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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINATION OF BEMPEDOIC ACID AND EZETIMIBE

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Abstract

A simple, rapid, precise, sensitive and reproducible reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the quantitative analysis of Bempedoic acid and Ezetimibe in pharmaceutical dosage form. Chromatographic separation of Bempedoic acid and Ezetimibe was achieved on Waters Alliance-e2695 by using Inertsil ODS (250x 4.6mm, 5µ) column and the mobile phase containing Methanol: 0.1% Perchloric acid in the ratio of 10:90% v/v. The flow rate was 1.0 ml/min; detection was carried out by absorption at 232nm using a photodiode array detector at ambient temperature. The number of theoretical plates and tailing factor for Bempedoic acid and Ezetimibe were NLT 2000 and should not more than 2 respectively. % Relative standard deviation of peak areas of all measurements always less than 2.0. The proposed method was validated according to ICH guidelines. The method was found to be simple, economical, suitable, precise, accurate & robust method for quantitative analysis of Bempedoic acid and Ezetimibe study of its stability.

Keywords: RP-HPLC, Bempedoic acid and Ezetimibe, simultaneous estimation.

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Introduction

Bempedoic acid (8-hydroxy-2,2,14,14-tetramethylpentadecanedioic acid) is a member of the class of medications called adenosine triphosphate-citrate lyase (ACL) inhibitors. Its primary role is to inhibit cholesterol production by the liver [1]. By inhibiting the liver's capacity to create new cholesterol, the adenosine triphosphate-citrate lyase (ACL) inhibitor bempedoic acid lowers LDL-C levels. Prior to 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, an enzyme known as ACL is present in the cholesterol production pathway. Ezetimibe ((3R,4S)-1-(4-fluorophenyl)-3-[(3S)-3-(4-fluorophenyl)-3-hydroxypropyl]-4-(4-hydroxyphenyl)azetidin-2-one), a lipid-lowering agent and azetidinone derivative, blocks cholesterol absorption. By physically interacting with cholesterol transporters at the brush edge of the small intestine, ezetimibe limits the absorption of cholesterol and related phytosterols.

An RP-HPLC approach for the measurement of Bempedoic acid and Ezetimibe has been attempted, with the goal of developing a validated stability indicator. A review of the relevant literature uncovered a dearth of published analytical techniques, either alone or in conjunction with other medications. The objective is to create an RP-HPLC technique that is easy to utilize, quick and specific enough to measure Bempedoic acid and Ezetimibe in bulk and prescription dose forms. To ensure that the suggested approaches are legitimate, they must meet the analytical criteria outlined in the ICH recommendations. These criteria include system appropriateness, accuracy, precision, specificity, linearity, robustness, limit of detection and limit of quantification.

Materials and Methods

Bempedoic acid and Ezetimibe active pharmaceutical ingredients (API) were obtained from Spectrum Pharma Research Solutions, Hyderabad as gift samples along with their analytical reports. Methanol and Perchloric acid was obtained from Rankem and Commercial tablets of Bempedoic acid and Ezetimibe were procured from the local drug market.

Chromatographic condition

The mobile phase consisted of Methanol: 0.1% Perchloric acid (10:90) at a flow rate of 1.0 ml/min. Inertsil C-18 column (4.6 x250mm, 5µ particle size) was used as the

stationary phase. Although the Bempedoic acid and Ezetimibe have different λ max, but considering the chromatographic parameter, sensitivity and selectivity of method for both drugs, 232 nm was selected as the detection wavelength for PDA detector [2].

Preparation of standard stock solution

Transfer 180 milligrammes of bempedoic acid and 10 milligrammes of ezetimibe (the working standard) into a 100 millilitre clean, dry volumetric flask. Add the diluent and sonicate until the chemical is fully dissolved. Fill the flask to the specified capacity using the same solvent (Stock solution). Pipette Transfer 5 millilitres of each stock solution to a 50 millilitre vacuum flask and fill to the top with diluent. (180ppm of Bempedoic acid, 10ppm of Ezetimibe).

Sample preparation

Transfer 302 milligrammes of the Bempedoic acid and Ezetimibe sample to a 100 millilitre clean, dry vacuum flask. Before centrifuging for 30 minutes to dissolve the diluent, add it to the mixture and sonicate it for up to 30 minutes. Use the same solvent to get the volume up to the mark. A 0.45 micron injection filter is then used to further filter it (Stock solution). Pipette Transfer 5 millilitres of each stock solution to a 50 millilitre vacuum flask and fill to the top with diluent. (180ppm of Bempedoic acid, 10ppm a of Ezetimibe)

Method validation

System suitability tests

The maximum allowable tailing factor for peaks in standard solution caused by Bempedoic acid and Ezetimibe is 2.0. There should be no fewer than 2000 theoretical plates for the peaks of Bempedoic acid and Ezetimibe in the standard solution. The resolution of the peaks in the standard solution corresponding to Bempedoic acid and Ezetimibe must not be lower than 2.

Linearity

By appropriate aliquots of the standard Bempedoic acid and Ezetimibe solutions with the mobile phase, six working solutions ranging between 45-270 µg/mL and 2.5-15 µg/mL respectively were prepared. Each experiment was performed in triplicate according to optimized chromatographic conditions3. The peak areas of the chromatograms were plotted against the concentration of Bempedoic acid and Ezetimibe to obtain the calibration curve.

Accuracy

Recovery studies by the standard addition method were performed with a view to justify the accuracy of the proposed method4. Previously analyzed samples of Bempedoic acid and Ezetimibe to which known amounts of standard Bempedoic acid and Ezetimibe corresponding to 50%, 100% and 150% of label claim were added. The accuracy expressed as the percentage of analyte recovered by the proposed method.

Precision

Precision was determined as repeatability and intermediate precision, in accordance with ICH guidelines5. The repeatability and intermediate precision were determined by analyzing the samples of Bempedoic acid and Ezetimibe. Determinations were performed on the same day as well as well as on consequent days.

Limit of detection and the limit of quantification

Limit of detection (LOD) and limit of quantification (LOD) of Bempedoic acid and Ezetimibe were determined by calibration curve method6. Solutions of both Bempedoic acid and Ezetimibe were prepared in linearity range and injected in triplicate. Average peak area of three analyses was plotted against concentration. LOD and LOQ were calculated by using following equations. LOD = (3.3 ×Syx)/b, LOQ= (10.0×Syx)/b.

Where Syx is residual variance due to regression; b is slope.

Robustness

The robustness of the method was performed by deliberately changing the chromatographic conditions7. The organic strength was varied by ±5%, column temperature was varied by ±5°C and the flow rate ±0.1mL.

Degradation Studies

Preparation of stock

After carefully weighing Bempedoic acid and Ezetimibe, put the mixture to a 10 millilitre clean, dry volumetric flask. Add the diluent and sonicate for 30 minutes to dissolve. Centrifuge for 30 minutes to thoroughly dissolve the sample. Finally, add enough of the same solvent to fill the flask to the mark. A 0.45 micron injection filter is then used to further filter it (Stock solution).

Acid degradation, Alkali degradation, Thermal degradation, Peroxide degradation, Reduction degradation, Photolytic degradation and Hydrolysis degradation were carried out (Table 9) [8, 9].

Result and Discussion

Method development

Initially reverse phase liquid chromatography separation was tried to develop using various ratios of Methanol: 0.1% Perchloric acid as mobile phases, in which both the drugs did not responded properly, and the resolution was also poor. With Methanol: 0.1% Perchloric acid (10:90) both drugs eluted with better separation at a flow rate of 1.0 ml/min. Inertsil C-18 column (4.6 x150mm, 5µ particle size) was used as the stationary phase was selected to improve resolution and the tailing of both peaks were reduced considerably and brought close to 1. To analyze both drugs detection were tried at various wavelengths from 210nm to 280nm. The wavelength at which both Bempedoic acid and Ezetimibe showed maximum absorption at 232nm was selected as the detection wavelength for PDA detector. The retention times were found to about 2.5 min and 3.9 min for Bempedoic acid and Ezetimibe, respectively. The obtained chromatogram was shown in the figure 1.

Method Validation

System suitability: System suitability parameters such as number of theoretical plates, retention time and peak tailing were determined. The results obtained were shown in table 1.

Linearity: Bempedoic acid and Ezetimibe were showed a linearity of response between 45-270 µg/mL and 2.5-15 µg/mL (Figure 2 & Figure 3) and the linearity were represented by a linear regression equation.

Accuracy: The percentage recoveries of Bempedoic acid and Ezetimibe were 99.9% and 99.5%, respectively. These results were summarized in table 2 & 3.

Repeatability: Six replicates of standard concentrations were analyzed in same day for repeatability and results were found within acceptable limits. These results were summarized in table 4.

Intermediate Precision: Six replicates of standard concentrations were analyzed on two different days and by two analysts for day to day and analyst to analyst variation and results were found within acceptable limits. These results were summarized in table 5.

Robustness: As per ICH norms, small, but deliberate variations, by altering the Flow rate, column temperature and concentration of the mobile phase were made to check the method's capacity to remain unaffected. It was observed that there were no marked changes in chromatograms, which demonstrated that the developed method was robust in nature (Table 6 & 7).

LOD and LOQ: LOD and LOQ for Bempedoic acid were 0.54 µg/mL and 1.8 µg/mL and for Ezetimibe were 0.03 µg/mL and 0.1 µg/mL respectively.

Tablet Analysis: Content of Bempedoic acid and Ezetimibe found in the tablets by the proposed method are shown in Table 8. The low values of RSD indicate that the method is precise and accurate.

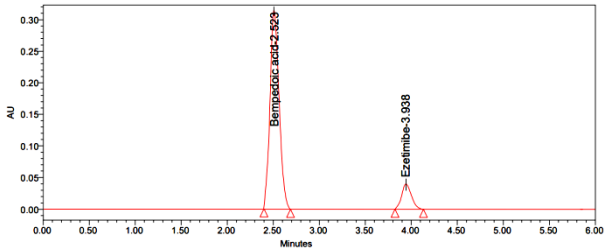

Figure 1. Optimized chromatogram

Table 1: System suitability of Bempedoic acid and Ezetimibe

S.no	Parameter	Bempedoic acid	Ezetimibe
1	RT	2.523	3.938
2	Theoretical plates	12375	14621
3	Tailing factor	1.17	1.14
4	Resolution	----	7.60
5	%RSD	0.17	0.48

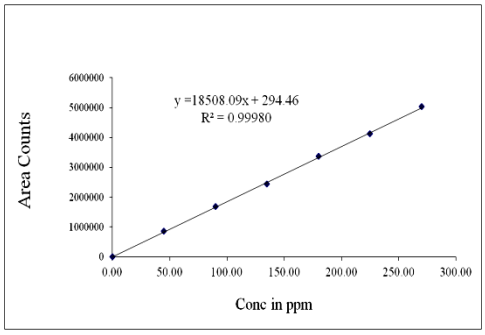

Figure 2. Calibration curve of Bempedoic acid

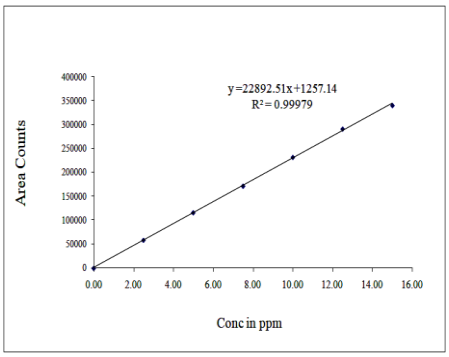

Figure 3. Calibration curve of Ezetimibe

Table 2: Results of Recovery Experiments of Bempedoic acid

% Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	1652822	90.00	88.86	98.7	99.4
	1675478	90.00	90.07	100.1	
	1665937	90.00	89.56	99.5	
100%	3351471	180.00	180.18	100.1	99.9
	3347241	180.00	179.95	100.0	
	3338687	180.00	179.49	99.7	
150%	5068617	270.00	272.49	100.9	100.3
	5017314	270.00	269.73	99.9	
	5021436	270.00	269.95	100.0	

Table 3: Results of Recovery Experiments of Ezetimibe

%Concentration	Response	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	118482	5.00	5.01	100.2	100.2

	117364	5.00	4.97	99.4	
	119241	5.00	5.05	101.0	
100%	236742	10.00	10.02	100.2	99.5
	234576	10.00	9.93	99.3	
	233781	10.00	9.90	99.0	
150%	354787	15.00	15.02	100.1	100.0
	353271	15.00	14.95	99.7	
	355484	15.00	15.05	100.3	

Table 4: Repeatability data of Bempedoic acid and Ezetimibe

S. No.	Bempedoic acid Response	Ezetimibe Response
1	3315977	235286
2	3337574	236871
3	3357354	234783
4	3334856	234874
5	3329669	237136
6	3318983	237584
Average	3332402	236089
Standard Deviation	14917.731	1246.549
%RSD	0.45	0.53

Table 5: Intermediate Precision for Bempedoic acid and Ezetimibe

S. No.	Bempedoic acid Response		Ezetimibe Response	
	Day-1	Day-2	Day-1	Day-2
1	3357379	3341873	236312	233846
2	3335559	3324310	236871	238197
3	3346358	3355946	235154	234230
4	3342652	3332011	236245	235506
5	3354768	3319745	237719	236944
6	3333883	3340258	234652	235519
Average	3345100	3335691	236159	235707
Standard Deviation	9681.643	13165.744	1118.251	1640.175
%RSD	0.29	0.39	0.47	0.70

Table 6: Robustness results of Bempedoic acid

Parameter	Bempedoic acid					
	Condition	Retention time(min)	Response	Tailing	Plate count	%RSD
Flow rate Change (mL/min)	Less flow (0.9ml)	2.705	3148626	1.20	12428	0.50
	Actual (1.0ml)	2.523	3348271	1.17	12375	0.17
	More flow (1.1ml)	2.451	3462472	1.13	12264	0.31
Organic Phase change	Less Org (9:91)	2.896	3058547	1.18	12487	0.41
	Actual (10:90)	2.528	3347520	1.15	12348	0.17
	More Org (11:89)	2.267	3648556	1.10	12194	0.25

Table 7: Robustness results of Ezetimibe

Parameter	Ezetimibe						
	Condition	Retention time (min)	Response	Resolution	Tailing	Plate count	%RSD
Flow rate Change (mL/min)	Less flow (0.9 ml)	4.126	223564	7.69	1.18	14715	0.15
	Actual (1.0 ml)	3.938	235032	7.60	1.14	14621	0.48
	More flow (1.1 ml)	3.744	254817	6.96	1.11	14578	0.53
Organic Phase change	Less Org (9:91)	4.338	214256	7.98	1.17	14770	0.61
	Actual (10:90)	3.933	236772	7.63	1.12	14683	0.48
	More Org (11:89)	3.619	267248	7.32	1.09	14514	0.36

Table 8: Assay of Bempedoic acid and Ezetimibe

Brand	Medication	Response	Avg sample area (n=2)	Std. Conc. (µg/ml)	Sample Conc. (µg/ml)	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
BEMPETOL-EZ	Bempedoic acid	3352168	3346687	180	180	180	99.8	179.92	100.0
		3341206							
	Ezetimibe	233163	234231	10	10	10	99.7	9.91	99.1

Table 9: Forced Degradation results for Bempedoic acid and Ezetimibe

Degradation	Bempedoic acid					Ezetimibe				
	Response	% Assay	% Deg	Purity Angle	Purity Threshold	Response	% Assay	% Deg	Purity Angle	Purity Threshold
Control	3347135	100	0	0.599	7.724	235991	100	0	1.656	6.522
Acid	2897265	86.5	13.5	0.584	7.728	204779	86.7	13.3	1.627	6.524
Alkali	2929838	87.5	12.5	0.583	7.732	210160	89.0	11.0	1.634	6.537
Peroxide	2858515	85.4	14.6	0.571	7.739	201543	85.4	14.6	1.648	6.587
Reduction	3009295	89.9	10.1	0.576	7.714	216460	91.7	8.3	1.685	6.575
Thermal	3266413	97.5	2.5	0.558	7.784	233251	98.8	1.2	1.666	6.579
Photolytic	3334724	99.6	0.4	0.582	7.721	228018	96.6	3.4	1.671	6.553
Hydrolysis	3307236	98.8	1.2	0.524	7.736	230150	97.5	2.5	1.664	6.565

Conclusion

The HPLC approach that has been devised for the measurement of certain medications is quick, easy, precise, accurate, reliable, and cost-effective. The solvents and mobile phase are cheap, easy to make, dependable, sensitive, and quick to prepare. The sample recoveries were consistent with the promises made on the labels, which means that the formulation receivers did not interfere with the estimate. This means that the medications may be routinely tested in labs. It has been concluded that the suggested methods, which are both simple and brief, would be the most useful for analysis because the system validation parameters of the HPLC method have demonstrated satisfactory, accurate, and repeatable results (without recipient interference, of course). The present study found that the RP-HPLC stability indicating test technique was easy to use, yielded correct results, was highly specific, and did not interact with either the placebo or degradation products. Therefore, they may be used for the normal evaluation of Bempedoic acid and Ezetimibe.

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Not Declared.

Conflict of Interest

No Conflict of interest

Informed Consent and Ethical Statement

Not Applicable.

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