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Research Study *Open Access*

A new validated RP-HPLC method for the analysis of Bempedoic acid and Ezetimibe in bulk drug samples

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ABSTRACT

A short selective, precise, accurate and sensitive for the estimation of bempedoic acid and ezetimibe was done by RP-HPLC. The chromatographic separation was clear at the flow rate of 1 ml/min, at UV detection of 232 nm. The assay for bempedoic acid and ezetimibe were found to be 99.95 and 100.24 respectively. which shows that the method is useful for routine analysis. The linearity of bempedoic acid and ezetimibe was found to be direct with a relationship coefficient of 0.999 and 0.999, which appears that the strategy is competent of creating great affectability. The LOD and LOQ for bempedoic acid was found to be 2.9 and 10.03 and LOD and LOQ for ezetimibewas found to be 3.0 and 10.1. The vigor restrain for versatile stage variety and stream rate variety are well inside the constrain, the % debasement comes about are in limits. Which appears that the strategy is having great framework reasonableness and accuracy beneath given set of conditions. From the recovery and the studies, showing acceptable limits, it can be concluded that this can be employed for estimation of bempedoic acid and ezetimibe in its dosage forms.

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Keywords: bempedoic acid, ezetimibe, Validation, RP-HPLC.

INTRODUCTION

Nexlizet (bempedoic acid and ezetimibe) is an adenosine triphosphate-citrate lyase (ACL) inhibitor and a cholesterol absorption inhibitor combination indicated as an adjunct to diet and maximally tolerated statin therapy for the treatment of adults with heterozygous familial hypercholesterolemia or established atherosclerotic cardiovascular disease who require additional lowering of LDL-C. The recommended dosage of Nexlizet, in combination with maximally tolerated statin therapy, is one tablet orally once daily.

Nexlizet contains bempedoic acid and ezetimibe and lowers elevated LDL-C through complementary mechanisms of action by inhibiting cholesterol synthesis in the liver and absorption in the intestine. One tablet of Nexlizet contains 180 mg of bempedoic acid and 10 mg of ezetimibe.[1-3]

Bempedoic acid is a prodrng. It is activated to thioester with coenzyme A in the liver. The activated substance (Bempedoic acid-CoA) is an adenosine triphosphate-citrate lyase (ACL) inhibitor that lowers low-density lipoprotein cholesterol (LDL-C) by inhibition ofcholesterol synthesis in the liver.[4 – 9] Bempedoic acid is a white to off-white crystalline powder that is highly soluble in ethanol, isopropanol and pH 8.0 phosphate buffer, and insoluble in water and aqueous solutions below pH 5. Bempedoic acid is

an adenosine triphosphate-citrate lyase (ACL) inhibitor that lowers low-density lipoprotein cholesterol (LDL-C) by inhibition of cholesterol synthesis in the liver. ACL is an enzyme upstream of 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase in the cholesterol biosynthesis pathway. Bempedoic acid and its active metabolite, ESP15228, require coenzyme A (CoA) activation by very long-chain acyl-CoA synthetase 1 (ACSVL1) to ETC-1002-CoA and ESP15228-CoA, respectively. ACSVL1 is expressed primarily in the liver. Inhibition of ACL by ETC-1002-CoA results in decreased cholesterol synthesis in the liver and lowers LDL-C in blood via upregulation of low-density lipoprotein receptors.

Ezetimibe is a lipid-lowering compound that inhibits intestinal cholesterol and phytosterol absorption. The discovery and research of this drug began in the early 1990s, after the intravenous administration of radiolabelled ezetimibe in rats revealed that it was being localized within enterocytes of the intestinal villi - this prompted studies investigating the effect of ezetimibe on intestinal cholesterol absorption.[10-14]Ezetimibe is a white, crystalline powder that is freely to very soluble in ethanol, methanol, and acetone and practically insoluble in water. Ezetimibe reduces blood cholesterol by inhibiting the absorption of cholesterol by the small intestine. The molecular target of ezetimibe has been shown to be the sterol transporter, Niemann-Pick C1-Like 1 (NPC1L1), which is involved in the intestinal uptake of cholesterol and phytosterols. Ezetimibe localizes at the brush border of the small intestine and 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 LDL receptors, resulting in clearance of cholesterol from the blood.

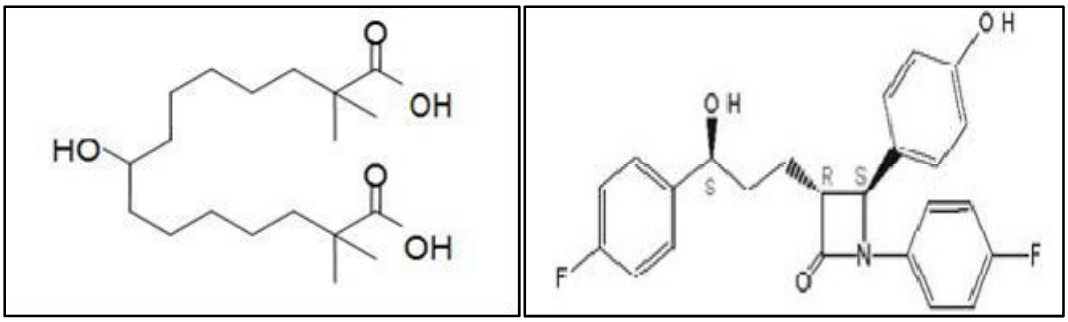

Fig.1: Structure of Bempedoic Acid and Ezetimibe

Only few methods were reported for the simultaneous estimation of bempedoic acid and ezetimibeby HPLC.[15-18] Hence we had made an attempt to develop a simple, accurate and precise RP-HPLC method for the simultaneous estimation of bempedoic acid and ezetimibe.

METHODOLOGY

Gift samples of bempedoic acid and ezetimibe were received from Startech lab, Hyderabad, whereas water, methanol for HPLC, acetonitrilefor HPLC and phosphoric acid were purchased from Merck.

Instrumentation

Waters HPLC was used for the separation of bempedoic acid and ezetimibe. UV/VIS spectrophotometer (LABINDIA UV 12.500^{+}) was used for detection. Instruments such as; pH meter used was of Adwa — AD 10100 and weighing machine was of Afcoset ER-1000A.

Preparation of buffer

Accurately weighed 20.214 g ofDisodium hydrogen phosphatetaken in 1000 ml of HPLC water,add 3.394 g of Monosodium phosphate to the solution and the volume was adjusted to pH 8.0 with HCl. Last arrangement was sifted through 0.44 µm Film channel and sonicate it for 10 mins.

Preparation of mobile phase

Precisely measured 200 ml (20%) of over buffer and800 ml (80%) of Methanol HPLC were blended and degassed in an ultrasonic water shower for 10 minutes and after that sifted through 0.45 µ channel beneath vacuum filtration and used as the diluent.

Standard Solution Preparation

Precisely weigh and exchange 18 mg of bempedoic acid and 1 mg of ezetimibe working standard into a 100 ml clean dry volumetric jar include around 7 mL of Diluent and sonicate to break up it totally and make volume up to the stamp with the same dissolvable. (Stock solution) Further pipette 3 ml of the over stock arrangements into a 10ml volumetric jar and weaken up to the stamp with diluent.

Sample Solution Preparation

Precisely weigh 10 tablets pulverize in mortor and pestle and exchange proportionate to 18 mg of Bempedoic Acid and 1 mg Ezetimibe test into a 100 mL clean dry volumetric jar include almost 7 mL of Diluent and sonicate it up to 15

mins to break up it totally and make volume up to the check with the same dissolvable. At that point it is Sifted through 0.45 micron Infusion channel. (Stock solution) Further pipette 3ml of bempedoic acid and ezetimibe from the over stock arrangement into a 10ml volumetric jar and weaken up to the stamp with diluent.

Method development and optimisation

Due to the significant difference in the physical and chemical properties of bempedoic acid and ezetimibe, several mobile phases and columns were initially trialed in order to have both eluents on the same chromatogram. The suitability of the column and the mobile phase used in the optimized method have been decided based upon the basis of the selectivity, sensitivity as well as acceptable chromatographic parameters of the produced peaks in terms of peak sharpness, peak symmetry, tailing factor and resolution between the two peaks. We used the mobile phase as a solvent for all samples to ensure minimum noise and to eliminate any unwanted solvent peaks.

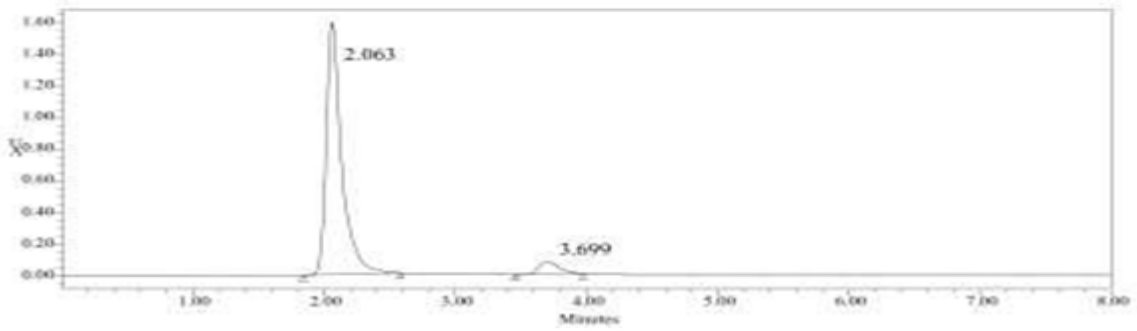

Fig.2: Standard Chromatogram of Bempedoic Acid And Ezetimibe

RESULTS AND DISCUSSION

Method Validation

The optimized method for simultaneous determination of bempedoic acid and ezetimibehas been validated as per International Conference of Harmonisation (ICH) guidelines Q2 (R1) for evaluating system suitability, specificity, precision, accuracy, linearity, limit of detection (LOD), limit of quantitation (LOQ) and robustness.[19]

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and UV detector.
Temperature	:	Ambient
Column	:	Chromosil C18 Column (250mm x 4.6mm)5µg
pH	:	8.0
Mobile phase	:	20% buffer 80% Methanol
Flow rate	:	1 ml per min
Wavelength	:	232 nm
Injection volume	:	20 µl
Run time	:	8 min.

System Suitability

Following figure 2 for the crests due to bempedoic acid and ezetimibe in Standard arrangement ought to not be more than 2.0 Theoretical plates for the bempedoic acid and ezetimibe crests in Standard arrangement ought to not be less than 2000. Resolution for the bempedoic acid and ezetimibecrests in standard arrangement ought to not be less than 2**.**

Table 1: Results of system suitability parameters

S.No	Name	Retention time(min)	Area (µV sec)	Height (µV)	USP resolution	USP tailing	USP plate Count
1	Bempedoic Acid	2.5	124505	213642	1.2	4673.4	
2	Ezetimibe	3.9	13084951	545666 0	1.3	6090.3	

Linearity

The standard stock solution of Bempedoic Acidis diluted in the concentration range of (180–900 µg/ml). Triplicates of such concentration range were prepared and plotted on a calibration curve. (Fig.3,4) The standard stock solution of Ezetimibe is diluted in the concentration range of (10–50 µg/ml). Triplicates of such concentration range were prepared and plotted on a calibration curve. Slope, intercept and correlation coefficient of the calibration curves (peak

area versus concentration) were determined to ensure linearity of the analytical method. (Table 2)

Table 2: Results of Linearity of Bempedoic Acid and Ezetimibe

S. No.	Bempedoic Acid		Ezetimibe	
	Concentration (µg/ml)	Area	Concentration (µg/ml)	Area
1	180	668934	10	66510
2	360	956781	20	94701
3	540	1313873	30	124802
4	720	1563458	40	152731
5	900	1867084	50	179732

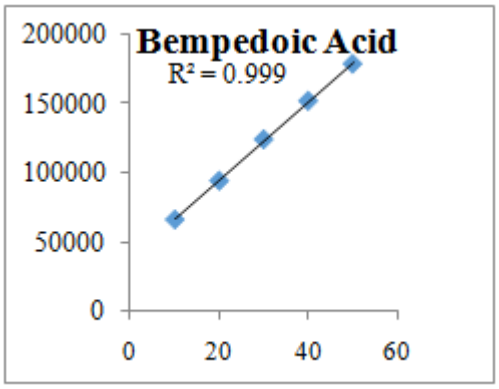

Fig 3: Calibration graph for Bempedoic Acid

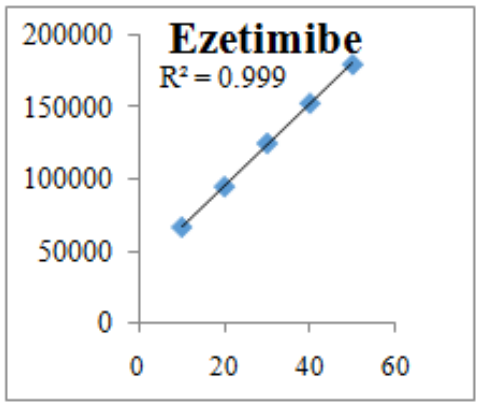

Fig 4: Calibration graph for Ezetimibe

Accuracy

Accuracy of the proposed method was confirmed with bempedoic acid and ezetimibeseparately at 3 different levels 50%, 100% and 150%, the determinations of these 3 levels have been recorded to obtain the mean and % recovery. (Table 3,4)

Table-3 Accuracy (recovery) data for Bempedoic Acid

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	9.0	9.036	100.7%	99.84%
100%	1304258	18.0	18.003	100.0%	
150%	1854608	54.0	54.224	98.780%	

Table-4 Accuracy (recovery) data for Ezetimibe

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	65800	0.50	0.53	100.8%	100.51%
100%	124353	1	10.10	100.01%	
150%	177940	15.0	15.45	99.68%	

LOD and LOQ

The LOD and LOQ arrangements was arranged infused, for three times and measured the region for all three infusions in HPLC. The %RSD for the zone of six reproduce infusions was found to be inside the required limits. (Table 5,6)

Table-5 Results of LOD

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Bempedoic Acid	52	152	2.9
Ezetimibe	52	156	3

Table-6 Results of LOQ

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Bempedoic Acid	52	522	10.03
Ezetimibe	52	524	10.1

CONCLUSION

The presented validated method is rapid, economic, simple, accurate, sensitive, robust, specific and linear. It can be used for routine analysis of bempedoic acid and ezetimibe in combination products.

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