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DEVELOPMENT AND VALIDATION OF A METHOD FOR SIMULTANEOUS DENSITOMETRIC ESTIMATION OF ATORVASTATIN CALCIUM AND EZETIMIBE AS THE BULK DRUG AND IN TABLET DOSAGE FORMS

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SUMMARY

Atorvastatin calcium is a selective HMG-CoA reductase inhibitor and ezetimibe has lipid-lowering activity. Both are potential anti-lipidaemic agents used in combination to reduce the amount of cholesterol and triglycerides in systemic circulation. This paper describes a simple, precise, and accurate HPTLC method for simultaneous quantification of these compounds as the bulk drug and in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminium plates precoated with silica gel 60 F_{254}, with toluene–methanol 8:2 (*v*/*v*) as mobile phase. Densitometric evaluation of the separated zones was performed at 240 nm. The two drugs were satisfactorily resolved with R_F values 0.23 ± 0.01 and 0.39 ± 0.01 for atorvastatin calcium and ezetimibe, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (0.4–2.4 μg/zone for both atorvastatin calcium and ezetimibe), precision (intra-day *RSD* 1.16–1.25% and inter-day *RSD* 1.16–1.44% for atorvastatin calcium, and intra-day *RSD* 0.47–0.63% and inter-day *RSD* 0.47–0.88% for ezetimibe), accuracy (98.51 ± 0.23% for atorvastatin calcium and 99.01 ± 0.15% for ezetimibe), and specificity, in accordance with ICH guidelines. The method can be used for analysis of ten or more formulations on a single plate and is a rapid and cost-effective quality-control tool for routine simultaneous analysis of atorvastatin calcium and ezetimibe as the bulk drug and in tablet formulations.

INTRODUCTION

Atorvastatin calcium ([*R*-(*R**,*R**)]-(2-(4-fluorophenyl)-β,δ-dihydro-

xy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino)carbonyl]-1*H*-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate) is a synthetic lipid-lowering agent. It is a selective competitive inhibitor of the enzyme HMG-CoA reductase, which catalyses the conversion of HMG-CoA to mevalonate, an important rate-limiting step in cholesterol biosynthesis [1,2]. Ezetimibe (1-(4-fluorophenyl)-3(*R*)-[3-(4-fluorophenyl)-3(*S*)-hydroxylpropyl]-4(*S*)-(4-hydroxyphenyl)-2-azetidinone) is another lipid-lowering agent [3].

Literature review reveals that methods have been reported for analysis of atorvastatin calcium by high-performance liquid chromatography (HPLC) [4–7] and high-performance thin-layer chromatography (HPTLC) [8], and for estimation of ezetimibe by HPLC [9], either alone or in combination with other drugs, but no HPTLC method has yet been reported for simultaneous estimation of atorvastatin calcium and ezetimibe. The purpose of this research was to establish and validate, in accordance with International Conference on Harmonization (ICH) guidelines [10], a simple, accurate, economical, and reproducible procedure for quantitative TLC analysis of atorvastatin calcium and ezetimibe as the bulk drug and in tablet dosage forms.

EXPERIMENTAL

Chemicals and Reagents

Working standards of pharmaceutical grade atorvastatin calcium (batch no. ATV-0509074) and ezetimibe (batch no. EZ- 082050) were obtained as generous gifts from Mepro Pharmaceuticals Wadhwan (Gujarat, India) and Hetero Drugs Erragadda (Hyderabad, India), respectively. Assay of the samples showed they contained 99.02% and 99.30% atorvastatin calcium and ezetimibe, respectively. Fixed-dose combination tablets (Bitorva) containing 10 mg atorvastatin calcium and 10 mg ezetimibe were procured from Hetero Drugs (Solan (H.P.) India).

Chemicals and reagents of analytical-grade were purchased from Merck Chemicals, Mumbai, India.

Preparation of Standard and Sample Solutions

Atorvastatin calcium and ezetimibe (10 mg each) were weighed accurately, transferred to two 10-mL volumetric flasks, and dissolved in 10 mL methanol. Both solutions (2 mL) were further diluted with methanol to furnish final concentrations of 0.2 μg μL^{-1}. For analysis of the tablet

dosage form, twenty tablets, each containing 10 mg atorvastatin calcium and 10 mg ezetimibe, were weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to 10 mg atorvastatin calcium and 10 mg ezetimibe was accurately weighed and dissolved in 10 mL methanol. The solution was centrifuged for 15 min at 600 rpm, filtered through Whatman no. 41 filter paper, and the residue was washed with methanol. The volume of the filtrate was adjusted to 10 mL with the same solvent. This solution (2 mL) was further diluted with methanol so the concentration was the same as that of the final standard solution.

Thin-Layer Chromatography

Analysis was performed on 20 cm × 20 cm aluminium plates precoated with silica gel 60 F_{254}. Before use, plates were washed with methanol, activated in an oven at 105°C for 20 min, then left to cool at room temperature. Standard solutions of atorvastatin calcium and ezetimibe (0.2 μg μL^{-1}, 4 μL) were applied to pre-washed activated plates, as 6-mm bands, 6 mm apart, under a stream of nitrogen, by means of a Camag Linomat IV

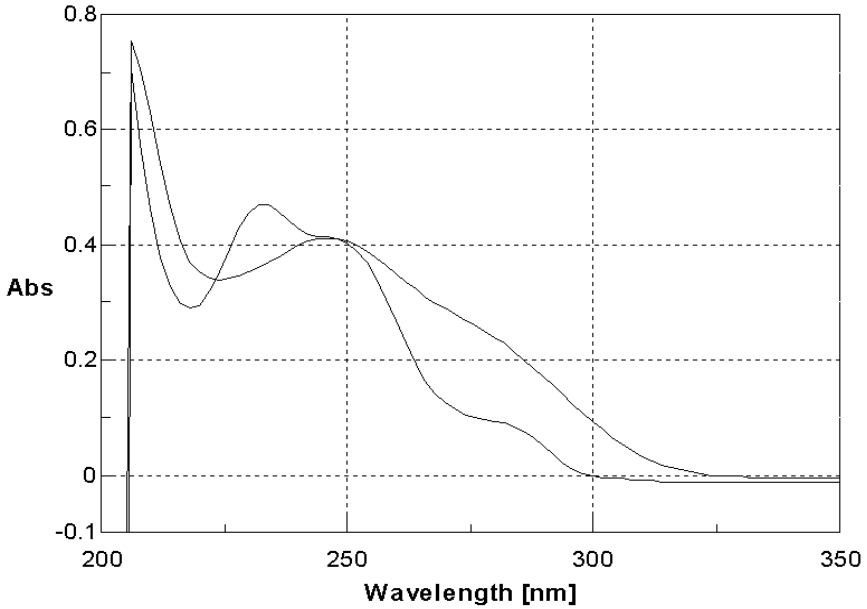

Fig. 1

Overlaid UV spectra of atorvastatin calcium and ezetimibe

automated spray-on band applicator equipped with a Hamilton 100-µL syringe. The plates were developed, with 20 mL toluene–methanol 8:2 (*v*/*v*) as mobile phase, in a Camag twin-trough chamber previously saturated with mobile phase vapour for 20 min. The development distance and time were 15 cm and 25 min, respectively. After development the plates were removed from the chamber and dried in air. Densitometry was performed at 240 nm (Fig. 1), in reflectance mode, with a Camag TLC Scanner 3 using CATS 4 software incorporating track-position optimization. The slit dimensions were 6.00 mm × 0.45 mm.

For preparation of a calibration plot, 2, 4, 6, 8, 10, and 12 µL of standard solutions of atorvastatin calcium and ezetimibe (0.2 µg μL^{-1}) were applied to the TLC plates. Analysis was then performed as described above.

Assay of the Marketed Formulation

Assay of the marketed formulation was performed by the method established in this work. Standard and sample solutions (6 µL) were applied to the plate and analysis was performed as described above. The amounts of atorvastatin calcium and ezetimibe per tablet were calculated by comparing the areas obtained from standard and sample (Table I).

Table I

Results from assay of atorvastatin calcium and ezetimibe in the formulation Bitorva (Hetero Drugs, Solan (H.P.) India)

Drug	Label claim (mg/tablet)[a]	Amount found (mg)	Drug content (%)[a]	*RSD* (%)
Atorvastatin calcium	10	9.82	98.62 ± 0.50	0.50
Ezetimibe	10	9.90	99.05 ± 0.30	0.30

[a]Average ± standard deviation from three determinations

Validation

The method was validated for linearity, accuracy and specificity, intra-day and inter-day precision, repeatability of measurement of peak area, and repeatability of sample application, in accordance with ICH guidelines [11,12] (Table II). Accuracy was evaluated by conducting a recovery study at three different levels – 80, 100, and 120%. Intra-day precision was determined by analysis of standard solutions of atorvastatin calcium and ezetimibe in range 0.4–2.4 µg/zone three times on the same day. Inter-day

precision was determined by analysis of similar standards on three different days over a period of one week. Relative standard deviation (*RSD*) was calculated for both series of analyses. The robustness of the method was studied, during method development, by determining the effects of small variations of mobile phase composition (±2%), duration of plate pre-washing, chamber saturation period, development distance, and scanning time (10% variation of each).

Table II

Summary of validation data

Method characteristic	Atorvastatin calcium	Ezetimibe
Linear range (μg)	0.4–2.4	0.4–2.4
Correlation coefficient (*r*)	0.9995	0.9999
Standard deviation (*n* = 6)	1.16	0.50
Accuracy (%, *n* = 6)	98.51 ± 0.23	99.01 ± 0.15
Repeatability of sample application (*RSD*, %, *n* = 6)	1.50	0.61
Repeatability of measurement of peak area (*RSD*, %, *n* = 6)	1.02	1.76
Precision (*RSD*, %)		
Inter-day (*n* = 3)	1.16–1.44	0.47–0.88
Intra-day (*n* = 3)	1.16–1.25	0.47–0.63
Specificity	Specific	Specific

RESULTS AND DISCUSSION

Tablet powder was extracted with methanol because both atorvastatin calcium and ezetimibe are freely soluble in this solvent. Centrifugation for 15 min at 600 rpm facilitated complete extraction of atorvastatin calcium and ezetimibe from the tablet matrix. A variety of mobile phases, for example chloroform–methanol, chloroform–toluene–acetic acid, and benzene–methanol–toluene mixtures, were investigated for separation of atorvastatin calcium and ezetimibe from their impurities and from excipients in the formulation. Toluene–methanol 8:2 (*v*/*v*) was found to result in the best peak shape. Atorvastatin calcium and ezetimibe were satisfactorily resolved with R_F values 0.23 ± 0.01 and 0.39 ± 0.01, respectively (Fig. 2). Pre-saturation of the TLC chamber with mobile phase vapour for 30 min ensured more reproducible migration of the drugs and better resolution.

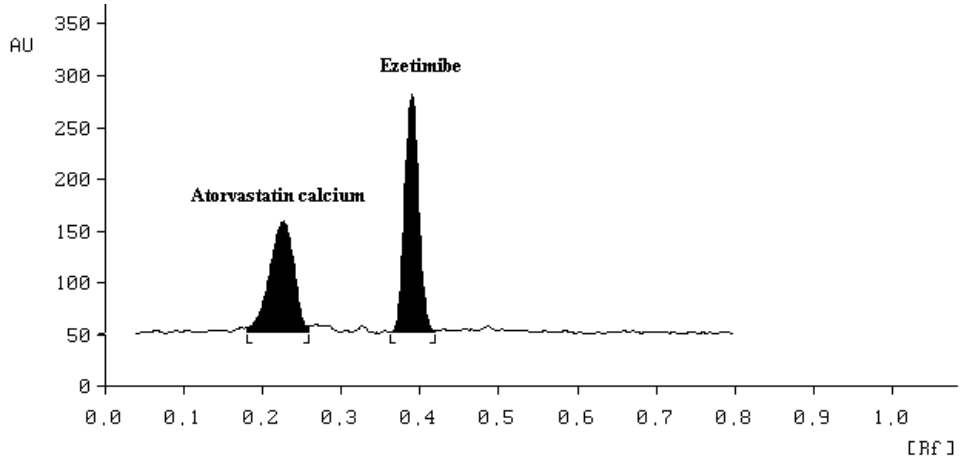

Fig. 2

Densitogram obtained from atorvastatin calcium and ezetimibe

As recommended by International Conference on Harmonization (ICH) [10], calibration plots were established for atorvastatin calcium and ezetimibe standards using six concentrations (0.4, 0.8, 1.2, 1.6, 2.0, and 2.4 μg per band). The correlation coefficients for the plots were 0.9995 for atorvastatin calcium and 0.9999 for ezetimibe. Results from the recovery study indicated the method enabled accurate quantification of the drugs in the tablet dosage form (Table III).

Table III

Recovery of atorvastatin calcium and ezetimibe from the formulation Bitorva (Hetero Drugs, Solan (H.P.) India)

Drug	Label claim (mg/tablet)	Amount added (%)	Total amount (mg)	Amount recovered (mg)	Recovery (%)[a]
Atorvastatin calcium	10	80	18	17.7	98.58 ± 0.27
		100	20	19.6	98.25 ± 0.20
		120	22	21.7	98.70 ± 0.22
Ezetimibe	10	80	18	17.8	99.16 ± 0.25
		100	20	19.8	99.00 ± 0.21
		120	22	21.7	98.87 ± 0.23

[a]Average value ± standard deviation of six determinations

The intra-day and inter-day relative standard deviations were in the ranges 1.16–1.25% and 1.16–1.44% for atorvastatin calcium and 0.47–0.63% and 0.47–0.88% for ezetimibe. These small values indicate the method is precise. Relative standard deviation (*RSD*) for measurement of peak area was 1.02% and 1.76% for atorvastatin calcium and ezetimibe, respectively, and *RSD* for repeatability of sample application was 1.50% and 0.61% for atorvastatin calcium and ezetimibe, respectively. *RSD* for measurement of peak area and sample application were better than the specifications of the instrument, indicating proper functioning of the TLC system. To confirm the specificity of the method, atorvastatin calcium and ezetimibe were applied to a TLC plate and developed and scanned as described above. Excipients present in the formulation did not interfere with the peaks of atorvastatin calcium and ezetimibe. The spectra acquired for atorvastatin calcium and ezetimibe extracted from the tablet were also compared with those acquired from atorvastatin calcium and ezetimibe standards; correlation was good. When small changes were made to the method conditions there were no marked changes in chromatographic behaviour, indicating the method is robust.

CONCLUSION

It is shown above that the new TLC–densitometric method achieved accuracy, reproducibility, repeatability, linearity, and selectivity that compares favourably with those of HPLC, HPTLC, spectrophotometry, and other methods reported regularly in the literature. The results also meet ICH guidelines [11,12] for validation of pharmaceutical TLC methods. The proposed TLC method is less expensive, simpler, more rapid, and more flexible than HPLC.

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