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Validation of an Isocratic HPLC Assay of Gabapentin in Pharmaceutical formulations and Stress test for Stability of Drug Substance

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ABSTRACT

This paper describes the validation of an isocratic HPLC method for the assay of Gabapentin Capsule and the evaluation of the stability of drug substance after stress test by Photodiode array detection. the HPLC separation was achieved on a Beckman ultra sphere C_{18}, 4.6 mm × 25 cm is suitable using mobile phase water: acetonitrile: Methanol: phosphate buffer solution (55:35:10:0.1) was used . The flow rate was 1ml/min and detection was set at 210 nm. Chromatograph of the standard solution, and record the peak responses as directed under procedure: the column efficiency is not less than 6000 theoretical plates, the tailing factor Gabapentin is not more than 2.0, and the relative standard deviation for replicate injections is not more than 1.0%. Chromatograph the resolution solution, and record the peak responses as directed under procedure: the resolution, R, between Gabapentin and Gabapentin related compound A is not less than 1.5.

Keywords: Gabapentin Capsule, Validation, RP-HPLC, Stability.

INTRODUCTION

Gabapentin (GBP) is described as 1-(aminomethyl)cyclohexanacetic acid) (Fig.1) with a molecular formula of C9H17NO2 and a molecular weight of 171.24, is a new antiepileptic drug which is a structural analogue of neurotransmitter of gamma amino butyric acid (GABA).GBP , unlike GABA has a cyclohexane molecule system and is able to penetrate through Blood brain barrier .GBP is used for the treatment of partial onset seizures with or without secondary generalized tonic-clonic convulsions in clinical practice. .After oral administration, GBP is well absorbed and reaches maximal plasma concentration within 2-3 h. the elimination half life of the drug is 5-7h after a single oral dose of 200-400 mg. GBP is not metabolised and mainly excreted by kidney. The drug does not bind plasma proteins. Pharmacokinetics of GBP is not affected by foods and other drugs. [1-2].

Several pharmacokinetic or therapeutic drug monitoring studies have been reported for the determination of GBP in human biological fluids [3-12]. For gas chromatography (GC) [13],

different detection methods are reported such as flame ionization [14-15] and mass spectrometry (MS) [16] .these methods require derivatization of GBP to improve the volatility and to avoid column interaction . High performance liquid chromatography (HPLC) is also used for this purpose with spectrophotometer [17] and spectroflourometric [18-24] detection. 2, 4, 6 – trinitrobenzene-sulphonic acid (TNBS) [25-27] and phenyl isothiocynate (PITC) [3] are used in spectrophotometric detection while o-pthaldiaaldehyde (OPA) [28-30**]** is used in spectroflourometric detection as derivatizing reagents. The purposes of this study was the development of a simple HPLC method with UV detection for Gabapentin assay in capsule and evaluate the stability of drug substance after stress tests by photodiode array detection.

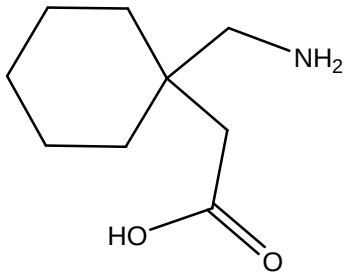


Figure 1 Chemical structure of Gabapentin

MATERIALS AND METHODS

Reagents and Chemicals

HPLC grade acetonitrile, Methanol, Phosphoric acid is obtained from Merck. Water was deionised and purified by a Milli- Q water purification system was used to prepare the mobile phase and sample and standard solutions. A reference standard of Gabapentin was procured from Sun Pharma.

Chromatographic equipment and conditions

The development and validation of the assay is performed on Waters Alliance HPLC system with UV-VIS Detector which include following parts: Fluidics management system (Model: 2695) includes seal wash, degasser, sample heater/cooler and Column heater .UV-VIS Detector (Model No: 2487)-Dual wavelength absorbance detector. Empower chromatography data system (Software). The analytical column 4.6 mm × 25 cm that contains 5 µm packing L1 (Beckmann ultra sphere). The mobile phase consisted of a water: acetonitrile: Methanol: phosphate buffer solution (55:35:10:0.1) was used. The flow rate was 1ml/min and detection was performed at 210 nm

Procedure for determination of Gabapentin in Reference Standard

Dissolve about 150 mg Gabapentin working standard, accurately weighed, in 15 ml mobile phase and sonicate for 15 mins and then the volume was made up to 25 ml with mobile phase .A chromatograms obtained from reference solution is presented in Fig 2 .

Procedure for determination of Gabapentin in Sample Solutions

Weigh and powder the content of 20 capsules, transfer a portion of powder equivalent to 150 mg of Gabapentin into a 25 ml volumetric flask, add about 15 ml of mobile phase and sonicate for 15 minutes, dilute with mobile phase to the volume, centrifuge at 3000 rpm for 10 minutes. A chromatograms obtained from reference solution is presented in Fig 3.

Figure 2: Chromatograms of Gabapentin Reference Standard

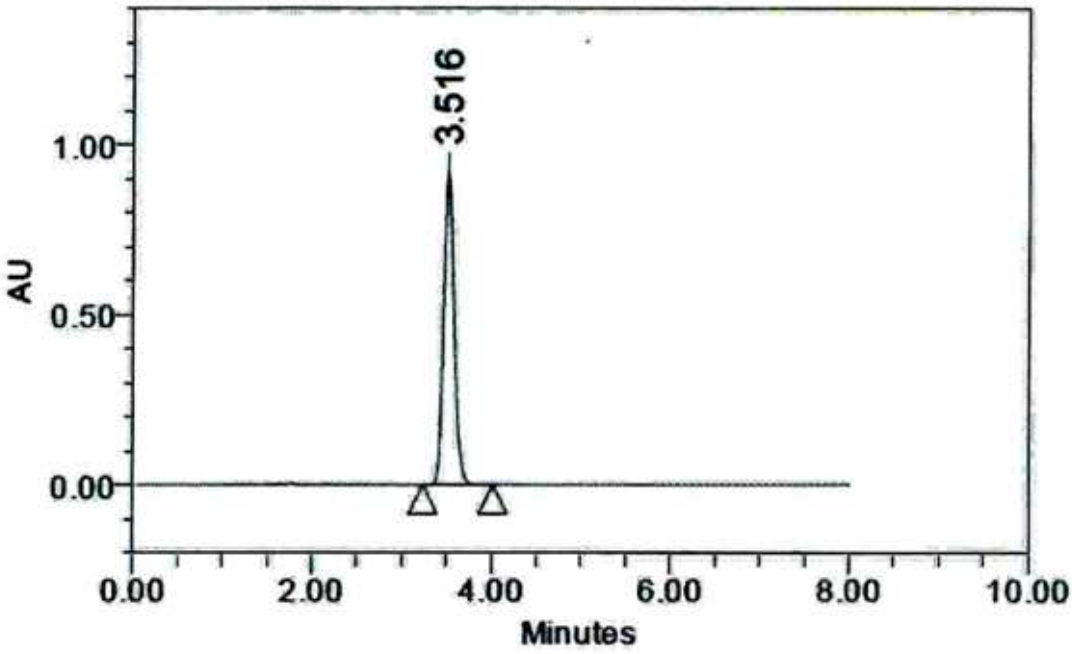
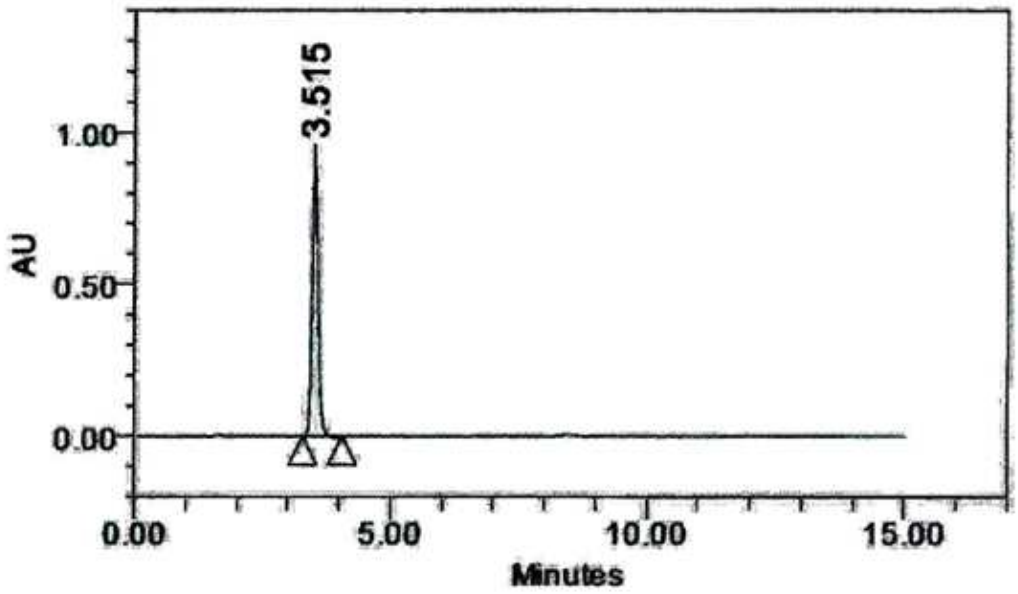


Figure 3 : Chromatograms of Gabapentin Capsule



Method validation

The Method was validated for Specificity, precision (repeatability and intermediate precision), accuracy.

Stress test for Stability

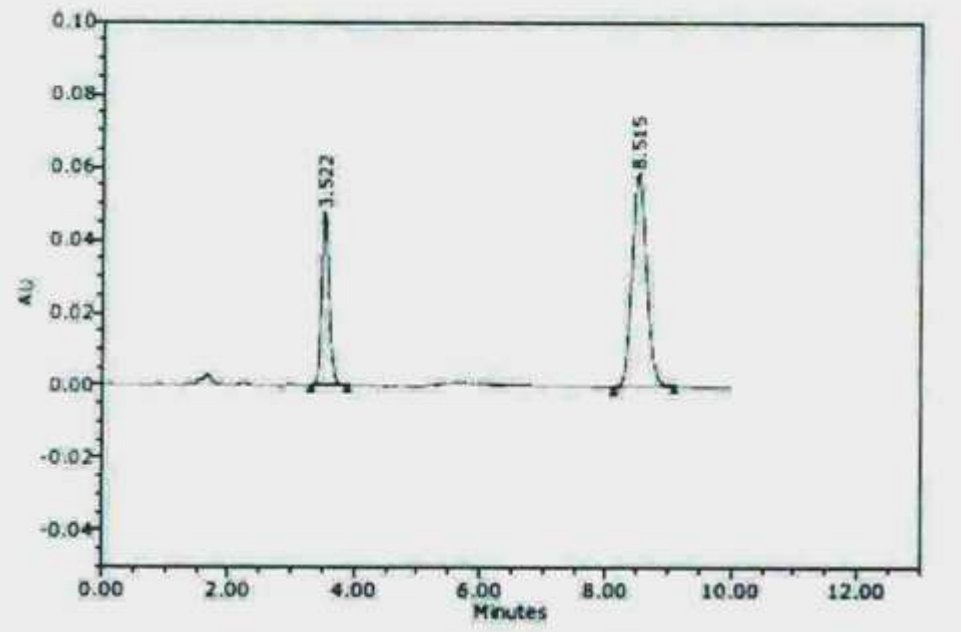
Accelerated degradation studies were performed to provide an indication of the stability of the drug and specificity of the method. Gabapentin reference standard was stressed under conditions that cause degradation included the following Placebo Degradation Preparation, Degradation Products Preparations, Drug Substances Degradation Preparations which involved (Decomposition in solid state and Photolysis in solid state)

RESULTS AND DISCUSSION

System Suitability solution

Having optimised the efficiency of a chromatographic separation the quality of 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 <2000. In all cases the relative standard deviation (R.S.D) for the analyte peak area for two consecutive injections was <2.0 %. A chromatograms obtained from reference substance solution is presented in Fig 4.

Figure 4: Resolution Solution of Gabapentin and Releated A



Specificity

The excipients in the Capsule contained the following in active ingredients: Lactose anhydrous (pharmatose), Maize starch, and Purified talc as excipients Chromatograms of Placebo Capsule solution showed no interference with the main Peak. Chromatograms showed indicated that no excipients or impurities interfered with Gabapentin peak. (Refer figure No. 3 for Sample and 5 for placebo).

Range of linearity:

The linearity of the method was evaluated by a calibration curve in the range of 0.5-50µg/ml of the day (n=5). A calibration curve was constructed by plotting the absorbance versus final concentration of Gabapentin, which showed a linear response showing the linear dynamic range over the concentration range 0.5-50µg/ml.

Figure 5: Chromatogram of Placebo in Capsule formulation

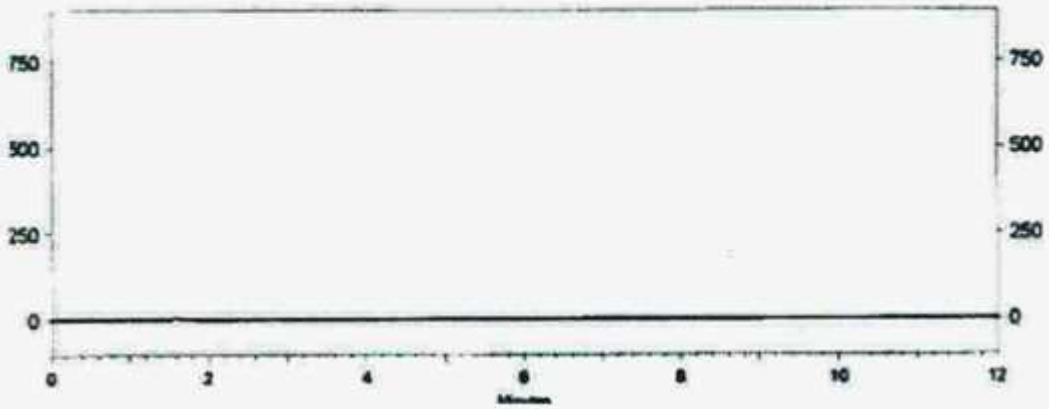


Table 1: Results of Precision:

Strength	Sample No.	Conc. (mg/100 ml)	% Recovery ≤5%	CV (≤5%)	CL (≤mean ± 10%)
NERVZ 100	1	11.75	100.3	0.5	100.5±0.6
	2	11.35	100.8		
	3	12.10	101.3		
	4	10.95	99.9		
	5	11.00	100.5		
	6	11.25	100.0		
NERVZ 300	1	33.90	100.7	0.4	101.1±0.5
	2	35.70	101.6		
	3	31.20	101.0		
	4	35.50	100.8		
	5	33.90	100.9		
	6	33.70	101.8		
NERVZ 400	1	47.10	100.3	0.7	101.0±0.7
	2	47.90	100.1		
	3	46.50	101.9		
	4	43.80	101.2		
	5	44.00	100.9		
	6	45.40	101.5		

Precision (Repeatability):

Method precision was demonstrated by the assay of a series of six samples for different strength of Gabapentin Capsule 100 , 300 and 400mg , prepared by transferring Gabapentin standard with placebo preparation described above , on three consecutive days , the repeatability , Mean , CV and recovery were calculated (Table1)

Table 2: Results of Accuracy:

Analyst	Conc. (mg/100ml)	% Recovery	% Bias (≤5%)
Analyst 1	317.0	102.7	+ 2.7
	626.6	101.0	+ 1.0
	946.4	99.5	-0.5
Analyst 2	285.0	100.6	+0.6
	625.6	101.3	+1.3
	950.0	99.9	-0.1
Analyst 3	313.0	102.9	+2.9
	623.3	100.5	+0.5
	952.0	99.4	-0.6

Accuracy:

The Accuracy of the method was evaluated by determination of the recovery of Gabapentin by three Analysts at three levels concentration. capsule sample solution were spiked with Gabapentin standard solution , corresponding to 75% to 125 % from 3.17-9.5mg/ml. the result showed good recoveries ranging from 99.4% to 102.9. The mean recovery data obtained for each level as well as for all levels combined (Table 2) were within the limits.

The Table below showed the characteristics used during the work with their limits and values obtained. (Table 3) Characteristics, their limits and values obtained

From the table, all the values obtained are complying with the limits, so the method is valid to be used for the determination of Gabapentin in Capsule formulation.

Table 3: Characteristics, their limits and values obtained

Characteristic	Limit	Value obtained		
		NERVZ 10	NERVZ 20	NERVZ 5
Precision:				
-Coefficient of Variance (CV)	≤5%	0.5	0.4	0.7
-Confidence limit (CL)	≤mean ±10%	100.5±0.6	101.1±0.5	101.0±0.7
Specificity:				
-% Interference	≤10%	0.0%	0.0%	0.0%

Stress test and its Evaluation:

Temperature Stress

No degradation for working standard of Gabapentin and its placebo preparation was observed when incubated in closed vials at 65ºC and in open vials at 40ºC/75%RH and 50ºC/75%RH for one month.

Acid Stress
No degradation products of Gabapentin reference substance was observed after refluxing for 48 hours at 65ºC.

Base Stress
Degradation products were observed in Gabapentin reference standard after for 4 hours with 1M. Sodium Hydroxide, due to a high instability of Gabapentin in basic media.

Peroxide stress
No degradation of Gabapentin was observed for reference substance stressed in 10% H_2O_2.

Table 4: Result of Placebo and Placebo Degradation Preparation Specificity Test

Sample/Storage	Response (peak area)	% Interference (≤ 2%)
Placebo at 25°C	0.0	0.0
Placebo at 65°C for 7 days	---	0.0

Table 5 Results of Specificity Test for Drug Substances and Degradation Preparations

(Stability Indicating):

Degradation Condition/ Time	% Recovery	% Drop (≥ 10%)
1 M HCl/65°C/48 hours	73.7	26.3%
1 M NaOH/65°C/4 hours	73.9	26.1%
10% H_2O_2/ 65°C/48 hours	54.6	55.4%
Daylight (Solid)/1 month	101.1	-
UV light (Solid) 1 month	101.5	-
Solid /65/ 1 month	101.3	-
Solid 40°C/75%RH/ 1 month	102.6	-
Solid / 50°C/75%RH/ 1 month	101.9	-

Photolysis in Solid State:
No degradation products of Gabapentin reference substance was observed after exposed to UV light and daylight for one month (Table No.6) for Degradation time and Recovery for different

Stress condition) Result of Placebo and Placebo Degradation Preparation Specificity Test (Table No.4) Also Results of Specificity Test for Drug Substances and Degradation Preparations. (Table No 5).

CONCLUSION

The Proposed method for the assay of Gabapentin in Capsules is very simple and rapid. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drugs from its degradation products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulation

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