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A VISIBLE SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION OF BEMPEDOIC ACID IN BULK AND TABLET DOSAGE FORMS

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ABSTRACT

A Visible Spectrophotometric method has been developed and validated for the quantitative measurement of Bempedoic acid in bulk and tablet dosage forms. The process is based on the production of a colored complex in the presence of Ferric chloride between Bempedoic acid and MBTH (3-methyl-2-benzothiazolinone Hydrazone). In this reaction,the carboxylic group reacts with MBTH to form a hydrazone derivative. This derivative is then oxidized by Ferric chloride to form a green to bluish color complex, which can be measured spectrophotometrically.

At 663 nm, the coloured complex was spectrophotometrically measured. The correlation coefficient (r2) of 0.999 revealed that the approach is linear in the concentration range of 10-60 µg/mL. It was discovered that the limits of detection (LOD) and quantitation (LOQ) were 2.169µg/mL and 6.572 µg/mL, respectively. For both intra-day and inter-day precision studies, the approach was proven to be precise with a percent relative standard deviation (%RSD) less than 2.0%. Recovery experiments were used to assess the method's accuracy, and it was discovered that the average percentage of recovery fall between 98.0 and 102.0%. The results of the suggested approach were compared to

those of the reference method for the accurate determination of Bempedoic acid in tablet dosage forms. The proposed technique outcomes were found to be in strong agreement with those of the reference approach. The technique was discovered to be simple, precise, accurate, and sensitive; it may be utilised for regular analysis of Bempedoic acid in dosage forms such as tablets and bulk.

Keywords: Bempedoic acid, Visible spectrophotometry, MBTH (3-methyl-2-benzothiazolinone Hydrazone), Validation, Tablet dosage forms

INTRODUCTION :

BEMPEDOIC ACID, 8-hydroxy-2, 2, 14, 14-tetramethyl penta decanedioic Acid, is a medication used to treat refractory hypercholesterolemia together with dietary changes and/or other treatment. It belongs to ACL inhibitors (adenosine triphosphate citrate lyase).

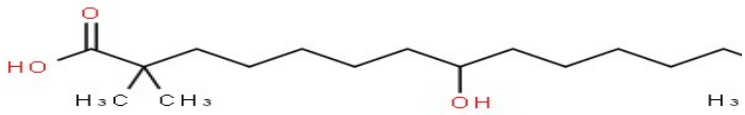


Figure 1: Structure of Bempedoic Acid

Bempedoic acid works by preventing the synthesis of cholesterol by inhibiting adenosine triphosphate-citrate lyase (ACL), which increases the expression of LDL receptors and raises plasma levels of low-density lipoproteins (LDL-C). It is an intracellularly activated prodrug for oral delivery **[1]**.

Literature Review reveals that there are very few HPLC and UPLC methods were done for the estimation of Bempedoic acid in bulk and formulation along with other drugs like Ezetimibe, and Atorvastatin **[2-7]**. The present study is more beneficial than the other methods as it is using less sophisticated equipment and economical solvents.

3-methyl-2-benzthiazolinone hydrazone (MBTH) has been used as an oxidative coupling reagent in the Development of Visible Spectrophotometric methods for the determination of many pharmaceuticals **[8, 9]**.

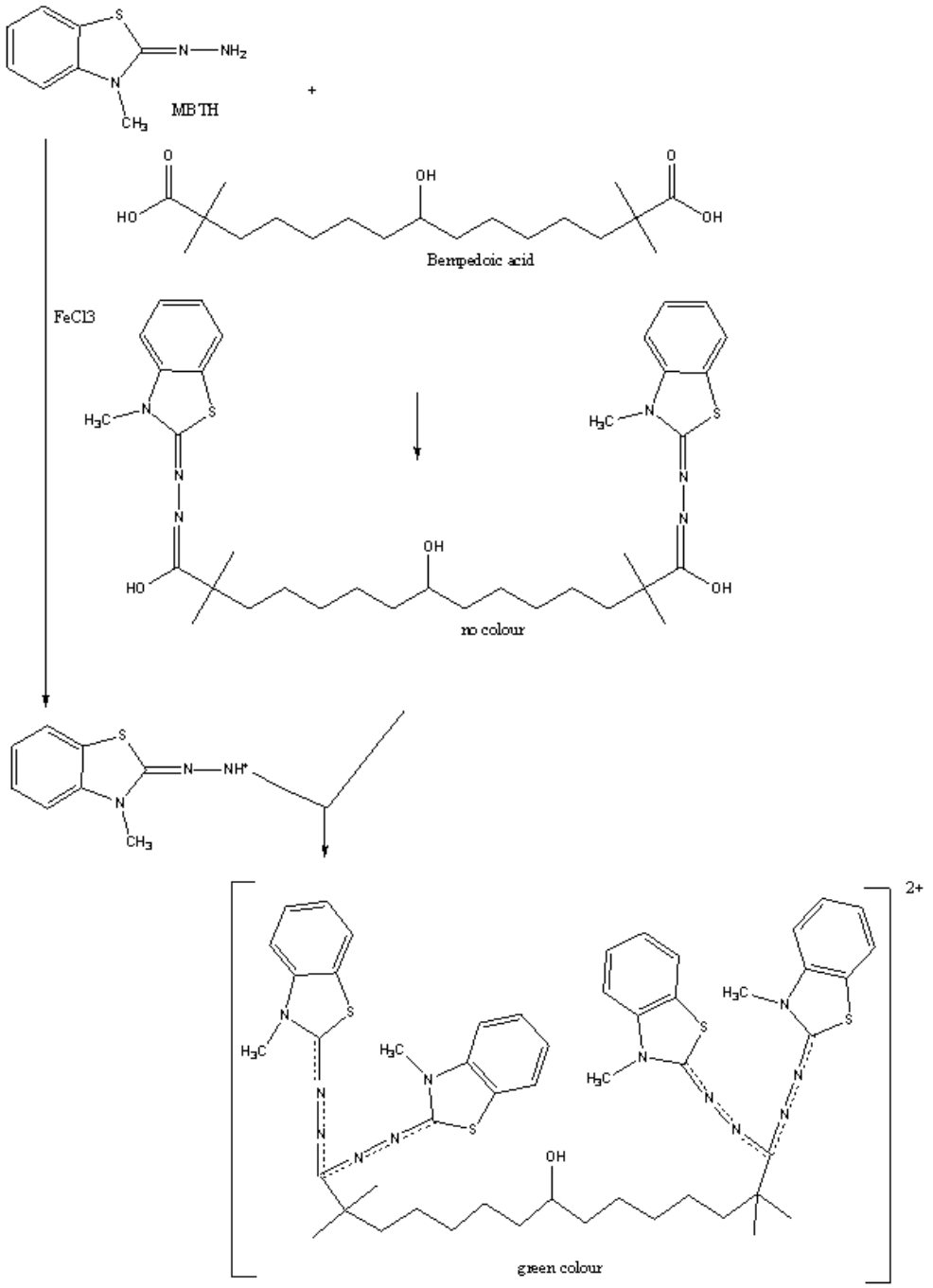
According to the literature survey, no validation and method development indicating the Visible Spectrophotometric method was reported on Bempedoic acid. In the present study a new simple, precise, rapid, and economical method was developed and validated for Bempedoic acid in bulk and tablet dosage forms using MBTH (3-methyl-2-Benzothiazolinone Hydrazone) as a reagent in UV/Visible Spectrophotometer and the solvent

used in this method is ethanol which is environmentally preferable green solvent.

Chemistry of the colored species:

The process is based on the production of a colored complex in the presence of Ferric chloride between Bempedoic acid and MBTH (3-methyl-2-benzothiazolinone Hydrazone). In this reaction,the carboxylic group reacts with MBTH to form a hydrazone derivative. This derivative is then oxidized by Ferric chloride to form a green to bluish color complex, which can be measured spectrophotometrically.

Reaction mechanism:



MATERIALS AND METHODS:

Reagents

Bempedoic Acid pure gift samples from ‘‘RESEARCH LAB FINE CHEM INDUSTRIES’’ and Bempedoic acid tablets were obtained from the online stores.

MBTH (3-Methyl-2-Benzothiazolinone Hydrazone): MOLYCHEM

Ferric chloride: RANKEM

Ethanol: Changshu Hongsheng Fine Chemical Co. Ltd

Distilled Water.

All chemicals substances were of analytical grade.

Instruments

The LABINDIA T60 UV-VIS Spectrophotometer, connected to a computer loaded with ‘‘microwave professionals 2.0’’ was employed for all spectrophotometric measurements.

Preparation of Reagents

Preparation of 0.5%w/v MBTH: Weighed accurately 0.05g of MBTH and transferred it into 10ml volumetric flask and dissolved it in a few ml of distilled water and made up to the mark with distilled water.

Preparation of 1%w/v Ferric chloride solution: Weighed accurately 0.5g of Ferric chloride and transferred into 50ml volumetric flask and dissolved it in few ml of distilled water and made up to the mark with distilled water**.**

Preparation of Standard Stock solution:

Dissolved 10mg of Bempedoic acid in 10ml volumetric flask with ethanol and was made upto the mark with ethanol. (1000µg/ml)

Selection of wavelength and determination of λ_{max}:

A concentration of 10µg/ml solution was prepared by diluting the working standard solution I (100µg/ml) into 10ml volumetric flask and added 2ml of 0.5% MBTH and 2ml of 1% Ferric chloride solution and was made up to the mark with ethanol and kept aside for 20mins for development of colour. The resulting-coloured complex was scanned in UV-Visible spectrophotometer from 400-800nm and λmax was found to be 663nm **(Figure 2)**.

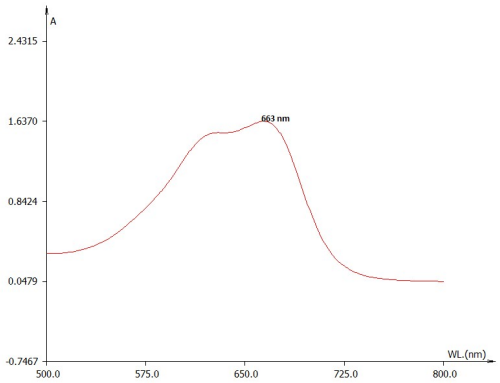


Figure 2: Absorption spectra of Bempedoic Acid

Parameter fixation of Bempedoic Acid

Table 1: Parameter fixation of Bempedoic Acid

Parameter	Range Fixation	Optimum condition
Volume of $FeCl_3.6H_2O$	1-2.5ml	2ml
Volume of MBTH	1-2.5ml	2ml
Order of addition	Bempedoic acid-MBTH-$FeCl_3$	Color intensity is maximum with this order of addition
Stability	0 to 48 hours (Bench Top & Refrigeration)	24 hours at bench top 48 hours at refrigeration

Method Developed:

Fresh aliquots of Bempedoic acid ranging from 0.1ml-0.6ml (1ml=100µg/ml) were transferred into a series of 10ml volumetric flasks to provide a final concentration ranging from 10-60µg/ml. To each flask 2ml $FeCl_3$ and 2ml of MBTH reagent were added and solution was made up to mark with the ethanol. Then the solution was kept aside for 20 minutes. The absorbance of the colored chromagen was measured at 663nm against the reagent blank in 1 Cm cuvette. The amount of the Bempedoic acid present in the sample was computed from its calibration curve.

Assay and Procedure for Pharmaceutical formulation:

Twenty tablets were taken, weighed, and pulverised. The powder equivalent to 100mg was taken, dissolved in a 10ml ethanol, sonicated for 20 minutes, and filtered through Whatmann filter paper No.41. From the filtrate 1ml was taken and transferred into 10 ml volumetric flask and made upto mark to get 1000µg/ml. From this solution, 1ml was transferred into a 10ml volumetric flask and made up to the mark to get 100µg/ml and was analysed at 663nm. Six replicates were analysed and the average values were presented in **Table 2**.

RESULT AND DISCUSSION:

Assay of Tablets:

Validation of the method [10, 11]:

The method was validated in accordance with the current ICH guidelines.

Specificity: The blank was scanned in the range 200-800nm and it was found that there was no interference of blank with sample λmax as shown in **(Figure 3 and 2)**.

Table 2: Application of the proposed method for the analysis of dosage form containing Bempedoic acid

Tablet	Drug	Labelled claim(mg)	Amount found(mg)	% Recovery
Bemdac	Bempedoic Acid	180	178.24	99.02%

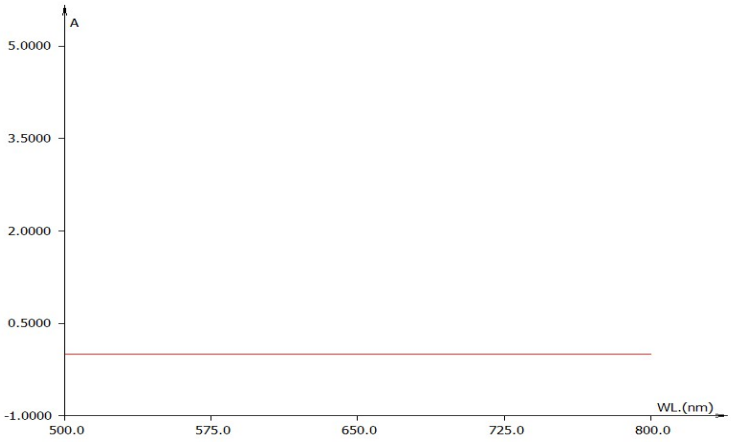


Figure 3: Blank peak

Linearty and Range:

The calibration graphs created by plotting absorbance values versus final concentrations (µg/ml) were identified as being linear over concentration ranges of 10 to 60 µg/ml, and the linear regression equation y =0.012x + 0.13 with correlation coefficient 0.999 was determined to be linear. The absorbance and final concentrations values represented in **Figure 4**.

The Range was found to be 60 µg/ml and 10 µg/ml.

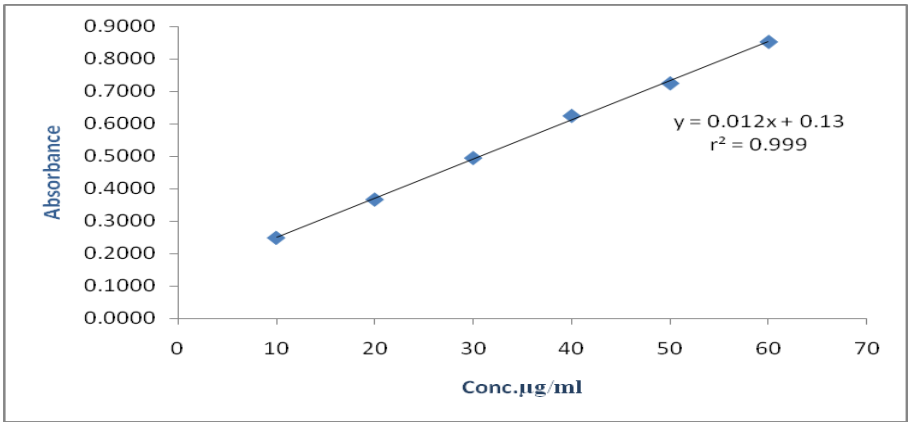


Figure 4: Calibration curve of Bempedoic Acid

Table 3: Optical characteristics of Bempedoic Acid

Parameters	Method
Lamda max	663nm
Beer's law limits(µg/ml)	10-60µg/ml
Molar absorbitivity(lit/mol^{-1}cm^{-1}	4.3 x 10^3
Sandell's sensitivity (µg^2/cm 0.001 abs units)	0.08333
Regression equation(Y)	Y=0.012x+0.13
Slope(b)	0.012
Intercept(a)	0.13
Correlation coefficient(r)	0.999
Assay % recovery	99.02%
Precision % RSD	Intra day- 0.10
	Inter day- 0.203
LOD	2.169µg/ml
LOQ	6.572µg/ml

Precision:

The method's repeatability was evaluated by scanning a 30µg/ml solution six times and recording the absorbance. The resultant result was then computed to get mean, standard deviation and %RSD. The Relative standard deviations were below 2%.

Table 4: Intraday Precision

Concentration(µg/ml)	Absorbance		
	Morning	Afternoon	Evening
30	0.5336	0.5400	0.5386
30	0.5337	0.5401	0.5385
30	0.5335	0.5399	0.5387
30	0.5301	0.5400	0.5386
30	0.5325	0.5401	0.5385
30	0.5328	0.5402	0.5388
Mean	0.5327	0.54005	0.538617
Standard Deviation	0.001361	0.000105	0.000117
%RSD	0.26	0.019	0.022
%RSD for 18 observations	0.1		

Table 5: Interday Precision

Concentration(µg/ml)	Absorbance	
	Day 1	Day2
30	0.5112	0.5119
30	0.5101	0.5108
30	0.5098	0.5089
30	0.5093	0.5093
30	0.5093	0.5085
30	0.5092	0.5092
Mean	0.509817	0.509767
Standard Deviation	0.000763	0.001305
%RSD	0.150	0.256
%RSD for 12 observations	0.203	

Accuracy: Three concentration levels (80%, 100%, and 120%) were spiked in order to conduct an accuracy investigation. Triplicate samples were scanned for each level, and the percentage of recovery was calculated. The % Recovery was between 98-102%.

Table 6: Accuracy data for Bempedoic Acid

Level of addition	Formulation amount(µg)	Amount added(µg)	Amount found (µg)	% recovery
80%	30	24	23.97	99.86%
	30	24	23.95	
	30	24	23.98	
100%	30	30	29.36	97.95%
	30	30	29.59	
	30	30	29.20	
120%	30	36	35.29	98.17%
	30	36	35.36	
	30	36	35.35	

DISCUSSION:

Optimal operating conditions employed in the approach were established by adopting variation of one variable at a time (OVAT) method. For the final dilution of the colored species, the effects of several parameters

including time, volume and strength of the reagents, the sequence in which they were added, and solvent, was investigated. As they form compounds with the medication Bempedoic acid, we have chosen the reagents MBTH (3-Methyl-2-Benzothiazolinone and Hydrazone) and Ferric chloride for this approach.

The best solvent for the last dilution was discovered to be ethanol. Alternative solvents like water and propylene glycol don't improve the color's intensity in any way. The created approach, which has the formula y=0.012x+0.13 and a linearity of 0.999, can be utilized for routine analysis.

The precision falls within the %RSD 0.10 & 0.203 range. Sandell's sensitivity and molar absorptivity were determined to be 0.08333 (lit/mol-'cm) and 4.3 x 103g/cm 0.001 abs units, respectively. The robust in range of %RSD 0.228, 1.624, 1.246 and 1.657. With this developed procedure, the maximum recovery is 99.02%.

CONCLUSION:

According to ICH criteria, the current analytical procedure has been validated. The visual technique created for Bempedoic acid estimation. The technique is based on the production of stable green coloured chromogen by Bempedoic acid with MBTH in the presence of $FeCl_3$, which has a 663 nm absorption maximum and follows Beer's law in the concentration range of 10 to 60 µg/ml.

The analytical approach was determined to be particular, accurate, linear, strong, and possessing stability suggesting features. The current analytical technique can be applied to the desired outcome.

The results of the proposed procedure and those of the reference approach were found to be in excellent agreement. It was found that the method was easy to use, precise, accurate, and sensitive; it could be used to regularly analysis of bempedoic acid in bulk and dosage forms.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation**.**

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