C_{ATV} = A2\ ay1 - A1\ ay2 / ax2\ ay1 - ax1ay2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

C_{EZM} = A1 ax2 – A2 ax2/ ax2 ay1 – ax1ay2…….... (2)

Where, A1 and A2 are absorbance of mixture at 232.5 nm and 246.0 nm; ax1 and ax2, absorptivities of Atorvastatin calcium at 232.5 nm and 246.0 nm, respectively; ay1 and ay2 absorptivities of Ezetimibe at 232.5 nm and 246.0 nm, respectively. C_{ATV} and C_{EZM} are concentration of Atorvastatin calcium and Ezetimibe in mixture. The absorptivities reported are the mean of six independent determinations (Table 1).

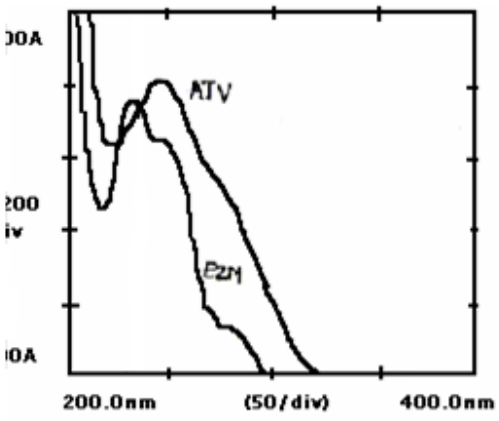

Fig 3. Overlain Spectrum of Atorvastatin calcium And Ezetimibe in Methanol. ATV is Atorvastatin calcium, EZM is Ezetimibe (each 15 μg.mL^{-1}) taken on UV – Vis spectrophotometer (SHIMADZU 1601)

2.3. HPLC method:

LC system used consisted of pump (model SHIMADZU; LC – 10 AT *vp*) with universal loop injector (Rheodyne 7725 i) of injection capacity 20 μL.Detector consists of Photodiode array detector SPD-10 A*vp*, SHIMADZU; the column used was Luna C 18 (5 μm, 25 cm X 4.6 mm i.d.) phenomenex, USA, at ambient temperature.

Different mobile phases were tested in order to find the best conditions, for separating both the drugs simultaneously. The optimal composition of mobile phase was determined to be Ammonium acetate buffer pH 5.0: Acetonitrile: Triethylamine (50:50:0.2, v/v). The flow rate was

set to 1 mL.min^{-1} and UV detection was carried out at 240 nm. Ibuprofen was used as an internal standard.

Stock solution was prepared by dissolving 10 mg of Atorvastatin calcium and Ezetimibe in 100 mL volumetric flask with methanol. 10 mg of Ibuprofen (internal standard) was taken in separate 100 mL volumetric flask and dissolved in methanol.

Table 1. Absorptivity Values at 232.5 nm (λ_{max} of Ezetimibe) and 246.0 nm (λ_{max} of Atorvastatin calcium)

	Absorptivity at 232.5 nm		Absorptivity at 246.0 nm	
	Atorvastatin calcium	Ezetimibe	Atorvastatin calcium	Ezetimibe
*Mean	ax1= 347.48	ay1= 494.97	ax2= 452.08	ay2= 388.96
± S.D.	1.15	0.48	0.51	0.44

* Absorptivity values are the mean of six determinations. S.D. is standard deviation. ax1 and ax2 absorptivities of Atorvastatin calcium at 232.5 nm and 246.0 nm, respectively; ay1 and ay2 absorptivities of Ezetimibe at 232.5 nm and 246.0 nm, respectively.

All solutions were stored at + 5^{0}C in the dark; these solutions were shown to be stable during the period of study.

From the above stock solutions, dilutions were made in the concentration range of 8–22 μg.mL^{-1} of Atorvastatin calcium and Ezetimibe, respectively and each concentration contains 50 μg.mL^{-1} of Ibuprofen (internal standard). A volume of 20 μL of each sample was injected into column. All measurements were repeated three times for each concentration and calibration curve was constructed by plotting the peak area ratios of analyte to internal standard vs. the corresponding drug concentration.

2.4. Analysis of Pharmaceutical Dosage Forms

To determine the content of Atorvastatin calcium and Ezetimibe simultaneously in tablets (label claim: 10 mg Atorvastatin calcium and 10 mg Ezetimibe, film coated); twenty tablets were weighed; their average weight determined and were finely powdered. The correct amount of powder was dissolved in methanol by stirring for 30 min. The excipients were separated by filtration. After filtration, an appropriate amount of internal standard was added and diluted up to mark with methanol. Appropriate aliquots were subjected to above methods and the amount of Atorvastatin calcium and Ezetimibe were determined. The results are reported in Table 2.

Table 2. Analysis data of tablet formulations

Parameters	UV – spectrophotometry		HPLC	
	Atorvastatin	Ezetimibe	Atorvastatin	Ezetimibe
Label Claim	10	10	10	10
*Drug content	100.16	99.89	101.13	101.63
± S. D.	0.4621	0.3080	0.7468	0.3321
% R.S.D.	0.4614	0.3083	0.7385	0.3268

* Value for Drug content (%) are the mean of five estimations; S.D. is standard deviation and R.S.D. is relative standard deviation

2.5. Recovery studies

To check the accuracy of the developed methods and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition method, at 80, 100 and 120 % level. From the total amount of drug found, the percentage recovery was calculated. The results are reported in Table 3.

3. Results and Discussion

Both, UV spectrophotometric and HPLC methods were found to be simple, accurate, economic and rapid for routine simultaneous estimation of Atorvastatin calcium and Ezetimibe, in

tablet dosage forms. For UV spectrophotometric method, linearity was obtained in concentration range of 5 – 25 μg .mL^{-1}, for both the drugs; with regression 0.9998 and 0.9999, intercept – 0.0677 and – 0.0043 and slope 0.0457 and 0.0391 for Atorvastatin calcium and Ezetimibe, respectively. Recovery was in the range of 99 – 101 %; the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method.

Table 3. Recovery studies

UV – spectrophotometry			HPLC		
Excess Drug	*Recovery	% R.S.D.	Excess Drug	*Recovery	% R.S.D.
Atorvastatin calcium					
80	99.83	0.2953	80	99.37	0.9405
100	99.72	0.2026	100	100.11	0.0115
120	99.07	0.0672	120	100.58	0.9702
Ezetimibe					
80	100.69	0.2953	80	100.32	1.1238
100	100.43	0.1036	100	99.33	0.0232
120	99.52	0.1165	120	98.80	1.1243

* Recovery is mean of three estimations.

In HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase compositions were tried, to separate drugs and internal standard. Mobile phase and flow rate selection was based on peak parameters (height, tailing, theoretical plates, capacity factor), run time etc. The system with 20 mM ammonium acetate buffer pH 5.0: acetonitrile: triethylamine (50:50:02 v/v) with 1 mL.min^{-1} flow rate is quite robust. Ibuprofen was applied as an internal standard, neutralizing the error inherent in sample injection, eliminating random errors. A typical chromatogram for Atorvastatin calcium, Ezetimibe and Ibuprofen (internal standard) is shown in fig 4. The optimum wavelength for detection was 240 nm at which better detector response for drugs was obtained. The average retention times for

Atorvastatin calcium, Ibuprofen and Ezetimibe was found to be 6.639 ± 0.03, 9.010 ± 0.03 and 11.822 ± 0.02 min, respectively. According to USP XXIV (621), system suitability tests are an integral part of chromatographic method. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. The parameters obtained are shown in Table 4. The calibration was linear in concentration range of 2 – 22 μg .mL^{-1}, with regression 0.9993 and 0.9992, intercept – 0.1514 and – 0.2066 and slope 0.4330 and 0.4204 for Atorvastatin calcium and Ezetimibe, respectively. The low values of % R.S.D. indicate the method is precise and accurate. The mean recoveries were found in the range of 98 – 100 %.

Table 4. System suitability parameters

Parameters	Atorvastatin calcium	Ezetimibe
Tailing factor	1.34	1.21
Theoretical Plates	6092	7835
Resolution factor	2.19	6.08
Capacity factor	2.50	5.16

Sample – to sample precision and accuracy were evaluated using, three samples of three different concentrations, which were prepared and analyzed on same day. Day – to day variability was assessed using three concentrations analyzed on three different days, over a period of one week. These results show the accuracy and reproducibility of the assay. Thus, it was concluded that there was no significant difference on the assay, which was tested on an intra – day and inter – day basis. The % R.S.D. values reported in Table 5, shows that proposed methods provides acceptable intra – day and inter – day variation of Atorvastatin calcium and Ezetimibe.

Ruggedness of the proposed methods was determined by analysis of aliquots from homogeneous slot in different laboratories, by different analysts, using similar operational and environmental conditions; the % R.S.D. reported in Table 5 was found to be less than 2 %.

The proposed methods are accurate, simple, rapid and selective for the simultaneous estimation of Atorvastatin calcium and Ezetimibe in tablet dosage form by internal standardization method. Hence, it can be conveniently adopted for the routine quality control analysis in the combination

formulations. As the drug combination is available in market, hence, work is toward development of an analysis.

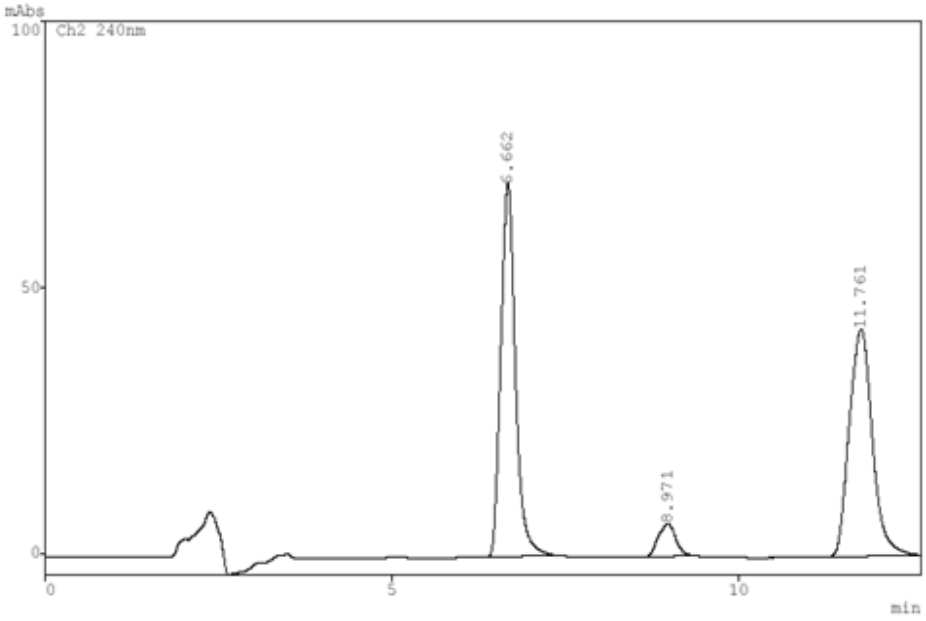

Fig 4. Chromatogram of Standard Atorvastatin calcium (10 μg.mL^{-1}); (R_t 6.662), internal standard (Ibuprofen) (50 μg.mL^{-1}); (R_t 8.971) and Ezetimibe (10 μg.mL^{-1}); (R_t 11.761) measured at 240 nm, mobile phase Ammonium acetate buffer (pH 5): Acetonitrile: Triethylamine (50:50:0.2 v/v).

Table 5. Summary of repeatability, precision and ruggedness

Parameter	UV – spectrophotometry		HPLC	
	Atorvastatin	Ezetimibe	Atorvastatin calcium	Ezetimibe
Repeatability	1.52	0.09	0.62	0.57
Precision				
Intra-day	1.07	0.13	0.29	0.43
Inter-day	0.17	0.14	0.56	1.55
Ruggedness				
Analyst 1	0.68	0.52	0.37	0.87
Analyst 2	0.32	0.58	0.34	1.56

n is the number of repetitions

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