THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

B.Lakshmi et al, The *Experiment, August, 2012, Vol.1 (2), 84-92*

RP-HPLC METHOD DEVELOPMENT FOR THE QUANTIFICATION OF GABAPENTIN IN FORMULATIONS

ABSTRACT

A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Gabapentin in tablet dosage form. Isocratic elution at a flow rate of 1 ml/min was employed on a symmetry Zodiac C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: Acetonitrile: Ortho phosphoric acid 65:33:2 % (V/V/V). The UV detection wavelength was 216nm and 20µl sample was injected. The retention time for Gabapentin was 3.7 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Gabapentin in tablet dosage form and bulk drug.

KEY WORDS: Gabapentin, RP-HPLC, UV detection, recovery, precise, 216 nm, Recovery

INTRODUCTION

Gabapentin [1-(amino methyl)-cyclohexaneacetic acid] is a cyclic GABA [gamma - amino butyric acid] analogue (Fig 1). Although, it is structurally related to GABA, gabapentin has no direct GABA mimetic effect. Gabapentin is originally developed for the treatment of epilepsy. It is widely used to relieve pain, especially neuropathic pain. It is well tolerated in most patients, has a relatively mild side effect profile and passes through the body unmetabolized. Its exact mechanism of action is unknown, but its therapeutic action on neuropathic pain is thought to involve voltage- gated N - type calcium ion channels. It is thought to bind to the α2∂, subunit of the voltage-dependent calcium channel in the central nervous system.

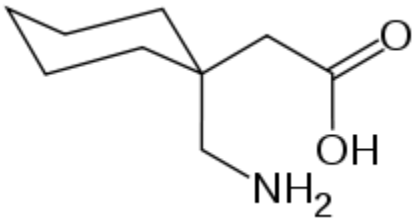


Figure 1: Structure of Gabapentin

Gabapentin has been found to be effective in prevention of frequent migraine headaches . It may be effective in reducing pain spasticity in all multiple sclerosis. It has also had success in treating certain instances of complex Regional Pain Syndrome. It has also been found to help patients with post - operative chronic pain Symptoms of this include a tingling sensation near or around the area where the operation was performed

Most of the HPLC assay procedures for the determination of gabapentin are based on the same approach, involving a simple automated O - phthaldehyde (OPA) derivatization followed by HPLC separation in acidic mobile phases and fluorometric detection. Although the derivatization step is simple and rapid, the OPA - derivative was only stable for 25 min and, therefore, less suitable for routine clinical monitoring. Most of the analysis of gabapentin was depend on derivatization with other reagent. In these cases, the

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

B.Lakshmi et al, The *Experiment, August, 2012, Vol.1 (2), 84-92*

derivatization condition was time consuming and the stability of the reaction products depends on experimental conditions such as pH, temperature and reaction time.

EXPERIMENTAL

Materials

Working standard of Gabapentin was obtained from well reputed research laboratories. Acetonitrile, Methanol,OPA was purchased from E. Merck (Mumbai, India).

Apparatus

A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system, Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), a manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

Determination of wavelength of maximum absorbance

The standard solutions of Gabapentin were scanned in the range of 200 -400 nm against mobile phase as a blank. Gabapentin showed maximum absorbance at 216 nm. So the wavelength selected for the determination of Gabapentin was 216 nm.

Chromatographic equipment and conditions

The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system Rheodyne manual sample injector with switch (77251), Analytical column Chromosil 100-5 C18. 250×4.6mm, manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software was used.

The mobile phase consisted of a Methanol: Acetonitrile: OPA 65:33:2 (v/v/v). Injections were carried out using a 20 µl loop at room temperature (20 + 2 °C) and the flow rate was 1 ml/min. Detection was performed at 216 nm with 10 min runtime.

Standard and sample solutions

A 10 mg amount of Gabapentin reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. From standard solution, by the serial dilution The required concentrations including standard concentration of 120 ppm was prepared.

A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Gabapentin was accurately weighted and quantitatively transferred into a 100 ml volumetric flask. Approximately 25 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 120 ppm.

Method validation

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

RESULTS AND DISCUSSION

System Suitability

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates. The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2500. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table.1. Standard chromatogram was given in Figure.2

S.NO	Mobile phase	Methanol: Acetonitrile: OPA 65:33:2 (v/v/v)
1	Pump mode	Isocratic
2	pH	4.2
3	Diluents	Mobile phase
4	Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
5	Column Temp	Ambient
6	Wavelength	216 nm
7	Injection Volume	20 µl
8	Flow rate	1 ml/min
9	Run time	10 minutes
10	Retention Time	3.7 minutes

Table.1 System suitability parameters

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

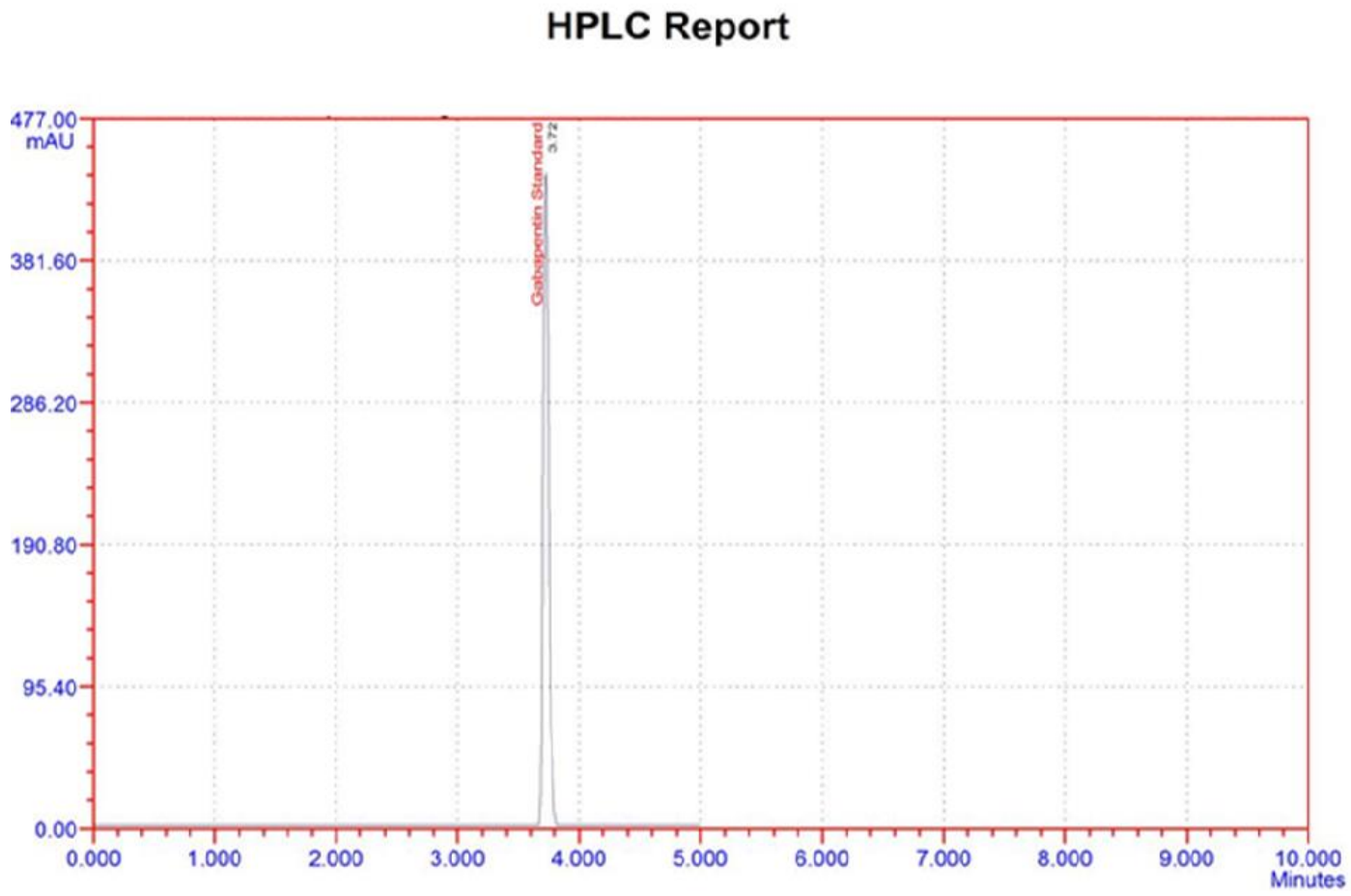


Figure.2

Range of linearity

Standard curves were constructed daily, for three consecutive days, using seven standard concentrations in a range of 30, 60, 90, 120, 150 and 180 ppm for Gabapentin. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 11122.29 + 7310.505x (r^2= 0.999). Linearity values can shown in Table: 2

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

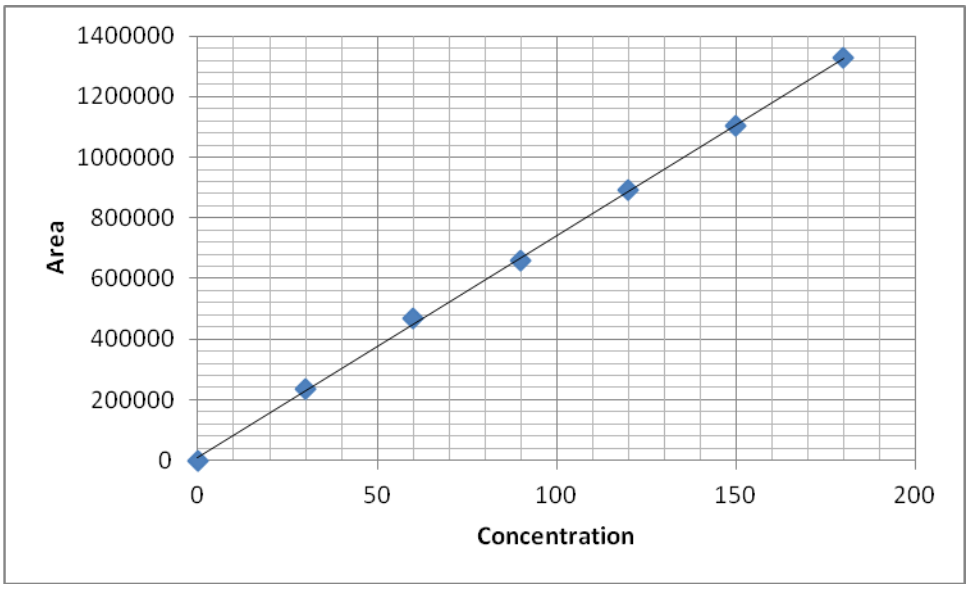
ISSN 2319-2119

RESEARCH ARTICLE

B.Lakshmi et al, The *Experiment, August, 2012, Vol.1 (2), 84-92*

LEVEL	CONCENTRATION OF GABAPENTIN IN PPM	PEAK AREA
Level 1	0	0
Level 2	30	234918
Level 3	60	467365
Level 4	90	659982
Level 5	120	891820
Level 6	150	1101962
Level 7	180	1327427
Range 30 ppm to 180 ppm	SLOPE INTERCEPT CORREALATION COEFFICIENT	7310.505 11122.29 0.999784

Table.2



Graph.1

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

Precision

To study precision, six replicate standard solutions of Gabapentin (120 ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table.3 and Table.4.

Precision Results for Gabapentin:

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTER DAY RSD (Acceptance criteria ≤ 2.0%)
Gabapentin	120	1	891820	0.256
		2	891195	
		3	892671	
		4	891562	
		5	891095	
		6	897107	

Table.3

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTRA DAY RSD (Acceptance criteria ≤ 2.0%)
Gabapentin	120	1	892781	0.29637
		2	891098	
		3	894371	
		4	897819	
		5	895687	
		6	891209	

Table.4

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

Limit of Detection and Limit of Quantification:

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 2.5 ppm dilution Peak was not clearly observed, based on which 2.5 ppm is considered as Limit of Detection and Limit of Quantification is 7.5 ppm.

Parameter	Measured Value
Limit of Quantification	8.25 ppm
Limit of Detection	2.5ppm

Table.5

Ruggedness:

Ruggedness was performed by using six replicate injections of standard and sample solutions of concentrations which were prepared and analyzed by different analyst on three different days. Ruggedness also expressed in terms of percentage relative standard deviation.

Sample (µg/ml)	Area
1	897166
2	890783
3	897621
4	891195
5	899432
6	891004
RSD	0.441895

Table.6

Robustness

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

S.NO	Parameter	Change	Area	% of Change
1	Standard	……	285700.5	……
2	MP	MeOH :ACN:OPA 85:13:2 45:53:2	 897181 890277	 0.60 0.17
3	P^H	4.4 4.0	892987 885656	0.13 0.69
4	WL	214nm 218nm	901782 889271	1.11 0.28

THE EXPERIMENT
INTERNATIONAL JOURNAL OF SCIENCE AND TECHNOLOGY

ISSN 2319-2119

RESEARCH ARTICLE

B.Lakshmi et al, The *Experiment, August, 2012, Vol.1 (2), 84-92*

Table.7

Recovery

Recovery test was performed at 3 different concentrations i.e. 90ppm, 120ppm, 150ppm. Results are given in table.7

% Recovery					
	Target Conc., (ppm)	Spiked conc, (ppm)	Final Conc, (ppm)	Conc., Obtained	% of Recovery
50%	60	30	90	89.27	99.78
	60	30	90	89.71	99.52
	60	30	90	89.56	99.27
100%	60	60	120	120.47	100.51
	60	60	120	121.47	101.22
	60	60	120	120.39	100.32
150%	60	90	150	149.61	99.74
	60	90	150	151.74	101.16
	60	90	150	152.91	101.94

Table.8

Formulation Analysis

S.NO	Tablet	Dosage	Sample conc	Sample estimated	% of Drug Estimated in Tablet
1	Neurontin	100mg	120ppm	118.72	98.93

Table 9: formulation results

CONCLUSION

The proposed method for the assay of Gabapentin in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

REFERENCES:

1. Frye, A. Mark. J. Clinical Psychopharmacology. 2000; 20:607-614.
2. Mack, Alicia. J. Managed Care Pharmacy. 2003; 9:559-568.
3. P. N. Patsalos. Seizure. 1994; 3:163-170.
4. M. J. McLean, Gabapentin: chemistry, absorption, distribution and excretion. In: R. Levy, R. Mattson, B. Meldrum, eds. Antiepileptic Drugs, New York, Raven Press Ltd. 1995; 843-849.
5. M. M. Kushnir, J. Crosset, P. I. Brown, F. M. Urry. J. Anal. Toxicol. 1999; 23:1-6.
6. C. E. Wolf, J. J. Saady, A. Poklis, J. Anal. Toxicol. 1996; 20:498-501.

7. D. C. R. Borrey, K. O. Godderis, V. I. L. Engerlrelst, D. R. Bernard, M. R. Langlois, Clin. Chim. Act. 2005; 354:147-151.
8. T. M. Chen, J. J. Contario, J. Chromatogr. 1984; 314:495-498.
9. T. M. Chen, R. R. Fike, Chromatography. 1990; 11:4-5.
10. S. George, L. Gill, R.A. Braithwaite, Clin. Biochem. 2000; 37:338-342.
11. Z. Zhu, L. Neirinck, J. Chromatogr. 2002; 779:307-312.
12. S. M. Cetin, S. Atmaca, J. Chromatogr. 2004; 1031:237-242.
13. T. A. C. Vermeij, P. M. Edelborek, J. Chromatogr. B. 2004; 810:297-303.
14. L. L. Garcia, Z. K. Shihabi, K. Oles, J. Chromatogr. B. 1995; 669:157-162.
15. F. M. Lin, H. S. Lin, H. S. Kou, S. M. Wu, S. H. Chen, H. L. Wu, Anal. Chim. Act. 2004; 523:9-14.
16. J. Al - Zehouri, S. Al - Madi, F. Belal, Arzeneim Forsch. 2001; 51:97-103.
17. N. Olgun, S. Erturk, S. Atmaca, J. Pharm. Biomed. Anal. 2002; 29:1-5.
18. F. Belal, H. Abdine, A. Al - Majed, N. Y. Khalil, J. Pharm. Biomed. Anal. 2002; 27: 253-260.
19. "The United states Pharmacopoeia, 30th revision, Asian Edition, United states pharmacopoeial convention, Inc., Twin brook Parkway, Rockville, MD. 2007: 2200.
20. F. Feigl. Spot tests in organic analysis. Chapter 3: Preliminary (Exploratory tests), 6th ed., Elsevier publishing company, Amsterdam. 117-118.
21. International Conference on Harmonization of technical requirements for the registration of pharmaceuticals for Human Use (ICH) Q2A. Validation of Analytical Methods (Definitions and Terminology) (October 1994).
22. ICH: Q2B Analytical Validation Methodology (November 1996).
23. A. Gupta, A. B. Ciavarella, V. A. Sayeed, M. A. Khan, P. J. Faustino, J. Pharm. Biomed. Anal. 2008; 46:181-186.

B.Lakshmi, Kallam Haranadhreddy Institute of Technology, Guntur

Prof. K.Saraswathi, Retd S.V.University

Prof T.V.Reddy , Prof Malla reddy College of Engineering, Secunderabad**.**

Email: lakshmi_bumi@yahoo.com