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Ezetimibe: An Overeview of Analytical Methods for the Drug Substance

Ms. Rinku Dhainje, Prof Tejaswini Kande, Ms. Varsha Dhanle, Ms. Pallavi Damle, Prof. Naziya Sayyad, Prof. Bhagyshri Shelar, Prof. Kiran Ghule, Prof. Nitin Neherkar

PDEAS, SUCOPSRC, Karadi, Pune, Maharashtra, India

ABSTRACT

A Simple and reproducible spectrophotometric method has been developed for the determination of Ezetimibe in bulk and in dosage forms. The λmax of Ezetimibe was found to be 234 nm. Linearity for this method lies in the range of 5-20 µg/ml. The proposed method is sensitive, accurate, reproducible and useful for the routine determination of Ezetimibe in tablet dosage form. No interference was observed from the excipients.

KEYWORDS: *Ezetimibe, UV Spectrophotometer*

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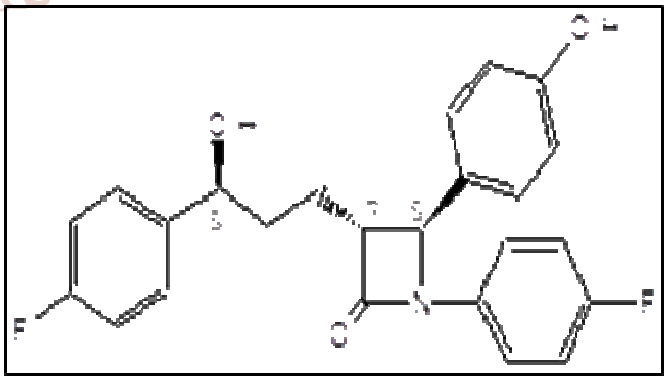
INTRODUCTION

Ezetimibe is a new Class of lipid lowering drug, which differs from other classes of cholesterol reducing compounds. It is chemically as (3R, 4S)1-(4-Flourophenyl-3 Hydroxy propyl]-4-(Hydroxy pheny-2-azetidinone1.

Ezetimibe is also known by the brand name Ezetrol. It can also be mixed with simvastatin, a type of statin. This is known by the brand name Inegy."

You may also be prescribed ezetimibe if you cannot take cholesterol-lowering medicines called statins, or if statins do not work for you.

STRUCTURE



CHEMICAL NAME - (3R, 4S) 1-(4-Flourophenyl-3 Hydroxy propyl]-4-(Hydroxy pheny-2-azetidinone1.

GENERIC NAME - Ezetimibe

BRAND NAME – Ezetrol, Ezedoc 10mg

PROPERTIES

1	COLOUR	White Amorphous powder
2	PH	4.5
3	Mol. Formula	C24H21F2NO3
4	Mol. Weight	409.4
5	Solubility	Practically Soluble
6	Melting Point	164-166 °C
7	Vapour Pressure	1.5X10-14 mm Hg at 25 °C (est)
8	LogP	og Kow = 3.94 (est)

PHARMACOLOGY

Mechanism of Action

EZETROL (ezetimibe) is in a class of lipid-modifying compounds that inhibit the intestinal absorption of cholesterol and related plant sterols.

Ezetimibe has a mechanism of action that differs from other classes of cholesterol reducing compounds (eg statins, bile acid sequestrants [resins], fibric acid derivatives, and plant sterols.)

The molecular target of ezetimibe is the sterol transporter, Niemann-Pick C1-Like 1 (NPC1L1), which is responsible for the intestinal uptake of cholesterol and phytosterols.

Ezetimibe therefore inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. This causes a reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood. Ezetimibe does not increase bile acid excretion (like bile acid sequestrants) and does not inhibit cholesterol synthesis in the liver (like statins).

In a 2 week clinical study in 18 hypercholesterolaemic patients, EZETROL inhibited intestinal cholesterol absorption by 54 %, compared with placebo. By inhibiting the absorption of intestinal cholesterol, EZETROL reduces the delivery of cholesterol to the liver. Statins reduce cholesterol synthesis in the liver. Together these distinct mechanisms provide complementary cholesterol reduction. EZETROL, administered with a statin, reduces total C, LDL C, Apo B, and TG and increases HDL C in patients with hypercholesterolaemia, beyond either treatment alone.

Clinical studies demonstrate that elevated levels of total C, LDL C and Apo B, the major protein constituent of LDL, promote human atherosclerosis. In addition, decreased levels of HDL C are associated with the development of atherosclerosis. Epidemiologic studies have established that cardiovascular morbidity and mortality vary directly with the level of total C and LDL C and inversely with the level of HDL C. Like LDL, cholesterol-enriched triglyceride-rich lipoproteins, including very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), and remnants, can also promote atherosclerosis.

A series of preclinical studies was performed to determine the selectivity of ezetimibe for inhibiting cholesterol absorption. Ezetimibe inhibited the absorption of [14C] cholesterol with no effect on the absorption of triglycerides, fatty acids, bile acids, progesterone, ethinyl estradiol, or the fat soluble vitamins A and D.

Pharmacokinetics

Absorption

After oral administration, ezetimibe is rapidly absorbed and extensively conjugated to a pharmacologically active phenolic glucuronide (ezetimibe-glucuronide). Mean maximum plasma concentrations (Cmax) occur within 1 to 2 hours for ezetimibe-glucuronide and 4 to 12 hours for ezetimibe. The absolute bioavailability of ezetimibe cannot be determined as the compound is virtually insoluble in aqueous media suitable for injection.

Effect of Food on Oral Absorption

Concomitant food administration (high fat or non-fat meals) had no effect on the oral bioavailability of ezetimibe when administered as EZETROL 10 mg tablets. EZETROL can be administered with or without food.

Distribution

Ezetimibe and ezetimibe glucuronide are bound 99.7% and 88 to 92% to human plasma proteins, respectively.

Metabolism

Ezetimibe is metabolised primarily in the small intestine and liver via glucuronide conjugation (a phase II reaction) with subsequent biliary excretion. Minimal oxidative metabolism (a phase I reaction) has been observed in all species evaluated. Ezetimibe and ezetimibe-glucuronide are the major drug-derived compounds detected in plasma, constituting approximately 10 to 20% and 80 to 90% of the total drug in plasma, respectively. Both ezetimibe and ezetimibe-glucuronide are slowly eliminated from plasma with evidence of significant enterohepatic recycling. The half-life for ezetimibe and ezetimibe-glucuronide is approximately 22 hours.

Excretion

Following oral administration of 14C ezetimibe (20 mg) to human subjects, total ezetimibe accounted for approximately 93% of the total radioactivity in plasma. Approximately 78% and 11% of the administered radioactivity were recovered in the

faeces and urine, respectively, over a 10 day collection period. After 48 hours, there were no detectable levels of radioactivity in the plasma.

Method

UV – Spectrophotometer - The stock solution of Ezetimibe was prepared by dissolving 50 mg of pure drug in ethanol in a 50ml volumetric flask. It was diluted as and when required. The absorbance of 10-µg/ml was measured against a solvent blank between 200-400 nm. A graph was plotted and the absorption maximum was determined as 234 nm, which is shown in fig 1. A calibration curve was obtained at 234 nm for a series of concentrations in the range of 5-20 µg/ml. It was found to be linear and hence, suitable for the estimation of the drug. The slope, intercept correlation coefficient and optical characteristics9 and summarized in Table 1.

Market sample analysis

Twenty tablets were weighed and finely powered. An accurately weighed portion of this equivalent to 50 mg of Ezetimibe was transferred in the 50 ml volumetric flasks containing about 25mlethanol. The contents were sonicated for 30 min with intermittent shaking to ensure the complete solubility of the drug and then filtered through 0.45µm membrane filter. The volume was made to the mark with ethanol. The results are shown in table 2.

Recovery studies

To study the accuracy and reproducibility10 of the proposed method, adding a known amount of drug to preanalysed sample at three levels and the percentage of recoveries were found out. The results are summarized in Table 3, which was found to be satisfactory.

Table 1: Optical characteristic and precision data

No.	Parameters	UV Method Value
1	Absorption Maximum (nm)	234
2	Beer's Law Limit (mcg/ml)	5-20
3	Sand ell's sensitivity (µg/cm2/0.001 abs. units)	0.0217
4	Molar Extinction Coefficient (L.mol-1cm-1)	188730
5	Correlation coefficient (r	0.9998
6	Slope (m)	0.04
7	Intercept (c)	0.0059
8	% RSD % Range of errors:	0.523
10	0.05 significance level	0.0021
11	0.01 significance level	0.0028

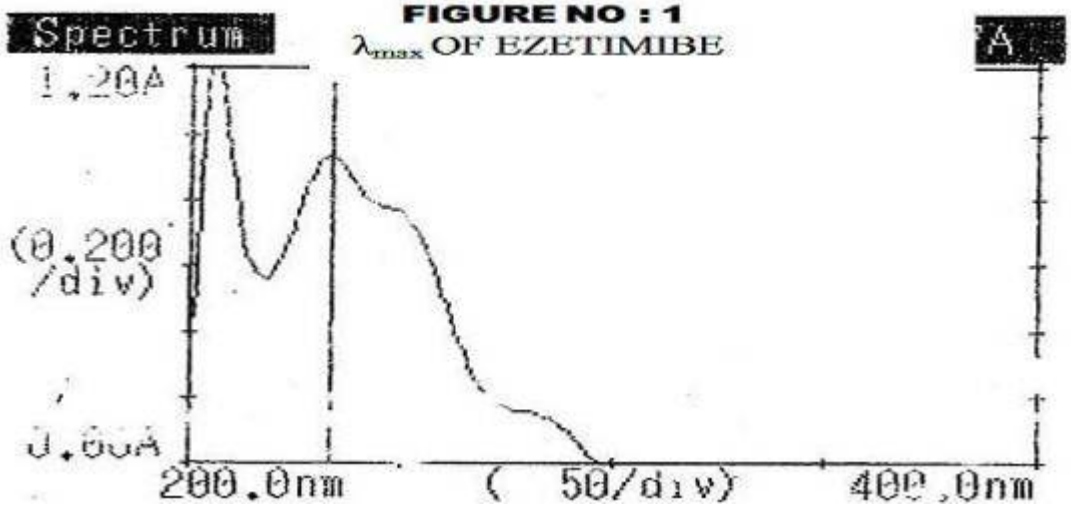
* For five replicate analysis with in Beer's law limits

Table 2: Results of Assay

Sample	Label Claim (mg)	UV-Method	
		Amount found (mg)	(%) R.S.D
Table -1	10	9.95	0.661
Table -2	10	9.98	0.583

Table 3: Recovery studies of Ezetimibe

Sample Concentration (µg/ml)	Fortified Concentration (µg/ml)	Percentage Recovery	
		Table A	Table B
10	8	99.11	98.0
	10	97.10	98.70
	12	96.95	97.50



Deviation indicates the reproducibility of the method. The method is useful for tablet formulation where there is no interference of excipients in the absorbance of Ezetimibe. Thus the proposed method was simple, accurate and reproducible and can be used for the routine analysis of Ezetimibe in bulk and in pharmaceutical dosage forms.

Major clinical applications of Ezetimibe

There is a wealth of evidence that ezetimibe reduces serum LDL-C levels. However, as detailed above, the

evidence regarding its effect on cardiovascular events is relatively new. This is at odds with statin therapy, for which the evidence base for both reducing LDL-C and reducing cardiovascular events is plentiful and well established. For this reason, statin therapy remains the first-line therapy for pharmacological LDL-C reduction, both in the context of secondary and primary prevention of cardiovascular disease.

Pharmacological lipid modification therapy is indicated for secondary prevention in patients who have proven cardiovascular disease. However, pharmacological lipid modification is only indicated for primary prevention when the patient is deemed to have a significant 10-year risk of developing cardiovascular disease. In the UK the threshold is set at a 10-year risk of cardiovascular disease of 10% or greater [NICE, 2014]. In the USA, the threshold is lower at a 10-year risk of cardiovascular disease of 7.5% or greater [Stone et al. 2014]. Modelling algorithms such as QRISK2 are routinely used as an assessment tool for predicting 10-year risk of cardiovascular disease.

Use of Ezetimibe

- It's used to treat high blood cholesterol. This is when you have too much of a fatty substance called cholesterol in your blood.
- Ezetimibe helps stop your body taking in cholesterol from food.
- It usually lowers cholesterol levels within 2 weeks.
- You can take this medicine with or without food.

INDICATIONS

1. Primary Hypercholesterolaemia

EZETROL administered alone, or with an HMG CoA reductase inhibitor (statin), is indicated as adjunctive therapy to diet in patients with primary (heterozygous familial and non-familial) hypercholesterolaemia.

2. Homozygous Familial Hypercholesterolaemia (HoFH)

EZETROL, administered with a statin, is indicated for patients with HoFH. Patients may also receive adjunctive treatments (e.g., LDL apheresis).

3. Homozygous Sitosterolaemia (Phytosterolaemia)

EZETROL is indicated for the reduction of elevated sitosterol and campesterol levels in patients with homozygous familial sitosterolaemia.

CONTRAINDICATIONS

- EZETROL is contraindicated in patients with hypersensitivity to any component of this medication.
- When EZETROL is to be administered with a statin, please refer to the Product Information for that particular statin.
- EZETROL in combination with fenofibrate is contraindicated in patients with gall bladder disease.
- Therapy with EZETROL in combination with a statin is contraindicated during pregnancy and lactation.
- The combination of EZETROL with a statin is contraindicated in patients with active liver disease or unexplained persistent elevations in serum transaminases.

PRECAUTIONS

When EZETROL is to be administered with a statin or fenofibrate, please refer to the Product Information for that particular product.

Liver Enzymes

In controlled co-administration trials in patients receiving EZETROL with a statin, consecutive transaminase elevations (□3 X the upper limit of normal [ULN]) have been observed. When EZETROL is co-administered with a statin, liver function tests should be performed at initiation of therapy and according to the recommendations of the statin (see ADVERSEEFFECTS).

In a controlled clinical study in which over 9,000 patients with chronic kidney disease were randomised to receive EZETROL 10 mg with simvastatin 20 mg daily (n=4,650) or placebo (n=4,620) (median follow-up period of 4.9 years), the incidence of consecutive elevations of transaminases (>3 X ULN) was 0.7% for EZETROL combined with simvastatin and 0.6% for placebo (see ADVERSEEFFECTS).

Skeletal Muscle

In clinical trials, there was no excess of myopathy or rhabdomyolysis associated with EZETROL compared with the relevant control arm (placebo or statin alone). However, myopathy and rhabdomyolysis are known adverse reactions to statins and other lipid-lowering drugs. In clinical trials, the incidence of CPK > 10 X ULN was 4 of 1674 (0.2%) patients administered EZETROL alone vs. 1 of 786 (0.1%) patients administered placebo, and for 1 of 917 (0.1%) patients co-administered EZETROL and a statin vs. 4 of 929 (0.4%) patients administered a statin alone.

Hepatic Insufficiency

Due to unknown effects of the increased exposure of ezetimibe in patients with moderate to severe hepatic insufficiency, EZETROL is not recommended in these patients (see Characteristics in Patients [Special Populations]).

Paediatric (10 to 17 Years of Age) Patients

Ezetrol has not been studied in patients younger than 10 years of age or in pre-menarchal girls. (See DOSAGE AND ADMINISTRATION)

The long-term efficacy of therapy with Ezetrol in patients below 17 years of age to reduce morbidity and mortality in adulthood has not been studied.

Fibrates

The co-administration of ezetimibe with fibrates, other than fenofibrate, has not been studied and is therefore not recommended. (See INTERACTIONS WITH OTHER MEDICINES).

Fenofibrate

Fibrates may increase cholesterol excretion from the bile, and ezetimibe increased cholesterol in the gallbladder bile in a preclinical study in dogs. Given the potential for cholelithiasis, and the numerically higher incidence of cholecystectomies in patients administered ezetimibe and fenofibrate in a clinical study (see CLINICAL TRIALSand ADVERSE EFFECTS sections), coadministration of ezetimibe and fenofibrate is not recommended in patients with pre-existing gallbladder disease (see CONTRAINDICATIONS).

Cyclosporin

Caution should be exercised when initiating ezetimibe in the setting of cyclosporin. Cyclosporin concentrations should be monitored in patients receiving EZETROL and cyclosporin (see INTERACTIONS WITH OTHER MEDICINES).

Anticoagulants

If EZETROL is added to warfarin, another coumarin anticoagulant or fluindione, the International Normalised Ratio (INR) should be appropriately monitored (See INTERACTIONS WITH OTHER MEDICINES).

Genotoxicity

Ezetimibe alone or in combination with a statin (simvastatin, lovastatin, pravastatin or atorvastatin) or fenofibrate did not cause gene mutation in bacteria or chromosomal damage in human peripheral lymphocytes or bone marrow cells in mice.

ADVERSE EFFECTS

Clinical studies of 8 to 14 weeks duration in which EZETROL 10 mg daily was administered alone, with a statin, or with fenofibrate in 3551 patients demonstrated: EZETROL was generally well tolerated, adverse reactions were usually mild and transient, the overall incidence of side effects reported with EZETROL was similar to that reported with placebo, and the discontinuation rate due to adverse experiences was comparable between EZETROL and placebo.

Taking 1 or 2 extra tablets is unlikely to harm you. But the amount of ezetimibe that can lead to overdose is different from person to person.

These common side effects may happen in more than 1 in 100 people

Safety of Ezetimibe

The side-effect profile of statins is well described and most notable for muscle toxicity (including myalgia, myopathy and rhabdomyolysis) and deranged liver enzymes [Hu et al. 2012]. The IMPROVE-IT trial demonstrated that the addition of ezetimibe to simvastatin did not increase the rates of elevated liver enzymes to a level greater than three times the upper limit of normal. Similarly the trial demonstrated that the addition of ezetimibe to statin therapy did not affect the number of patients with muscle-related events (rhabdomyolysis, myopathy, myalgia or elevated creatinine kinase levels). There was no evidence of any association between ezetimibe therapy and cancer or cancer deaths [Cannon et al. 2015].

Conclusion

Ezetimibe is a drug that reduces LDL-C by reducing intestinal cholesterol absorption. The IMPROVE-IT trial has demonstrated that ezetimibe significantly reduces the risk of major cardiovascular events in a group of high-risk patients with known cardiovascular disease and already low LDL-C levels, with an absolute risk reduction of 2%. Ezetimibe is safe to use and well tolerated. The clinical applications of ezetimibe are currently centred on its use in combination with a statin when total or LDL-C concentration is not appropriately controlled either after appropriate dose titration of initial statin therapy, when dose titration is limited by intolerance of statins, or when a change from an initial statin therapy is being considered. Ezetimibe monotherapy is recommended for primary hypercholesterolaemia in patients in whom statin therapy is contraindicated, or in patients who are unable to tolerate statin therapy. There is significant scope for further research into the role of ezetimibe, especially its role as monotherapy and in the primary prevention of cardiovascular disease. Additional areas of interest include the role of ezetimibe in 'high cholesterol absorbers' and the combination of ezetimibe with PCSK9 inhibitors.

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