ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Simultaneous Estimation of Bempedoic Acid and Ezetimibe by UPLC

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Article History:

Received on: 17 Aug 2020
Revised on: 14 Aug 2020
Accepted on: 17 Sep 2020

Keywords:

Bempedoic Acid,
Ezetimibe,
UPLC,
Validation,
Retention Time

ABSTRACT

A selective and novel method has been optimized for the evaluation of Bempedoic Acid and Ezetimibe in bulk samples by U.P.L.C. The principle analyzes were eluted with the conditions of mobile phase having the 0.1% orthophosphoric acid and acetonitrile (90:10 ratio) using the X bridge C18 (250 × 4.6 mm, 5μm) analytical column with the 1.0 ml/min flow rate and 10 μl sample volume at 290 nm in a photodiode array detector. The retention times of Bempedoic Acid and Ezetimibe were 0.27 min and 0.73 min with the total run time of 2 min. The curve indicates the correlation coefficient (r^2) was superior by having the value equal to 0.998 with a linear range of 30 n.g/m.l- 225 n.g/m.l for Bempedoic Acid and for Ezetimibe 150 n.g/m.l-1125 n.g/m.l. The correlation coefficient (r^2) found linear. The LOQ and LOD for the Bempedoic Acid and Ezetimibe found 2.5 n.g/m.l and 7.5 n.g/m.l and 2.9 n.g/m.l and 8.9 n.g/m.l. After the method optimization, the method was validated as per ICH guidelines. As per the results obtained in the method validation, there was no interference of the blank and carryover problem even at the LOQ level quantification. Both LOQ and LOD of this method was verified practically in the instrument with S/N ratio criteria. The results were found satisfactory.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4366

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INTRODUCTION

Bempedoic acid and ezetimibe is an inhibitor of adenosine triphosphateitrate lyase (ACL) (Sharma *et al.*, 2010; Mishra *et al.*, 2007) and a combination of cholesterol absorption inhibitors indicated as a dietary supplement and maximally tolerated statin therapy for the treatment of adults with heterozygous familial hypercholesterolemia or established atherosclerotic cardiovascular disease requiring additional lowering of LDL-C (Jain *et al.*, 2009; Ajmera *et al.*, 2012). Bempedoic acid is an adenosine triphosphate-citrate lyase (ACL) inhibitor. The molecular formula is C19H36O5, and the molecular weight is 344.5 grams per mole (Godse *et al.*, 2009; El-Moghazy *et al.*, 2009). Ezetimibe is in a class of lipid-lowering compounds that inhibits the intestinal absorption of cholesterol and related phytosterols. The empirical formula is C24H21F2NO3. Its molecular weight is 409.4 (Rajput and Raj, 2009; Goel *et al.*, 2013). Based on the literature survey there was no analytical method for this new formulation i.e. Bempedoic acid and Ezetimibe (Kothapalli

et al., 2007; Nagarsenker *et al.*, 2010). There are several methods were developed with other combination (Bhusari *et al.*, 2009; Oswald *et al.*, 2006). For the Bempedoic acid combination there was lack of sensitive analytical methods (Gajjar and Vishal, 2010; Kumar *et al.*, 2012) for the identification and quantification in bulk samples and there was no sensitive analytical method having the low detectability to quantify the product (ICH, 1996).

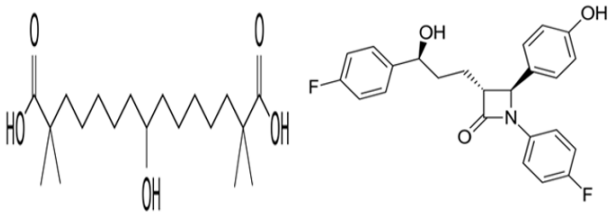

Figure 1: Chemical structures of A) Bempedoic acid B) Ezetimibe.

MATERIALS AND METHODS

Bempedoic acid and Ezetimibe Figure 1 were obtained as gift sample from MICRO LABS, Bangalore.

High purity acetonitrile, water (Milli — Q system, Millipore, Bedford, MA, USA), All chemicals were high purity grade.

Conditions of chromatography

Separation and estimation was performed using UPLC (Waters with PDA detector), X bridge C18 (250mm, 4.6 mm, 5μm) column used in the experiment.

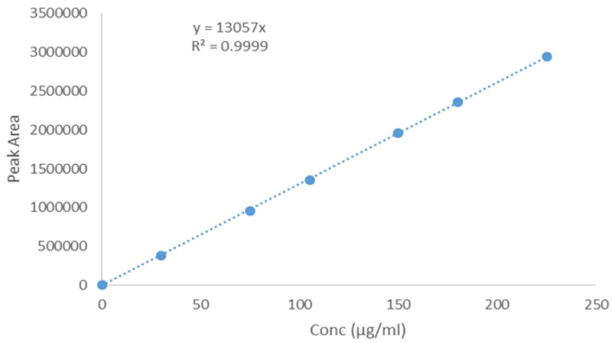

Figure 2: Linearity of Bempedoic Acid.

The mobile phase was prepared by mixing 0.1 percent Ortho Phosphoric Acid pH adjusted to 2.5 buffer: the ratio of (90:10) acetonitrilein was filtered and degassed and detection was at 290 nm.

The retention times of Bempedoic Acid and Ezetimibe hydro chloride were 0.27 min and 0.73 min with the total run time of 2 min.

Solution Preparation for standards

Standard stock solution of Bempedoic Acid and Ezetimibe pure drug (1mg / ml) was prepared by accurately weighing approximately 100 mg of the drug and transferred to a 100 ml volumetric flask and dissolved in a diluent.

Buffer preparation and Mobile phase

Accurately transferred 1ml of concentrated orthophosphoric acid in 1000ml of volumetric flask add about 900ml of milli water added to add 1ml of triethylamine and degas to the ultrasonic water bath for 10 minutes and finally make up the volume with water, then adjust the pH to 2.5 with dil. orthophosphoric acid. 0.1% OrthoPhosphoric Acid buffer solution (pH 2.5) and Acetonitrile in the ratio of 90:10 (v/v). Prepared the diluent by taking 2000 ml of water in 4000 ml mobile phase bottle and added 2000 ml of methanol and degassed to get 4000 ml of diluent.

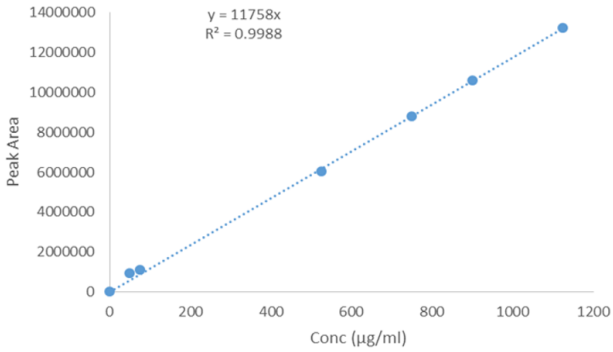

Figure 3: Linearity of Ezetimibe.

Method Validation

Specificity

To verify the method validation in terms of the selectivity and exactness injected triplicate preparations of 100 % concentrations of Bempedoic Acid and Ezetimibe. Then injected one blank also to prove the method was not having the carryover issue. The limit for the specificity is, it should pass the system suitability criteria and there should not be RT shift for all the three preparations.

Performance of the instrument

To verify the system producing the consistent results with the optimized method injected the standard for six times with the criteria of % RSD for retention time and area NMT 2.0%, theoretical plates NLT 3000 plates, tailing factor NMT 1.5 and resolution NLT 2.

Linearity & Range

The method linearity was verified with the five concentrations of 100% concentration as 30, 75, 105,

Table 1: Outcomes of precision.

Sample No	Area of Bempedoic Acid	Area of Ezetimibe
Mean	221195	455388.5
SD	194.57	4889.43
% RSD	0.09	1.07
	Interday precision data	
Mean	219216.33	458224.83
SD	4112.48	7111.48
% RSD	1.88	1.55

Table 2: Outcomes of Accuracy (Recovery).

Level of % recovery	Target Conc (n.g/m.l)	Amount of drug Spiked (n.g/m.l)	Amount found (n.g/m.l)	%Recovery	Mean Value	S.D	% R.S.D
			Bempedoic Acid				
80	180	144	144.29	100.20	99.62	0.86	0.86
			144.04	100.03			
			142.03	98.63			
100	180	180	180.04	100.02	100.04	0.02	0.02
			180.08	100.04			
			180.11	100.06			
120	180	216	214.68	99.39	100.20	0.85	0.85
			218.36	101.09			
			216.25	100.12			
			Ezetimibe				
80	900	720	721.45	100.20	99.62	0.86	0.87
			720.23	100.03			
			710.14	98.63			
100	900	900	900.23	100.03	100.05	0.02	0.02
			900.43	100.05			
			900.56	100.06			
120	900	1080	1078.32	99.84	100.35	0.99	0.99
			1096.18	101.50			
			1076.97	99.72			

150, 180, 225 n.g/m.l of Bempedoic Acid (30 to 225 n.g/m.l) and 50, 75 525, 750, 900, 1125 n.g/m.l of Ezetimibe (50 to 1125 n.g/m.l) with the acceptance criteria of regression coefficient (R^2) NLT 0.99.

Precision

After passing the specificity and system suitability criteria the method was verified for the system precision and method precision with the limit of % RSD for the retention time and area NMT 2%. The intermediate precision was verified on the next day with another column by following the limit as % RSD for the retention time and area should be NMT 2%.

Accuracy (Recovery)

To verify the method accuracy triplicate preparations were prepared at 80% and 100% and 120% level of 100% concentration (180 n.g/m.l of Bempedoic Acid and 900 n.g/m.l of Ezetimibe) by spiking the standard into the diluent. Calculated the recovery with the acceptance criteria 95-105%.

Robustness

To verify the method efficiency when the minor changes happened in the optimized method parameters like mobile phase composition and column temperature and flow and buffer pH this parameter was performed with the criteria, it should pass the system suitability criteria.

Limit of detection and Limit of quantification

The detection limit (LOD) is defined as the lowest

Table 3: Outcomes of the Robustness.

S No	Parameter	Condition	Bempedoic Acid		Ezetimibe	
			Area(n=3)	% change	Area(n=3)	% change
1	Standard	Standard conditions	221455	0	451134	0
2	Mobile Phase composition	92:8, %v/v	221351	0.1	450637	0.14
		88:12, %v/v	221247	1.33	470140	0.55
3	Mobile phase pH	2.7	221143	0.83	459643	0.11
		2.3	221039	0.52	459146	0.56
4	Wavelength	288 nm	220935	0.19	458649	0.07
		292 nm	220831	0.23	463485	1.51
5	Flow rate	1.2	220727	0.25	464988	0.07
		0.8	220623	0.18	466491	1.51

analyte concentration that can be reliably distinguished from the background levels. The quantification limit (LOQ) of an individual analytical procedure is the lowest amount of analyze which can be quantitatively determined with appropriate accuracy and precision.

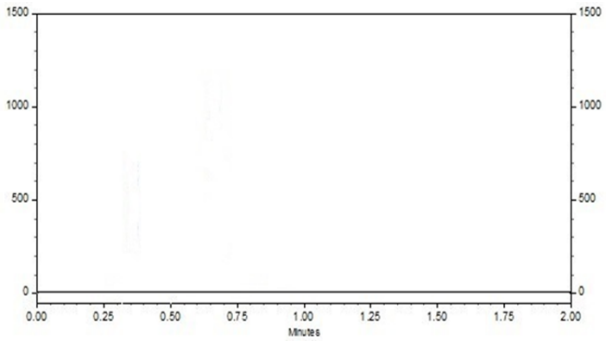

Figure 4: Blank chromatogram of Bempedoic Acid & Ezetimibe.

Solution Stability

Stability of the solution was assured using standard solutions and test stock. These stocks were prepared and stored for 36 hrs under room temperature and refrigerated conditions and differences were calculated for percentage.

Filter validation

To evaluate the impact of PVDF and Nylon filters on the assay results the samples were analyzed after passing through the filters.

RESULTS AND DISCUSSION

There was a clear separation and good resolution and without any carryover was achieved with this method as shown in the Figures 4 and 5. The system suitability acceptance criteria also found satisfactory as ICH guidelines. For the system precision parameter the %RSD of area for the Bempedoic acid and Ezetimibe achieved less than 2% against the limit. For the method precision parameter the %RSD of RT and area for the Bempedoic acid and Ezetimibe achieved against the limit NMT 2.0%.

The linearity parameter was quantified by peak area Vs concentration methodology. Different concentrations from 50 to 1125 n.g/m.l standard solutions and 30 to 225 n.g/m.l for Bempedoic acid and Ezetimibe were prepared and injected into the system. The calculated regression coefficient for Bempedoic acid and Ezetimibe are nearer to 0.998 as shown in the Figures 2 and 3.

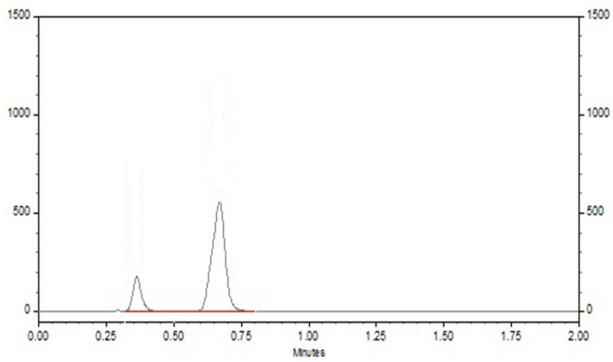

Figure 5: Chromatogram of Bempedoic Acid & Ezetimibe.

The method was verified for the ruggedness as inter day and intra day precision. For the intermediate precision parameter the %RSD of area for the Bempedoic acid and Ezetimibe achieved in day -1 as 0.617, 0.664 and on the next day 1.80, 1.50 against the limit NMT 2.0% as shown in the Table 1. The recovery for the 80%, 100% and 120% was more than 99% against the acceptance criteria 95-105% as shown in the Table 2.

To evaluate the method capability of producing precise results with the minor variations of flow, mobile phase composition, pH, column temperature variations as a robustness was performed. The results

were shown in the Table 3.

The results proving that the method was stable to produce consistent results with the minor variation of the method parameters. The compatibility of the filters were verified with the PVDF and Nylon filters.

The LOQ and LOD were identified by injecting the lower concentrations with the S/N ratio criteria,2.5n.g / ml for Bempedoic Acid and 7.5n.g/ m.l for Ezetimibe and 2.9n.g/ m.l for Bempedoic Acid and 8.9n.g/ m.l for Ezetimibe.

Standard solution are stable for 36 hours at 5°C as the percentage difference was found to be less than 2.0 percent in the area. Filter interference was performed of 0.45μ filters (Nylon, PVDF), and the percentage difference for sample solutions and standard solutions calculated against centrifuged samples and standard was found to be below 2.0 percent.

During method optimization initially organic solvents used as mobile phase with the water in different composition. But both compounds were not detected. Then started usage of buffer with organic solvent such as acetonitrile in different ratios and pH with the C_{18} column. Finally, the method was found optimized with the conditions of mobile phase 0.1 percent ortho Phosphoric Acid buffer system: Acetonitrile (90:10 v / v), wavelength 290 nm, flow rate of 1.0 ml/min, column temperature of 25°C, sample compartment temperature of 10°C, sample volume of 10 μl. With this method both actives i.e. Bempedoic Acid and Ezetimibe were having good resolution and symmetry.

CONCLUSIONS

Based on the results obtained in the developed method was very sensitive, accurate, linear and economical. Due to short time of chromatographic program more samples can be analyzed within the short period of time, which will be helpful in the industry at the time of multiple products manufacturing continuously. The method was met all the predefined acceptance criteria. With this method the sample of bulk and formulation samples can be analyzed. As the method having the capability of detecting the formulation, bulk shelf life samples also can be analyzed by using this method.

ACKNOWLEDGEMENT

The authors are thankful to Principal and chairman, MAM College of Pharmacy, Narasaraopet, Andhra Pradesh, India, for providing necessary research facilities for carrying out the research work.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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