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SIMULTANEOUS ESTIMATION OF BEMPEDOIC ACID AND EZETIMIBE BY HPLC

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ABSTRACT

The aim of the project is to develop a fast, simple, precise, and cost-efficient RP-HPLC method for measuring the amounts of Bempedoic Acid and Ezetimibe simultaneously in both pharmaceutical products and bulk samples. This technique has effectively achieved the separation of Bempedoic Acid and Ezetimibe in large quantities. The separation was performed using a Kromosil C18 150 x 4.6 mm, 5m analytical column at a wavelength of 280 nm. The mobile phase consisted of a mixture of Potassium dihydrogen orthophosphate, acetonitrile, and water in a ratio of 25:60:15. The pH of the buffer was adjusted to 6.0. The separation was carried out in isocratic elution mode with a flow rate of 1.2 ml/min. Bempedoic Acid had a retention time of 2.252 minutes, whereas Ezetimibe showed a retention duration of 2.987 minutes. Quantitative analysis of Bempedoic Acid and Ezetimibe was achieved using PDA detection at 280 nm using a linear calibration curve. The concentration ranges of 5-25 µg/ml (with a correlation coefficient of 0.9998) and 10-50 µg/ml (with a correlation coefficient of 0.9996) were used for reliable quantification. The limit of detection (LOD) for Bempedoic Acid was 0.1345 µg/ml, whereas the LOD for Ezetimibe was 0.2456 µg/ml. The proposed method is highly suitable for use in quality-control laboratories for the bulk and pharmaceutical quantitative analysis of pharmaceuticals, whether used individually or in combination. This technique is characterised by its simplicity and efficiency, while yet ensuring a high level of accuracy and precision.

Keywords: Bempedoic Acid, Ezetimibe and RP-HPLC.

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Introduction:

Bempedoic acid, a substance that inhibits ATP citrate lyase, decreases levels of low-density lipoprotein (LDL) cholesterol and is linked to a low occurrence of muscle-related negative events; its impact on cardiovascular outcomes is yet unclear. Ezetimibe is a pharmacological drug prescribed for the treatment of individuals with hyperlipidemia, a condition characterised by abnormal levels of lipids in the blood. The FDA granted approval to it in 2002. Ezetimibe is a frequently used nonstatin drug that reduces LDL-C levels by 13% to 20%.The user's text is. Ezetimibe functions as a suppressor of the absorption of cholesterol in the intestines. Patients diagnosed with primary hyperlipidemia, mixed hyperlipidemia, familial hypercholesterolemia (HoFH), and homozygous sitosterolemia (phytosterolemia) should aim to decrease their total cholesterol, low-density lipoprotein (LDL), apolipoprotein B (apo B), and non-high-density lipoprotein (HDL) levels.

Bempedoic acid and Ezetimibe are used together to treat hypercholesterolemia and ASCVD by decreasing lipid parameters and mitigating hsCRP levels. Multiple studies have used spectroscopic RP-HPLC and UPLC-MS techniques to quantify the amounts of ezetimibe and bempedoic acid, both alone and in conjunction with other medications. Hence, it was deemed valuable to create a highly accurate, precise, and cost-effective quick RP-HPLC technology to concurrently determine the levels of ezetimibe and bempedoic acid in tablet form [1-6]. The objective of the present work was to develop a rapid and accurate RP-HPLC assay method for the simultaneous measurement of Bempedoic Acid and Ezetimibe. The proposed methodology may be efficiently used for quality assurance.

Materials and Methods

Drug substance (Bempedoic Acid and Ezetimibe),Potassium dihydrogen orthophosphate(HPLC grade), Orthophosphoricc acid (HPLC grade, Water (HPLC grade) and acetonitrile (HPLC grade), HPLC system (Shimadzu SPD-20A, Tokyo, Japan).

Instrumentation

The HPLC studies were conducted using a Shimadzu SPD-20A HPLC system from Tokyo, Japan. The system included a separation module and a photodiode array detector, and the tests were performed in isocratic mode using an Autosampler. The data collection and processing were conducted with laboratory solution software. The separation was performed using a Kromosil C18 150 x 4.6 mm, 5m analytical column. The additional equipment used included a pH metre (Eutech), weighing balance (Shimadzu), and ultrasonicator (Unichrome, UCA701).

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Preparation of mobile phase

Mobile phase was prepared by mixing Potassium dihydrogen orthophosphate, acetonitrile and water in the ratio 0f 25:60:15 and the pH of the buffer was adjusted to 6.0 and was filtered through 0.45µ membrane.

Preparation of standard stock solution

Through the use of a digital microbalance, 5.0 mg of Bempedoic Acid and 10.0 mg of Ezetimibe were weighed into a volumetric flask of 10 millilitres. After adding seven millilitres of diluent, it was sonicated to dissolve it. After that, the solution was diluted to volume with the diluent, and lastly, it was diluted to a final volume by adding more diluent.

Chromatographic conditions

High Performance Liquid Chromatography equipped with PDA detector.

For Bempedoic Acid and Ezetimibe (isocratic)

Column	:	Kromosil C18 150 x 4.6 mm, 5m analytical column
Wavelength	:	280 nm
Injection Volume	:	20µl
Column Temperature	:	Ambient
Flow rate	:	1.2 ml/min

At 2.252 minutes, the BPA peak was found to have an area of 749156, with a tailing factor of 1.42. As shown in Fig. 1 and Table 1, the EZM peak was seen at 2.987 min with a peak area of 1098956, a tailing factor of 1.22, and a resolution of 2.25. This experiment was deemed optimal due to its positive outcomes and shorter retention duration. BPA has a retention time of around 2.252 minutes and EZM has a retention time of 2.987 minutes.

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Table 1: System suitability parameters

S.No.	Name of the Peak	Retention Time (Mins)	Peak Area	Tailing Factor	Resolution	Plate Count
01	Bempedoic Acid	2.252	749156	1.42	---	4242
02	Ezetimibe	2.987	1098956	1.22	2.25	5262

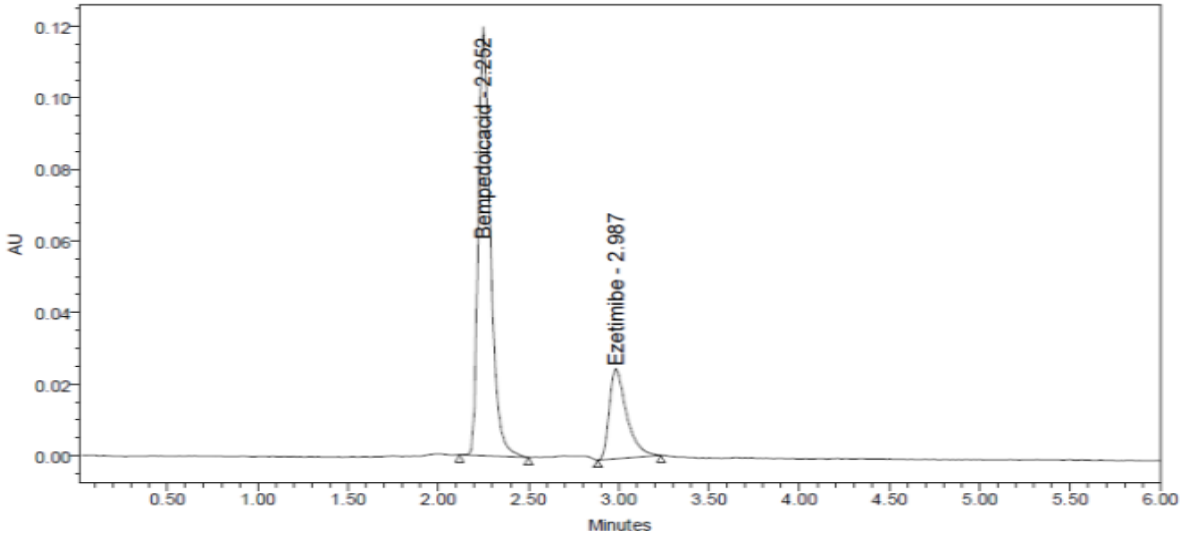

Fig.No. 01 : Typical Chromatogram of Bempedoic Acid and Ezetimibe

Preparation of sample solution

About 10 mg of sample was weighed into a 10 ml volumetric flask, and then 7 ml of diluent was added. The mixture was then sonicated to dissolve the material, and then diluted to volume with diluent. Further diluted to 10 ml with the diluent and filtered through 0.45µ Nylon syringe filter.

Procedure

Five injections of 20 µl each of active BPA and EZM standard solutions were performed. Chromatograms were obtained and peak responses were evaluated. The system's suitability was calculated by evaluating its parameters. The quantification of BPA and EZM in the sample was achieved by the analysis of the peak responses.

Method Validation

The present study examined many parameters to establish the validity of the HPLC methodology for quantifying BPA and EZM in accordance with the specified procedure,

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hence demonstrating its suitability for the intended use. The implementation of all validation criteria was done in compliance with the standards set by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH).

Linearity and Range

The concentrations of BPA and EZM that showed a linear relationship with peak area were (05 - 10µg/ml), (10 - 50µg/ml).Results are shown in (Fig.2 & 3), (Table 2 & 3), and the linearity of the calibration curve is confirmed by the high value of the correlation coefficient of the regression equation.

Table 2: Linearity data of BPA

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	5	374234
3	10	749156
4	15	1146543
5	20	1509675
6	25	1874535
Slope		**75294**
Intercept		**1186.8**
Regression		**0.9998**

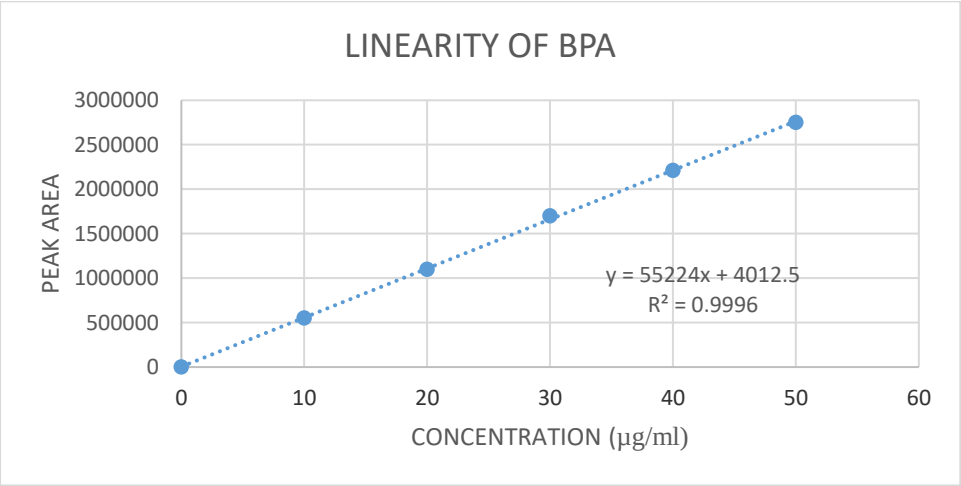

Fig.No. 02 : Linearity of Bempedoic Acid

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Table 3: Linearity data of EZM

S.No.	Concentration (µg/ml)	Peak Area
1	0	0
2	10	549765
3	20	1098956
4	30	1699765
5	40	2209587
6	50	2749645
Slope		**55224**
Intercept		**4012.5**
Regression		**0.9996**

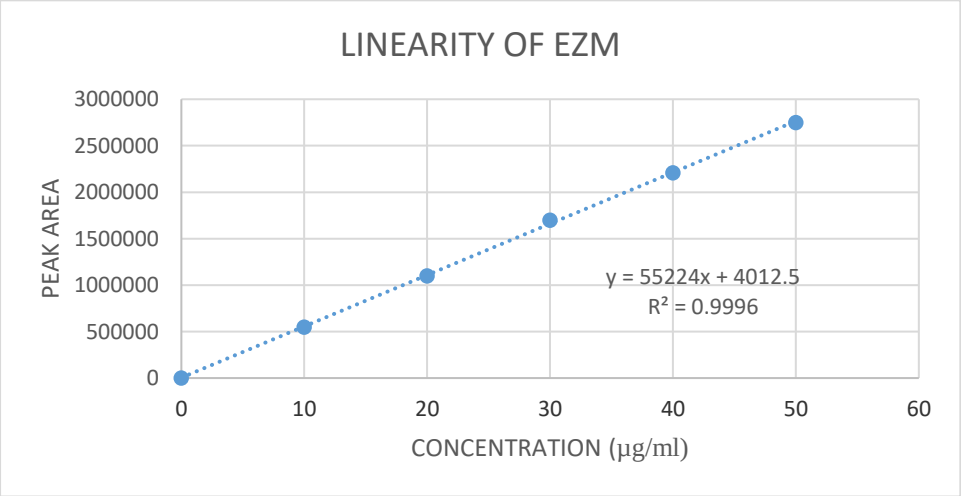

Fig.No. 03 : Linearity of Ezetimibe

Accuracy and Precision:

Accuracy as recovery was examined by spiking previously analyzed test solution with extra Standard drug at three different concentration levels. With a relative standard deviation (RSD) of less than 2%, we observed that the suggested technique is accurate for the simultaneous estimate of both BPA and EZM, with a recovery of 99.97 % for BPA and 100.24% for EZM, respectively. The high reproducibility and low RSD values show that the Method is reliable. (table-4& 5).

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Table 4: Precision data of BPA and EZM

Injection Number	BPA				EZM			
	Retention Time	Peak Area	Plate Count	Peak Symmetry	Retention Time	Peak Area	Plate Count	Peak Symmetry
1	2.252	2817649	8767	1.42	2.987	1098956	8967	1.22
2	2.25	2834862	9865	1.43	2.876	1097634	9654	1.21
3	2.255	2827654	9645	1.44	2.769	1096598	9873	1.2
4	2.253	2829743	8975	1.45	2.654	1078453	9427	1.23
5	2.252	2814378	9642	1.43	2.964	1087598	8769	1.22
6	2.254	2806539	9743	1.44	2.958	1085498	8845	1.34
Average	2.3	2821804			2.9	1090790		
Standard Deviation	0.0018	10707			0.1320	8218		
% RSD	0.08	0.38			4.60	0.75		

Table 5: Accuracy data of BPA and EZM

Sample Preparation No.	BPA Assay (%)	EZM Assay (%)
1	99.45	101.45
2	99.98	100.54
3	98.98	98.23
4	100.12	99.87
5	100.89	100.45
6	100.37	100.89
Mean	**99.97**	**100.24**
SD	**0.6752**	**1.1133**
RSD (%)	**0.67542**	**1.11070**

Robustness:

The results of the robustness analysis are shown in Table no.6. Both components exhibited comparable tailing factors, elution orders, resolutions, relative standard deviations, and recoveries. The analysis revealed that the relative standard deviation (RSD) of the peak sites was much below 2.0%.

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Table 6: Robustness data of BPA and EZM

Condition	Bempedoic Acid			Ezetimibe		
	% RSD	Tailing Factor	% Recovery	% RSD	Tailing Factor	% Recovery
1) Change in Flow rate						
Normal Condition (1.2 ml per minute)	0.12	1.42	99.23	0.23	1.21	99.21
Flow rate (1.0 ml per minute)	0.34	1.34	99.45	0.24	1.23	99.67
Flow rate (1.4 ml per minute)	0.54	1.22	100.23	0.34	1.22	98.89
2) Change in minor component in the mobile phase						
Normal Condition (Potassium dihydrogen orthophosphate, acetonitrile, and water in a ratio of 25:60:15)	0.45	1.45	100.34	0.56	1.22	99.89
(Potassium dihydrogen orthophosphate, acetonitrile, and water in a ratio of 30:55:15)	0.54	1.34	100.45	0.57	1.34	99.41
(Potassium dihydrogen orthophosphate, acetonitrile, and water in a	0.76	1.37	100.34	0.87	1.25	101.21

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ratio of 20:65:15)						
3) Change in Wave Length						
Normal:Wave Length 280 nm	0.23	1.45	100.49	0.34	1.22	100.45
Wave Length 285 nm	0.43	1.32	98.75	0.67	1.34	100.49
Wave Length 275 nm	0.76	1.41	100.72	0.78	1.21	100.93
4) Change in pH						
Normal:pH 6.0	0.34	1.67	99.89	0.56	1.23	99.92
pH 5.5	0.45	1.83	98.97	0.72	1.20	98.59
pH 6.5	0.72	1.23	98.83	0.92	1.19	100.83

Ruggudness:

Bempedoic Acid and Ezetimibe had respective mean peak areas of 749156and 1098956 with an RSD of 0.35 and 0.28%, respectively.

SUMMARY

In order to estimate BPA and EZM in bulk and in pharmaceutical, a new and verified RP-HPLC approach has been developed. Given the findings of the literature analysis, which revealed a scarcity of methods for estimating BPA and EZM in large quantities, there is an urgent need for a direct, cost-efficient, and precise solution to address this issue.

The concentrations of BPA and EZM were determined by injecting a mixture of Potassium dihydrogen orthophosphate, acetonitrile, and water (in a ratio of 25:60:15) with a pH of 6.0 onto a Kromosil C18 column measuring 150 x 4.6 mm and having a particle size of 5m. The flow rate was set at 1.2 ml/min, and the injection volume was 20 µl. The BPA peak had a retention period of 2.252 minutes, whereas the EZM peak had a retention duration of 2.987 minutes.

After its enhancement, the technique was validated according to ICH standards for system compatibility, linearity, sensitivity parameters, precision, accuracy, and resilience. All validation parameters returned values within acceptable limits. The relative standard

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deviation (RSD) values for the tests were less than 2. The recoveries ranged from 98% to 102%.

CONCLUSION

The proposed RP-HPLC technology offers a time-efficient and effortless approach that remains simple, rapid, precise, accurate, specific, resilient, and cost-effective. Therefore, it is a favoured method for the simultaneous determination of Bempedoic Acid and Ezetimibe. The implemented method was thoroughly verified in accordance with ICH guidelines in every aspect.

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