International Journal of Chemistry and Pharmaceutical Sciences
ISSN: 2321-3132 | CODEN (USA): IJCPNH
Available online at:http://www.pharmaresearchlibrary.com/ijcps

Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Pregabalin and Methylcobalamin in Formulation by QBD Approach

Aasadi Balaji*, G. Dharmamoorthy, G. Aruna, KV Nanda Kumar, P. Jaya Chandra Reddy

Krishna Teja Pharmacy College, Chadalawada Nagar, Renigunta Road Tirupati-517506

ABSTRACT

By considering the current regulatory requirement for an analytical method development, a simple, precise, accurate, linear and rapid Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for the simultaneous quantitative estimation of Pregabalin 75mg and Methylocobalamin 750µg in capsule has developed using analytical quality by design approach.The optimized method was achieved using Unisol C-18 (3µm, 4.6 ×150mm) column with mobile phase consisting of mixture of water and methanol (40:60v/v) with a flow rate of 0.6ml/min at 210nm. The developed method resulted in pregabalin eluting at 5.540min and methylcobalamin eluting at 2.593min. The method exhibited linearity over the range of 50-150µg/ml and 5-15µg/ml for pregabalin and methylcobalamin respectively. The method precision is exemplified by %RSD of 0.999 for both pregabalin and methylcobalamin. Accuracy studies revealed % mean recoveries during spiking experiments between 98 and 102. The limit of detection was obtained as 2.69µg/ml for pregabalin and 1.74µg/ml for methylcobalamin, while the limit of quantitation was obtained was 8.18µg/ml for pregabalin and 5.27µg/ml for methylcobalamin. There are no interfering peaks underperformed degradation conditions. Therefore, a simple, precise, accurate, linear and rapid RP-HPLC method was developed and validated as per ICH guidelines and hence can be applicable in routine analysis for tablets in various pharmaceutical industries.

Key Words: Pregabalin, Methylcobalamin, QbD, RP-HPLC, Validation.

ARTICLE HISTORY: Received 09 Nov 2019, **Accepted** 29 Dec 2019, **Available Online** 27 February 2020

Citation: Aasadi Balaji, *et al.* Validation of Stability Indicating RP-HPLC Method for the Simultaneous Estimation of Pregabalin and Methylcobalamin in Formulation by QBD Approach r.*International Journal of Chemistry and Pharmaceutical Sciences,* 8(2), 2020: 32-38.

CONTENTS:

Corresponding author
Aasadi Balaji
Krishna Teja Pharmacy College,
Chadalawada Nagar, Renigunta Road
Tirupati-517506

JOURNAL QR-CODE

1. Introduction

Pregabalin chemically known as (3S)-3-(amino methyl)-5-methylhexanoic acid. Pregabalin is an anticonvulsant drug used for neuropathic pain, as an adjunct therapy for partial seizures, and in generalized anxiety disorder. It was designed as a more potent successor to gabapentin. Pregabalin is marketed by Pfizer under the trade name Lyrica.Pregabalin binds with high affinity to the alpha2-delta site (an auxiliary subunit of voltage-gated calcium channels) in central nervous system tissues. Although the mechanism of action of pregabalin is unknown, results with genetically modified mice and with compounds structurally related to pregabalin (such as gabapentin) suggest that binding to the alpha2-delta subunit may be involved in pregabalin's anti-nociceptive and anti-seizure effects in animal models. In vitro, pregabalin reduces the calcium-dependent release of several neurotransmitters, possibly by modulation of calcium channel function. Although pregabalin is a structural derivative of inhibitory neurotransmitter gamma-amino butyric acid (GABA), it does not bind directly to GABA or benzodiazepine receptors. The sodium channels, opiate receptors, and cyclooxygenase enzymes are not involved with the mechanism of pregabalin.

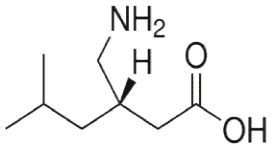

Fig 1: Chemical structure of Pregabalin

Methyl cobalamine chemically known as (10S,12R,13S, 17R, 23R,24R,25R,30S,35S,36S,40S,41S, 42R,46R)-30,35, 40-tris(2-carbamoylethyl)-24,36,41-tris(carbamoylmethyl)-46-hydroxy-12-(hydroxymethyl)-1,5,6,17,23, 28,31, 31,36, 38, 41,42-dodecamethyl-15,20-dioxo-11,14,16-trioxa-2λ^5, 9,19, 26,43λ^5,44λ^5,45λ^5-heptaaza-15λ^5-phospha-1-cobalt adodecacyclo [27.14.1.1^{1},^{34}.1^{2},^{9}.1^{10}, ^{13}.0^{1},^{26}.0^{3},^{8}.0^{23},^{27}.0^{25}, ^{42}.0^{32},^{44}.0^{39},^{43}.0^{37},^{45}]heptatetraconta-2(47),3,5,7,27, 29(44), 32,34(45),37,39(43) -decaene-2,43,44,45-tetrakis(ylium)-1,1,1-triuid-15-olate. Mehylcobalamin (mecobalamin, MeCbl, or MeB12) is a cobalamin, a form of vitamin B12. Cyanocobalamin stimulates reticulocytes, thus playing important role in hematopoiesis in that, together with folic acid and involved in formation of deoxyribonucleotides from ribonucleotides.

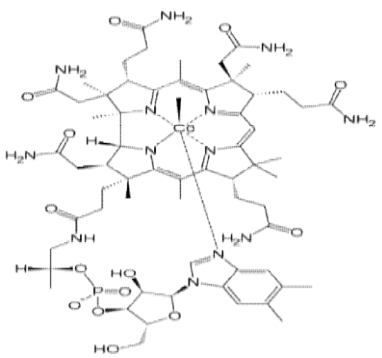

Fig 2: Chemical structure of Methylcobalamin

A literature review surveys scholarly articles, books and other sources relevant to a particular issue or an area of research. Basically, various journals are searched to collect the literature. For the present work, the articles about the developed HPLC method for the simultaneous estimation of Pregabalin and Methylcobalamin in a formulation are collected and referred. From those articles and collected information, a method was designed which varies from the previous methods in that literature and that method will be developed.

2. Methodology

Instruments used: The list of instruments used in the course of experimental work is as follows.

Materials: The experimental work involves several chemicals. Chemicals used presently are listed below:

Selection of Detection wavelength:

The sensitivity of the HPLC method depends upon the proper selection of wavelength. Drug solution of 100 µg/ml was scanned over the range of 200-400 nm in UV region using different solvents like water, acetonitrile, hexane and methanol. It was observed that the formulation showed maximum absorbance in methanol, water at 210 nm and hence methanol and water was used as solvent and 210 nm was used as maximum wavelength for detection of pregabalin and methyl cobalamin for further study.

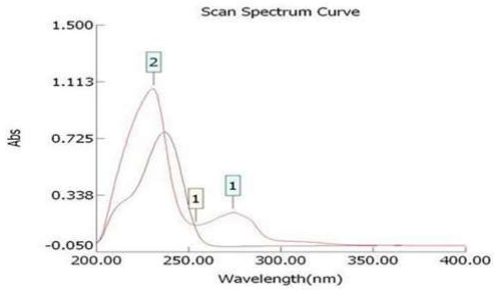

Fig 3: Overlay spectrum of Pregabalin and Methylcobalamin

Method development

Selection of mobilephase:

Experiments were conducted with mobile phase consisting of water and methanol and trails were conducted taking different combinations of mobile phases to achieve maximum possible theoretical plates, least possible tailing factor and retention time. Based on this data, the best separation was obtained with water and methanol (40:60) mobile phase composition.

Preparation of Standard stock solutions: From the formulation, each capsule is weighed and from that amount of power required for 100mg pregabalin is calculated. From that, a solution containing 1000 mcg/ml pregabalin and 100 mcg/ml methylcobalamin is prepared.

Preparation of Standard working solutions (100% solution): 10 ml from each stock solution was pipette out and taken into a 100 ml volumetric flask and made up with diluent. (100µg/ml pregabalin and 10 µg/ml methyl cobalamin)

Preparation of Dilutions: Dilute the working standard solution (100µg/ml) by pipetting 2, 4, 6 and 8 ml of 100 µg/ml solution into 10 ml volumetric flasks and make up the volume with diluents. This gives dilutions of 20, 40, 60 and 80 µg/m solutions respectively.

Chromatographic conditions:

Mobile phase : Water and Methanol taken in the ratio 40:60
Flow rate : 0.6 ml/min
Column : Unisol C18 (3µm, 4.6 ×150mm)
Detector wave length: 210nm
Column temperature : 30°C
Injection volume : 10µL
Run time : 10 min
Inference : Peak obtained for both Pregabalin and Methylcobalamin was good with excellent peak characteristics and it was eluted at 5.540 min and 2.593min for pregabalin and methylcobalamin respectively. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

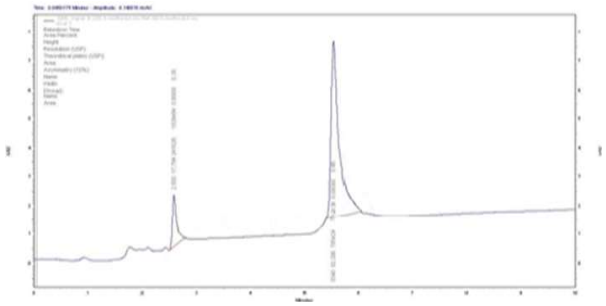

Fig 4: Optimized chromatogram for Pregabalin and Methylcobalamin

3. Results and Discussion

Method validation

Method validation was carried on accordance with ICH guidelines Q_2 (R_1). The method validation parameters like Specificity, Linearity, Accuracy, Precision, LOD & LOQ, robustness and System suitability.

System Suitability: The system suitability parameters were determined by preparing standard solutions of pregabalin and methylcobalamin combination in 10:1 ratio and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Specificity:

The system suitability parameters were determined by preparing standard solutions of pregabalin and methylcobalamin combination in 10:1 ratio and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

Linearity:

Linearity were found by preparing various dilutions from the working standard solution and recording their responses at the optimized set of chromatographic conditions. The calibration plots were constructed between concentrations versus peak areas and the linearity was found in the range from 50µg/ml to 150µg/mland 5µg-15µg of Pregabalin and Methylcobalamin. The regression equation and correlation coefficient were calculated. And the correlation coefficient was found to be 0.9997 and 0.999. (NLT 0.9988).

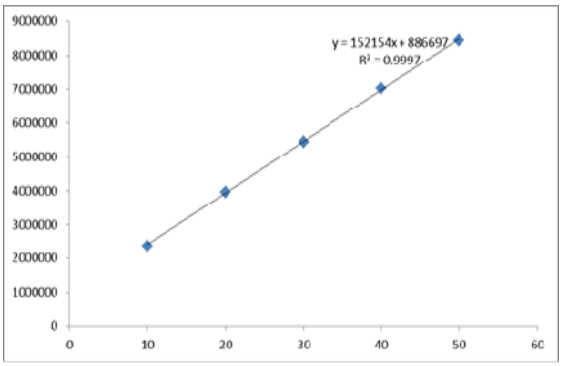

Fig 5: Calibration curve of Pregabalin

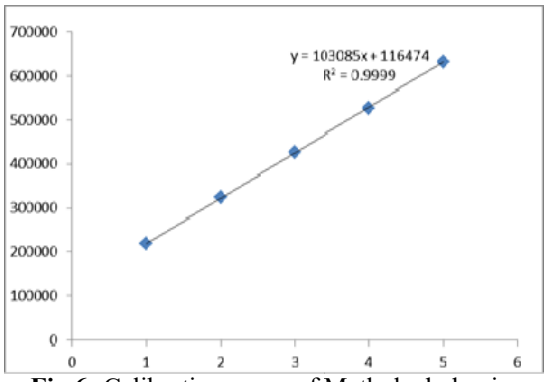

Fig 6: Calibration curve of Methylcobalamin

Accuracy: To the preanalyzed sample three different amounts of 50%, 100% and 150 % of working standard was added, at each level 3 replicate samples were prepared and samples were analyzed to determine percentage recovery from the sample. Percentage recovery is calculated for all nine readings from the ratio of amount of drug found .Further statistical parameters such as percentage recovery are calculated.

Precision: The precision was determined for pregabalin and methylcobalamin in formulation in terms of intraday precision and interday precision. Sample solution of 30µg/ml was prepared and injected into the system six times in different time intervals within a day (intraday) and at 6 different days (interday).Statistical parameters such as mean, standard deviation and percentage relative standard deviation are calculated.

Intermediate precision/Ruggedness

The intermediate precision study was performed for five injections of Pregabalin and Methylcobalamin. Each standard injection was injected into chromatographic system. The area of each standard injection was used for calculation of % RSD.

LOD & LOQ: The LOD value was found at 2.69µg/ml for pregabalin and 1.74µg/ml for methylcobalamin where the signal to noise ratio is found to be 3:1 and the LOQ value was found at 8.18µg/ml for pregabalin and 5.27µg/ml for methylcobalamin with a signal to noise ratio of 10:1.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.6ml/min), Flow plus (1.0ml/min), mobile phase minus, mobile phase plus, was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

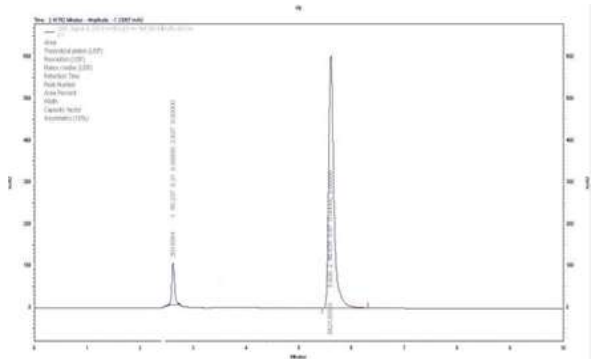

Fig 7: Chromatogram for flow rate variation (0.6 ml/min)

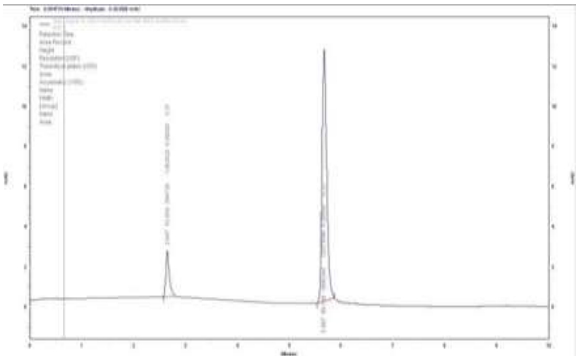

Fig 8: Chromatogram for flow rate variation (1.0 ml/min)

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of pregabalin and methylcobalamin combination in 10:1 ratio and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined.

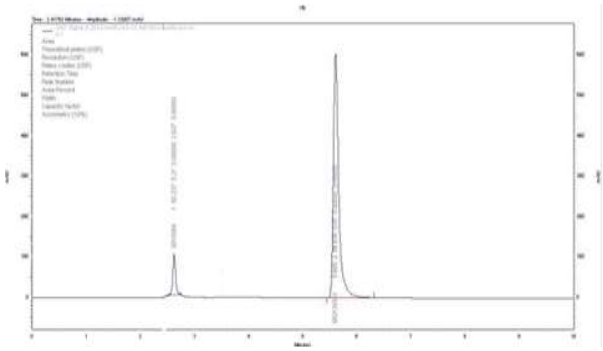

Fig 9: System suitability Chromatogram of injection 1

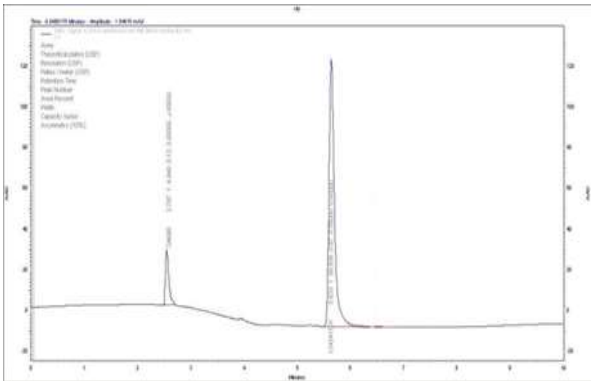

Fig 10: System suitability Chromatogram of injection 2

Table 1: List of Instruments

Instrument	Model No.	Software	Manufacturer's name
HPLC Alliance	Waters 2695	Empower	Waters
PDA Detector	Waters 996		
UV double beam spectrophotometer	UV 3000	UV Win 5	Lab India
Digital weighing balance	BSA224SCW	-	Satorius
pH meter	AD102U	-	Lab India
Ultra sonicator	SE60US	-	-
Suction pump	VE115N	-	-

Table 2: List of Chemicals

S.No.	Chemical	Manufacturer	Grade
1	Water	Merck	HPLC Grade
2	Methanol	Merck	HPLC Grade
3	Acetonitrile	Merck	HPLC Grade

4	NaOH	Merck	A.R
5	HCl	Merck	A.R
6	Pregabalin & Methylcobalamine	-	-
7	Prelin M-75mg Cap	Local Pharmacy	-

Table 3: Accuracy results of Pregabalin

% Level	Standard amount(mcg/ml)	Spiked amount(mcg/ml)	Amount found(mcg/ml)	% Recovery	Mean %Recovery
50%	30	15	44.9	99.9	99.8
	30	15	44.6	99.6	
	30	15	45	100	
100%	30	30	60	100	99.9
	30	30	59.9	99.9	
	30	30	59.7	99.8	
150%	30	45	74.9	99.9	99.9
	30	45	74.6	99.7	
	30	45	75	100	

Table 3: Accuracy results of Methylcobalamine

% Level	Standard amount(mcg/ml)	Spiked amount(mcg/ml)	Amount found(mcg/ml)	% Recovery	Mean %Recovery
50%	3	1.5	4.49	99.4	99.5
	3	1.5	4.45	99.1	
	3	1.5	4.5	100	
100%	3	3	6.01	100	99.5
	3	3	5.88	99	
	3	3	6.03	100	
150%	3	4.5	7.5	100	99.8
	3	4.5	7.47	99.6	
	3	4.5	7.49	99.9	

Acceptance Criteria: The % Recovery for each level should be between 98.0 to 102%.

Table 4: Repeatability results for Pregabalin

S. No	Retention time	Area
1	5.54	785424
2	5.48	784321
3	5.55	784341
4	5.56	786521
5	5.54	785561
6	5.58	784321
Mean	5.54.4	785083.17
S.D	0.0342	910.86
%RSD	0.61	0.11

Table 6: Repeatability results for Methylcobalamin

S. No	Retention time	Area
1	2.593	241628
2	2.596	241742
3	2.61	240946
4	2.594	241346
5	2.593	241564
6	2.594	241594
Mean	2.597	241470

S.D	0.0066	287.44
%RSD	0.26	0.12

Table 8: Intermediate precision results for Methylcobalamin

S. No	Retention time	Area
1	2.593	241628
2	2.594	242742
3	2.593	241546
4	2.596	241694
5	2.592	240642
6	2.596	241946
Mean	2.594	241699.7
S.D	0.0017	677.43
%RSD	0.06	0.28

Table 9: LOD & LOQ

Drug	LOD	LOQ
Pregabalin	2.69	8.18
Methylcobalamin	1.74	5.27

Table 10: Robustness data for Pregabalin and Methylcobalamin

S.No.	Condition	%RSD	
		Pregabalin	Methylcobalamin
1	Flow rate (-) 0.6ml/min	1.08	1.03
2	Flow rate (+) 1.2ml/min	0.58	1.01
3	Mobile phase (-) 40:60	0.72	0.23
4	Mobile phase (+) 70:30	0.72	0.26

Table 11: System suitability parameters for Pregabalin & Methylcobalamin

S.No.	Retention time(R_t)		Tailing factor		Theoritical plates	
	Pregabalin	MCN	Pregabalin	MCN	Pregabalin	MCN
1	5.54	2.593	1.41	1.14	10509	6757
2	5.54	2.593	1.43	1.09	9896	6724
3	5.54	2.593	1.38	1.12	9959	6596
4	5.54	2.593	1.42	1.08	10324	6973
5	5.54	2.593	1.39	1.13	10102	6762
6	5.54	2.593	1.43	1.12	9936	6636

4. Conclusion

A simple, rapid, reliable, robust and optimized reversed phase high performance liquid chromatographic method for the simultaneous estimation of Pregabalin and Methylcobalamin in formulation was successfully developed and validated as per International Conference on Harmonization guidelines. The optimized method was achieved using Unisol C-18 (150 mm × 4.6 mm × 3 µm) column with mobile phase consisting of mixture of water and methanol (40: 60 v/v) with a flow rate of 0.6ml/min at 210nm.The developed method was found linear over the concentration range of 50-150µg/ml for Pregabalin. The detection limit was found to be 2.69µg/ml and 1.74µg/ml and quantitation limit was found to be 8.18µg/ml and 5.27µg/ml for Pregabalin and Methylcobalamin Respectively.There are no interfering peaks underperformed degradation conditions. Therefore, a sensitive, accurate and stability indicating method was developed with high degree of practical utility.It can be concluded that the developed reverse phase HPLC isocratic method is accurate, precise, linear, rugged and robust. Accordingly, the method can be used for the routine analysis of Pregabalin and Methylcobalamin in capsules.

5. References

[1] B.k Sharma et al. Instrumental methods of chemical analysis, Introduction to analytical chemistry, 23rd Edition Goel publication , Meerut, (2007)

[2] Lindholm.J et al. Development and Validation of HPLC Method for Analytical and Preparative purpose. Acta Universitatis Upsaliensis. 2004: Pg .13-14.

[3] Rashmin. An introduction to analytical Method Development for Pharmaceutical formulations. Indoglobal Journal of Pharmaceutical Sciences. 2012: Vol.2, Issue 2, Pg 191-196.

[4] Malvia R, Bansal V, Pal O.P and Sharma P.K. et al. A Review of High Performance Liquid Chromatography. Journal of Global Pharma technology (2010)

[5] Douglas A Skoog, F. James Holler, Timothy A. Niemen et al. Principles of Instrumental Analysis. Pg 725-760.

[6] Dr.S. Ravi Shankar et al. Text book of Pharmaceutical analysis, Fourth edition, Pg 13.1-13.2

[7] David G.Watson et al. Pharmaceutical Analysis, A text book for Pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited; 2nd Ed., Pg 221-232.

[8] Remingtonn et al. The Sciences and Practise of Pharmacy, 20th Edition (2000)

[9] Connors Ka et al. A Textbook of Pharmaceutical Analysis, Wiley intersciences Inc; Delhi. 1994: 3rd Ed, Pg 373-421.

[10] Gurdeep R.Chatwal and Sham K .Anand et al. Instrumental Methods of Chemical Analysis. 2007: Pg 2.566-2.638.

[11] David G. Watson et al. Pharmaceutical Analysis: A text book for pharmacy students and Pharmaceutical Chemists. Harcourt Publishers Limited. 2nd Ed.,Pg-267-311

[12] Nasal.A, Siluk.D, and Kaliszan.R. et al. Chromatographic Retention Parameters in Medicinal Chemistry and Pharmacology. Pubmed. 2003: Vol.10, Issue 5 Pg no-381-426.

[13] Ashok Kumar, Lalith Kishore, navpreet Kaur , Anroop Nair et al. Method Development and Validation for Pharmaceutical Analysis. International Pharmaceutica Sciencia. 2012: Vol 2, Issue 3, Jul-Sep.

[14] Kaushal.C, Srivatsava.B et al. A Process of Method Development: A Chromatographic Approach. J Chem Pharm Res. 2010: Vol.2, Issue 2, 519-545.

[15] Vibha Gupta, Ajay Deep Kumar Jain, N.S.Gill, Kapil et al. Development and Validation of HPLC method. International Research Journal of Pharmaeutical and Applied Sciences. 2012: Vol 2, Issue 4.

[16] Hokanson GC et al. A life cycle approach to the validation of analytical methods during Pharmaceutical Product Development. Part 1: The Initial Validation Process. Pharm Tech. 1994: 92-100

[17] Green JM et al. A Practicle guide to analytical method validation, Anal Chem. 1996: 305A-309A

[18] ICH, Validation of analytical procedures: Text and Methodology. International Conference on Harmonization, IFPMA , Geneva , (1996)

[19] Ewelina rutkowska, Karolina paj k and Krzysztof J”ewiak et al. Lipophilicity: Methods of determination and its role in medicinal chemistry Acta Poloniae Pharmaceutica n Drug Research. 2013: Vol. 70 No.1 pp. 3n18.

[20] IUPAC. Compendium of Chemical Terminology, 2nd edn. (The Gold Book). PAC69, 1137 (1997). Glossary of terms used in computational drug design (IUPAC Recommendations.

[21] K. D. Tripathi et al. Essentials of Medical Pharmacology, 6th Edition, Jaypee brother’s medical publishers (P) LTD, p-254-255.

[22] Indian Pharmacopoeia, Indian Pharmacopoeial Commission, Controller of Publication, Government of India, Ministry of health and Family Welfare, Ghaziabad, India. 2010: 1657-1658.

[23] British Pharmacopoeia, The British Pharmacopoeial Commission, the stationary office, UK, London. 2011: 1408-1409.

[24] http://www.drugbank.ca/drugs/DB00331

[25] M. Schweitzer, M. Pohl, M. Hanna-Brown, P. Nothercote, P. Borman, G. Hasen, K. Smith, J. Larew et al. Implications & Opportunities of Applying QbD Principles to Analytical Measurements. Pharm. Tech. 2010: Vol.34, issue2.

[26] P. Nothercote, P. Borman, M.Chatfield, D.Thompson, K. Truman et al. The Application of QbD to Analytical Methods. Pharm. Tech 2007.

[27] F.G. Vogt, A.S. Kord et al.REVIEW Development of Quality-By-Design Analytical Methods. J. Pharm. Sci. 2010: 100 797-812.

[28] Benoit Viollet, Bruno Guigas, Nieves Sanz Garcia, Jocelyne Leclerc, Marc Foretz, and Fabrizio Andreelli et al. Cellular and molecular mechanisms of Pregabalin: An overview, Clincal Science (London). 2012: 122(6): 253–270.

[29] Kasawar G.B and Farooqui M.N, 2010. Development and validation of HPLC method for the determination of pregabalin in capsules. Indian Journal of Pharmaceutical Sciences, 72 (4), 517-519.

[30] Baheti K.G and Galande V.R. et al. Validated Simultaneous Estimation of Gabapentin in the Presence of Methylcobalamin in Tablet by HPTLC Method. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011: 2 (3), 1199-1202.

[31] Vijaya Lakshmi.G and Yerramsetty Gowtham et al. RP-HPLC method development and validation for the determination of methylcobalamin and pregabalin in combined capsule dosage form. Int. J. Res. Pharm. Sci. 2013: 4(1), 25-29.

[32] Saravanan.J., Shajan A., Joshi N.H., Varatharajan R. and Valliappan K. et al. A Simple and validated RP-HPLC method for the estimation of methylcobalamin in bulk and capsule dosage form. International Journal of Chemical and Pharmaceutical Sciences. 2010: 1 (2), 13-16.