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Research article *Analytical research*

Simultaneous estimation of new analytical method development and validation of mecobalamin and gabapentin by high-performance liquid chromatography

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ABSTRACT

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Mecobalamin and Gabapentin was done by RP-HPLC. The Phosphate buffer was p^H 3 and the mobile phase was optimized with consists of Methanol: Phosphate buffer (pH-3) mixed in the ratio of 70:30 % v/ v. A Phenomenex Luna column C18 (4.6 x 150 mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1 ml/min. The linearity range of Mecobalamin and Gabapentin were found to be from 16-80µg/ml, 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

Keywords: Mecobalamin and Gabapentin

INTRODUCTION

Analytical chemistry[1]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purposes of chemical analysis are together and interpret chemical information that will be of value to society in a wide range of contexts. Quality control in manufacturing industries, the monitoring of clinical and environmental samples, the assaying of geological specimens, and the support of fundamental and applied research are the principal applications. Analytical chemistry involves the application of a range of techniques and methodologies to obtain and assess qualitative, quantitative and structural information on the nature of matter.

- **Qualitative analysis** is the identification of elements, species and/or compounds present in sample.
- **Quantitative analysis** is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

Structural analysis is the determination of the spatial arrangement of atoms in an element or molecule or the identification of characteristic groups of atoms (functional groups). An element, species or compound that is the subject of analysis is known as analyte. The remainder of the material or sample of which the analyte(s) form(s) a part is known as the matrix.

The gathering and interpretation of qualitative, quantitative and structural information is essential to many aspects of human endeavour, both terrestrial and extra-terrestrials. The maintenance of an improvement in the quality of life throughout the world and the management of resources heavily on the information provided by chemical analysis. Manufacturing industries use analytical data to monitor the quality of raw materials, intermediates and finished products. Progress and research in many areas is dependent on establishing the chemical composition of man-made or

natural materials, and the monitoring of toxic substances in the environment is of ever increasing importance. Studies of biological and other complex systems are supported by the collection of large amounts of analytical data. Analytical data are required in a wide range of disciplines and situations that include not just chemistry and most other sciences, from biology to zoology, butte arts, such as painting and sculpture, and archaeology. Space exploration and clinical diagnosis are two quite desperate areas in which analytical data is vital. Important areas of application include the following.

Quality control

(**QC**) in many manufacturing industries, the chemical composition of raw materials, intermediates and finished products needs to be monitored to ensure satisfactory quality and consistency. Virtually all consumer products from automobiles to clothing, pharmaceuticals and foodstuffs, electrical goods, sports equipment and horticultural products rely, in part, on chemical analysis. The food, pharmaceutical and water industries in particular have stringent requirements backed by legislation for major components and permitted levels of impurities or contaminants. The electronic industry needs analyses at ultra-trace levels (parts per billion) in relation to the manufacture of semi-conductor materials. Automated, computer-controlled procedures for process-stream analysis are employed in some industries.

Monitoring and control of pollutants

The presence of toxic heavy metals (e.g., lead, cadmium and mercury), organic chemicals (e.g., polychlorinated biphenyls and detergents) and vehicle exhaust gases (oxides of carbon, nitrogen and sulphur, and hydrocarbons) in the environment are health hazards that need to be monitored by sensitive and accurate methods of analysis, and remedial action taken. Major sources of pollution are gaseous, solid and liquid wastes that are discharged or dumped from industrial sites, and vehicle exhaust gases.

MATERIALS AND METHODS

Mecobalamin from Sura labs, Gabapentin from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK). Acetonitrile for HPLC from Merck, Phosphate Buffer Finar chemicals ,Citric Acid Finar chemicals

HPLC method development

Mobile Phase Optimization

Initially the mobile phase tried was Water: Methanol and ACN: Phosphate Buffer ACN: Methanol with varying proportions. Finally, the mobile phase was optimized to phosphate buffer (pH 3), Methanol in proportion 30:70 v/v respectively.

Optimization of Column

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

Optimized chromatographic conditions

Instrument used	:	Waters HPLC with auto sampler and PDA detector 996 model.
Temperature	:	Ambient
Column	:	Phenomenex Luna C18 (4.6×250mm) 5 µ
Buffer	:	Phosphate buffer (pH-3)-Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000ml. Adjust the pH 3 by using ortho phosphoric acid.
pH	:	3
Mobile phase	:	Methanol: Phosphate Buffer pH3 (70:30v/v)
Flow rate	:	1 ml per min
Wavelength	:	230 nm
Injection volume	:	10 µl
Run time	:	6 min.

Validation

Preparation of buffer and mobile phase

Preparation of Phosphate buffer (pH-3)

Dissolve 0.9g of anhydrous di hydrogen phosphate and 1.298 g of Citric acid mono hydrate in sufficient water to produce 1000mL .Adjust the p H 3 by using ortho phosphoric acid.

Preparation of mobile phase

Accurately measured 700 ml (70%) of Methanol and 300 ml of Phosphate buffer pH3(30%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase : Methanol: Phosphate Buffer pH3 (70:30v/v)

Column : Phenomenex Luna C18 (4.6×250mm) 5 µ
Flow rate : 1 ml/min
Wavelength : 230 nm
Column temp : Ambient
Sample Temp : Ambient
Injection Volume : 10 µl
Run time : 6 minutes

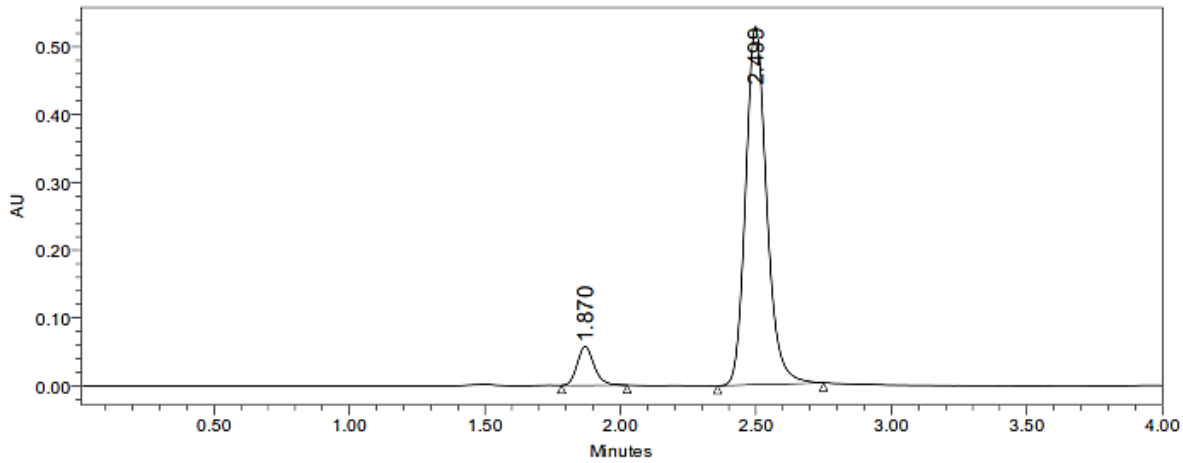

Fig 1: chromatogram for trail 5

Table1: peak results

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Gabapentin	1.870	5664027	299752			2314
2	Mecobalamin	2.499	5033532	210321	4.6	1.3	2921

From the above chromatogram it was observed that the Gabapentin and Mecobalamin peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.
Retention time of Gabapentin–1.870min
Retention time of Mecobalamin – 2.499min

Optimized Chromatogram (Sample)

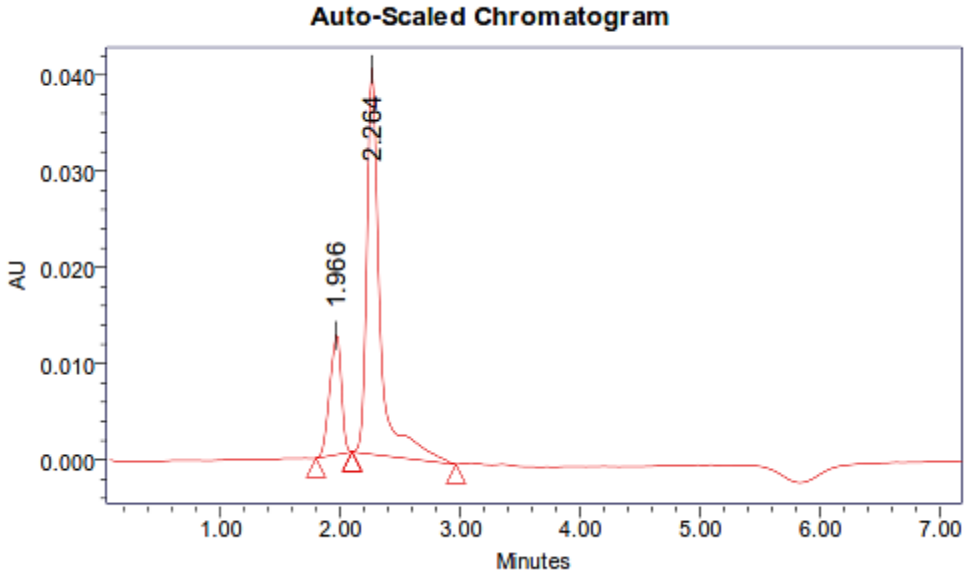

Fig 2: chromatogram for trail 4

Table 2: peak results for trail 4

S.No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Gabapentin	1.966	175068	15332		1.13	936
2	Mecobalamin	2.264	792682	36779	8.06	0.97	863

Assay (Standard)

Table 3: Showing assay standard results

S.no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Gabapentin	1.866	2762937	399854		1.3	2300.1	1
2	Mecobalamin	2.496	2534375	210326	4.6	1.3	2937.7	1
3	Gabapentin	1.866	2774613	386542		1.3	2344.7	2
4	Mecobalamin	2.497	2526189	226741	4.7	1.3	3008.8	2
5	Gabapentin	1.868	2776429	364121		1.3	2344.2	3
6	Mecobalamin	2.498	2546248	231494	4.7	1.3	2990.7	3

.

Assay (Sample)

Table 4: Showing assay sample results

S.no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Gabapentin	1.870	2732203	294531		1.3	2314	1
2	Mecobalamin	2.495	2507543	216321	4.6	1.3	2954	1
3	Gabapentin	1.873	2751843	286473		1.3	2369	2
4	Mecobalamin	2.499	2509101	216354	4.6	1.3	2944	2
5	Gabapentin	1.874	2744776	312684		1.3	2329	3
6	Mecobalamin	2.501	2515628	206571	4.6	1.3	2990	3

Table 5: Showing assay results

S.No	Name of compound	Label claim	Amount taken(from combination tablet)	%purity
1	Gabapentin	300mg	299.9	99.9 %
2	Mecobalamin	500mg	499.9	99.9%

The retention time of Gabapentin and Mecobalamin was found to be 1.8mins and 2.4mins respectively. The % purity of Gabapentin and Mecobalamin in pharmaceutical dosage form was found to be 99.9% and 99.9% respectively.

Linearity

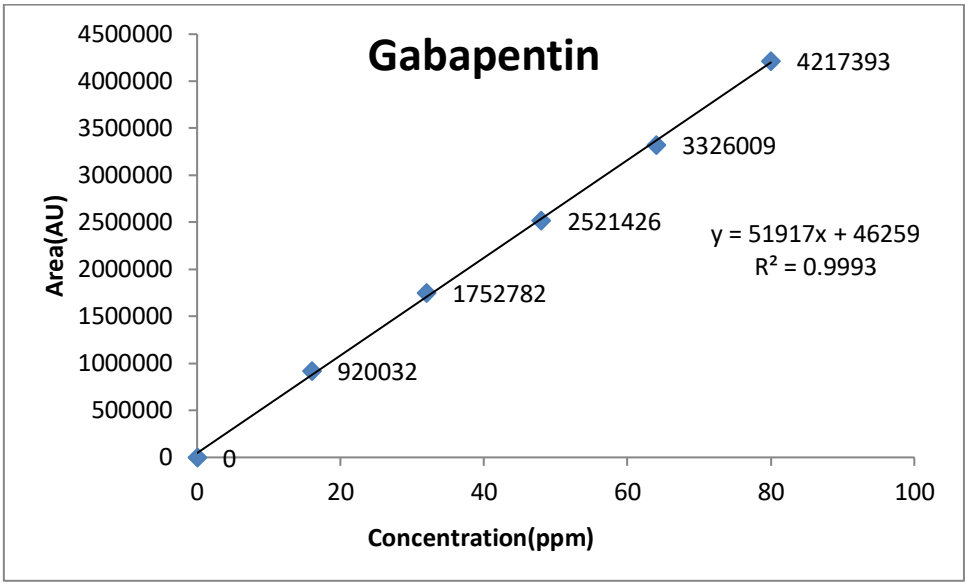

Fig 3: calibration graph for Gabapentin

Linearity Results: (for Gabapentin)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	10	892464
2	II	20	1866364
3	III	30	2777423
4	IV	40	3709213
5	V	50	4601317
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.999

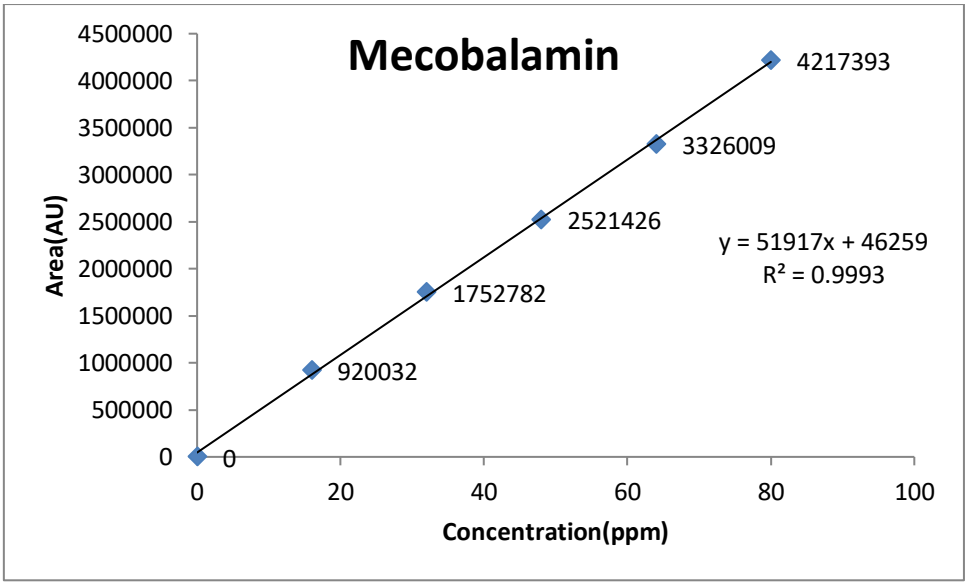

Fig 4: Calibration graph for Mecobalamin

Linearity Results: (for Mecobalamin)

S.No	Linearity Level	Concentration(ppm)	Area
1	I	16	920032
2	II	32	1752782
3	III	48	2521426

4	IV	64	3326009
5	V	80	4217393
Correlation Coefficient			0.999

Correlation coefficient should be not less than 0.99.

Intermediate precision

Table 6 Results of Intermediate precision for Gabapentin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Gabapentin	1.869	2781856	294651	2647	1.3
2	Gabapentin	1.872	2761510	284123	2781	1.3
3	Gabapentin	1.872	2748811	274561	2984	1.3
4	Gabapentin	1.873	2790831	281241	2475	1.3
5	Gabapentin	1.874	2785112	286471	2647	1.3
6	Gabapentin	1.872	2781932	294512	2489	1.3
Mean			2775009			
Std. Dev			16222.05			
% RSD			0.5			

Table 7: Results of Intermediate precision for Mecobalamin

Sno	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Mecobalamin	2.497	2536301	211541	2495	1.4	4.6
2	Mecobalamin	2.499	2541972	206141	2694	1.4	4.6
3	Mecobalamin	2.500	2521259	198641	2785	1.4	4.7
4	Mecobalamin	2.500	2537081	206741	2947	1.4	4.6
5	Mecobalamin	2.500	2549869	209487	2742	1.4	4.6
6	Mecobalamin	2.500	2536301	193481	2914	1.4	4.6
Mean			2537131				
Std. Dev			9370.087				
% RSD			0.3				

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

Accuracy

Table 8: Accuracy (recovery) data for Gabapentin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2771991	15	14.9	98%	99.1%
100%	5664027	30	29.99	99.9%	
150%	8337191	45	44.95	99.6%	

- *The % Recovery for each level should be between 98.0 to 102.0%.*

Table 9: Accuracy (recovery) data for Mecobalamin

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2426681	24	23.9	98%	98.8%
100%	5033532	48	47.92	99.2%	
150%	7419721	72	71.9	99.3%	

- *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.

Robustness

Table 10: System suitability results for Gabapentin

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2231.8	1.3
2	1.0	2344.7	1.3
3	1.1	2071.6	1.3

***** *Results for actual flow (1.0 ml/min) have been considered from Assay standard.*

Table 11: System suitability results for Mecobalamin

S.No	Flow Rate (ml/min)	System Suitability Results	
		USP Plate Count	USP Tailing
1	0.9	2953.6	1.3
2	1.0	3008.8	1.3
3	1.1	2704.0	1.3

***** *Results for actual flow (1.0ml/min) have been considered from Assay standard.*

CONCLUSION

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Mecobalamin and Gabapentin was done by RP-HPLC. The Phosphate buffer was p^H 3 and the mobile phase was optimized with consists of Methanol: Phosphate buffer (pH-3) mixed in the ratio of 70:30 % v/ v. A Phenomenex Luna column C18 (4.6 x 150mm, 5µm) or equivalent chemically bonded to porous silica particles was used as stationary phase. The solutions were chromatographed at a constant flow rate of 1 ml/min. The linearity range of Mecobalamin and Gabapentin were found to be from 16-80µg/ml, 10-50µg/ml respectively. Linear regression coefficient was not more than 0.999, 0.999.

The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 99.9-99.9% of Mecobalamin and Gabapentin. LOD and LOQ were found to be within limit.

The results obtained on the validation parameters met ICH and USP requirements. It inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

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REFERENCES

1. Dr. Kealey, Haines PJ. Analytical chemistry. 1st ed. Bios Publisher; 2002. P. 1-7.
2. BraithWait A, Smith FJ. Chromatographic methods. 5th ed. Kluwer Academic Publishers; 1996. P. 1-2.
3. Weston A, Phyllisr. Brown, HPLC principle and practice. 1st ed. Academic press; 1997. P. 24-37.
4. Kazakevich Y, Lobrutto R. HPLC for pharmaceutical scientists. 1st ed. Wiley Interscience A JohnWiley & Sons, Inc Publishing House; 2007. P. 15-23.
5. Chromatography [online]. Wikipedia. Available from: http://en.wikipedia.org/wiki/Chromatography.
6. Meyer VR. Practical high-performance liquid chromatography. 4th ed. England: John Wiley & Sons Ltd; 2004. P. 7-8.
7. Sahajwalla CG a new drug development. Vol. 141. New York: Marcel Dekker, Inc; 2004. P. 421-6.
8. Introduction to column [online]. Available from: http://amitpatel745.topcities.com/index_files/study/columncare.pdf.
9. Detectors used in HPLC (online). Available from: http://wiki.answers.com/Q/What_detectors_are_used_in_HPLC.
10. Detectors [online]. Available from: http://hplc.chem.shu.edu/NEW/HPLC_Book/Detectors/det_uvda.html.
11. Detectors [online]. Available from: http://www.dionex.com/enus/webdocs/64842-31644-02_PDA-100.pdf.
12. Detectors [online]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8867705.
13. Detectors [online]. Available from: http://www.chem.agilent.com/Library/applications/59643559.pdf.
14. Detectors [online]. Available from: http://hplc.chem.shu.edu/new/hplcbook/detector.
15. Draft ICH. Guidelines on Validation of Analytical Procedures Definitions and terminology. Fed Regist. 1995;60:1126.
16. Code. Q2B, validation of analytical procedures; methodology. ICH harmonized tripartite guidelines. 1996:1-8.
17. Introduction to analytical method validation [online], available from. Available from: http://www.standardbase.hu/tech/HPLC%20validation%20PE.pdf.
18. Data elements required for assay validation [online] available from. Available from: http://www.labcompliance.com/tutorial/methods/default.aspx.
19. Snyder LR practical HPLC method development. 2nd ed. New York: John Wiley & sons; 1997. P. 180-2.

20. Skoog DA, West DM, Holler FJ. Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers; 1994. P. 1-5.
21. Sharma BK. Instrumental method of chemical analysis Meerut; 1999. P. 175-203.
22. Breaux J, Jones K. Understanding and implementing efficient analytical method development and validation. Pharm Technol. 2003;5:110-4.
23. Willard H, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th ed CBS publisher and distributors. New Delhi; 1991. P. 436-9.