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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PRAVASTATIN AND BEMPEDOIC ACID IN BULK AND PHARMACEUTICAL FORMULATION

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ABSTRACT
A simple, rapid, accurate and precise reverse phase high performance liquid chromatographic technique has been progressed for the determination of Pravastatin and Bempedoic acid in absolute form and tablet. Separation of drug peaks were achieved on a Gemini C18 1µ10 Å, LC Ea 150mm x 4.6mm; 5µm column using a mixture of 0.025mM KH_2PO4 P^H 2.5 and Acetonitrile (60:40) as the mobile phase at rate of flow 1.0ml/min, the recognition was accomplished at 272nm. The retention time of the Pravastatin and Bempedoic caid was 2.398 and 4.801min correspondingly. The approach generates linear reaction in the concentration series of 6-30µg/ml of Pravastatin and 36-270µg/ml of Bempedoic acid. The precision for the assay was below 2.0%RSD. The %recovery for the accuracy was in between 99-101%. The method will be useful in the quality check assessment of Pravastatin and Bempedoic acid in bulk and pharmaceutical formulations.

KEYWORDS: Pravastatin, Bempedoic acid, RP-HPLC, Validation, Pravastatin and bempedoic acid tablets.

INTRODUCTION

Bempedoic acid goes to the category of long-chain molecule fatty acid. It is an alpha omega dicarboxylic acid. It is sold under the brand names of Nexletol and Nexlizet. Chemically, 8-hydroxy,2,2,14,14-tetramethylpentadecanedoic acid, having atomic formula of $C_{19}H_{36}O_5$, its Chemical framework is shown "Fig. 1". It is white crystallin fine particles, soluble in DMSO and methanol. Bempedoic acid is deemed as remedy for the management of excessive cholesterol levels[1,2] along with_other lipid lowering agents greatly with statins. It belongs to the first line class of ATP Citrate synthase inhibitor recommended one per day for lowering LDL cholesterol levels in Statins refractory.[3,4] It is indicated as an assistant curative for elders with Fredrickson class2a hyperlipidemia or atherosclerotic cardiovascular aliment that necessitate extra lowering of LDL.[5,6] The amalgamation of Bempedoic with statins is also instructed with nourishment organization and tremendously endorsed statin therapy to treat high LDL-C levels.[7]

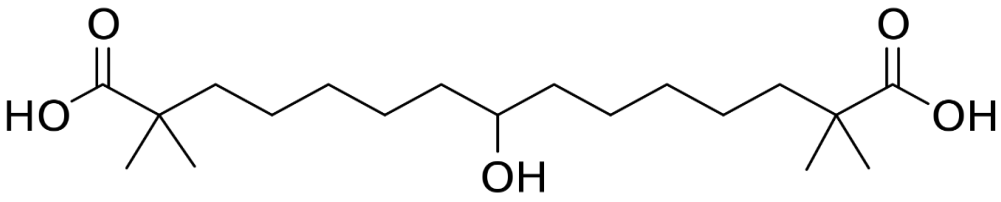

Fig.1 Structure of Bempedoic acid.

Bempedoic acid is a congener. It is make active to the thioester coenzyme A, publicized in "Fig. 2" with a very lengthy chain acyl-CoA synthase in the liver to inhibit ATP citrate lyase, concerned in the synthesis of cholesterol demanding of HMG-CoA reductase, which is blocked by statins.[8,9]

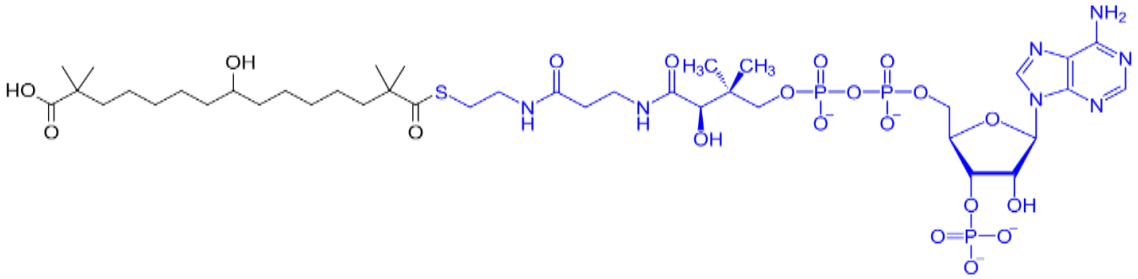

Fig.2: Bempedoyl- CoA; active metabolite.

Pravastatin is a carboxylic ester, advertised in the trade name Pravachol. Chemically, it is 3R,5R)-3,5-Dihydroxy-7-[(1S,2S,6S,8S,8aR)-6-hydroxy-2-methyl-8-{[(2S)-2-methylbutanoyl] oxy}-1,2,6,7,8,8a-hexahydro-1-naphthalenyl] enanthic acid**,** having a distinct formulary of $C_{23}H_{36}O_7$, its Chemical structure is shown "Fig. 3"**.** It is half white crystallin pulverized, soluble in DMSO, ethanol and dimethyl formamide. It belongs to a group of HMG-CoA reductase inhibitors, predominantly utilized for hypercholesterolemia, hyperlipidaemia and mixed dyslipidaemia and the avoidance of cardiovascular disorder at extreme hazard and doctoring unusual lipids. It is proposed designate consumed by diet changes, exercise, and weight loss. pravastatin use in the America was authorized by the FDA on 24 April 2006.[10,11]

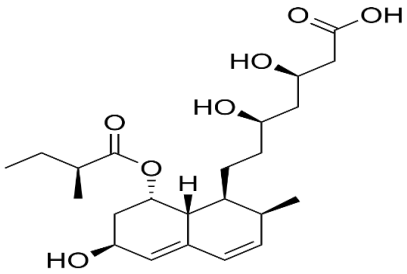

Fig.3: Structure of Pravastatin.

Pravastatin is a revokable competing inhibitor interfere with hydroxy methyl glutaryl-CoA reductase as it plays a key role in the rate-controlling step during the biosynthesis of cholesterol. Pravastatin reduces the production of extremely low-density lipoproteins, predecessor to bad cholesterol. These declines raise the total cellular LDL receptors; thus, its acceptance accelerates and coming out of circulation.[12]

Pravastatin and Bempedoic acid and their metabolites can be detected in human plasma and urine by LCMS-MS.[13-15] Strength representing studies of pravastatin by HPLC and Stress degradation studies of Bempedoic acid by UPLC.[16,17] So, liquid chromatographic methods with UV detection have been realized as the most appropriate for the determination of Pravastatin and Bempedoic acid. Various techniques were advertised for the quantification of either Pravastatin[18-20] or Bempedoic acid alone[21] or in association with other medicaments in bulk and therapeutic formulations or synthetic mixture.[22-30] The previous literature review revealed no articles concerned with the concurrent evaluation of Pravastatin and Bempedoic either in bulk or unit doses. So, Reverse phase-High performance liquid chromatography Approach aimed at the estimation of these two drugs in tablets has been established and validated.

MATERIALS AND METHODS

Materials: Pravastatin and Bempedoic acid active compounds were acquired as a contribution sample from Hetero drugs Pvt. Ltd, Hyderabad, India. Mono potassium phosphate and Hydrochloric acid of A.R level, Acetonitrile and purified aquatic of HPLC rank were bought from Merk Ltd. Bhiwandi, Bombay, Bharat. The 0.45µ nylon filters were bought from Millipore.

Methods: The HPLC apparatus Waters sampler separation module HPLC; 2695/2487 equipped with a high-pressure quaternary pump, photo diode assemblage discoverer and 10µL size injector loops. The arrangement was interlaced with Empower 2 software for examining and administering files. UV-VIS spectrophotometer- Schimazdu 1800; with special bandwidth of 2mm and 10mm and corresponding quartz cells interwoven with UV win 6 Software for determining absorbance. The analytical column used was Gemini 5 µm C18 1µ10 Å, 150 x 4.6 mm, Ea; Phenomenex, Part # 00F-4435-E0. Other equipments like analytical balance- Metlertolido; MA104/A was used for weighing the materials. P^H meter-Polmon; LP139 SA and ultra sonicator-Polmon; LP139 SA.

Selection of Solvent: Pravastatin and Bempedoic caid exhibited high solubility in Ethanol, Water and ACN. To assess the stability of working solutions in their relevant solvents, 2 diverse solutions having 20µg/ml of Pravastatin and 180µg/ml Bempedoic caid were analyzed using a UV spectrophotometer. The drugs were stable at room temperature for 24 hr and at freezing condition for 48 hr. Based on the solubility study and stability study, Water and ACN were selected as a solvent for additional aid of process progress followed by optimization of method parameters by RP-HPLC method. The combination of 60:40 of Water and ACN was selected as a solvent for future preparation of working standard solutions during RP-HPLC method development and validation.

Selection of Wavelength: The standard solution of Pravastatin (20µg/ml), and Bempedoic caid (180µg/ml) were scanned separately in the range of 200 - 400nm.

Selection and Optimization of Mobile phase for simultaneous determination of Pravastatin and Bempedoic acid

Any analytical method started applying universally employed LC, plus similarly obtainable C-18 columns. The organic form was preferred based on the responsiveness of the manner, the period mandatory for the assessment, accessible liquids and comfort of formation. The streamline of the mobile phase was taken based on different specifications like period of retention, abundance of theoretical plates and resoluteness.

The mobile phase comprised of a combination of 0.025mM KH2PO4 in water PH 2.5 and Acetonitrile in proportion of 60:40. Samples were analyzed using the following parameters. Flow rate; 1ml/min, injection volume; 50 µl, run time; 10 min, column oven temperature; 25^{o}c and detection wavelength; 272.0 nm.

Preparation of buffer: Precisely weighed 100gm of Potassium dihydrogen Ortho phosphate was shifted to 1000ml of measuring flask, around 800ml of milli-Q water was poured, sonicated for degassing the pH was attuned to 2.5 with 0.1M hydrochloric acid and finally make up to 1000ml.

Preparation of mobile phase: 0.025mM KH_2PO4 buffer P^H 2.5 and Acetonitrile were mixed in proportion of 60:40. The gas was removed from mixture in an ultrasonic thermal tub for 5 min. and cleaned via 0.45µm screen under vacuum.

Preparation of Primary stock solutions (200µg/ml Pravastatin & 1800µg/ml Bempedoic acid): 20mg and 180mg of accurately weighed Pravastatin & Bempedoic acid were dissolved individually in laboratory flask by means of 3/4th of diluent, vortexed for 10 minutes and volume was made up to 100ml with diluent.

Preparation of Standard stock solution (20µg/ml and 180µg/ml):10ml of every stock solution was sucked into a 100ml graduated flask and made up with diluent.

Preparation of Pravastatin and Bempedoic acid Sample stock solution (205µg/ml and 1802 µg/ml):20 tablets were balanced, mean quantity of pill was considered, mass corresponding to 20.5mg of pravastatin and 180.2mg of Bempedoic acid were shifted into a 100 ml laboratory flask, diluent was poured and sonicated for 25 min, finally the capacity was completed and filtered by HPLC filters.

Preparation of Sample working Solution (100% solution): 10ml of streamed sample stock solution was passed on to 100ml volumetric flask and made up using diluent. (20.5µg/ml of Pravastatin and 180.2µg/ml of Bempedoic acid).

Method Validation

System suitability: Evenness of column by organic level, the blank preparation (twice) and six multiple injections of 20ppm of Pravastatin and180ppm of Bempedoic acid standard solutions were injected through an auto injector into the optimized chromatography process and the chromatograms were documented. The relevant parameters were measured.

Specificity: Analytical technique specificity was decided by inoculating a mobile phase, Pravastatin and bempedoic acid standard and sample preparations into the chromatography system in similar investigational circumstances. The chromatograms were judged.

Linearity

Various dilute solutions of 2 drugs were prepared from respective stock solutions over the concentration level of 30 to 150%. seven concentration points were injected in replicate throughout the range. The linearity of Pravastatin and Bempedoic acid were assessed by measuring the peak area response.

Accuracy

By utilizing the standard addition method, It was calculated through recovery tests and was accomplished via spiking known amount of Pravastatin and Bempedoic acid at Quantitation limit, 50%, 100%, and 150% of 20ppm and 180ppm in Sample Preparation. Each level was analyzed 3 times and the % of standard recovered was estimated. for each of the 2 drugs as part of the recovery study.

Precision

Repeatability (Intra-day precision) plus system precision (inter-day precision) were assessed by spiking 6 repeated insertions of solutions of 20.5ppm and 180.2ppm as per similar experimental conditions. The peak area of Pravastatin and Bempedoic acid was measured, concentrations and % assay were determined. The mean peak response, percentage assay and % RSD were calculated.

Robustness

It was proven by finding retention time of Pravastatin and Bempedoic acid under minor but purposely modified chromatographic situations like movement rate, organic solvent constitution, wavelength and column heat on minimal and eminent side of the original evaluates.

Limit of Detection and Quantitation: The LOD and LOQ limits of Pravastatin and Bempedoic acid were determined by the signal-to-noise ratio (S/N) method. These proportions are 3:1 and 10:1. Solution of Pravastatin and Bempedoic acid were made around its quantitation limit (QL) concentration and injected in six

replicates. The LOD was determined by following equation.

LOD = 3.3 × (SD/Slope)

Where, SD = Standard deviation of the Y- intercepts

Slope = Mean slope.

In the same way, LOQ was estimated using the below equation.

LOQ = 10 × (SD/Slope)

Where, SD = Standard deviation of the Y- intercept

Slope = Mean slope.

RESULTS

Solubility Study: Solubility tests for pravastatin and Bempedoic acid were performed in various solvents. Careful observations of solubility were made for the most used and most suitable solvents. Pravastatin was freely soluble in HPLC grade water. Bempedoic caid was sparingly soluble in HPLC grade water. Additionally, both drugs were freely soluble in acetonitrile. Based on over observation, mixture of acetonitrile and water was chosen as a conventional solvent for extend method development and Authentication.

Optimized wavelength selection: 272nm was selected as a common wavelength for further process development and optimization of the method parameters using HPLC equipment.

Optimized chromatographic condition for RP-HPLC method for simultaneous determination of Pravastatin and Bempedoic acid

Various trials were conducted to attain the simultaneous determination of 2 drugs by RP-HPLC, and the best beneficial outcomes were obtained under these optimized conditions. A mobile phase of 0.025mM KH_2PO4 and CH_3CN in the percentage of 60:40 % v/v was initiate to be the greatest appropriate mobile phase for best resolution of Pravastatin and Bempedoic acid. Mobile phase was pumped all about the Gemini 5 µm C18 1µ10 Å, LC Ea column owning a length of 150mm x 4.6mm; and 5µm with a run speed of 1 ml/min. The column was at a 25^0c and was equilibrized by pushing the mobile phase for minimum 30 minutes preceding the interpolate of test solution. 10µl of authenticate and trial fluids were loaded in six replicates separately in injection port of the instrument and the response for the drug was measured. The recognition of peak was observed at 272nm. The duration was programmed at 10 min. Under these improved chromatographical provisions, the RT for the Pravastatin and Bempedoic acid were 2.398min and 4.801min. The regular spectrum of standard solutions is given in figures 4.

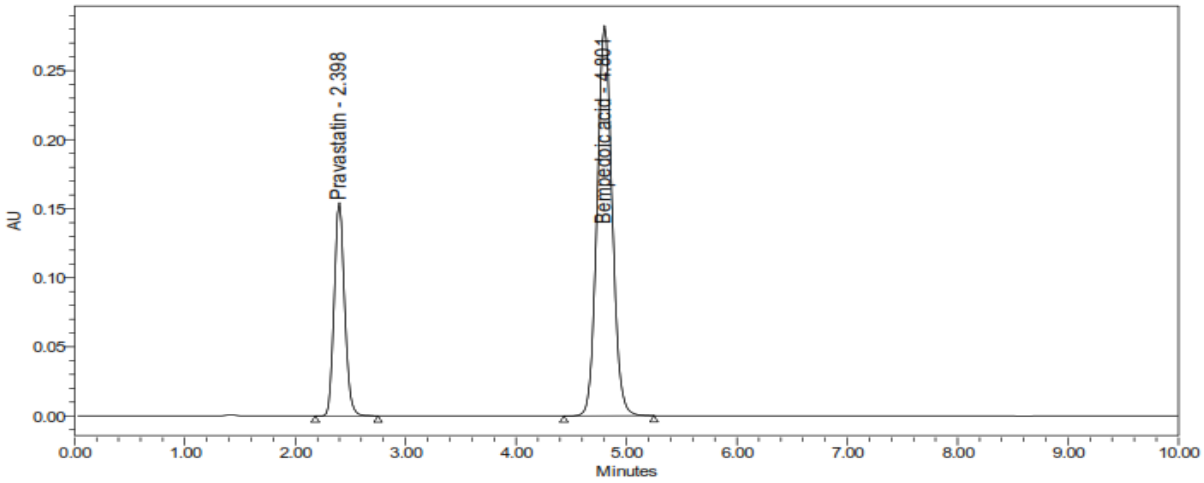

Fig. 4: Optimized Chromatogram of Standard.

Assay: six replicate injections of research solutions of Pravastatin and Bempedoic acid were injected through an auto injector into the optimized chromatography process and the chromatograms were documented. From the peak response, the % assay of Pravastatin and Bempedoic acid in drug dosage was learnt designate 102.86% and 100.69%. Assay results are summarised in Table 1.

Table 1: Peak results for Assay sample of Pravastatin and Bempedoic acid.

Injection No	Pravastatin		Bempedoic caid	
	RT (min)	Peak Area	RT (min)	Peak area
1	2.410	1014931	4.847	2682438
2	2.414	1030320	4.857	2722361
3	2.416	1016411	4.861	2686194
4	2.416	1016802	4.862	2688727
5	2.414	1015447	4.859	2683571
6	2.415	1017590	4.862	2689562

Validation: Authentication of analytical technique confirms the quality of the method if it fulfils the conditions of the process. The projected method was validated aggreging to ICH standards for following recommendations.

System suitability: the consequences are presented in Table 2 and Peak summary is revealed in Figure 5. The results have been demonstrated with respect to %RSD, USP plate count and USP peak tailing. The % relative standard deviation for the six repeated insertions was < 2%.

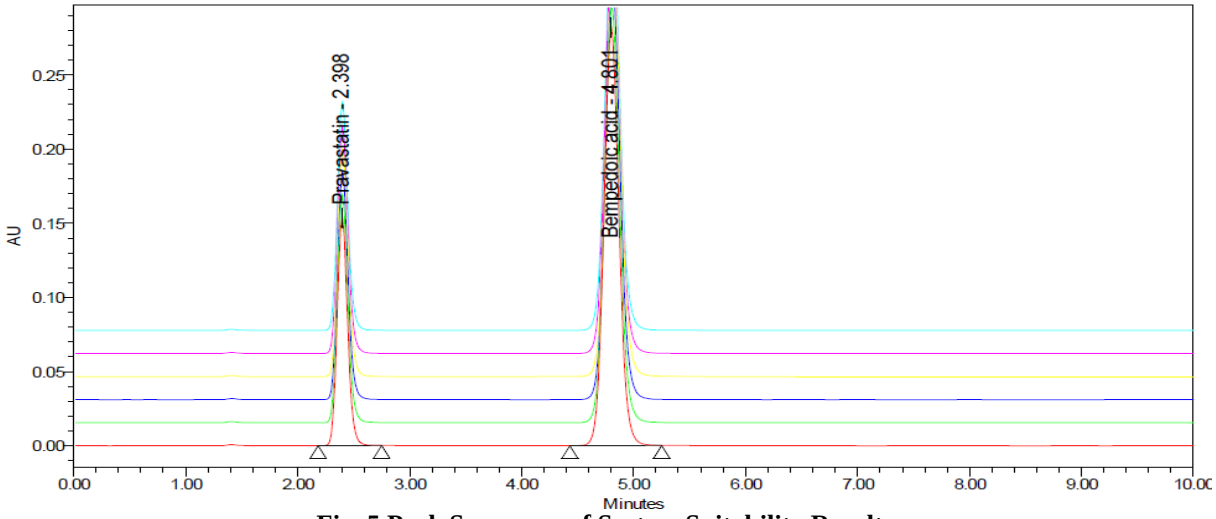

Fig. 5 Peak Summary of System Suitability Results.

Table 2: System Suitability Results of Pravastatin and Bempedoic acid.

Injection No	Pravastatin					Bempedoic acid					
	RT (min)	Peak Area	% Area	Peak height	USP tailing	RT (min)	Peak response	% Area	Peak height	USP resolution	USP tailing
1	2.398	1013074	27.45	153775	1.12	4.801	2677432	72.56	281637	11.12	1.12
2	2.400	1012908	27.44	153509	1.13	4.805	2677902	72.55	281222	12.02	1.13
3	2.401	1013447	27.44	153737	1.12	4.812	2679287	72.56	281510	11.89	1.12
4	2.402	1012289	27.45	153444	1.13	4.816	2675070	72.55	280887	12.51	1.13
5	2.401	1010773	27.45	153188	1.13	4.811	2671690	72.55	282294	12.76	1.13
6	2.400	1013254	27.45	153859	1.12	4.810	2678595	72.55	282205	12.51	1.12
Mean	2.400					4.809					
Std. Dev.	0.001					0.005					
%RSD	0.001					0.11					

Specificity: it was observed from the chromatogram of blank, no interference at the retention times of Pravastatin and Bempedoic acid. These are 2.398 and 4.801min. A chromatogram of blank is shown from Figure 6.

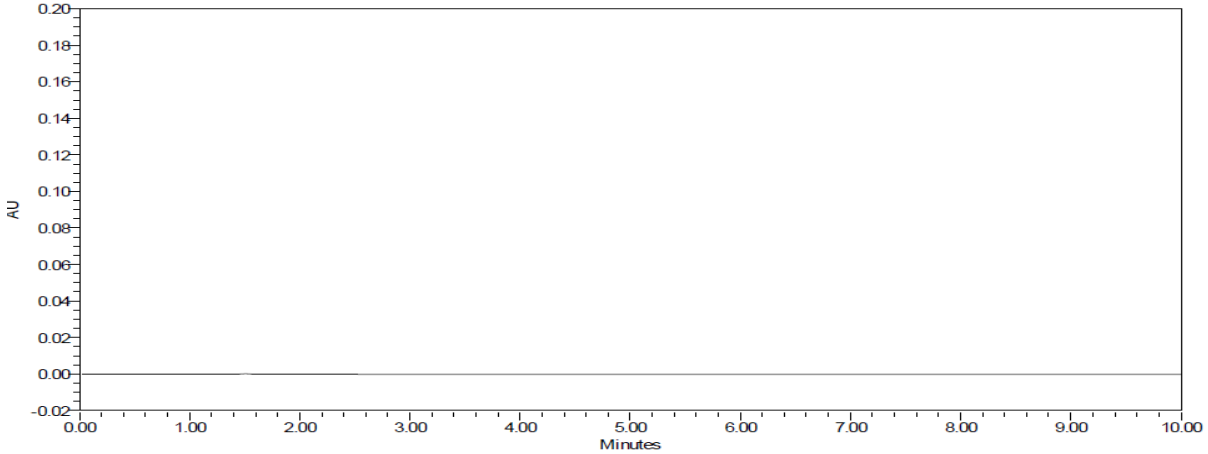

Fig. 6: A typical Chromatogram of blank.

Linearity: A direct correlation attained amongst the peak response & concentration of Pravastatin and Bempedoic acid for LC method as proven in Fig.7 and 8 and was confirmed by the value of regression coefficient(r^2). The values are exhibited in Table 3.

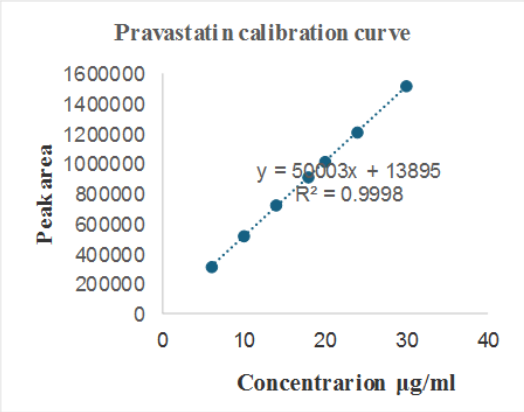

Fig. 7: Calibration curve for Pravastatin.

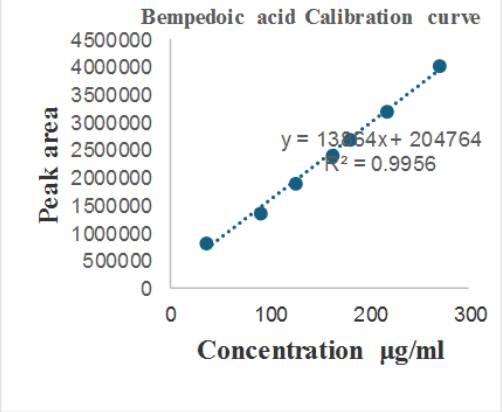

Fig. 8: Calibration curve for Bempedoic acid.

Table 3: Chromatography Data for Linearity study of Pravastatin and Bempedoic acid.

S.No	Preparation Level (%)	Pravastatin		Bempedoic acid	
		Concentration (µg/ml)	Mean peak area	Strength (µg/ml)	Average peak response
1	30	6	312794	36	823814
2	50	10	514903	90	1363742
3	70	14	721298	126	1911097
4	90	18	908211	162	2407410
5	100	20	1015433	180	2688385
6	120	24	1204500	216	3190290
7	150	30	1520450	270	4021841
	Slope	5000.3		1386.4	
	y-intercept	13895		204764	
	R^2	0.9998		0.9956	

Accuracy: The accuracy investigation was performed in the form of % recovery of spiked samples of separate drugs in a mixture. The recovery findings are reviewed in Table 4.

Table 4: Accuracy results for Pravastatin and Bempedoic acid.

Accuracy level	Pravastatin				Bempedoic acid			
	Amount Spiked (ppm)	Amount recovered (ppm)	% Recovery	Mean	Amount Spiked (ppm)	Amount recovered (ppm)	% Recovery	Mean
50%	10	9.974	99.740		90	90.612	100.68	
100%	20	19.985	99.925	100.512	180	180.502	100.278	100.302
150%	30	30.562	101.873		270	269.867	99.950	

Precision: The percentage RSD was found to be < 2. The outcomes are reviewed in the Table5.

Table 5: Results of Precision for Pravastatin and Bempedoic acid.

Injection No	Repeatability						Intermediate precision	
	Pravastatin			Bempedoic acid			Pravastatin	Bempedoic acid
	Peak area	Concentration (ppm)	% Assay	Peak expanse	Concentration (ppm)	% Assay	Peak region	Peak area
1	1014931	20.50	102.49	2682438	180.59	100.33	1013254	2679287
2	1030320	20.81	104.05	2722361	183.28	101. 82	1010773	2671690
3	1016411	20.53	102.64	2786184	180.84	100.47	1012289	2677902
4	1016802	20.54	102.68	2788727	181.01	100.56	1012908	2675070

5	1015477	20.51	102.54	2723571	180.67	100.36	1013074	2677432
6	1017590	20.55	102.76	2729562	181.07	100.59	1013447	2678595
Mean	1018583.5		102.86	2692142.1		100.68	1012624	2676662.7
Std. Dev.	2606.180		0.26434	6736.512		0.25227	989.53	2828.88
% RSD	0.25586		0.25699	0.255022		0.25057	0.10	0.11

Robustness: The tailing factor is less than 2.0. Therefore, the procedure was strong, and findings are briefed in Table 7.

Table 7: Results of Robustness for Pravastatin and Bempedoic caid.

Parameter	Pravastatin			Bempedoic acid			
	Retention Time (min)	Peak area	USP tailing	Retention Time (min)	Peak area	USP resolution	USP tailing
Actual Flow - 1ml/min	2.410	1014931	1.05	4.847	2682438	9.24	1.03
Less Flow - 0.9ml/min	2.429	918046	1.05	4.906	2682438	9.29	1.03
More Flow -1.1ml/min	2.429	1214512	1.05	4.912	2684986	9.31	1.03
Less mobile phase 55:45	2.430	925847	1.05	4.909	2452146	9.29	1.03
More mobile phase 65:35	2.428	1211086	1.05	4.905	3205803	9.20	1.03
Less Wavelength-270 nm	2.430	918437	1.05	4.912	2434986	9.31	1.03
More Wavelength-274 nm	2.428	1218672	1.05	4.904	3221101	9.22	1.03
Less Column Temperature-23_0c	2.424	1218672	1.05	4.890	4046136	9.45	1.03
More Column Temperature-27^0c	2.425	1532438	1.05	4.894	4046186	9.09	1.04

Limit of Detection and Quantitation: The limits for the Pravastatin are originated to be 0.402 & 1.219ppm and for the Bempedoic acid are 17.235 and 52. 228ppm. The chromatograms are displayed in Fig.9 and 10.

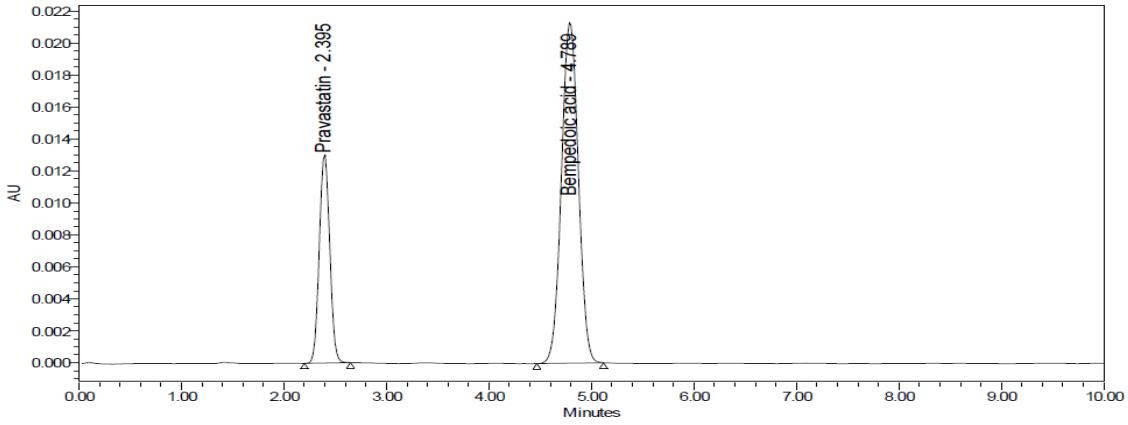

Fig. 10: LOD Chromatogram of Pravastatin and Bempedoic acid.

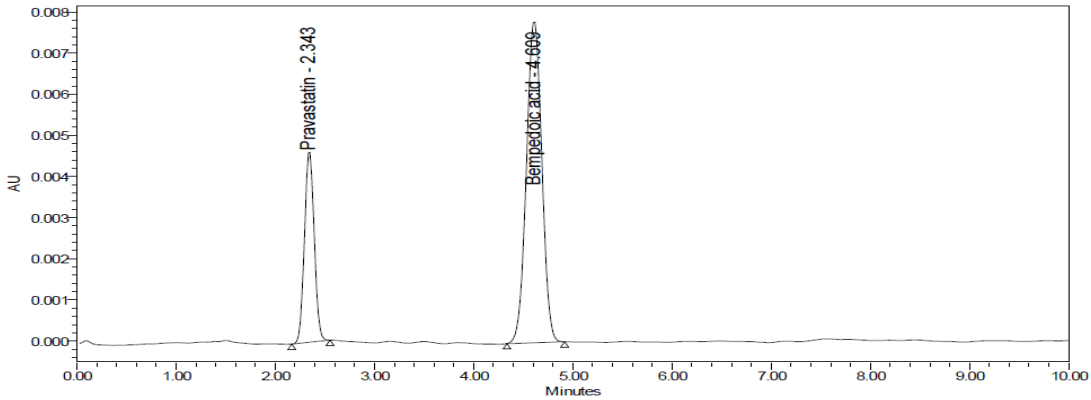

Fig. 11: LOQ Chromatogram of Pravastatin and Bempedoic acid.

DISCUSSION

When implementing the RP-HPLC method for a two drug mixture, the choice of the mobile phase and solvent performs a fundamental role in the complete method development. In this case, all two drugs exhibit different properties in terms of solubilities, pH, and pKa. Consequently, the simultaneous development of a technique for this dual mixture is attainable only through the utilization of a buffer with optimized pH and a isocratic program to achieve proper resolution of 2 drugs concurrently. Solubility testing of Pravastatin and Bempedoic caid was performed in different solvents. It was observed that a appropriate conventional solvent for the simultaneous determination of the selected binary mixture is a blend of HPLC-grade water and acetonitrile in an optimized ratio of 60:40, respectively. Besides, it was observed that the working standard of the binary mixture remains entirely stable in the selected solvent during the experimental measurements at ambient temperature. The mobile phase was selected to get proper peak shape with sufficient height, theoretical plates, resolution and purity. The assortment of mobile phases also includes selection of buffer, pH of buffer or water, selection of solvent and buffer to solvent ratio. Various combinations of diluent were studied to improve mobile phase. A variability of buffers like phosphate buffers and citrate buffers with different pH were tried as per the basic properties of two selected APIs. Experiments involving a various range of pH values and varying ratios of the aqueous to organic phases were conducted to get simultaneous resolution of the chosen binary drug mixture, all while meticulously considering the fundamental characteristics of the two drugs and evaluating chromatograms from several trial runs. Finally, the simultaneous determination of two drugs was accomplished through isocratic programming and the consumption of a Phosphate buffer with a pH of 2.5. In this method, pravastatin and Bempedoic caid were eluted by maintaining a same ratio of the aqueous phase as the buffer, and acetonitrile to facilitate the elution. The optimized method generated the best results in conditions of all the validation parameters of analytical technique along with system suitability of the developed method. All the results of various parameters were found to be within the acceptance criteria and standard limits.

CONCLUSION

Founded on the outcomes, it is decided that both drugs have good resolution with limits of system suitability and short analysis time 10 mi. The intended technique is easy, quick, reasonable, accurate, linear, precise and robust. Hence, it was productively utilized for regular concurrent analysis of Pravastatin and Bempedoic acid in bulk and pills.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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