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ORIGINAL ARTICLE **OPEN ACCESS**

New Analytical Method Development and Validation for Estimation of Pregabalin and Mecobalamin in Bulk and Tablet by RP-HPLC

K. Chaitanya Prasad[1]*, Sana Khanam[1], Ramya Sri. S[2]
[1]Department of Pharmaceutical analysis, Samskruti College of Pharmacy, Affiliated to JNTUH University, Hyderabad 501301, Telangana, India
[2]Department Of Pharmacy, University College of Technology, Osmania University, Hyderabad – 500 007, Telangana, India
Corresponding Author's Email Id- chaitanyaprasadpharmacy@gmail.com

ABSTRACT
A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for simultaneous determination of Mecobalamin and Pregabalin in pure form and marketed combined pharmaceutical dosage forms. A column having Symmetry (C18) (150mm x 4.6mm, 5µm) in isocratic mode with mobile phase containing Methanol: Phosphate Buffer (pH-3.8) (28:72v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 218 nm. The retention time (min) and linearity range (ppm) for Mecobalamin and Pregabalin were (1.794, 3.440min) and (10-30, 10-50), respectively. The method has been validated for linearity, accuracy and precision, robustness and limit of detection and limit of quantitation. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.86µg/ml and 2.58µg/ml for Mecobalamin and 1.28µg/ml 3.84µg/ml for Pregabalin respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of Mecobalamin and Pregabalin in bulk form and marketed combined pharmaceutical dosage forms.
Keywords: *Mecobalamin and Pregabalin, RP-HPLC, Validation, Accuracy, Precision.*

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INTRODUCTION

Analysis may be defined as the science and art of determining the composition of materials in terms of the elements or compounds contained in them. In fact, analytical chemistry is the science of chemical identification and determination of the composition (atomic, molecular) of substances, materials and their chemical structure.

Chemical compounds and metallic ions are the basic building blocks of all biological structures and processes which are the basis of life. Some of these naturally occurring compounds and ions (endogenous species) are present only in very small amounts in specific regions of the body, while others such as peptides, proteins, carbohydrates, lipids and nucleic acids are found in all parts of the body. The main object of analytical chemistry is to develop scientifically substantiated methods that allow the qualitative and quantitative evaluation of materials with certain accuracy. Analytical chemistry derives its principles from various branches of science like chemistry, physics, microbiology, nuclear science and electronics. This method provides information about the relative amount of one or more of these components [1].

Every country has legislation on bulk drugs and their pharmaceutical formulations that sets standards and obligatory quality indices for them. These regulations are presented in separate articles relating to individual drugs and are published in the form of book called "Pharmacopoeia" (e.g. IP, USP, and BP). Quantitative chemical analysis is an important tool to assure that the raw material used and the intermediate products meet the required specifications. Every year number of drugs is introduced into the market. Also quality is important in every product or service, but it is vital in medicines as it involves life.

There is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. This happens because of the possible uncertainties in the continuous and wider usage of these drugs, report of new toxicities and development of patient resistance and introduction of better drugs by the competitors. Under these conditions standard and analytical procedures for these drugs may

not be available in Pharmacopoeias. In instrumental analysis, a physical property of the substance is measured to determine its chemical composition. Pharmaceutical analysis comprises those procedures necessary to determine the identity, strength, quality and purity of substances of therapeutic importance [2].

Pharmaceutical analysis deals not only with medicaments (drugs and their formulations) but also with their precursors i.e. with the raw material on which degree of purity and quality of medicament depends. The quality of the drug is determined after establishing its authenticity by testing its purity and the quality of pure substance in the drug and its formulations.

Quality control is a concept which strives to produce a perfect product by series of measures designed to prevent and eliminate errors at different stages of production. The decision to release or reject a product is based on one or more type of control action. With the growth of pharmaceutical industry during last several years, there has been rapid progress in the field of pharmaceutical analysis involving complex instrumentation. Providing simple analytical procedure for complex formulation is a matter of most importance. So, it becomes necessary to develop new analytical methods for such drugs. In brief the reasons for the development of newer methods of drugs analysis are:

1. The drug or drug combination may not be official in any pharmacopoeias.
2. A proper analytical procedure for the drug may not be available in the literature due to Patent regulations.
3. Analytical methods for a drug in combination with other drugs may not be available.
4. Analytical methods for the quantitation of the drug in biological fluids may not be available.
5. The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable [1, 2].

DIFFERENT METHODS OF ANALYSIS

The following techniques are available for separation and analysis of components of interest.

Spectral methods

The spectral techniques are used to measure electromagnetic radiation which is either absorbed or emitted by the sample.

E.g. UV-Visible spectroscopy, IR spectroscopy, NMR, ESR spectroscopy, Flame photometry, Fluorimetry.2

Electro analytical methods

Electro analytical methods involved in the measurement of current voltage or resistanceas a property of concentration of the component in solution mixture.

E.g. Potentiometry, Conductometry, Amperometry [2-3].

Chromatographic methods

Chromatography is a technique in which chemicals in solutions travel down columns or over surface by means of liquids or gases and are separated from each other due to their molecular characteristics.

E.g. Paper chromatography, thin layer chromatography (TLC), High performance thin layer chromatography (HPTLC), High performance liquid chromatography (HPLC), Gas chromatography (GC) [2].

MATERIAL AND METHODS

Mecobalamin from Sura labs, Pregabalin from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

HPLC METHOD DEVELOPMENT [3-6]:

TRAILS

Preparation of standard solution:

Accurately weigh and transfer 10 mg of Mecobalamin and Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.

Further pipette 0.2ml of the above Mecobalamin and 0.3ml of the Pregabalin stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure:

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization:

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: Phosphate Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer in proportion 28:72 (pH-3.8) v/v respectively.

Optimization of Column:
The method was performed with various columns like C18 column, Symmetry and Zodiac column. Symmetry (C18) (150mm x 4.6mm, 5µm) Column was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS [7-10]:

Instrument used : Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature : Ambient
Column : Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Buffer : Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.
pH : 3.8
Mobile phase : Methanol: Phosphate Buffer (28:72%v/v)
Flow rate : 1ml/min
Wavelength : 218nm
Injection volume : 20 µl
Run time : 8 min

METHOD VALIDATION [11]

PREPARATION OF BUFFER AND MOBILE PHASE:

Preparation of Potassium dihydrogen Phosphate (KH_2PO_4) buffer (pH-3.8):
Dissolve 6.8043 of potassium dihydrogen phosphate in 1000 ml HPLC water and adjust the pH 3.8 with diluted orthophosphoric acid. Filter and sonicate the solution by vacuum filtration and ultra sonication.

Preparation of mobile phase:
Accurately measured 280 ml (28%) of Methanol, 720 ml of Phosphate buffer (72%) were mixed and degassed in digital ultra sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:
The Mobile phase was used as the diluent.

VALIDATION PARAMETERS [12-21]

SYSTEM SUITABILITY
Accurately weigh and transfer 10 mg of Mecobalamin and 10mg of Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.2ml of the above Mecobalamin and 0.3ml of the Pregabalin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure:
The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

SPECIFICITY STUDY OF DRUG:

Preparation of Standard Solution:
Accurately weigh and transfer 10mg of Mecobalamin and 10mg of Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.2ml of the above Mecobalamin and 0.3ml of the Pregabalin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Preparation of Sample Solution:
Take average weight of one Tablet and crush in a mortor by using pestle and weight 10 mg equivalent weight of Mecobalamin and Pregabalin sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.
Further pipette 0.2ml of the sample solution from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.
The mean and percentage relative standard deviation were calculated from the peak areas.

Procedure:
Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUG SOLUTIONS FOR LINEARITY:

Accurately weigh and transfer 10 mg of Mecobalamin and 10mg of Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (10ppm of Mecobalamin & 100ppm of Pregabalin):

Pipette out 0.1ml of Mecobalamin and 0.1ml of Pregabalin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (15ppm of Mecobalamin & 200ppm of Pregabalin):

Pipette out 0.15ml of Mecobalamin and 0.2ml of Pregabalin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (20ppm of Mecobalamin & 300ppm of Pregabalin):

Pipette out 0.2ml of Mecobalamin and 0.3ml of Pregabalin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (25ppm of Mecobalamin & 400ppm of Pregabalin):

Pipette out 0.25ml of Mecobalamin and 0.4ml of Pregabalin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (30ppm of Mecobalamin & 500ppm of Pregabalin):

Pipette out 0.3ml of Mecobalamin and 0.5ml of Pregabalin stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure:

Inject each level into the chromatographic system and measure the peak area.

Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

REPEATABILITY

Preparation of Mecobalamin and Pregabalin Product Solution for Precision:

Accurately weigh and transfer 10 mg of Mecobalamin and 10mg of Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Mecobalamin and 0.3ml of the Pregabalin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

INTERMEDIATE PRECISION:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:

DAY 1:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

DAY 2:

The standard solution was injected for Six times and measured the area for all Six injections in HPLC. The %RSD for the area of Six replicate injections was found to be within the specified limits.

ACCURACY:

For preparation of 50% Standard stock solution:

Accurately weigh and transfer 10 mg of Mecobalamin and 10mg of Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.1ml of the above Mecobalamin and 0.15ml of the Pregabalin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 100% Standard stock solution:

Accurately weigh and transfer 10 mg of Mecobalamin and 10mg of Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.2ml of the above Mecobalamin and 0.3ml of the Pregabalin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

For preparation of 150% Standard stock solution:
Accurately weigh and transfer 10 mg of Mecobalamin and 10mg of Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.3ml of Mecobalamin and 0.45ml of Pregabalin from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:
Inject the Three replicate injections of individual concentrations (50%, 100% and 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Mecobalamin and Pregabalin and calculate the individual recovery and mean recovery values.

ROBUSTNESS [10-14:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results. .

For preparation of Standard solution:
Accurately weigh and transfer 10 mg of Mecobalamin and 10mg of Pregabalin working standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 0.2ml of the above Mecobalamin and 0.3ml of the Pregabalin stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Effect of Variation of flow conditions:
The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

Effect of Variation of mobile phase organic composition:
The sample was analyzed by variation of mobile phase i.e. Methanol: Phosphate Buffer was taken in the ratio and 33:64, 23:77 instead (28:72), remaining conditions are same. 20µl of the above sample was injected and chromatograms were recorded.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase ratio : Methanol: Phosphate Buffer (pH-3.8) (28:72v/v)
Column : Symmetry (C18) (150mm x 4.6mm, 5µm) Column
Column temperature : Ambient
Wavelength : 218nm
Flow rate : 1.0ml/min
Injection volume : 20µl
Run time : 8minutes

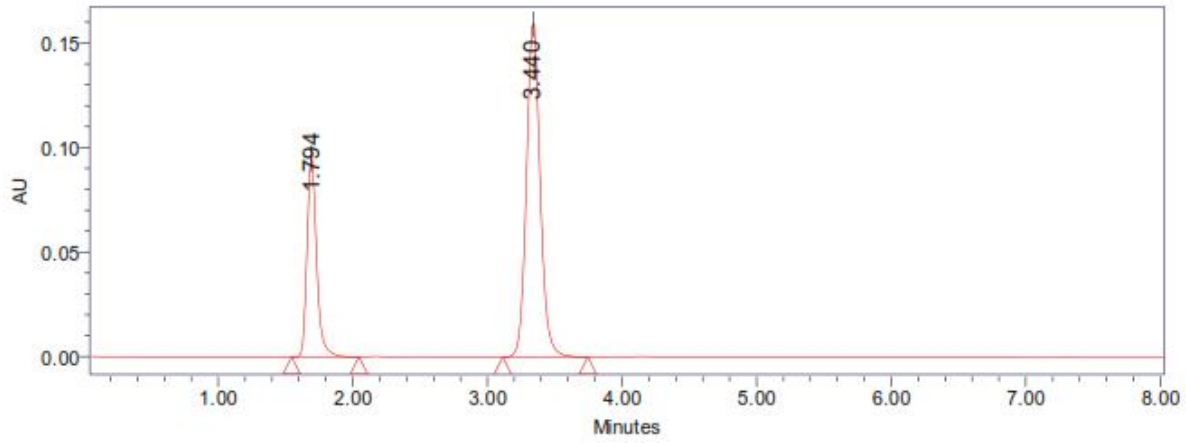

Fig.No.1: Optimized Chromatogram (Standard)

Table No 1: Optimized Chromatogram (Standard)

S.No.	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Mecobalamin	1.794	545265	7462	1.09	7564
2	Pregabalin	3.440	7768545	43652	1.12	8695

Optimized Chromatogram (Sample)

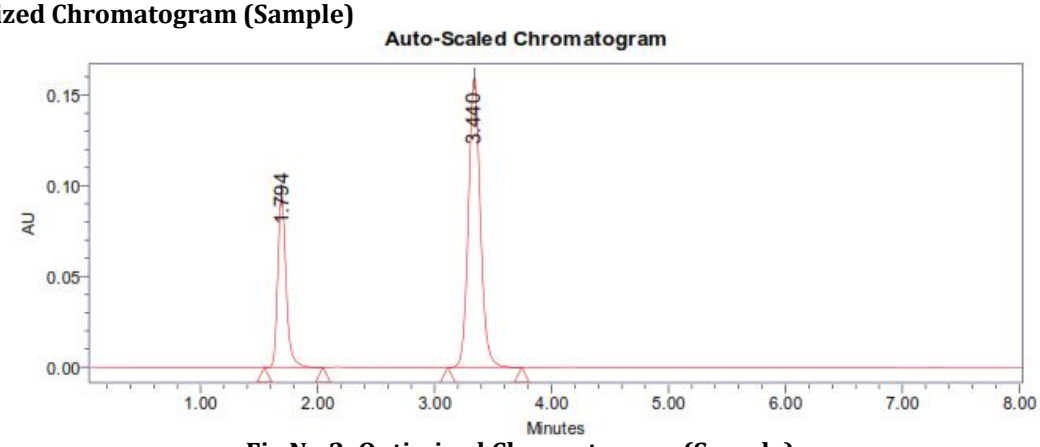

Fig.No.2: Optimized Chromatogram (Sample)

Table No. 2: Optimized Chromatogram (Sample)

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Mecobalamin	1.794	558659	7584	1.10	7659
2	Pregabalin	3.440	7856985	44658	1.13	8743

Acceptance Criteria:

- Theoretical plates must be not less than 2000.
- Tailing factor must be not less than 2.
- It was found from above data that all the system suitability parameters for developed method were within the limit.

METHOD VALIDATION

Blank:

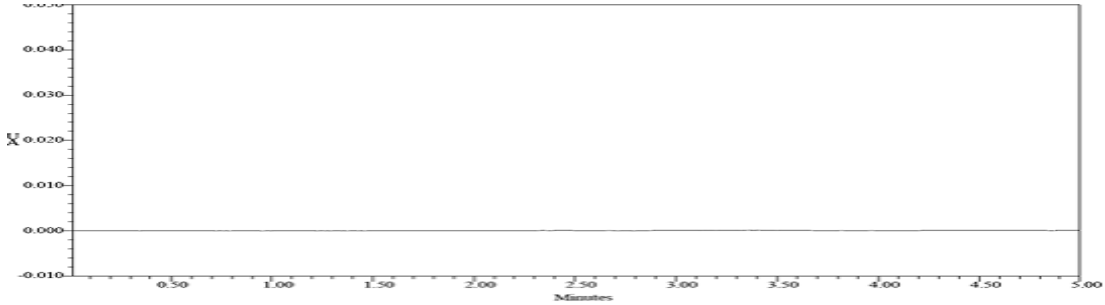

Fig.No.3: Chromatogram showing blank (mobile phase preparation)

SPECIFICITY

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components.

Analytical method was tested for specificity to measure accurately quantities Mecobalamin and Pregabalin in drug product.

Assay (Standard):

Table No. 3: Peak results for assay standard of Mecobalamin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Mecobalamin	1.788	545698	7458	7595	1.09
2	Mecobalamin	1.792	548765	7469	7548	1.10
3	Mecobalamin	1.793	548965	7428	7563	1.09
4	Mecobalamin	1.788	548783	7495	7592	1.10
5	Mecobalamin	1.787	548752	7461	7543	1.09
Mean			548192.6			
Std. Dev.			1397.209			
% RSD			0.254876			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Table No 4: Peak results for assay standard of Pregabalin

S.No	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pregabalin	3.438	7785698	43652	8652	1.12
2	Pregabalin	3.446	7786354	43698	8674	1.13
3	Pregabalin	3.444	7786942	43587	8692	1.13
4	Pregabalin	3.465	7785464	43698	8649	1.12
5	Pregabalin	3.465	7785986	43568	8625	1.12
Mean			7786089			
Std. Dev.			581.3667			
% RSD			0.007467			

Acceptance Criteria:

- %RSD of five different sample solutions should not more than 2.
- The %RSD obtained is within the limit, hence the method is suitable.

Assay (Sample):

Table No.5: Peak results for Assay sample of Mecobalamin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count	Injection
1	Mecobalamin	1.794	556985	75895	1.10	7698	1
2	Mecobalamin	1.791	558742	75468	1.10	7682	2
3	Mecobalamin	1.791	559683	75426	1.11	7649	3

Table No.6: Peak results for Assay sample of Pregabalin

S.No	Name	RT	Area	Height	USP Tailing	USP Plate Count
1	Pregabalin	3.440	7856859	44586	1.14	8759
2	Pregabalin	3.442	7826594	44658	1.15	8726
3	Pregabalin	3.434	7854879	44859	1.14	8794

LINEARITY

Table No-7: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY FOR MECOBALAMIN:

Concentration µg/ml	Average Peak Area
10	292985
15	430752
20	565265
25	693487
30	821584

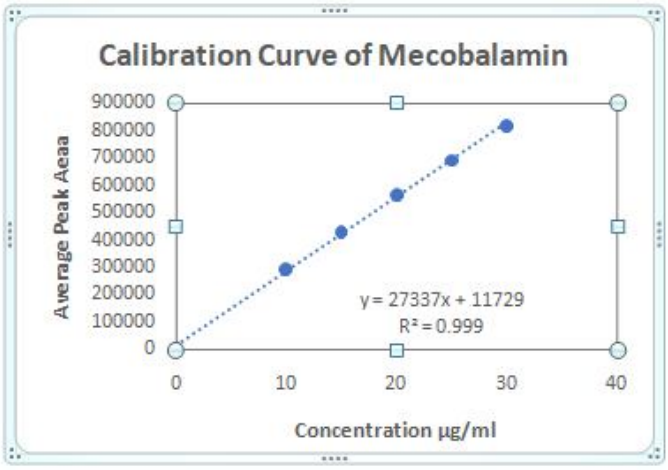

Fig.No.4: Chromatogram showing linearity level

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Mecobalamin is a straight line.

Y = mx + c

Slope (m) = 27337

Intercept (c) = 11729

Correlation Coefficient (r) = 0.999

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 11729. These values meet the validation criteria.

Table No. 8: CHROMATOGRAPHIC DATA FOR LINEARITY STUDY FOR PREGABALIN:

Concentration µg/ml	Average Peak Area
100	2828756
200	5485784
300	7999859
400	10656542
500	13085985

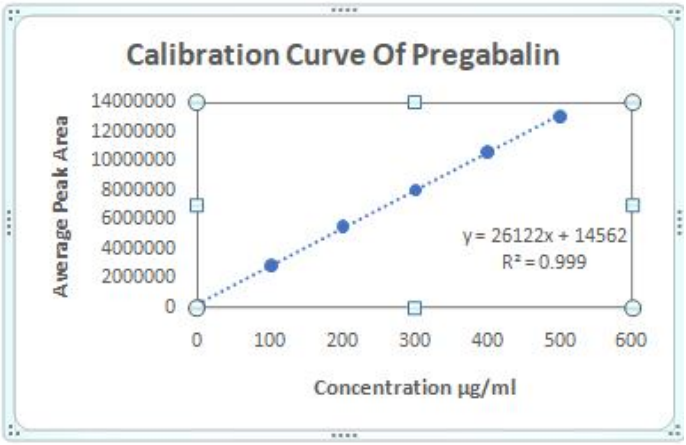

Fig.No.5: Chromatogram showing linearity level

LINEARITY PLOT:

The plot of Concentration (x) versus the Average Peak Area (y) data of Pregabalin is a straight Line.

Y = mx + c

Slope (m) = 26122

Intercept (c) = 14562

Correlation Coefficient (r) = 0.9994

VALIDATION CRITERIA: The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

CONCLUSION: Correlation Coefficient (r) is 0.99, and the intercept is 14562. These values meet the validation criteria.

PRECISION:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

REPEATABILITY

Table 9: Results of Repeatability for Mecobalamin:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Mecobalamin	1.792	548698	7458	7569	1.10
2	Mecobalamin	1.791	548955	7485	7546	1.10
3	Mecobalamin	1.790	548745	7469	7592	1.09
4	Mecobalamin	1.790	549856	7463	7519	1.10
5	Mecobalamin	1.789	546587	7495	7535	1.09
Mean			548568.2			
Std.dev			1202.217			
%RSD			0.2191554			

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Table No. 10: Results of Repeatability for Pregabalin:

S. No.	Peak Name	Retention time	Area (µV*sec)	Height (µV)	USP Plate Count	USP Tailing
1	Pregabalin	3.435	7768958	43659	8659	1.12
2	Pregabalin	3.428	7765984	43856	8647	1.13
3	Pregabalin	3.419	7785469	43658	8675	1.12
4	Pregabalin	3.414	7785498	43549	8652	1.12
5	Pregabalin	3.408	7769852	44526	8692	1.13
Mean			7775152			
Std.dev			9539.236			
%RSD			0.122689			

Acceptance Criteria:

- %RSD for sample should be NMT 2.
- The %RSD for the standard solution is below 1, which is within the limits hence method is precise.

Intermediate precision:

Table No. 11: Results of Intermediate precision day1 for Mecobalamin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Mecobalamin	1.787	556985	75986	7695	1.11
2	Mecobalamin	1.789	558649	75986	7642	1.12
3	Mecobalamin	1.789	557847	75689	7683	1.12
Mean			557827			
Std. Dev.			832.1803			
% RSD			0.149183			

Acceptance Criteria:

%RSD of three different sample solutions should not more than 2

Table No. 12: Results of Intermediate precision day 1 for Pregabalin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pregabalin	3.482	7856982	44586	8758	1.13
2	Pregabalin	3.477	7845285	44758	8769	1.14
3	Pregabalin	3.477	7854633	44986	8728	1.13
Mean			7852300			
Std. Dev.			6187.659			
% RSD			0.078801			

Acceptance Criteria:

- %RSD of three different sample solutions should not more than 2.

Day 2:

Table No. 13: Results of Intermediate precision Day 2 for Mecobalamin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Mecobalamin	1.790	536598	7365	7459	1.08
2	Mecobalamin	1.789	534875	7358	7436	1.07
3	Mecobalamin	1.793	534698	7349	7482	1.08
Mean			535390.3			
Std. Dev.			1049.608			
% RSD			0.196045			

Acceptance Criteria:

%RSD of three different sample solutions should not more than 2

Table No. 14: Results of Intermediate precision Day 2 for Pregabalin

S.No.	Peak Name	RT	Area (µV*sec)	Height (µV)	USP Plate count	USP Tailing
1	Pregabalin	3.474	7698521	42568	8582	1.11
2	Pregabalin	3.473	7685985	42698	8546	1.10
3	Pregabalin	3.478	7645897	42365	8574	1.10
Mean			7676801			
Std. Dev.			27487.83			
% RSD			0.358064			

Acceptance Criteria:

- %RSD of three different sample solutions should not more than 2.

ACCURACY:

Table No. 15: The accuracy results for Mecobalamin

% Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	286080.7	10.035	10	100.350%	100.291%
100%	561215	20.100	20	100.500%	
150%	833959.7	30.077	30	100.023%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Table No.16: The accuracy results for Pregabalin

%Concentration (at Specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	408328	15	15.074	100.493%	100.163%
100%	798306.3	30	30.003	100.010%	
150%	1189915	45	44.994	99.986%	

Acceptance Criteria:

- The percentage recovery was found to be within the limit (98-102%).

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

LIMIT OF DETECTION FOR MECOBALAMIN AND PREGABALIN

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

$$\mathbf{LOD = 3.3 \times \sigma / s}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Mecobalamin =0.86µg/ml

Pregabalin=1.28µg/ml

QUANTITATION LIMIT FOR MECOBALAMIN AND PREGABALIN

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

$$\mathbf{LOQ = 10 \times \sigma / S}$$

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Result:

Mecobalamin =2.58µg/ml

Pregabalin= 3.84µg/ml

ROBUSTNESS

Table No. 17: Results for Robustness -Mecobalamin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	545265	1.794	7564	1.09
Less Flow rate of 0.8mL/min	625486	1.867	7856	1.13
More Flow rate of 1.0mL/min More Flow rate of 0.9mL/min	526548	1.744	7425	1.12
Less organic phase (about 5 % decrease in organic phase)	536548	1.831	7265	1.06
More organic phase (about 5 % Increase in organic phase)	514875	1.874	7169	1.08

Table No. 18: Results for Robustness-Pregabalin

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 0.9mL/min	7768545	3.440	8695	1.12
Less Flow rate of 0.8mL/min	7985695	3.721	8948	1.13
More Flow rate of 1.0mL/min	7458642	3.097	8452	1.12
Less organic phase (about 5 % decrease in organic phase)	7685421	6.242	8365	1.10
More organic phase (about 5 % Increase in organic phase)	7569864	2.402	8254	1.09

Acceptance Criteria:

The Tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Mecobalamin and Pregabalin in bulk drug and pharmaceutical dosage forms.

Mecobalamin (hydrochloride) is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF) methylene chloride, which should be purged with an inert gas. The solubility of

Mecobalamin (hydrochloride) in ethanol is approximately 15 mg/ml and approximately 30 mg/ml in DMSO and DMF, slightly soluble in methanol, chloroform and water, sparingly soluble in water; soluble in alcohol and in dichloromethane. Pregabalin is freely soluble in water and both basic and acidic solutions, sparingly soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide.
Methanol: Phosphate Buffer (pH-3.8) (28:72v/v) was chosen as the mobile phase. The solvent system used in this method was economical.
The %RSD values were within 2 and the method was found to be precise.
The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods.
This method can be used for the routine determination of Mecobalamin and Pregabalin in bulk drug and in Pharmaceutical dosage forms.

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