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Development And Validation Of RP-HPLC Method For Simultaneous Estimation of Piracetam and Mecobalamin in Bulk and its Pharmaceutical Formulations

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Abstract
A new, simple, rapid, selective, precise, and accurate isocratic reverse-phase high-performance liquid Chromatography assay method has been developed for simultaneous estimation of Piracetam and Mecobalamin tablet formulations. The separation was achieved by using column Phenomenex Luna C18, 250x4.6mm, 5µm, in mobile phase consisted of pH 6.0 phosphate buffer and Acetonitrile and methanol in the ratio of 40:50:10 v/v/v. The flow rate was 1.0 mL/min, column oven temperature 25° C, sample cooler temperature 25°C and the injection volume was 20µL, and detection was performed at 215 nm using a photodiode array detector (PDA), Run time 7minutes. The retention time of Piracetam and Mecobalamin was noted to be 2.218 minutes and 3.68 minutes, respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: *Liquid Chromatography, Piracetam, Mecobalamin, combined dosage forms, Simultaneous estimation, Validation*

I. Introduction

Piracetam (2-oxo-1-pyrrolidine acetamide) is universally recognized as the 'Smart' or 'nootropic' drugs Figure.:1. It is a water-soluble cyclic derivative of the neurotransmitter GABA. [1-4]. This drug is responsible for improving memory and cognition, improve blood flow and supply of oxygen to the brain, delay brain aging, support stroke recovery, and improve Down syndrome, Alzheimer's, dementia, dyslexia, and is also used for schizophrenia treatment. It improves cognitive function without leading to sedation or stimulation and also protects the cerebral cortex against hypoxia. For the analysis of piracetam in biological fluids, various methods were developed like thin layer densitometric determination, capillary electrophoresis, and micellar electrokinetic chromatography methods [5-6]. In Indian Pharmacopoeia [7] and British Pharmacopoeia 2003, a liquid chromatography method is mentioned for estimation piracetam as a bulk drug [8]. The piracetam impurities using TLC and FT-IR spectroscopy were also determined [9-10].

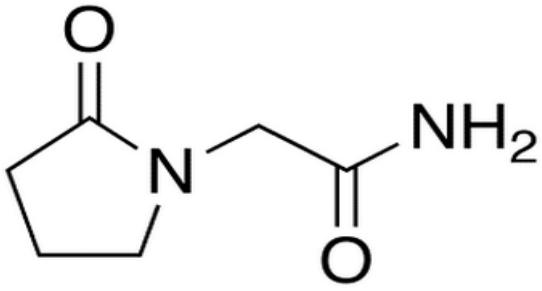

Figure.1: Structure of Piracetam

Methylcobalamin is carbamide; cobalt(3+);[5-(5,6-dimethylbenzimidazol-1-yl)-4-hydroxy-2-(hydroxymethyl) oxolan-3-yl]-1-[3-[(4Z,9Z,14Z)-2,13,18-tris(2-amino-2oxoethyl)- 7,12,17-tris(3-amino-3-oxopropyl)-3,5,8,8,13,15,18,19-octamethyl- 2,7,12,17-tetrahydro-1H-corrin-21-id-3-yl] propanoylamino] propane-2-yl phosphate chemically with molecular formula C63H91CON13O14P and official in Japanese Pharmacopoeia (XIV) [11]. The chemical structure of Methylcobalamin is shown in Figure:2. Methylcobalamin is one of the co enzymatically active cobalamin derivatives. It is an essential nutrient linked to human growth, cell development and an important component of several enzymes which is involved in the metabolism of

certain amino acids. MeCbl had been used to prepared by partial synthesis up to 1962 and was found to be an active constituent in enzymatic synthesis of methionine by extract of an Escherichia coli mutant [12].

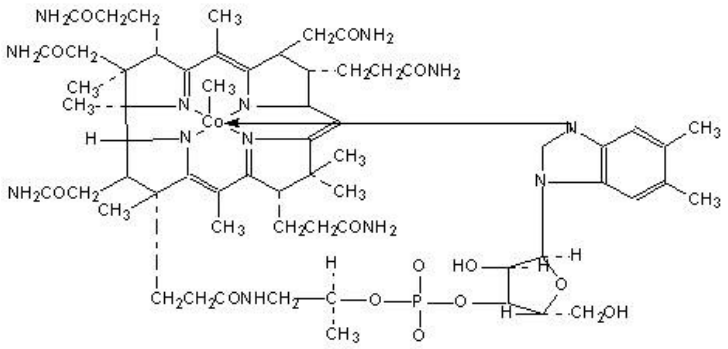

Figure.2: Structure of Mecobalamin

Literature survey reveals that few analytical methods have been reported for the estimation of Piracetam and Mecobalamin in the pharmaceutical dosage form. The objective of this work was to develop a new rapid, novel, and economical RP-HPLC method which can be used as a stability-indicating assay for combination drug product of Piracetam and Mecobalamin.

II. Experimental

A. Chemicals and Reagents

Milli-Q Water, Acetonitrile, Methanol (HPLC Grade), and Potassium dihydrogen phosphate monohydrate (AR Grade), orthophosphoric acid (GR Grade) were obtained from Qualigens Ltd., Mumbai. All other chemicals of analytical grade were procured from local sources unless specified. All dilutions were performed in standard class-A, volumetric glassware.

B. Instrumentation and Chromatographic Conditions

Waters 2489 U.V-Visible detector/2695 Separation Module, equipped with Empower 2 software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Metller Toledo Model) were used in the present assay.

Preparation of solutions:

Buffer Preparation:

Weighed 6.8gms of Potassium dihydrogen Ortho phosphate accurately and transferred into 1000ml of volumetric flask. Added a small amount of HPLC grade water and shook it until it dissolved. After that, the volume made up to the mark with the same HPLC water. The pH was adjusted to 6.0 with orthophosphoric acid. Filtered the above solution through the 0.45µ membrane filter and degassed.

Mobile Phase Preparation:

Prepared a filtered and degassed mixture of Buffer (pH=6.0): Acetonitrile: methanol in the ratio of 400:500:100 % volume/volume/volume.

Diluent:

The mobile phase is used as diluent.

Preparation of standard solution :

Accurately weighed and transferred 100mg of Piracetam and 0.125mg of Mecobalamin working standard into a 100ml clean, dry volumetric flask, added about 70ml of Diluent, and sonicated to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipetted 0.4ml Piracetam and Mecobalamin of the above stock solution into a 10ml volumetric flask and diluted up to the mark with diluent.

Preparation of sample solution:

Ten tablets were weighed and ground to a fine powder. Accurately weigh and transfer equivalent to 10 mg of Piracetam and Mecobalamin sample into a 100ml clean, dry volumetric flask and add about 70mL of Diluent and sonicated to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 0.4ml of Piracetam and Mecobalamin from the above stock solution-1 into a 10ml volumetric flask and dilute up to the mark with diluent.

Chromatographic conditions

Phenomenex Luna C18, 250X4.6mm, 5µm, Column was used for analysis at 25°C column temperature and sample cooler 25°C. The mobile phase consisted of pH 6.0 phosphate buffer and acetonitrile and methanol in the ratio of 400:500:100 % volume/volume/volume. The mobile phase was pumped through the column at a flow rate of 1.0mL/min. The sample injection volume was 20 µL. The photodiode array detector was set to a wavelength of 215nm for the detection, and the chromatographic runtime was 7 minutes.

III. Method development

To develop a suitable and robust LC method for the determination of Piracetam and Mecobalamin, different mobile phases were employed to achieve the best separation and resolution. The method development was started with Waters symmetry C18 (250x4.6 mm, 5 µm.) with the following mobile phase. Methanol:Acetonitrile: water (40:40:20 v/v/v). Detector wavelength 215 nm, column temperature 25° C, Injection volume 20µL, and Flow rate 1.0 ml/min used. Peak shape was not good due to asymmetry (tailing factor) So. Another trial was taken with a buffer in place of water.

For the next trial was changed with pH 6.0 buffer in place of water remaining chromatographic conditions are the same. The resolution between the Piracetam and Mecobalamin peak is less. Hence, further trials were carried out.

For the next trial, the column was changed to Phenomenex Luna C18 (250x4.6mm, 5µm) from Waters symmetry C18, (250x4.6 mm, 5µm.) remaining chromatographic conditions are same. Peak shape was satisfactory in both standard and sample preparations. The retention time of Piracetam and Mecobalamin were found to be 2.218 and 3.680 minutes acceptable. The chromatogram of Piracetam and Mecobalamin standard using the proposed method is shown in **(Fig-3.)** System suitability results of the method are presented in **Table-1.**

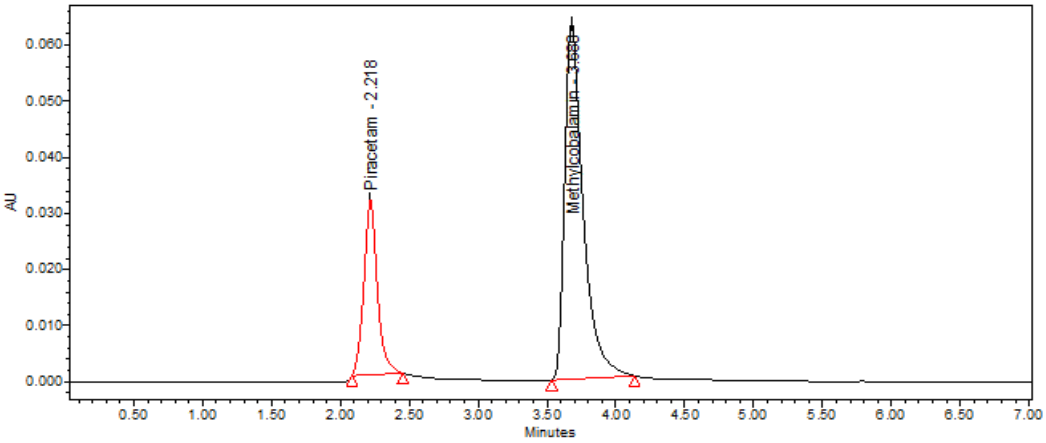

Figure 3: A typical HPLC Chromatogram standard

IV. Method validation

The developed RP-LC method extensively validated for assay of Piracetam and Mecobalamin using the following Parameters.

A. Specificity & System suitability: Blank and Placebo interference

A study to establish the interference of blank and placebo were conducted. Diluent and placebo were injected into the chromatograph in the defined above chromatographic conditions, and the blank and placebo chromatograms were recorded. Chromatogram of Blank solution **(Figure:3)** showed no peaks at the retention time of Piracetam and Mecobalamin peak. This indicates that the diluent solution used in sample preparation do not interfere in the estimation of Piracetam and Mecobalamin in tablets. Similarly, a Chromatogram of the Placebo solution **(Figure:4)** showed no peaks at the retention time of Piracetam and Mecobalamin peak. This indicates that the Placebo used in sample preparation do not interfere in the estimation of Piracetam and Mecobalamin in Piracetam and Mecobalamin tablets. The chromatogram of Piracetam and Mecobalamin Blank using the proposed method is shown in **Figure:4**. The chromatogram of Piracetam and Mecobalamin Placebo using the proposed method is shown in **Figure:5**.

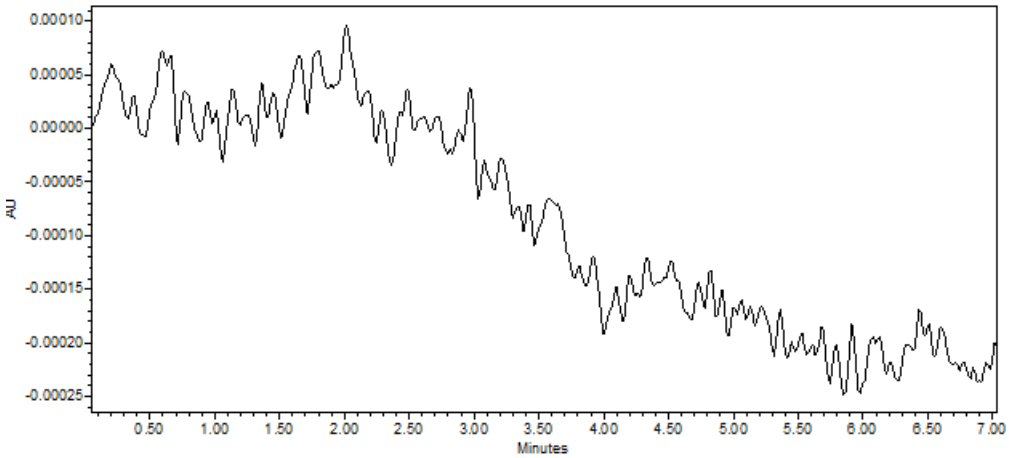

Figure 4: Typical Chromatogram of Blank

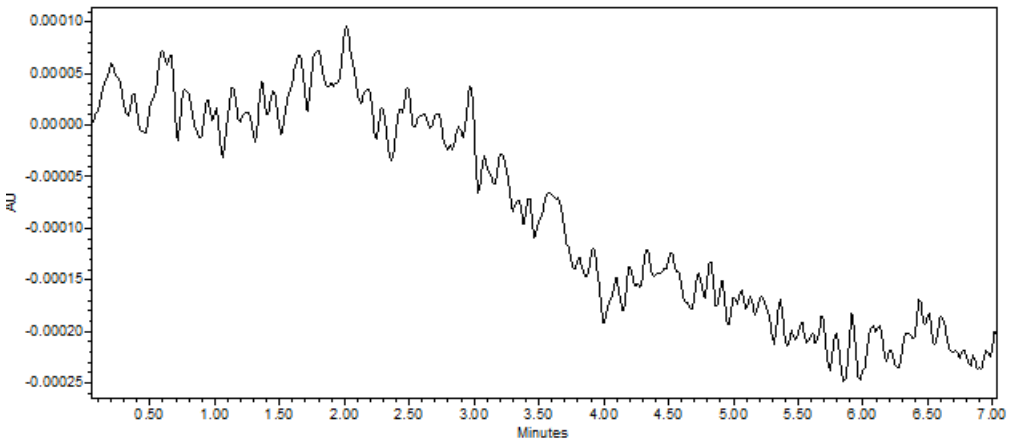

Figure 5: Typical Chromatogram of placebo

Table 1.0: System suitability parameters

Parameters	Piracetam	Mecobalamin
Resolution	6.87	
Retention time (min)	2.216	3.682
No. of Theoretical plates	9495	10910
Tailing factor	1.12	1.67

B. Method Precision:

The precision of the test method was evaluated by doing an assay for six sample preparations as per the test method. The content of % label claim for Piracetam and Mecobalamin for each of the test preparation was calculated. The average content of the six preparations and %RSD for the six observations were calculated. The chromatogram as shown in **Figure: 1.5** and data were shown in **Table: 1.1.**

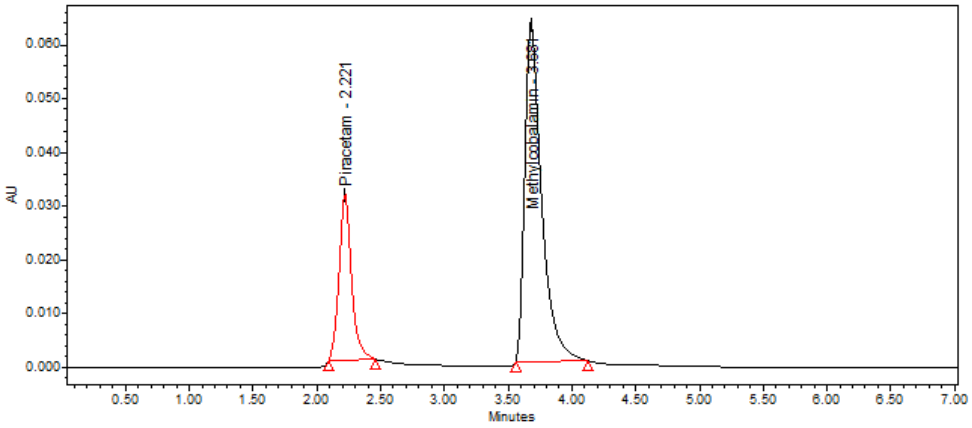

Figure: 1.5 Method precision sample chromatogram

Table 1.1: Method Precision results

Preparations	Piracetam	Mecobalamin
1	99.97	100.04
2	99.84	100.02
3	100.17	100.00
4	99.99	99.96
5	100.03	99.97
6	100.00	100.00
Mean	**100.00**	**100.00**
SD	**0.11**	**0.03**
% RSD	**0.11**	**0.03**

C. Intermediate Precision :

The intermediate precision of the test method was demonstrated by carrying out a method precision study in six samples, representing a single batch by two different analysts on two different days, different columns, different HPLC systems, and by the different analysts. These samples were prepared as per the test method. The % assay was calculated for each of these samples. The precision of the method was evaluated by computing the % Relative standard deviation of % assay of Piracetam and Mecobalamin. The chromatogram was shown in **Figure: 1.6,** and data were shown in **Table: 1.4.**

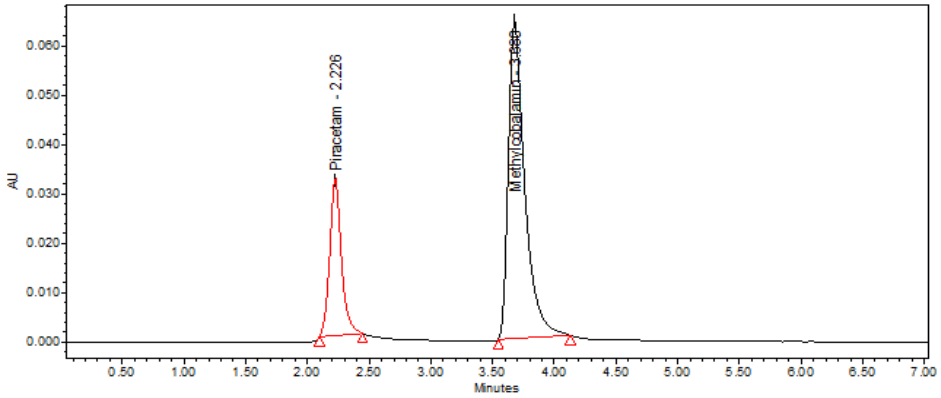

Figure: 1.6 Intermediate precision sample chromatogram

Table: 1.4 Intermediate Precision results

Preparations	Piracetam	Mecobalamin
1	100.11	100.03
2	100.11	99.95
3	99.97	100.04
4	99.84	100.02
5	100.17	101.56
6	99.99	101.48
Mean	**100.03**	**100.51**
SD	**0.12**	**0.78**
% RSD	**0.12**	**0.78**

- ❖ Overall and individual % of Assay are complies as per test method specification.
- ❖ The relative standard deviation of six assay preparations are 0.12% and 0.78%.

D. Accuracy:

The accuracy of the test method was demonstrated by preparing recovery samples of Piracetam and Mecobalamin at 50%, 100%, and 150% of the target concentration level. The recovery samples were prepared in triplicate for each concentration level 50 %, 100%, and 150%. The percentage recoveries with found in the range of 100.01 to 100.74 for Piracetam, and The percentage recoveries with found in the range of 99.89 to 100.60 for Mecobalamin. From the data obtained, which is given in **Table-:3 and Table-:4,** the method was found to be accurate.

Table 3: Recovery studies for Piracetam by the proposed method

S.No.	% spike level	% Recovery	%Mean recovery
1	50%	100.74	100.60
2		100.81	
3		100.24	
1	100%	100.07	100.74
2		101.2	
3		100.95	
1	150%	99.94	100.01
2		100.8	
3		99.3	

Table 4: Recovery studies for Mecobalamin by the proposed method

S.No.	% spike level	% Recovery	%Mean recovery
1	50%	100.74	100.10
2		100.12	
3		99.45	
1	100%	98.9	99.89
2		100.02	
3		100.74	
1	150%	100.00	100.60
2		99.99	
3		101.8	

E. Linearity of detector response:

The standard curve was obtained in the concentration range of 20-80 µg/ml for Piracetam and 0.025-0.1 µg/ml for Mecobalamin. The linearity of this method was evaluated by linear regression analysis. Slope, intercept, and correlation coefficient [r2] of the standard curve were calculated and given in **Figure-5** For Piracetam and **Figure-6** for Mecobalamin to demonstrate the linearity of the proposed method. From the data obtained, which is given in **Table-5** For Piracetam and **Table-6** for Mecobalamin, the method was found to be linear within the proposed range.

Table 5: Linearity studies for Piracetam

S.No	Linearity Level	Concentration ppm	Average area
1	10	20	116387
2	50	30	174042
3	80	40	232552
4	100	50	290506
5	120	60	348370
6	150	70	406620
7		80	464955
correlation coefficient			1.000
Slope			5809.5643
Intercept			12.0717

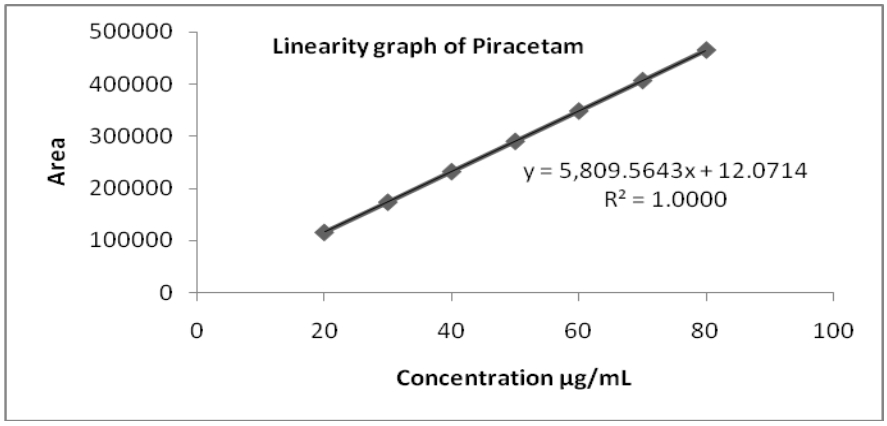

Figure 5: Calibration curve for Piracetam

Table 6: Linearity studies for Mecobalamin

S.No	Concentration ppm	Average area
1	0.025	320214
2	0.0375	480246
3	0.05	640249
4	0.0625	802259
5	0.075	960505
6	0.0875	1120828
7	0.1	1280011
correlation coefficient		1.000
Slope		12802317
Intercept		471.18

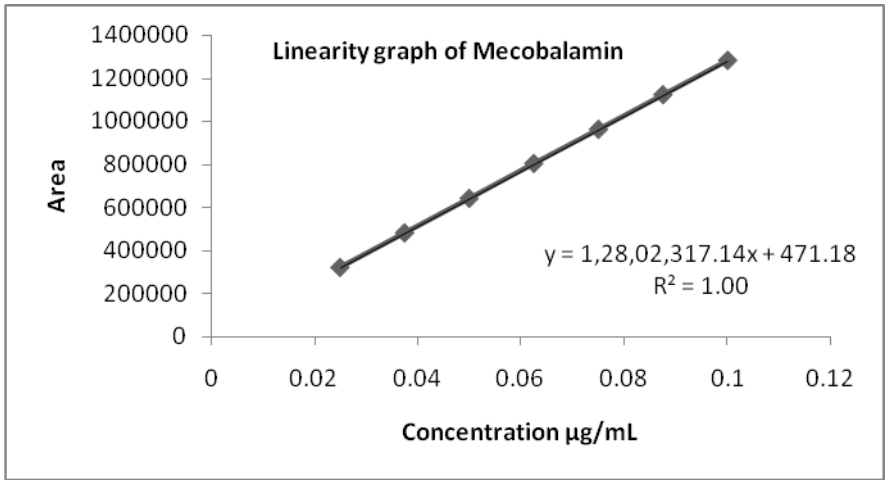

Figure 6: Calibration curve for Mecobalamin

V. Results & Discussion

An RP-HPLC method for simultaneous estimation of Piracetam and Mecobalamin was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. As there is no interference of blank and placebo at the retention time of Piracetam and Mecobalamin hence method was specific. Linearity was observed over a concentration range of 20.0-80.0µg/ml for Piracetam and 0.025-0.10µg/mL for Mecobalamin. The correlation coefficient Piracetam was found to be 1.000. and 1.000 for Mecobalamin.

The relative standard deviation for method precision was found to be 0.11% for Amlodipine Piracetam and 0.03% for Mecobalamin. Intermediate precision was found to be 0.12% for Piracetam and 0.78% for Mecobalamin.

The accuracy studies were shown as % recovery at 50 to 150% level for Piracetam. The limit of % recovered is shown in the range of 100.01% to 100.74%, and the results obtained were found to be within limits. Hence the method was found to be accurate. The accuracy studies were shown as % recovery at 50 to 150% level for Mecobalamin. The limit of % recovered is shown in the range of 99.89% to 100.60%, and the results obtained were found to be within limits. Hence the method was found to be accurate.

VI. Conclusion

The developed method was validated as per ICH Q2A (R1) guideline. The developed method was found to be specific because there was no interference from the placebo, matrix, and degradation products at the retention time of the analyte. In conclusion, the developed method can be used for routine quality control analysis and stability studies of Piracetam and Mecobalamin in combination products.

Acknowledgment

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Conflict of interests

The authors claim that there is no conflict of interest.

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