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RP-HPLC METHOD DEVELOPMENT AND VALIDATION OF SIMULTANIOUS DETERMINATION OF ATORVASTATIN & EZETIMIB IN BULK AND FORMULATION

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ABSTRACT

Simple, specific, economical and precise high performance liquid chromatographic method for the simultaneous determination of Ezetimibe and Atorvastatin in API (active pharmaceutical ingredient) and formulation has been developed and validated. Chromatography was carried out at 30°C on a pre packed Zorbax SB C_{18} (5 mm, 250×4.6 mm) column with the 0.02 M Potassium dihydrogen phosphate: Acetonitrile: Methanol (10:40:50, v/v/v) was used as the mobile phase. The UV detection was carried out at 236 nm. The results obtained showed good agreement with the declared contents. Ezetimibe and Atorvastatin separated in less than 10 min with good resolution and minimal tailing and without interference of excipients. The retention times of Ezetimibe and Atorvastatin were 5.7 min and 9.1 min, respectively. The method was linear in the range of 5–50 µg/ml for Ezetimibe concentration with a correlation co-efficient 0.999 and in the range 5–60 µg/ml for Atorvastatin concentrations having correlation co-efficient 0.9994 and the recovery was 99-102%. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The proposed method can be used for quantitative determination of Ezetimibe and Atorvastatin combination from API and formulations.

KEYWORDS: Ezetimibe, Atorvastatin, Zorbax SB C_{18}, dihydrogen phosphate.

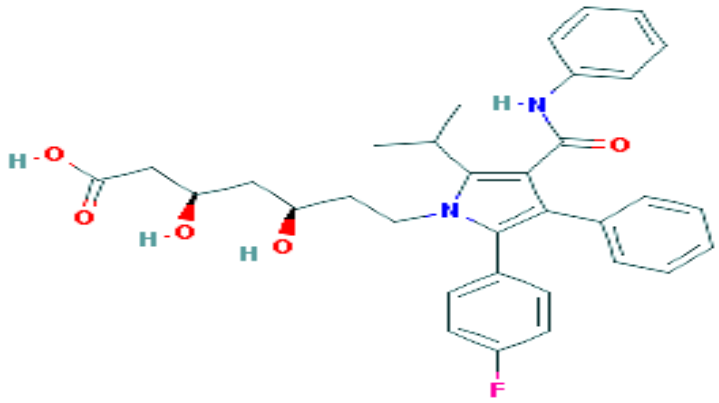
INTRODUCTION

High performance liquid chromatography (HPLC)

High Performance Liquid Chromatography (HPLC)[1-2] is a simple, fast, specific, precise and highly accurate analytical technique that is used for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial etc. In HPLC, separations are achieved by partition, adsorption or ion exchange, according to the nature of the interactions between the solute and the stationary phase, which may arise from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophobic forces or basing on the size of the particles.[3] Reversed phase HPLC (RP-HPLC or RPC) has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group such as $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are more non-polar, while polar molecules elute more readily. An investigator can increase retention time by adding more water to the mobile phase; thereby making the affinity of the hydrophobic analyte for the hydrophobic stationary phase stronger relative to the now more hydrophilic mobile phase. Similarly, an investigator can decrease retention time by adding more organic solvent to the eluent. Structural properties of the analyte molecule play an important role in its retention characteristics. In general, an analyte with a larger hydrophobic surface area (C-H, C-C, and generally non-polar atomic bonds, such as S-S and others) results in a longer retention time because it increases the molecule's non-polar surface area, which is non-interacting with the water structure. On the other hand, polar groups, such as -OH, $-NH_2$, COO^- or $-NH_3^+$ reduce retention as they are well integrated into water.

ATORVASTATIN

Atorvastatin has been extensively studied in the primary and secondary prevention of cardiovascular events, and may have some clinical advantages over various other statins in these respects. The principal primary prevention study of atorvastatin, ASCOT-LLA (Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm), revealed that atorvastatin reduced the relative risk of primary coronary heart disease (CHD) events by 36% (p = 0.0005) compared with placebo in patients with hypertension. Much published data confirm the secondary preventive benefits of atorvastatin in various clinical settings. The IDEAL (Incremental Decrease in End Points Through Aggressive Lipid Lowering) and TNT (Treating to New Targets) trials demonstrate the preventive efficacy of atorvastatin in patients with stable CHD. Relative to simvastatin (in the IDEAL trial) and low-dosage atorvastatin (in the TNT trial), intensive atorvastatin therapy (80 mg/day) reduced the risk of nonfatal myocardial infarction (MI) by 17-22% (p < or = 0.02). The ALLIANCE (Aggressive Lipid-Lowering Initiation Abates New Cardiac Events) and GREACE (Greek Atorvastatin and Coronary-heart-disease Evaluation) trials highlight the benefits of atorvastatin in the 'real world' setting in patients with stable CHD.



AIM OF WORK

The aim of the work of the thesis entitled “Studies on HPLC profile of some pharmaceutical compounds” is to develop analytical methods for drug quantification. It has been distinguish in three divisions as under

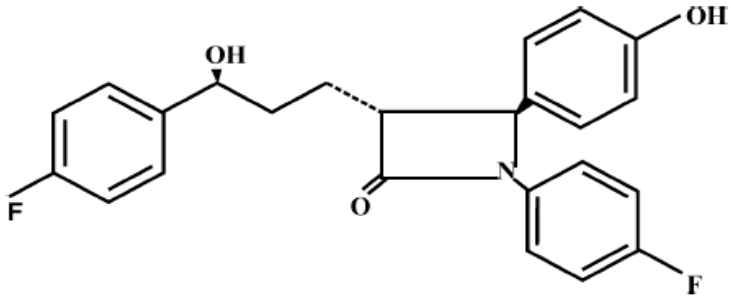
1. Selection of analytical technique for drug quantification

2. HPLC method development, method determination and method validation of dosage form of Ezetimibe
3. HPLC method development & method determination of several combination dosage forms of Ezetimibe with Statins and Fibrate and precision evaluation for the same

The research work performed in above divisions mainly addresses development of stability indicating HPLC methods for drug quantification followed by validating method. Validation activity is carried out according to ICH guidelines with pre-defined acceptance criteria. The strategy for drug quantification is applicable to dosage form and combination dosage forms of drugs.

EZETIMIBE

Ezetimibe is chemically 1-(4-fluorophenyl)-3(R)-[3-(4-fluorophenyl)-3(S)- hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone. Its molecular formula is $C_{24}H_{21}F_2NO_3$ and it has a molecular weight of 409.43 g mol^{-1}.

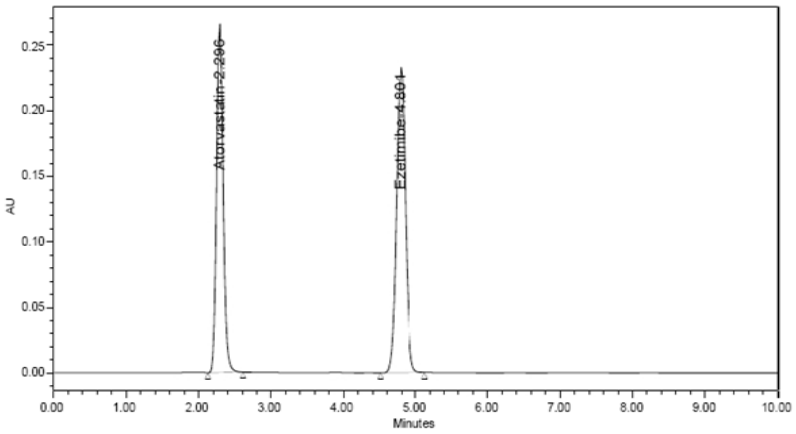


RESULTS AND DISCUSSION

Validation

1) SPECIFICITY

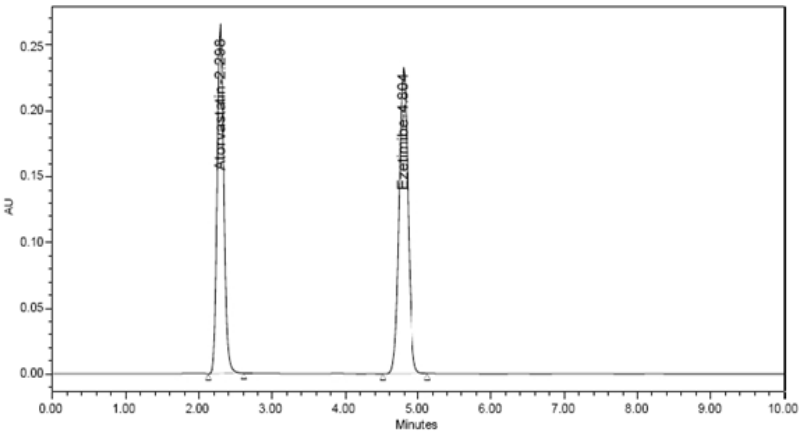
Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual analytical procedure may be compensated by other supporting analytical procedure(s). This definition has the following implications: Identification: to ensure the identity of an analyte. Purity Tests: to ensure that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc. Assay (content or potency): to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.



	Peak Name	RT	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Atorvastatin	2.296	1676835	45.45	264765		1.11	3656
2	Ezetimibe	4.801	2033445	54.55	232415	12.31	1.01	6928

EZETIMIBE

Weighed and transferred 10.0 mg of Ezetimibe working standard into 50 mL volumetric flask, added 50 mL of diluent and sonicated to dissolve and diluted to volume with diluent. Further transferred 1 mL of above solution into 10 mL volumetric flask and diluted to volume with diluents The chromatogram of Atorvastatin and Ezetimibe was shown in.



	Peak Name	RT	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Atorvastatin	2.298	1679955	45.44	264762		1.12	3641
2	Ezetimibe	4.804	2031241	54.56	232442	12.32	1.01	6922

Our study was discussed and the following charcters (PRECISION , LINEARITY , ACCURACY , RUGGEDNESS, ROBUSTNESS) untill it was reching the seventh stage

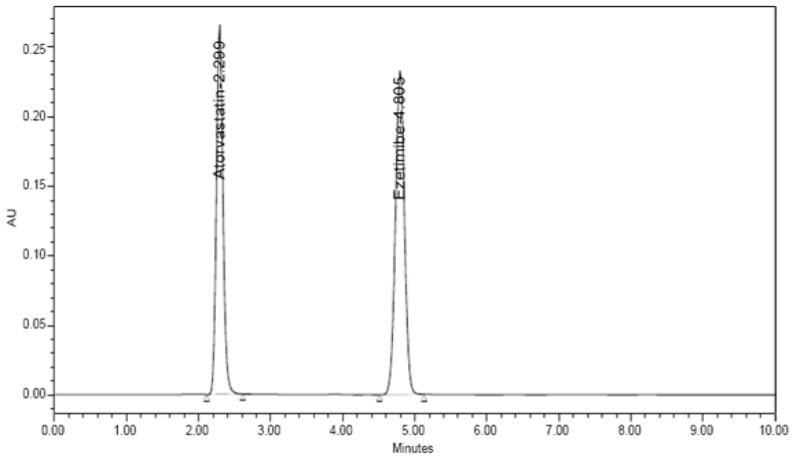
7) Assay :(Marketed Sample-Tablets)

Standard preparation

Transferred 1 mL of standard stock solution in to 10 mL volumetric flask and make up to volume with diluent.

Sample Preparation

Transferred sample quantitatively equivalent to 10 mg of Atorvastatin and 10 mg of Ezetimib in to 50 mL volumetric flask added 25 mL of diluent, sonicated to dissolve for 10 minutes and diluted to volume with diluent. filter the solution through filter paper. Diluted 1 mL of filtrate to 10 mL with mobile phase.



	Peak Name	RT	Area	% Area	Height	USP Resolution	USP Tailing	USP Plate Count
1	Atorvastatin	2.299	1681862	45.44	264695		1.09	3641
2	Ezetimibe	4.805	2027651	54.56	232375	12.32	0.98	6952

CONCLUSIONS

The present study was aimed at developing a simple, sensitive, precise and accurate HPLC method for the simultaneous analysis of Atorvastatin calcium and Ezetimibe from bulk samples and their tablet dosage forms. A non-polar C18 analytical chromatographic column was chosen as the stationary phase for the separation and simultaneous determination of Atorvastatin calcium and Ezetimibe. Mixtures of commonly used solvents like water, methanol and acetonitrile with or without buffers in different combinations were tested as mobile phases. The choice of the optimum composition is based on the chromatographic response factor, a good peak shape with minimum tailing. A mixture of buffer and acetonitrile in the ratio of 40:60 v/v was proved to be the most suitable of all the combinations since the chromatographic peak obtained was well defined, better resolved and almost free from tailing. The retention times of the Atorvastatin calcium and Ezetimibe were found to be 3.740 and 6.173 min respectively. The linearity was found satisfactory for both the drugs in the range 10 – 30 µg/mL . The regression equation of the linearity curve between concentrations of Atorvastatin calcium and Ezetimibe over its peak areas were found to be Y = 30.727X – 0.1187 (where Y is the peak area and X is the concentration of Atorvastatin caclcium in

µg/mL) and Y = 39.524X – 5.8813 (where Y is the peak area and X is the concentration of Ezetimibe in µg/mL) respectively. Precision of the method was studied by repeated injection of tablet solution and results showed lower %RSD values . This reveals that the method is 168 quite precise. The percent recoveries of the drug solutions were studied at three different concentration levels. The percent individual recovery and the %RSD at each level were within the acceptable limits. This indicates that the method is accurate. The absence of additional peaks in the chromatogram indicates non-interference of the commonly used excipients in the tablets and hence the method is specific. The deliberate changes in the method have not much affected the peak tailing, theoretical plates and the percent assay. This indicates that the present method is robust . The system suitability studies were carried out to check various parameters such as theoretical plates and tailing factor. The lowest values of LOD and LOQ as obtained by the proposed method indicate that the method is sensitive. The solution stability studies indicate that both the drugs were stable up to 24 hours. Therefore, the proposed method was simple, specific and sensitive and can be used for simultaneous analysis of Atorvastatin calcium and Ezetimibe in bulk samples and its tablet dosage forms.

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