METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF GABAPENTIN IN BULK FORM AND MARKETED PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

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ABSTRACT : *A New Analytical, simple, precise, accurate and robust high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the estimation of Gabapentin in bulk form and Marketed Pharmaceutical Dosage form. Chromatographic separation was optimized by gradient HPLC on a Symmetry C18, 250 mm x 4.6 mm and 5µm Column utilizing a mobile phase consisting Acetonitrile: Water in the ratio of 70: 30 v/v at a flow rate of 1 ml/min with UV detection at 240 nm. The retention time of Gabapentin was found to be 2.790min. Good linearity obtained over the range of 10µg/ml to 35µg/ml for Gabapentin. The correlation coefficient was found to be 0.998. The % RSD of precision for Gabapentin was found to be 0.7901. The % mean recovery was found to be 100.355% for Gabapentin. The results obtained for accuracy, precision, LOD, LOQ and ruggedness were within limits. The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for estimation of Gabapentin in bulk form and Marketed Pharmaceutical Dosage form. Thus the validated economical method was applied for the analysis of Gabapentin in bulk form and Marketed Pharmaceutical Dosage form.*

Key Words: Gabapentin, RP-HPLC, Accuracy, Precision, ICH Guidelines.

I.INTRODUCTION

Gabapentin is a structural analogue of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) that was first approved for use in the United States in 1993. It was originally developed as a novel anti-epileptic for the treatment of certain types of seizures - today it is also widely used to treat neuropathic pain. Gabapentin[1] has some stark advantages as compared with other anti-epileptics, such as a relatively benign adverse effect profile, wide therapeutic index, and lack of appreciable metabolism making it unlikely to participate in pharmacokinetic drug interactions. It is structurally and functionally related to another GABA derivative, Pregabalin. Gabapentin[2] is a synthetic analogue of the neurotransmitter gamma-aminobutyric acid with anticonvulsant activity. Although its exact mechanism of action is unknown, gabapentin appears to inhibit excitatory neuron activity. This agent also exhibits analgesic properties. Gabapentin is a unique anticonvulsant that is used as adjunctive therapy in management of epilepsy and for neuropathic pain syndromes. Therapy with gabapentin is not associated with serum aminotransferase elevations, but several cases of clinically apparent liver injury from gabapentin have been reported. Gabapentin[3] is a gamma-amino acid that is cyclohexane substituted at position 1 by amino methyl and Carboxymethyl groups. Used for treatment of neuropathic pain and restless legs syndrome. It has a role as an anticonvulsant, a calcium channel blocker, an environmental contaminant and a xenobiotic. It derives from a gamma-aminobutyric acid. The IUPAC Name of Gabapentin is 2-[1-(amino methyl) cyclo hexyl] acetic acid. The Chemical Structure of Gabapentin is in fig-1.

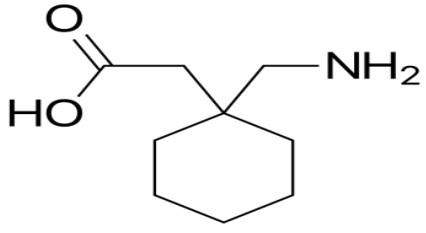

Fig-1: Chemical Structure of Gabapentin

II. EXPERIMENTAL

Table-1: List of Equipments

S.No.	Instruments/Equipments/Apparatus
1.	HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.
2.	ELICO SL-159 UV – Vis spectrophotometer
3.	High Precision Electronic Balance
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Vacuum Filtration kit (Labindia)
6.	Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column
7.	P^H Analyzer (ELICO)

Table-2: List of Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	HPLC grade water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
3.	Dipotassium hydrogen orthophosphate	96%	L.R.	Sd fine-Chem ltd; Mumbai
4.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
5.	Potassium dihydrogen orthophosphate	99.9%	L.R.	Sd fine-Chem ltd; Mumbai
6.	Ortho phosphoric acid	99.9%	L.R.	Sd fine-Chem ltd; Mumbai

Method Development:

HPLC Instrumentation & Conditions:

The HPLC system employed was HPLC WATERS with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer analysis:

10 mg of Gabapentin standard was transferred into 10 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Gabapentin, so that the same wave number can be utilized in HPLC UV detector for estimating the Gabapentin.

Mobile Phase Preparation:

A mixture of above Acetonitrile 700ml (70%) and 300 ml of HPLC grade water (30%) were mixed and degassed in ultrasonic water bath for 15 minutes and filtered through 0.45 µm filter under vacuum filtration.

Preparation of Standard solution: Working concentration should be around 20µg/ml. Accurately weighed around 10mg of Gabapentin working standard, taken into a 25 ml volumetric flask, then dissolved and diluted to

volume with the mobile phase to obtain a solution having a known concentration of about 1000 mcg/ml. Further dilutions have been made to get the final concentration of 20µg/ml.

Preparation of Test solution:

Diluted quantitatively an accurately measured volume of label claim solution with diluents to obtain a solution containing about a linear range.

Method Validation:

The developed method was validated as per ICH guidelines including the parameters specificity, linearity, precision, accuracy, Limit of detection, Limit of quantification and Robustness.

Specificity:

Specificity[4] determines the placebo interference of the related substances or the excipients like diluents, glidants, lubricants and binders in the process of determination of the drug. The excipients were spiked to the drug concentrations and interference was estimated.

Linearity:

Six linear[5] dilutions were prepared by transferring 0.1ml, 0.15ml, 0.20ml, 0.25ml, 0.30ml and 0.35ml from the standard stock solution in to six 10ml volumetric flasks and made up with diluents results in solutions with 10ppm, 15ppm, 20ppm, 25ppm, 30ppm and 35ppm of Gabapentin respectively in six volumetric flasks.

Precision:

Intraday Precision:

It is also called repeatability[6], sample working solution was prepared by multiple sampling from a homogeneous mixture six samples were prepared, injected and reported as %Relative standard deviation.

Inter Day Precision:

It is also called intermediate precision[7], day-day precision and analyst-analyst precision. Sample working solution was prepared by multiple sampling from a homogeneous mixture six samples were prepared and injected on the next day. It was expressed as % Relative standard deviation.

Accuracy:

Three levels of sample solution were prepared 80% (16ppm of Gabapentin), 100% (20ppm of Gabapentin) and 120% (24ppm of Gabapentin) and injected. The % recovery[8] was calculated and reported.

Limit of Detection (LOD):

Limit of detection[9] is the lowest concentration of the drug that can be detected at the detector level without necessary quantification.

Limit of Quantification (LOQ):

Limit of quantification[10] is the lowest concentration of the drug that can be quantified with an accuracy[11] and precision.

Robustness: Small deliberate changes[12] were made in the method like Mobile phase plus and mobile phase minus (5% of Organic solvent) Flow rate plus and flow rate minus (0.1%) temperature plus and minus (5%). And sample working solutions were injected and reported as %Relative standard deviation.

System Suitability:

System suitability[13] for that method was tested by five replicate injections of standard preparation. Plate count, tailing factor, resolution and %RSD were reported.

Assay of Gabapentin in Pharmaceutical Dosage form:

Twenty tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent[14] to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. The solution prepared was injected in five replicates into the HPLC system[15] and the observations were recorded.

A duplicate injection of the standard solution was also injected into the HPLC system and the peak areas were recorded. The data are shown in Table-8.

$$\text{Assay}^{16}\,\% = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution[17] of Standard solution

DT = Dilution of sample solution

P = Percentage purity[18] of working standard

III.RESULTS AND DISCUSSION

Method Development:

Optimized Chromatographic Conditions:

Column : Symmetry C_{18}, 250 mm x 4.6 mm and 5µm Column

Mobile Phase : Acetonitrile: Water = 70:30

Flow Rate : 1.0ml/minute

Wave length : 240 nm

Injection volume : 20 µl

Run time : 6.0 minutes

Column temperature : Ambient

Sampler Temperature : Ambient

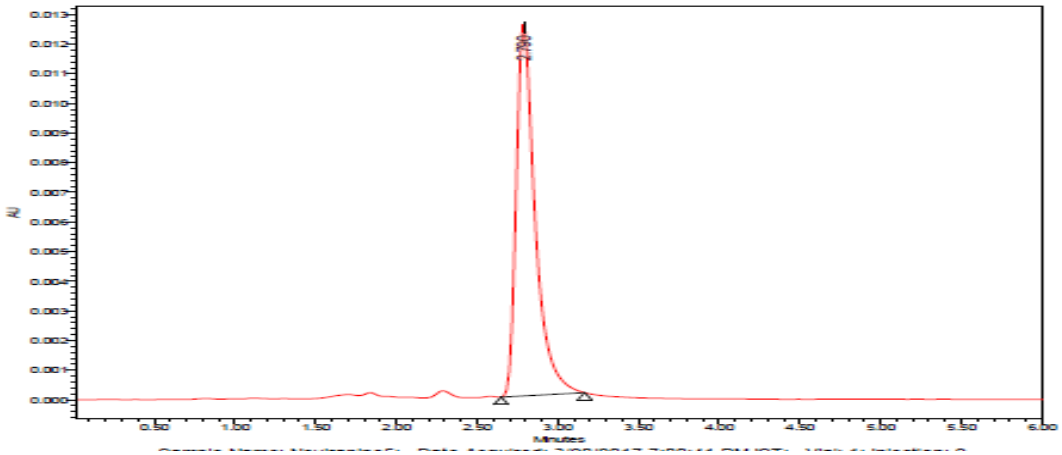

Fig-2: Optimized Chromatogram for Gabapentin

Method Validation:

1. Accuracy:

Recovery study:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Gabapentin were taken and added to the pre-analysed formulation of concentration 20µg/ml. From that percentage recovery[19] values were calculated. The results were shown in Table-3.

Table-3: Accuracy Readings

Sample ID	Concentration (µg/ml)		Peak Area	% Recovery of Pure drug	Statistical Analysis
	Amount Added	Amount Found			
S_1 : 80 %	16	15.90281	90132	99.39257	Mean= 100.285%
S_2 : 80 %	16	16.15674	91571	100.9796	S.D. = 0.811857
S_3 : 80 %	16	16.07733	91121	100.4833	% R.S.D.= 0.809548
S_4 : 100 %	20	20.01239	113421	100.062	Mean= 100.2387%
S_5 : 100 %	20	19.99828	113341	99.99138	S.D. = 0.368952%
S_6 : 100 %	20	20.13256	114102	100.6628	R.S.D.= 0.3680734
S_7 : 120 %	24	24.09233	136542	100.3847	Mean= 100.544%

S_8 : 120 %	24	24.10627	136621	100.4428	S.D. = 0.227462 %R.S.D. = 0.226231
S_9 : 120 %	24	24.19309	137113	100.8045	

2. Precision:

2. 1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Gabapentin (API). The percent relative standard deviation[20] was calculated for Gabapentin are presented in the Table-4.

Table-4: Results of Repeatability Studies

HPLC Injection Replicates of Gabapentin	Peak Area
Replicate – 1	126755
Replicate – 2	128743
Replicate – 3	125874
Replicate – 4	126784
Replicate – 5	127436
Replicate – 6	126343
Average	**126989.2**
Standard Deviation	**1003.395**
% RSD	**0.7901136**

2. 2. Intermediate precision:

2.2.1. Intra-assay & inter-assay:

The intra & inter day variation of the method[21] was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Gabapentin revealed that the proposed method is precise

Table-5: Results of intra-assay & inter-assay

Conc. Of Gabapentin (API) (µg/ml)	Observed Conc. Of Gabapentin (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
16	16.08	0.97	16.03	0.97
20	20.04	0.44	20.03	0.45
24	23.97	0.37	24.05	0.19

3. Linearity & Range:

The calibration curve showed good linearity[22] in the range of 0-35 µg/ml, for Gabapentin (API) with correlation coefficient (r^2) of 0.9986 (Fig-3). A typical calibration curve has the regression equation of y = 5667.x + 1077 for Gabapentin.

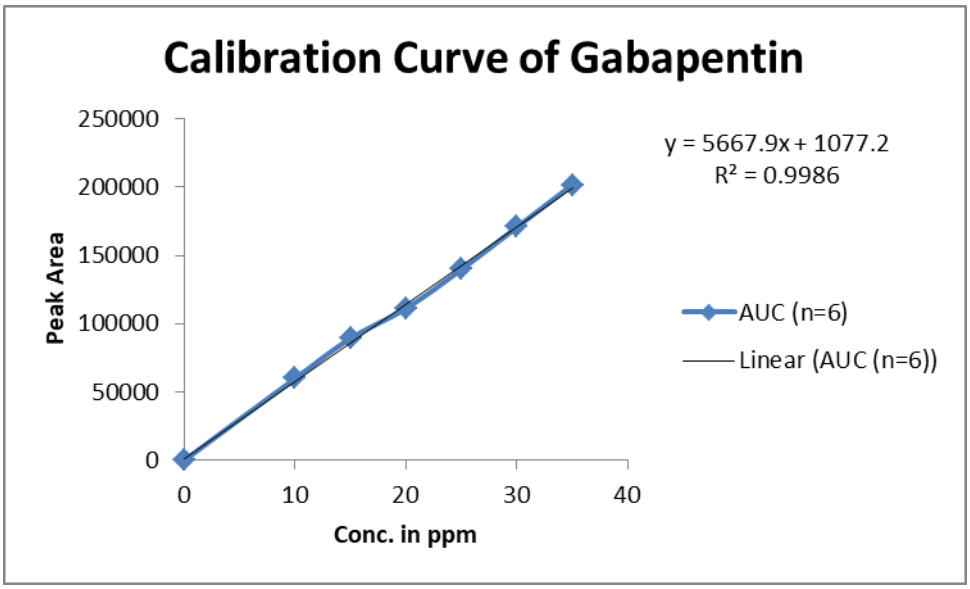

Fig-3: Calibration Curve of Gabapentin (API)

Table-6: Concentration of Gabapentin

CONC.	AUC (n=6)
0	0
10	59895
15	89302
20	111183
25	139851
30	170745
35	201734

4. Robustness:

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Temperature (±2^{0}C), Wavelength of detection (±2nm) & Acetonitrile content in mobile phase (±2%) studied to determine the robustness[23] of the method are also in favour of (Table-7, %RSD < 2%) the developed RP-HPLC method for the analysis of Gabapentin (API).

Table-7: Result of method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.09
Flow (0.9 ml/min)	0.07
Temperature (27^{0}C)	0.06

Temperature (23^0C)	0.14
Wavelength of Detection (242 nm)	0.24
Wavelength of detection (238nm)	0.28

5. LOD & LOQ:

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.06 & 0.18 µg/ml respectively.

6. Assay of Gabapentin in Pharmaceutical Dosage Form:

Table-8: Assay of Gabapentin Tablets

Brand Name of tablets	Labelled amount of Drug (mg)	Mean (±SD) amount (mg) found by the proposed method (n=6)	Mean (± SD) Assay (n = 6)
Neurontin Tablet	300	299.85 (±0.06)	100.02 (±0.48)

Result & Discussion: The assay of Neurontin tablets containing Gabapentin was found to be 100.02 %.

IV.CONCLUSION

In present study Gabapentin estimated by HPLC, good linearity obtained for Gabapentin (10µg/ml-35µg/ml) with Correlation coefficient of 0.9986. The results for precision, recovery system suitability, LOD and LOQ and ruggedness were within limits. Hence the method was successfully applied for HPLC-UV method for estimation of Gabapentin was novel, simple, precise, accurate, robust and cost-effective method. There is no HPLC method reported till now on selected drug. Hence the developed method was suitable for the routine analysis and quality control of pharmaceutical preparations containing Gabapentin either individually or in combination.

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