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A REVIEW ON VARIOUS ANALYTICAL METHODS DEVELOPED AND VALDATED FOR ESTIMATION OF ATORVASTATIN CALCIUM AND EZETIMIBE

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ABSTRACT

Atorvastatin calcium is an HMG-CoA reductase inhibitor and Ezetimibe is a cholesterol inhibitor. Atorvastatin calcium is used to lower the LDL and triglycerides level. It also lower the chances of heart attack and stroke. Ezetimibe is used to reduce the hyperlipidemia, alone or in combination with other cholesterol lowering agents. The combination is used together with proper diet to treat high cholesterol and triglycerides levels in the blood. The combination was approved by US-FDA in May 2013 under the trade name Liptruzet, Atozet. A survey of literature and review published in various analytical and pharmaceutical journals has been conducted and the methods which were developed and used for determination as single and combination in bulk drugs, in pharmaceutical formulation, and in biological fluids have been reviewed. This review covers the analytical methods including spectrophotometric methods, chromatographic methods including HPLC, RP-HPLC and HPTLC, liquid chromatography tandem mass spectroscopy were reported.

KEYWORD: Atorvastatin calcium, Ezetimibe, Hyperlipidemia, HPLC, UV, Liptruzet.

INTRODUCTION

Dyslipidemia is an abnormal level of cholesterol and other lipid, also called fats, in the blood. A major cause of ischemic disease is related to dyslipidemia. Dyslipidemia is disorder of the metabolism of lipoproteins, including lipoprotein over production and deficiency of

lipoproteins. If metabolic disorder is due to the genetic reasons, termed as the primary dyslipidemias and if metabolic disorder is due to some other disease is termed as the secondary dyslipidemia.[1]

Combination of the Atorvastatin and Ezetimibe was approved under the brand name Liptruzet. Liptruzet approved by FDA on May 3, 2013. Liptruzet (Atorvastatin and Ezetimibe) is a HMG-CoA reductase inhibitor and cholesterol absorption inhibitor combination indicated for the treatment of dyslipidemia. Liptruzet available in the following strength – Atorvastatin: Ezetimibe – 10mg:10mg, 20mg:10mg, 40mg:10mg and 80mg:10mg. Ezetimibe reduces blood cholesterol by inhibiting the absorption of cholesterol by the small intestine, while atorvastatin reduces the plasma cholesterol and lipoprotein levels by inhibiting LDL production in the liver.[2]

PHARMACOLOGY

Atorvastatin calcium

Atorvastatin is a synthetic reversible competitive inhibitor of 3-hydroxy-methylglutyryl-co-enzyme-A (HMG-CoA) reductase. The conversion of HMG-CoA to mevalonate is an early and rate-limiting step in the formation of endogenous cholesterol. The inhibition of cholesterol formation by HMG-CoA reductase inhibitors reduces intracellular stores of cholesterol. This results in upregulation of the number of low density lipoprotein (LDL) receptors which increases the clearance of LDL-cholesterol from plasma, thus restoring intracellular cholesterol homeostasis.

Plasma cholesterol levels may also be lowered by HMG-CoA reductase inhibitor inhibition of hepatic synthesis of very low density lipoproteins (VLDL) cholesterol, a precursor of LDL-cholesterol, causing reduced production of LDL-cholesterol.[3]

Ezetimibe

Ezetimibe reduces blood cholesterol by inhibiting the absorption of cholesterol by the small intestine.[7]

Circulating plasma levels of cholesterol are derived from two primary sources: cholesterol production from the liver and peripheral tissues, and the absorption of dietary and biliary cholesterol in gastrointestinal tract. Cholesterol synthesis begins with the conversion of acetyl-CoA to mevalonic acid, a reaction catalyzed by the enzyme HMG-CoA reductase.

Cholesterol synthesized by hepatocytes undergo esterification by acyl-CoA acyl transferase (ACAT) and is incorporated into apolipoprotein B (ApoB)-containing lipoproteins such as very low density lipoprotein (VLDL) via microsomal transfer proteins. Subsequent modification of VLDL with hydrolysis of triglycerides by the enzymes lipoprotein lipase and hepatic lipase produces intermediate-density lipoprotein (IDL) and LDL.[4]

ADVERSE EFFECT

Atorvastatin

Commonly reported side effect: Hemorrhagic stroke, arthralgia, diarrhea, and nasopharyngitis. Other side effects are urinary tract infection, insomnia, limb pain, muscle spasm, musculoskeletal pain, myalgia, and nausea.[8]

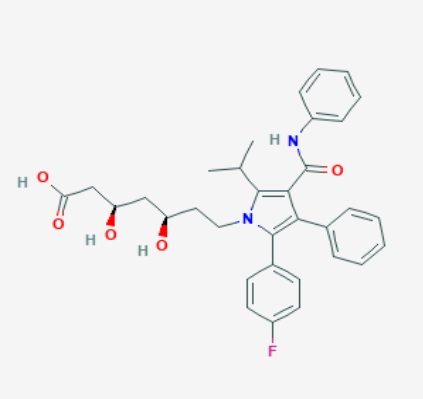
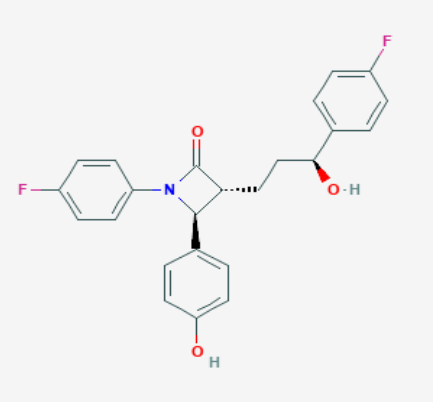
Ezetimibe

Most common adverse effect are abdominal fullness, black tarry stools, bleeding gums, boating, blood in urine and stools, chills, constipation, fast heartbeat, fever, darkened urine, gaseous abdominal pain, loss of appetite, light colored stools, nausea, skin rash, vomiting, yellow eyes and skin, abdominal pain, etc.[10]

Physicochemical property

Physicochemical properties of atorvastatin and ezetimibe are given in below table:[5,7]

Table 2: physicochemical properties of atorvastatin and ezetimibe.

Properties	ATORVASTATIN	EZETIMIBE
CAS NO	134523-00-5	163222-33-1
Structure		
Chemical formula	$C_{33}H_{35}FN_2O_5$	$C_{24}H_{21}F_2NO_3$
IUPAC NAME	(3R,5R)-7-[2-(4-fluorophenyl)-3-phenyl-4-(phenylcarbamoyl)-5-(propan-2-yl)-1H-pyrrol-1-yl]-3,5-	(3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one

	dihydroxyheptanoic acid.	
Molecular mass	558.64 g/mol	409.42g/mol
Characteristics	A white to off-white crystalline powder	A white, crystalline powder
Solubility	Slightly soluble in water while freely soluble in methanol	Freely soluble in ethanol, methanol and acetone.
Melting point	164-180°C	164-166 °C
Boiling point	722.2±60.0 °C at 760 mmHg	654.9±55.0 °C at 760 mmHg
PKa	9.48	9.48
logP	4.24/5.39	4.14/4.56
logS	-6	-4.7

Analytical method

Analytical method are define as the set of techniques that allows to know qualitatively and quantitatively the composition of any material and chemical state in which it is located.[7]

Analytical method validation parameter are as follows:

1. Linearity and range
2. Accuracy
3. Precision
4. Specificity
5. Limit of Detection
6. Limit of Quantification
7. Robustness
8. Ruggedness

Ultraviolet–Visible spectroscopy method

Ultraviolet-visible (Uv – Vis) spectroscopy method refers to absorption spectroscopy or reflectance spectroscopy in part of the ultraviolet and the full, adjacent visible spectral regions. Wavelength range for Uv region is 100-400nm and for the Vis region is 400-700nm.[13]

Table 3: Uv-Vis method development and validation for the Atorvastatin.

- Uv-Vis method development and validation for the Ezetimibe.

- Uv-Vis method development and validation for the combination of Atorvastatin and Ezetimibe

Sr. no.	Title	Description	Ref. no.
1	Spectrophoto -Metric Method of Estimatio of Atorvastatincalcium Using Sulfo-Phospho-Vanillin Reaction	λmax: - 414nm solvent: - Water r^2 = 0.99865	[14]
2	New Kinetic Spectrophoto Metric Method For Determination of Atorvastatin In Pure Pharmaceutical Dosage Form	λmax: - 566nm solvent: - Water r^2 = 0.9998	[15]
3	Extractive Spectrophoto Metric Determination of Atorvastatin In Bulk And Pharmaceutical Dosage Form	λmax: - 618.7nm solvent: - Water r^2= 0.9992	16
4	Spectrophoto Metric methods for atorvastatin calcium from tablet dosage form	λmax: - 240nm solvent: - Water r^2= 0.9999	[17]
5	Spectrophoto Metric determination of ezetimibe	λmax: - 510nm r^2= 0.9999 solvent: - Water	[18]
6	Development and validation of uv spectroscopic assay method of ezetimibe in bulk and drug formulations	λmax: - 230 nm r^2= 0.994 solvent: - Acetonitrile & water	[19]
7	Simple novel uv-spectroscopic method for estimation of ezetimibe in tablet dosage form	λmax: - 252 nm r^2= 0.998 solvent: - Ethanol: Glacial acetic acid(90:10)	[20]
8	Application of uv-spectrophoto- Metery an rp-hplc for simultaneous estimation of atorvastatin calcium and ezetimibe in pharmaceutical dosage form	λmax: - 232.5nm r^2 = 0.9998 & 0.9999 solvent: - Methanol	[21]
9	Simultaneous spectrophotometric determination of atorvastatin calcium and ezetimibe in tablet dosage form	λ = 227nm & 246.5nm r^2 =0.9992 & 0.9986 solvent: - Methanol	[22]
10	Spectrophotometric determination of atorvastatin and ezetimibe using 2,4-dnp in bulk and pharmaceutical dosage forms	λmax: - 479nm & 457nm r^2 =0.998 & 0.999 solvent: - Methanol	[23]

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is a form of a liquid chromatography, where separation occurs between a mobile phase and a stationary phase. It is the ability with

which the sample constituents will distributes themselves between the two phases that will affect the separation.[19]

HPLC is a technique used for separation, identification and quantification of each component in a mixture. It depends on pumps to pass a pressurized liquid solvent containing the sample mixture through a column crammed with a solid adsorbent. It is used to separate the complex mixtures of molecules encountered in chemical and biological system.[24]

Table 4: - HPLC method development and validation for the Atorvastatin.

- HPLC method development and validation for the Ezetimibe.
- HPLC method development and validation for the combination of Atorvastatin and Ezetimibe.

Sr. no.	Title	Description	Ref. no.
1	A VALIDATED REVERSED- PHASE HPLC METHOD FOR THE DETERMINATION OF ATORVASTATIN CALCIUM IN TABLET	Column: -LiChrospher^{R} 100RP-18(4mm*250mm, 5µm) Mobile phase: - 0.1% acetic acid solution: acetonitrile (45:55, v/v), pH=3.8 Flow rate: - 0.8mL/min Retention time: - 6.3 min Detection wavelength: - 246nm	[26]
2	STABILITY INDICATING HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF ATORVASTATIN CALCIUM IN PHARMACEUTICAL DOSAGE FORM	Column: - XTerra^{R} RP-18 (4.6mm*250mm, 5µm) Mobile p0hase: - Methanol: acetonitrile: buffer solution (pH6.85±0.05),(45:45:10) Flow rate: - 1.0mL/min Detection wavelength: - 246nm	[27]
3	STABILITY INDICATING RP-HPLC METHOD FOR ANALYSIS OF ATORVASTATIN IN BULK DRUG, MARKETED TABLET ANF NANOEMULSION FORMULATION	Column: - RP-18 column (4.6mm*250mm, 5mm) Mobile phase: - 0.05M sodium phosphate buffer: methanol (3:7, v/v), pH = 4.1 Flow rate: - 1.0mL/min Retention time: - 4.02 min Detection wavelength: - 247nm	[28]
4	VALIDATION OF HPLC METHOD FOR DETERMINATION OF ATORVASTATIN IN TABLETS AND FOR MONITORING STABILITY IN SOLID PHASE	Column: - C-18 column (4.6mm*250=mm, 5µm) Mobile phase: - Water: acetonitrile,(48:52, v/v), pH = 2.0 Flow rate: - 1.5mL/min Retention time: - 6.5 min Detection wavelength: - 245nm	[29]
5	DETERMINATION OF ATORVASTATIN IN HUMAN SERUM BY REVERSED PHASE	Column: - CLS-ODS C-18 column (4.6mm*150mm, 5µm)	[30]

	HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION	Mobile phase: - 0.05M sodium phosphate buffer: methanol (33:67, v/v), pH = 4.0 Flow rate: - 2.5mL/min Retention time: - 3.4 min Detection wavelength: - 247nm	
6	A SIMPLE AND RAPID HPLC METHOD FOR THE DETERMINATION OF ATORVASTATIN ON HUMAN PLASMA WITH UV DETECTION AND ITS APPLICATION TO PHARMACOKINETIC STUDIES	Column: - Nucleosil C-8 column (4.0mm*125mm, 5µm) Mobile phase: - Sodium dihydrogen phosphate buffer: acetonitrile (60:40, v/v), pH = 3.5 Flow rate: - 1.5mL/min Retention time: - 3.6 min Detection wavelength: - 245nm	[31]
7	HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ESTIMATION OF EZETIMIBE IN PHARMACEUTICAL FORMULATION TABLETS	Column: - C-18 analytical column (4.6mm*250mm, 5µm) Mobile phase: - Acetonitrile: ammonium acetate (75:25, v/v), pH = 3.0 Flow rate: - 1.0mL/min Retention time: - 3.6 min Detection wavelength: - 240nm	[32]
8	VALDATED RP-HPLC METHOD FOR ESTIMATION OF EZETIMIBE IN DIFFERENT TABLET DOSAGE FORM	Column: - C-18 analytical column (4.6mm*250mm, 5µm) Mobile phase: - Acetonitrile: methanol (50:50, v/v) Flow rate: - 1.0mL/min Retention time: - 4.959 min Detection wavelength: - 245nm	[33]
9	A STABILITY INDICATING RP-HPLC METHOD DEVELOPMENT FOR DETERMINATION OF EZETIMIBE IN TABLET DOSAGE FORM	Column: - Zorbax SB C18 column, (4.6mm*250mm, 5µm) Mobile phase: - 0.02N ortho phosphoric acid: acetonitrile (20:80, v/v), pH = 3.0 Flow rate: - 1.0mL/min Retention time: - 3.5 min Detection wavelength: - 232nm	[34]
10	DEVELOPMENT AND VALIDATION OF A REVERSED PHASE HPLC METHOD FOR THE DETERMINATION OF EZETIMIBE IN PHARMACEUTICAL DODSAGE FORMS	Column: - Kromasil 100 C18 column, (4.6mm*250mm, 5µm) Mobile phase: - Water: acetonitrile (30:70, v/v) Flow rate: - 0.5mL/min Retention time: - 6 min Detection wavelength: - 232nm	[35]
11	HPLC ANALYSIS FOR SIMULTANEOUS DETERMINATION OF ATORVASTATIN AND EZETIMIBE IN PHARMACEUTICAL FORMULATIOS	Column: - Inertsil ODS-3V column, (4.6mm*250mm, 5µm) Mobile phase: - 0.01M ammonium acetate buffer: acetonitrile (50:50, v/v) Flow rate: - 1.0mL/min	[36]

		Retention time: - 15.50 & 19.30 min Detection wavelength: - 254nm	
12	A RP-HPLC METHOD FOR SIMULATANEOUS ESTIMATION OF ATORVASTATIN AND EZETIMIBE IN PHARMACEUTICAL FORMULATIONS	Column: - Phenomenex C18, (4.6mm*250mm, 5µm) Mobile phase: - Water and 0.4%(v/v) TEA: acetonitrile (50:50, v/v); pH = 6.5, adjusted suing orthophosphoric acid Flow rate: - 1.0mL/min Retention time: - 3.42 & 6.90 min Detection wavelength: - 248nm	[37]
13	A VALIDATED STABILITY INDICATING RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF ATORVASTATIN CALCIUM AND EZETIMIBE HYDROCHLORIDE IN BULK AND TABLET DOSAGE FORM	Column: - X – terra C8, (4.6mm*150mm, 3.5µm) Mobile phase: - Phosphate buffer: acetonitrile (pH = 3.5) pH adjusted with orthophosphoric acid; (55:45, v/v) Flow rate: - 1.2mL/min Retention time: - 6.81 & 4.96 min Detection wavelength: - 232nm	[38]

High Performance Thin Layer Chromatography (HPTLC)

High Performance Thin Layer Chromatography (HPTLC) is enhanced type of the Thin Layer Chromatography (TLC). A number of enhancements can be made to the basic method of Thin Layer Chromatography(TLC) to automate the different steps, to increase the resolution achieved and allow more accurate quantitative measurement.[40]

Table 5: - HPTLC method development and validation for the Atorvastatin.

- HPTLC method development and validation for the Ezetimibe.

- HPTLC method development and validation for the combination of Atorvastatin and Ezetimibe

Sr. no.	Title	Description	Ref. no.
1	A SIMPLE AND SENSITIVE HPTLC METHOD FOR THE DETERMINATION OF CONTENT UNIFORMITY OF ATORVASTATIN CALCIUM TABLET	Stationary phase: - Precoated Silica gels F_{254} aluminum Mobile phase: - Benzene: methanol (7:3; v/v) Detection wavelength: - 236nm	[42]
2	HPTLC DETERMINATION OF ATORVASTATIN IN PLASMA	Stationary phase: - Precoated Silica gels $60F_{254}$ aluminium Mobile phase: - Toluene: methanol (7:3, v/v) Detection wavelength: - 280nm	[43]
3	APPLICATION OF A STABILITY INDICATING HPTLC METHOD FOR THE QUANTITATIVE DETERMINATION OF	Stationary phase: - Precoated Silica gels F_{254} aluminium Mobile phase: - Toluene: ethyl	[44]

	EZETIMIBE IN PHARMACEUTICAL DOSAGE FORM	acetate, (7:3; v/v) Detection wavelength: - 254nm	
4	DEVELOPMENT AND VALIDATION OF A METHOD FOR SIMULATANEOUS DENSITOMETRIC ESTIMATION OF ATORVASTATIN CALCIUM AND EZETIMIBE AS THE BULK DRUG AND IN TABLET DOSAGE FORMS	Stationary phase: - Precoated Silica gels F_{254} aluminium Mobile phase: - Toluene: methanol (8:2; v/v) Detection wavelength: - 240nm	[45]
5	HPTLC METHOD DEVELOPMENT, VALIDATION, AND STRESS DEGRADATION STUDIES FOR ATORVASTATIN AND EZETIMIBE IN MULTICOMPONENT TABLET DOSAGE FORM	Stationary phase: - Precoated Silica gels F_{254} aluminium Mobile phase: - Toluene: ethyl acetate: methanol (12:5:3; v/v/v) Detection wavelength: - 254nm	[46]

LIQUID CHROMATOGRAPHY – MASS SPECTROSCOPY (LC – MS)

Liquid chromatography – mass spectroscopy (LC – MS) is a technique that uses liquid chromatography or HPLC with the mass spectroscopy. LC-MS/MS is often utilized in laboratories for the qualitative and measurement of drug substance, drug product and biological samples.[47]

Table 6:- LC – MS method development and validation for the Atorvastatin.

- LC – MS method development and validation for the Ezetimibe.
- LS – MS method development and validation for the combination of Atorvastatin and Ezetimibe.

Sr.no.	Title	Description	Ref. no.
1	THE QUANTITATION OF ATORVASTATIN IN HUMAN PLASMA BY SOLID PHASE MICRO-EXTRACTION FOLLOWED BY LC-MS/MS AND ITS APPLICATION TO PHARMACOKINETICS STUDY	Ion transition for atorvastatin (*m/z*): - 559.2/440.1 Ion transition for fluvastatin (*m/z*): - 414.2/224.2 Column: - Higgins C18 column (3.0mm*100mm, 5µm) Mobile phase: - Ammonium acetate: acetonitrile (60: 40, v/v) Flow rate: - 0.6 ml/min Retention time: - 4.5±0.5 min	[49]
2	DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR THE DETERMINATION OF EZETIMIBE IN HUMAN PLASMA AND PHARMACEUTICAL FORMULATIONS	Ion transition for atorvastatin (*m/z*): - 392/161 Ion transition for fluvastatin (*m/z*): - 359.3/280 Column: - Phenomenex (Torrance, USA) Luna C18 column (150*4.5mm, 4µm) Mobile phase: - 0.02M phosphate buffer(pH=7): acetonitrile: methanol (40:55:5, v/v/v) Flow rate: - 1.0ml/min	[50]

		Retention time: - 1.05 & 1.09min	
3	LC-MS/MS SIMULTANEOUS DETERMINATION OF ATORVASTATIN ANF EZETIMIBE IN HUMAN PLASMA	Ion transition for atorvastatin (*m/z*): - 422.0/290.0 Ion transition for fluvastatin (*m/z*): - 408.0/271.0 Column: - Zorbax Eclipse plus (USA) C18 column (4.6*100mm, 3.5µm) Mobile phase: - 0.2% formic acid in water: acetonitrile (30:70, v/v) Flow rate: - 0.6ml/min Retention time: - 2.680 & 3.361 min	[51]

CONCLUSION

The detailed review of the study highlights the analytical method for the determination of Atorvastatin calcium, Ezetimibe and its combination in bulk drug, pharmaceutical dosage forms and biological samples. HPLC and spectrophotometric method were found to be most widely used for Atorvastatin calcium, Ezetimibe and its combination. HPLC method is frequently used due to its high sensitivity, specificity and better separation for the qualitative and quantitative determination. The other analytical methods like HPTLC and LC-MS/MS is also used for the determination of Atorvastatin calcium, Ezetimibe and its combination in serum, pharmaceutical dosage form and also in stability studies.

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