ISSN 2063-5346

# STABILITY INDICATING RS METHOD DEVELOPMENT AND VALIDATION OF PREGABILIN BY UPLC

**Raman S G[1], Sundhararajan R[2], Vijayakumar AR[3], Pradeepraj D[4], Parthiban R[5], Mahesh PG[6]**

**Article History: Received: 01.02.2023 Revised: 07.03.2023 Accepted: 10.04.2023**

## Abstract

The objective of the work is to develop a stability indicating RP UPLC related substances method for the determination of PREGABALIN and its related impurities.For that several trials have been carried out using EMPOWER software.

Present study includes: Development of stability indicating related substances by UPLC using EMPOWER software (WATERS).Forced degradation studies according to ICH Guidelines. Validation of the method according to ICH Guidelines.

**Results:** The resolution between pregabalin and amide impurity was found to be 3.2, and peak tailing of pregabalin was found to be 1.2 and %R.S.D was found to be 0.41. Significant degradation was observed at room temperature and after 3,6,12, 24 Hrs.respectively. The results obtained in the solid state stability study indicate that Pregabalin is stable upon exposure to white fluorescent light. The results obtained in the force degradation study Pregabalin was stable at different Stress condition. The specificity of the method was confirmed and the method is stability indicating. The specificity of the method was confirmed and the method is stability indicating. LOQ and LOD values of all the impurities were found to be within the acceptable criteria. Linearity of Pregabalin pharma results**:** R2 values were found to be 0.999, 0.999, 0.990, and 0.999 for Amide, Lactum N-Methyl and Pregabalin respectively.LOQ Precision The %RSD was found to be 0.87, 2.60, 2.80, and 1.40 for Pregabalin, Amide, Lactum, and N-Methyl Respectively. Robustness the resolution between Amide and Pregabalin was found to be >2, peak tailing of Pregabalin was found to be 1.0. In flow variation and temperature variation the RRT remains same.

**Conclusion:** The developed method was successfully validated for its linearity, range, precision, accuracy and specificity in accordance with the requirements of ICH guidelines.The results of the study showed that, the proposed UPLC method was simple, rapid, precise, accurate and stability indicating, which can be used for the routine analysis for the determination of Pregabalin and its related impurities.

**Keywords:** Pregabalin; UPLC; ICH guidelines; photo diode array (PDA) detector.

[1]Mohamed Sathak AJ College of Pharmacy, Sholinganallur, Chennai-600119, Tamilnadu, India.
[2]Mohamed Sathak AJ College of Pharmacy, Sholinganallur, Chennai-600119, Tamilnadu, India
[3]Sree Balaji Medical College and Hospital, BIHER, Chromepet, Chennai-600044. Tamil Nadu, India.
[4]School of Pharmacy, Sri Balaji Vidyapeeth Deemed to be University,Puducheery, India.
[5]Surya School of Pharmacy, Vikravandi, Tamilnadu, India.
[6]School of Pharmaceutical Sciences, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai 600117.

**Address for correspondence :** S.G.Raman, M. Pharm., Associate Professor, Mohamed Sathak AJ College of Pharmacy, Sholinganallur, Chennai-600119. Tamilnadu. Email. rtanush2015@gmail.com

**DOI:10.31838/ecb/2023.12.s1-B.429**

## 1. INTRODUCTION

Ultra performance liquid chromatography (UPLC) is a recent technique in liquid chromatography, which enables significant reductions in separation time and solvent consumption. Literature indicates that UPLC system allows about nine fold decrease in analysis time as compared to the conventional HPLC system using sub-2µm particle size analytical columns, and about threefold decrease in analysis time in comparison with 3-5µm particle size analytical columns without compromise on overall separation. Reducing these separation times without reducing the quality of the separation would mean that important analytical information could be generated more quickly. These particles operate at elevated mobile phase linear velocities to affect dramatic increase in resolution, sensitivity and speed of analysis. As it is very well known from Van Demeter equations, the efficiency of chromatographic process is proportional to particle size decrease. According to this model describing band broadening, it describes relationship between height equivalent of theoretical plate (HETP) and linear velocity, one of the terms (path dependent term), is dependent on a diameter of particle packed into the analytical column. Smaller particle diameter can significantly reduce HETP which results in higher efficiency and the flatter profile of Van Demeter curve. Consequently, the mobile phase flow-rate increase does not have negative influence to the efficiency as it could be observed at 10 or 5 µm particles. The negative aspect of small particle packed columns used in HPLC is, however, high back-pressure generating.

The UPLC system is connected with specially designed Acquity UPLC columns containing X-Terra sorbent of second generation. The hybrid material utilizes bridged ethylsiloxane/silica hybrid (BEH) structure, particle size is only 1.7 µm. BEH technology ensures the column stability under the high pressure and through wider pH range (1–12) comparing to generation one X-Terra sorbent or conventional stationary phases. Acquity UPLC columns are available with C18, Shield RP18, C8 and Phenyl stationary phases. High pore volume HPLC particles do not possess the mechanical stability necessary to withstand the high pressures inherent of UPLC separations. This mechanical limitation led Waters material scientists to develop a silica particle designed for high mechanical stability and appropriate morphology necessary to provide long column lifetimes and UPLC efficiencies at high pressures. The 1.8 µm High Strength Silica (HSS) particle is the first and only 100% silica particle designed, tested and intended for use in applications up to 15,000 psi [1034 bar].

## 2. MATERIAL &METHODS

### OPTIMIZED METHOD

Buffer: 0.01M Sodium dihydrogen ortho phosphate buffer with, pH 7adjusted with NaOH

**Mobile phase A** : Buffer:ACN(98:2)

**Mobile Phase B:** ACN

**Chromatographic parameters:**

Column : Halo C18 (50x2.1mm, 2.7um) .

Detector : 210nm

Flow rate : 0.3ml/min

Injector volume : 4µL

Column oven temp : 40°C

Run time : 15min

Diluent : Water : MeOH (20:80 v/v).

**Gradient run:**

| Time(min) | Flow rate(ml/min) | %A | %B |
|---|---|---|---|
| 0.0 | 0.3 | 100 | 0 |
| 2.0 | 0.3 | 100 | 0 |
| 8.0 | 0.3 | 70 | 30 |
| 10.0 | 0.3 | 70 | 30 |
| 10.5 | 0.3 | 100 | 0 |
| 15.0 | 0.3 | 100 | 0 |

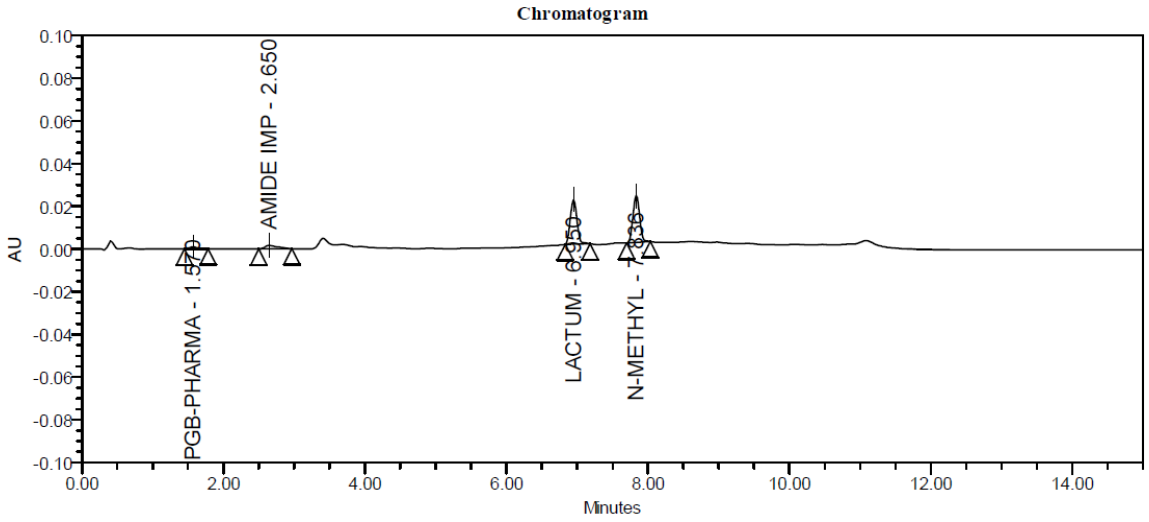



**Fig-1 chromatogram optimized method**

***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | S_N_Ratio |
|---|---|---|---|---|---|---|---|
| 1 | PGBPHARMA | 1.57 | 6812 | 2.35 | 1.1 | | 24.2 |
| 2 | AMIDE IMP | 2.65 | 16161 | 5.58 | 1.5 | 3.6 | 37.3 |
| 3 | LACTUM IMP | 6.96 | 129433 | 44.66 | 1.0 | 14.9 | 511.7 |
| 4 | N-METHYL IMP | 7.85 | 137386 | 47.41 | 1.0 | 5.0 | 543.2 |

| **MATERIALS ,EQUIPMENTS REAGENTS:** | **SUPPLIERS:** |
|---|---|
| Pregabalin and impurities | Mylan Laboratories ltd, |
| Sodium dihydrogen phosphate | Rankem |
| Methanol | Rankem |
| Acetonitrile | Rankem |
| NaOH | Rankem |
| Filter membrane | Whattman paper(0.22µ) |
| Sonicator | PCI ultra bath sonicator |
| Milli-Q water | Milli-Q gradient |

**PREPARATIONS:**

**MOBILE PHASE PREPARATIONS:**

**Mobile phase A:**

**Buffer preparation**: 1.56 gm of Sodium dihydrogen phosphate salt was accurately weighed transferred into 1 litre milliQ water, sonicated to dissolve and pH 7 adjusted with dilute sodium hydroxide and finally filtered through 0.22 µm filter paper.

Mobile phase A: Buffer: ACN (98:2) (%v/v).

Accurately measured 980ml of buffer and 20 ml of Acetonitrile, and were transferred in to a mobile phase container mixed thoroughly, finally sonicated to degas.

**Mobile phase B:** Acetonitrile: 100 %

Acetonitrile, was transferred into mobile phase container, mixed thoroughly Sonicated to Degass.

**PREPARATION OF SOLUTIONS:**

**Diluent:** : MEOH: WATER (20:80) Accurately measured methanol and water

200ml, 800ml respectively with 1litre measuring cylinder and were transferred in to mobile phase container, mixed thoroughly.

**1N HCL:** 85ml accurately measured amount of concentrated HCL (33%) analytical grade was diluted to dissolve with Milli-Q-water, sonicated to mix, made up to 1000ml.

**1N NaOH:** 40gm accurately Weighed amount of NaOH was diluted to dissolve with Milli-Q-water, sonicated to mix, made up to 1000ml.

**5% $H_2O_2$:** 5ml of 30% $H_2O_2$ was dissolved with Milli-Q-water, and, made up to 30ml

**Column Washing Solution (ACN: Water 50:50%v/v)**

500mL of milliQ water & 500ml of ACN were accurately measured individually with 500ml measuring cylinder, were taken and filtered through 0.22µ filter, and mixed thoroughly to get solution of 50:50(% v/v.)

**Strong needle wash solution (ACN: Water 90:10%v/v)**

100 ml of Milli-Q water, 900ml of ACN were accurately measured individually with 1000ml Measuring cylinder, filtered through 0.22µ filter, and mixed thoroughly to get final solution of 100:900% v/v of Water: Acetonitrile.

**Weak needle wash solution (ACN: Water 10:90%v/v)**

900 ml of Milli-Q water, 100ml of ACN were accurately measured individually with 1000ml measuring cylinder, filtered through 0.22µ filter, and mixed thoroughly to get final solution of 900:100% v/v of Water: Acetonitrile

**Standard stock solution (15mg/ml):** Accurately weighed and transferred about 150mg of standard into a 10ml volumetric flask, dissolved in and diluted to volume with diluent.

**Stock solution: All** individual impurities of 15mg each and standard 15 mg individually weighed, transferred to 100ml volumetric flasks and make up the volume with diluent

**Reference solution**: From Stock -1 solution taken 10ml, made up to 100 ml with diluent and final concentration was 0.015mg /ml (0.1% w.r.t. test concentration).

**Sample Solution Spiked at Specification Level:** Accurately weighed and transferred about 150 mg of standard into10ml volumetric flask and make up the volume with (0.1%) Reference solution.

**LOQ solution:** Prepared Amide Impurity, Lactum Impurity ,N-methyl impurities of 10mg each and standard 10 mg individually weighed, transferred to 10 ml volumetric flask and from above stock solutions diluted 200µL,50µL,50µL and 600µL respectively into 100 ml volumetric flask and make up to 100 ml with diluent.

**LOD solution:** Pipetted out from 3.3ml from LOQ solution into 10ml volumetric flask and made up to volume with diluent.

**For Accuracy (sample preparations):**

**LOQ solution:** Accurately weighed and transferred about 150mg of sample into a 10ml volumetric flask, dissolved in and diluted to volume with LOQ solution.

**40% spike solution:** Accurately weighed and transferred about 150mg of sample into a 10ml volumetric flask, added 4ml of reference solution, dissolve and diluted to volume with diluent.

**100% spike solution:** Accurately weighed and transferred about 150mg of sample into a 10ml volumetric flask, dissolved in and diluted to volume with Reference solution.

**150% spike solution:** Accurately weighed and transferred about 150mg of sample into a 10ml volumetric flask, dissolved and made up to volume with Stock solution.

**For Linearity (sample preparations):**

**Linearity stock solution:** Accurately weighed and transferred (amide impurity) 15mg, (lactum impurity) 15mg, (N—methyl Impurity) 15mg, & (Pregabalin standard) 15mg in to 100ml volumetric flask and diluted to 100ml with diluent .

**40% solution:** Accurately transferred about 0.4ml of linearity solution in to 10 ml volumetric flask and diluted to volume with diluent.

**60% solution:** Accurately transferred about 0.6ml, of linearity solution in to 10 ml volumetric flask and diluted to volume with diluent.

**80% solution:** Accurately transferred about 0.8ml of linearity solution in to 10 ml volumetric flask and diluted to volume with diluent.

**100% solution:** Accurately transferred about 1ml of linearity solution in to 10 ml volumetric flask and diluted to volume with diluent.

**120% solution:** Accurately transferred about 1.2ml of linearity solution in to 10 ml volumetric flask and diluted to volume with diluent.

**150% solution:** Accurately transferred about 1.5ml, of linearity solution in to 10 ml volumetric flask and diluted to volume with diluent.

**Degradation Study (Sample Solution Preparations)**

**Degradation study:** Analyze the impurities and pregabalin individually as per the method to verify the retention times. In order to assess the stability indicating nature of the UPLC method, Pregabalin samples were stressed by acid, base, hydrogen peroxide, heat and UV radiation. The degraded samples were analyzed using a photodiode-array detector for determining the peak purity and related substances.

**Sample Preparation:**

**Stock Solution:** Accurately weighed and transferred about 1.5 gm of Pregabalin into a 100ml volumetric flask, dissolved in and diluted to volume with diluent.

**Control sample:** The control sample for this study was prepared by diluting 5.0 mL of stock solution to 10 ml with diluent.

- **Acid Hydrolysis:**

**At 60°C:**

Transferred 5 mL of stock solution into a 10 mL volumetric flask, added 0.2 mL of 1.0 N hydrochloric acid solution. Kept the solution at 60°C for 3, 6, 12, 24 hours, then neutralize with 0.2 mL of 1.0 N sodium hydroxide solution and diluted to 10 mL with diluent.

- **Base Hydrolysis**

**At 60°C:**

Transferred 5 mL of stock solution into a 10 mL volumetric flask, added 0.2 mL of 1.0 N sodium hydroxide solution. Kept the solution at 60°C for 3, 6, 12,24hours, then neutralized with 0.2 mL of 1.0 N hydrochloric acid solution and diluted to 10 mL with diluent.

- **Oxidation**

**At 60°C:**

Transferred 5 mL of stock solution into a 10 mL volumetric flask, added 0.2 mL of 5% hydrogen peroxide solution.Kept the solution at 60°C for three hours, then diluted to 10 mL with diluent.

**UV Degradation:**

Transfered 5 mL of stock solution into a 10 mL volumetric flask .Exposed the solution to UV light with an integrated near ultraviolet energy of not less than 200 Watt hours/square meter , then diluted to 10 mL with diluent.

❖ **Heat Degradation:**

Transfered 5.0 mL of stock solution into a 10 mL volumetric flask. Kept the solution at 60°C

Temperature for3, 6, 12, 24 Hours then diluted to 10 mL with diluent.

➢ **Solid state stability:**

**Sample preparation:**

**Control sample**

Accurately weighed and transferred about 150mg of Pregabalin sample into a 10 mL volumetric flask, dissolved in and diluted up to volume with the diluent.

❖ **Exposure to UV light:**

Taken some quantity of sample, spread in a Petri dish and exposed to UV light with energy of not less than 200 Wh/sq mt. After exposure, accurately weighed and transferred about 150 mg of sample into a 10 mL volumetric flask, dissolved in and diluted up to volume with diluent.

❖ **Exposure to White fluorescent light:**

Taken some quantity of sample, spread in a Petri dish and exposed to White fluorescent light with an overall illumination of not less than 1.2 million lux hours. After exposure, accurately weighed and transferred about 150 mg of sample into a 10 mL volumetric flask, dissolved in and diluted to volume with diluent.

❖ **Heat Degradation:**

Taken about some quantity of sample, spread in a Petri dish and exposed to 105**°**C for 24Hrs. After exposure, accurately weighed and transferred about 150mg of sample into a 10 mL volumetric flask, dissolved in and diluted to volume with diluent.

## 3. RESULTS

**1. SYSTEM SUITABILITY:**

Reference solution at 100% level is injected 6 times and analyzed.

**Figure -2: Chromatogram of System suitability**

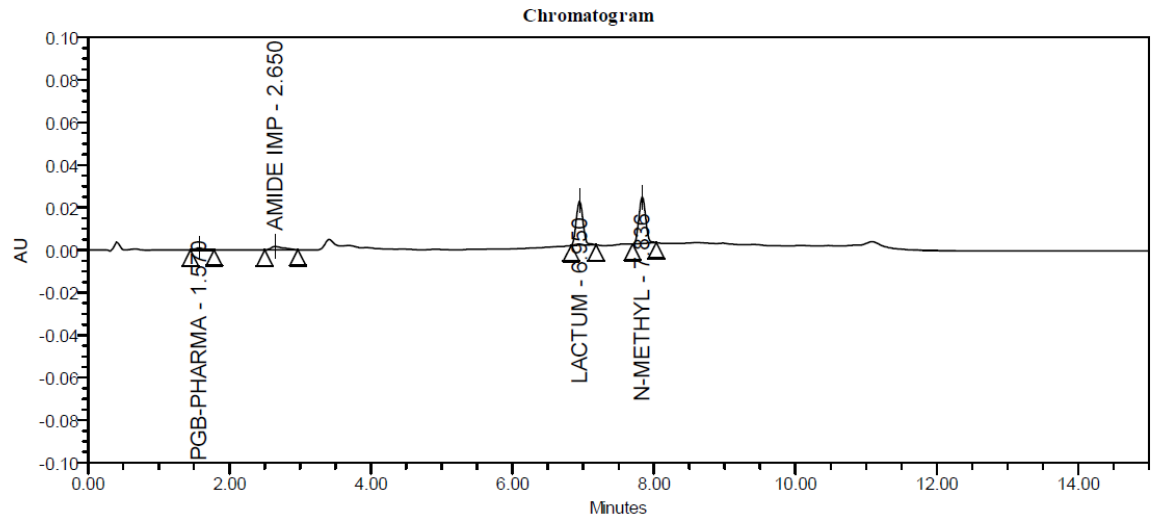


**Table-1: System suitability**

| S.no | Peak area of Pregabalin |
|---|---|
| 1 | 7588 |
| 2 | 7532 |
| 3 | 7564 |
| 4 | 7511 |
| 5 | 7568 |
| 6 | 7588 |
| **Average** | 7558.5 |
| **SD** | 31.0467 |
| **%RSD** | 0.41 |

| | |
|---|---|
| **Resolution between Pregabalin and Amide** | 3.2 |
| **Peak tailing of Pregabalin** | 1.2 |

System Suitability Peak Results

**Acceptance criteria:**

The resolution between Pregabalin and amide impurity Peak should be not < 2.

Peak tailing of pregabalin should be <2

% R.S.D For six replicate injections should be less than 2.0

**Conclusion:**The resolution between pregabalin and amide impurity was found to be 3.2, and peak tailing of pregabalin was found to be 1.2 and %R.S.D was found to be 0.41

**1.5.2 SPECIFICITY:**

**Fig-3 chromatogram of blank**

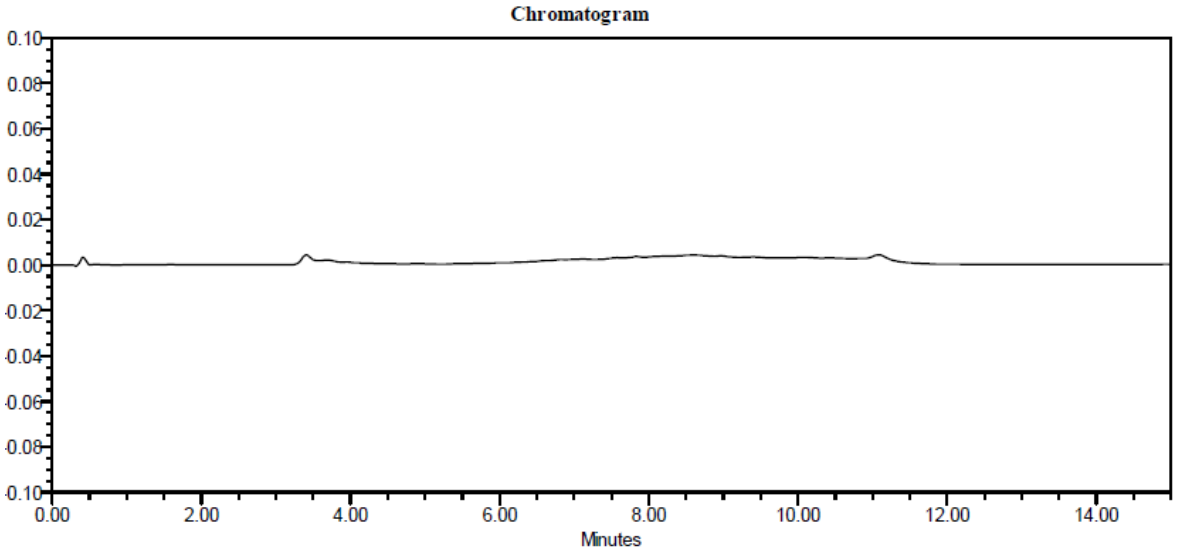
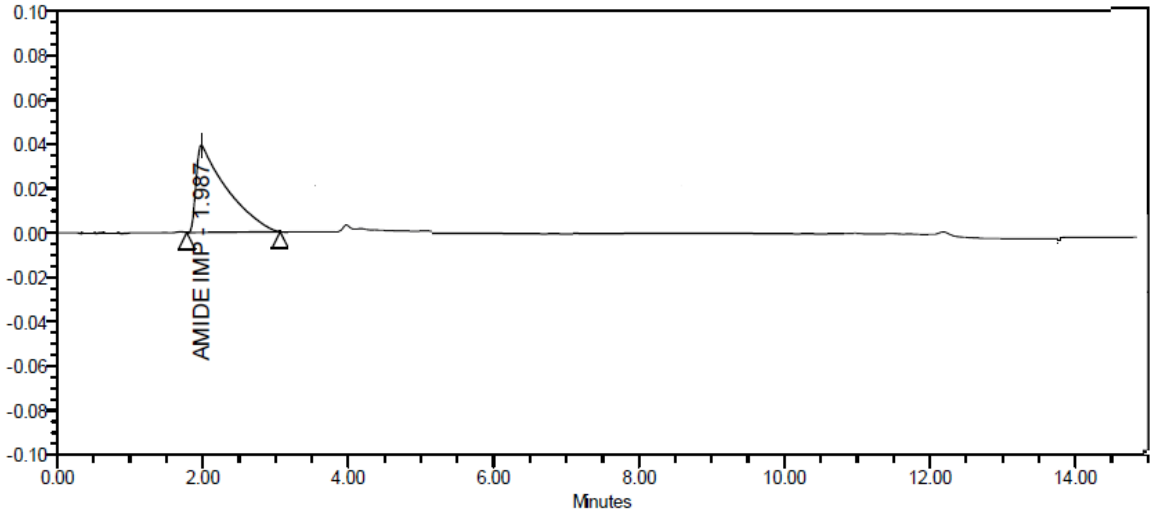


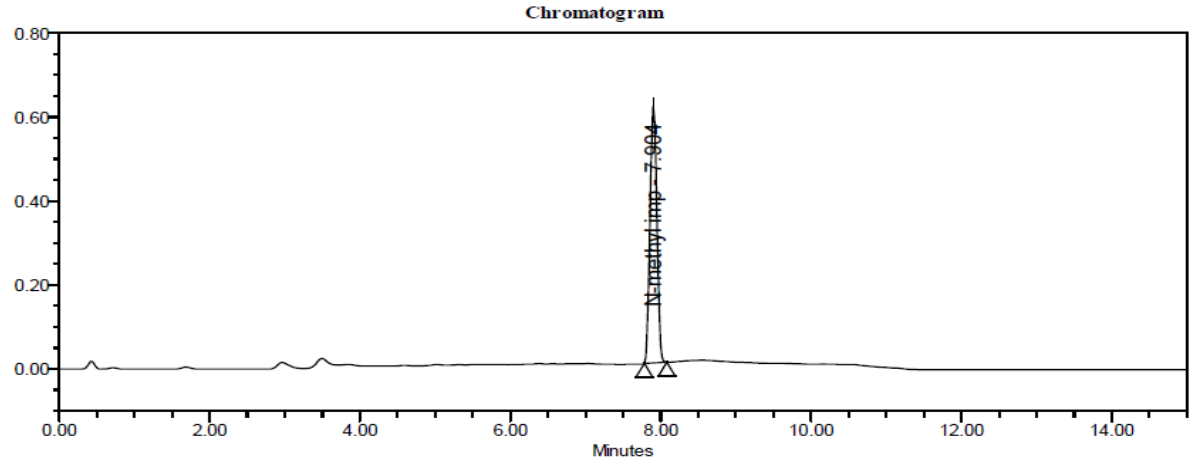
**Fig 4 chromatogram of AMIDE impurity**




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | Purity_Test | Purity1 Angle | Purity1 Threshold |
|---|---|---|---|---|---|---|---|---|
| 1 | AMIDE IMP | 1.98 | 181457 | 100.00 | 0.9 | Pass | 5.101 | 39.350 |

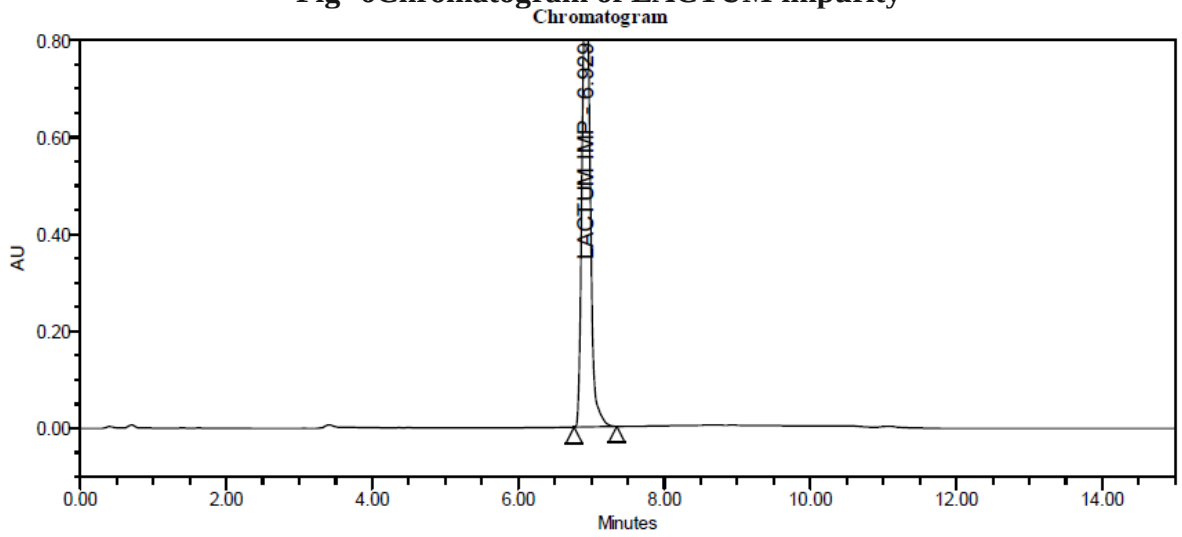
**Fig-5 Chromatogram of N-Methyl impurity**




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | Purity_Test | Purity1 Angle | Purity1 Threshold |
|---|---|---|---|---|---|---|---|---|
| 1 | N-methyl imp | 7.90 | 3919756 | 100.00 | 1.0 | Pass | 1.779 | 3.610 |

**Fig -6Chromatogram of LACTUM impurity**




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | Purity_Test | Purity1 Angle | Purity1 Threshold |
|---|---|---|---|---|---|---|---|---|
| 1 | LACTUM IMP | 6.93 | 7611712 | 100.00 | 1.2 | Pass | 4.179 | 25.599 |

**Fig-7 Chromatogram of PREGABALIN**

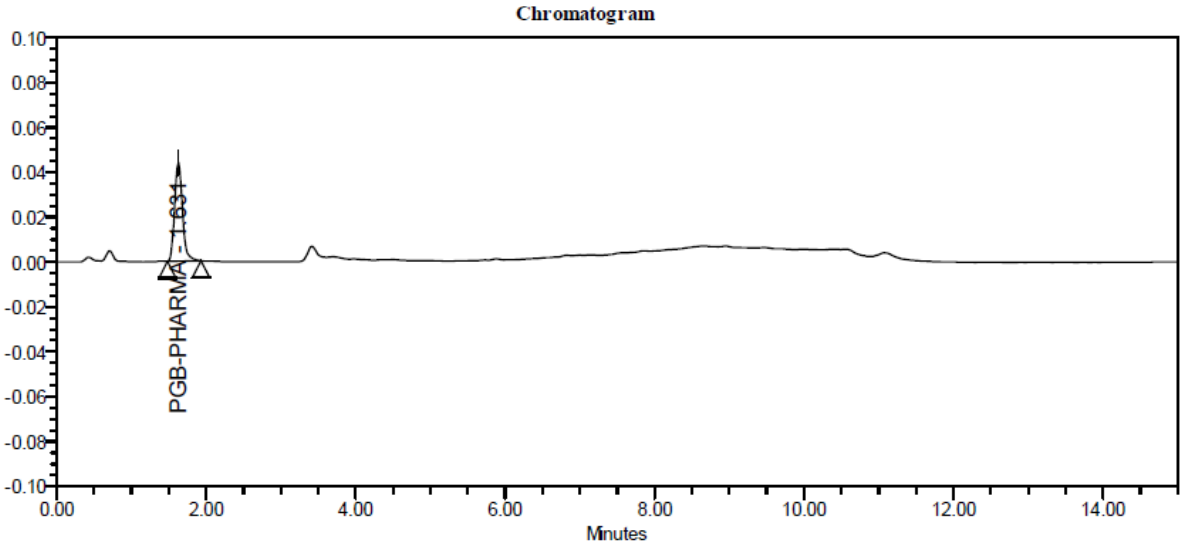


**Fig -8Chromatogram of the 0.1% reference solution:**

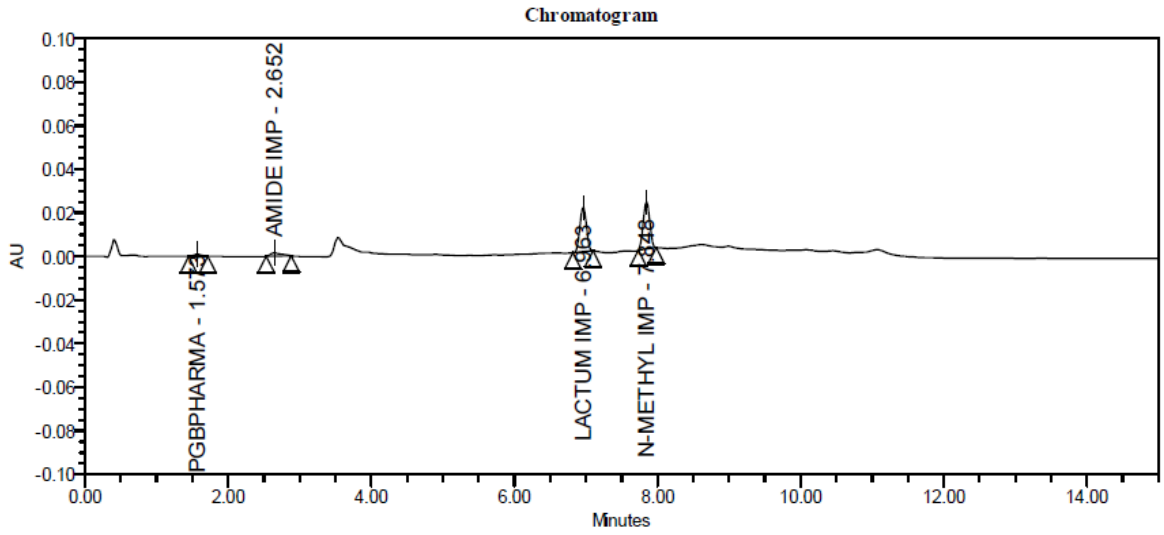



**Table: 0.1% Reference solution peak result**

*Peak Results*

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | S_N_Ratio |
|---|---|---|---|---|---|---|---|
| 1 | PGBPHARMA | 1.57 | 6812 | 2.35 | 1.1 | | 24.2 |
| 2 | AMIDE IMP | 2.65 | 16161 | 5.58 | 1.5 | 3.6 | 37.3 |
| 3 | LACTUM IMP | 6.96 | 129433 | 44.66 | 1.0 | 14.9 | 511.7 |
| 4 | N-METHYL IMP | 7.85 | 137386 | 47.41 | 1.0 | 5.0 | 543.2 |

| Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|
| 24.582 | 90.000 | Pass |
| 19.440 | 90.000 | Pass |
| 2.846 | 63.708 | Pass |
| 4.739 | 90.000 | Pass |

**Fig-9 peak purity of Lactum impurity**

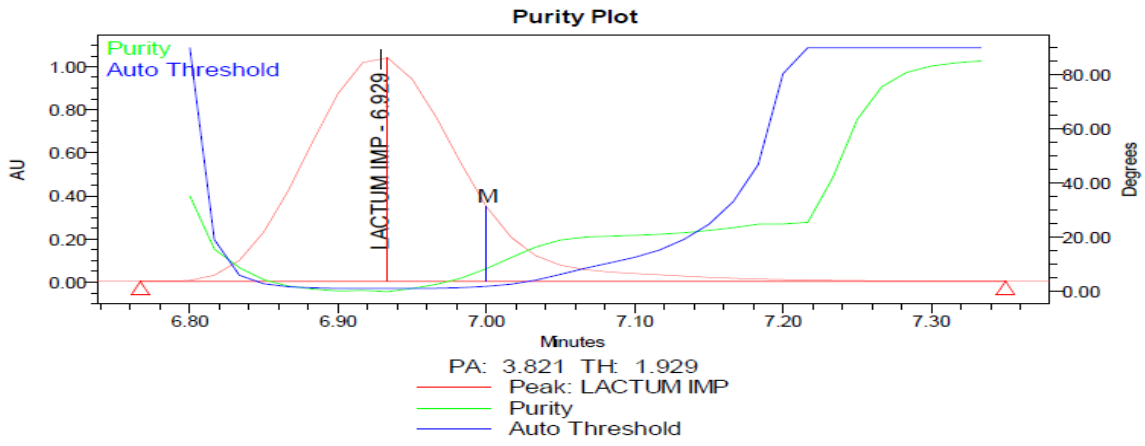


**Fig -10 Purity plot of the PREGABALIN**

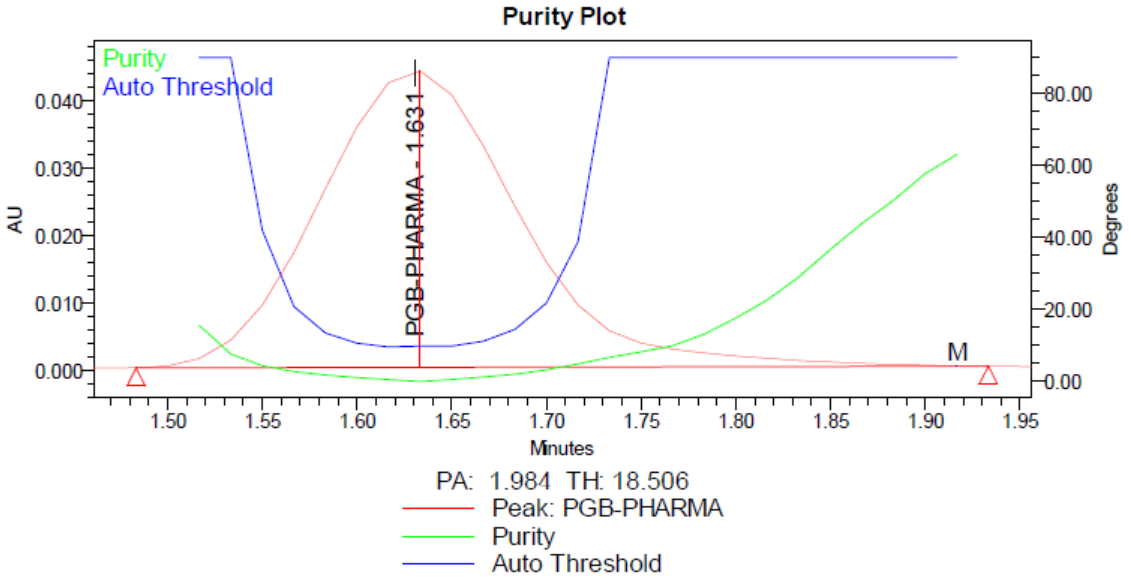



**Fig-11 Purity plot of AMIDE IMPURITY**

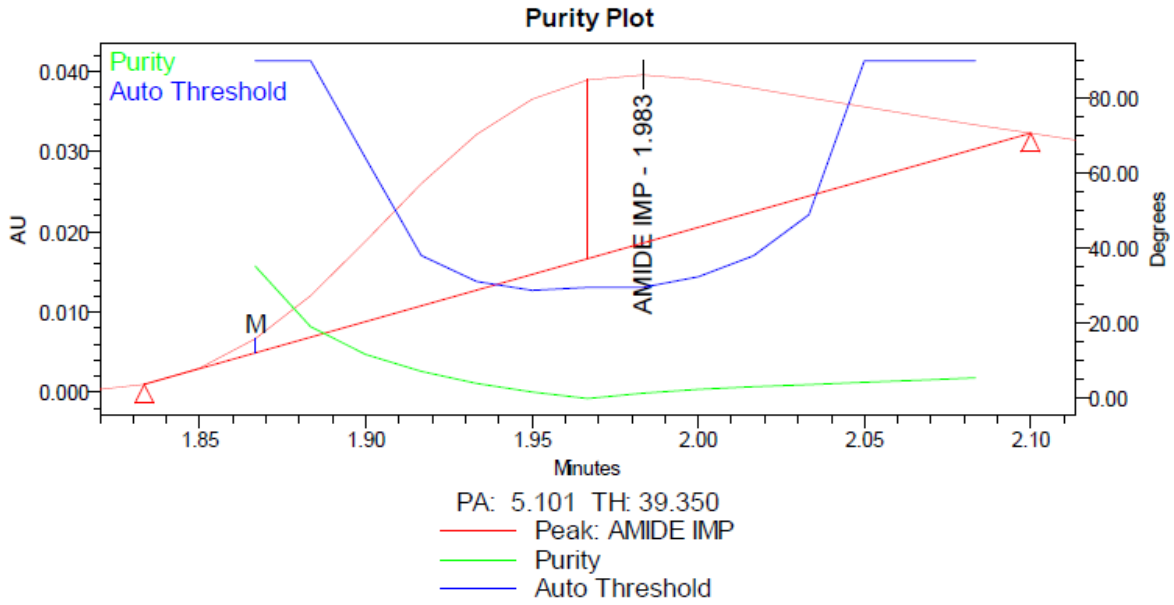


**Fig- 12 Purity plot of N-METHYL IMPURITY**

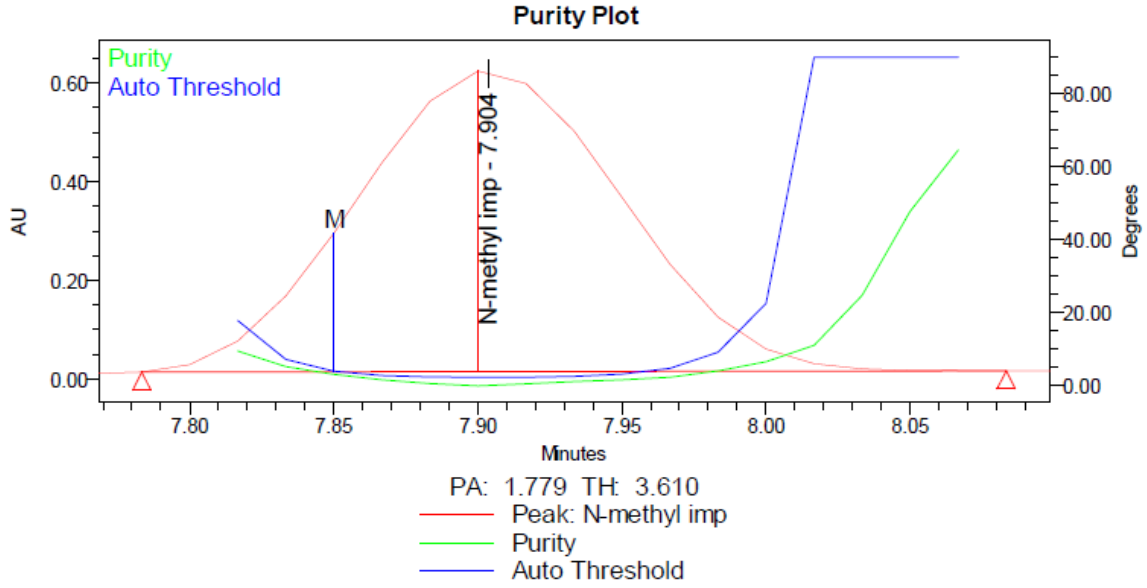
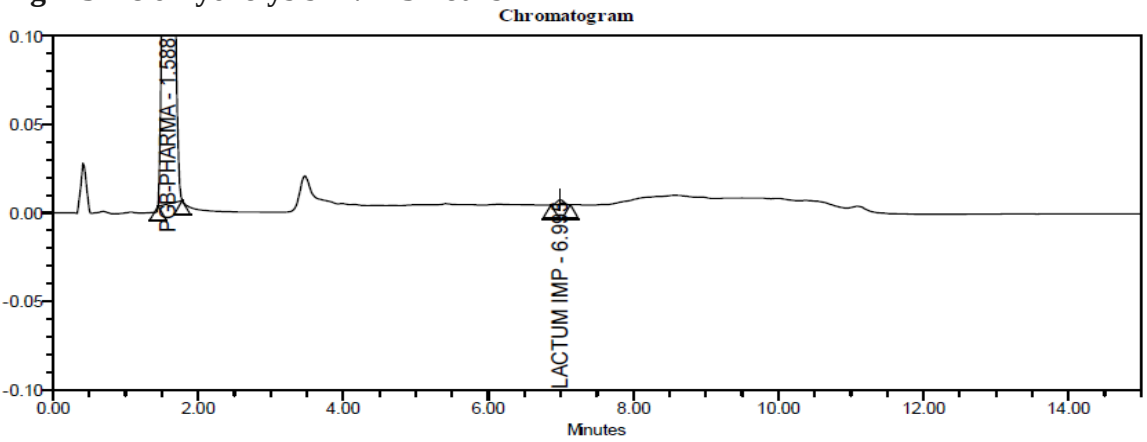



**Table-2 Degradation Study : AFTER 3 HOURS**

| Degradation condition | 1NHCl | | 1N NaOH | | 5% $H_2O_2$ | | Purity test |
|---|---|---|---|---|---|---|---|
| | API (%area) | % Degradation | API (%area) | % Degradation | API (%area) | % Degradation | |
| Acid hydrolysis at 60 °C for 3 Hrs | 99.55 | 0.45 | NA | NA | NA | NA | Pass |
| Base hydrolysis at 60 °C for 3Hrs . | NA | NA | 99.56 | 0.44 | NA | NA | Pass |
| Oxidation at RT at 60 °C for 3 Hrs | NA | NA | NA | N.A | 99.56 | 0.44 | Pass |

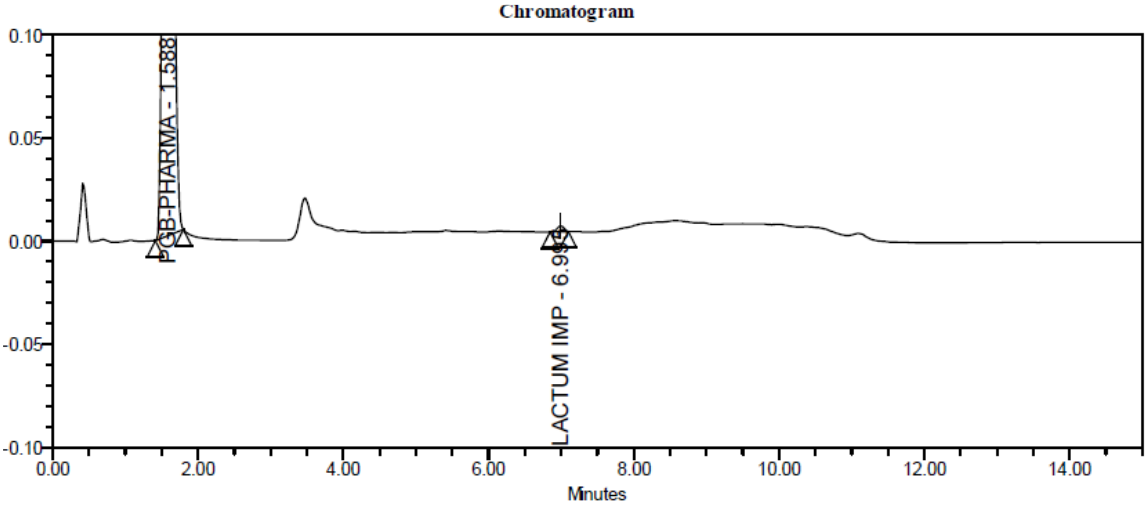
**Fig -13 Acid hydrolysis A/F 3 hours**




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.59 | 4066285 | 99.55 | 1.1 | | 2.975 | 4.809 | Pass |
| 2 | LACTUM IMP | 6.99 | 18392 | 0.45 | 1.0 | 26.4 | 19.214 | 90.000 | Pass |

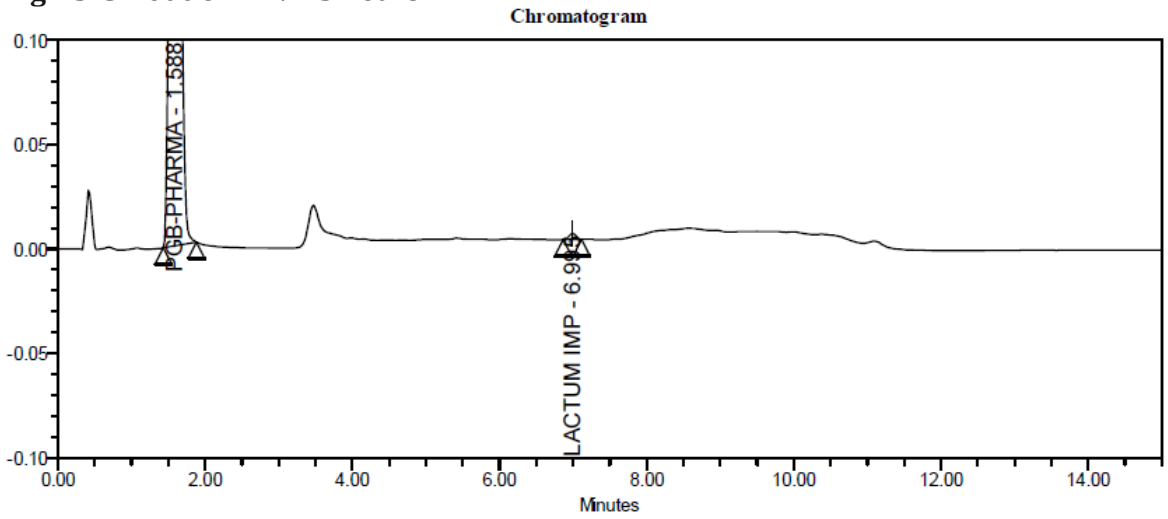
**Fig – 14 Base hydrolysis A/F 3 hours**




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.59 | 4114047 | 99.56 | 1.1 | | 3.033 | 5.118 | Pass |
| 2 | LACTUM IMP | 6.99 | 18057 | 0.44 | 0.9 | 26.5 | 17.267 | 90.000 | Pass |

**Fig-15 Oxidation A/F 3 hours**



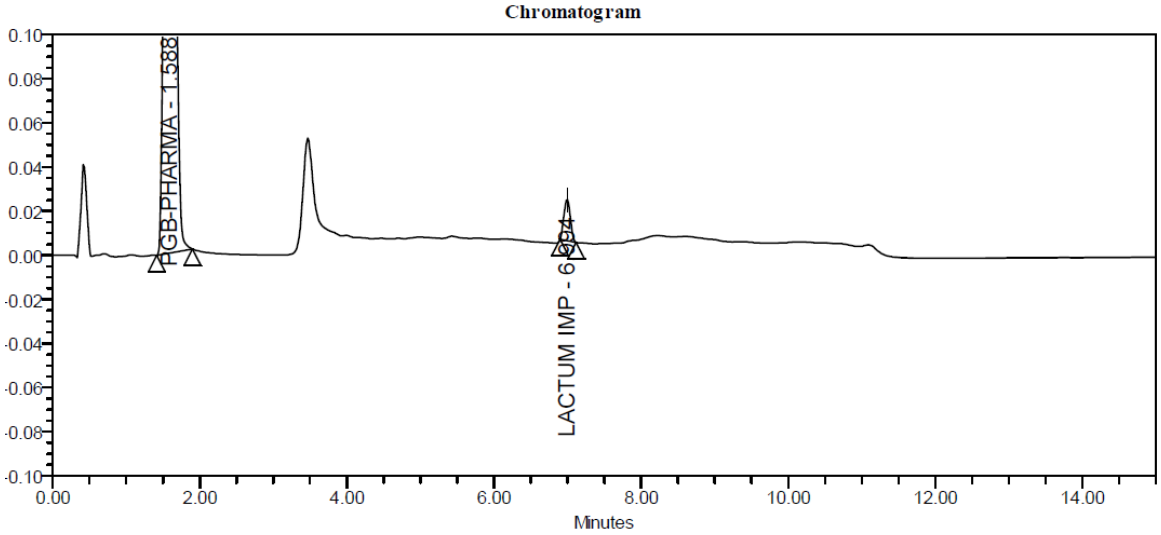

*Peak Results*

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.59 | 4150973 | 99.56 | 1.1 | | 3.185 | 5.534 | Pass |
| 2 | LACTUM IMP | 6.99 | 18318 | 0.44 | 1.0 | 26.3 | 16.935 | 90.000 | Pass |

**Table-3 Degradation Study: AFTER 6 HOURS**

| Degradation condition | 1NHCl | | 1N NaOH | | 5% $H_2O_2$ | | Purity test |
|---|---|---|---|---|---|---|---|
| | API (%area) | % Degradation | API (%area) | % Degradation | API (%area) | % Degradation | |
| Acid hydrolysis 60°C 6 Hrs | 97.41 | 2.59 | NA | NA | NA | NA | Pass |
| Base hydrolysis at 60°C for 6Hrs . | NA | NA | 97.45 | 2.55 | NA | NA | Pass |
| Oxidation at RT 60 °C for 12 Hrs | NA | NA | NA | N.A | 99.86 | 0.14 | Pass |

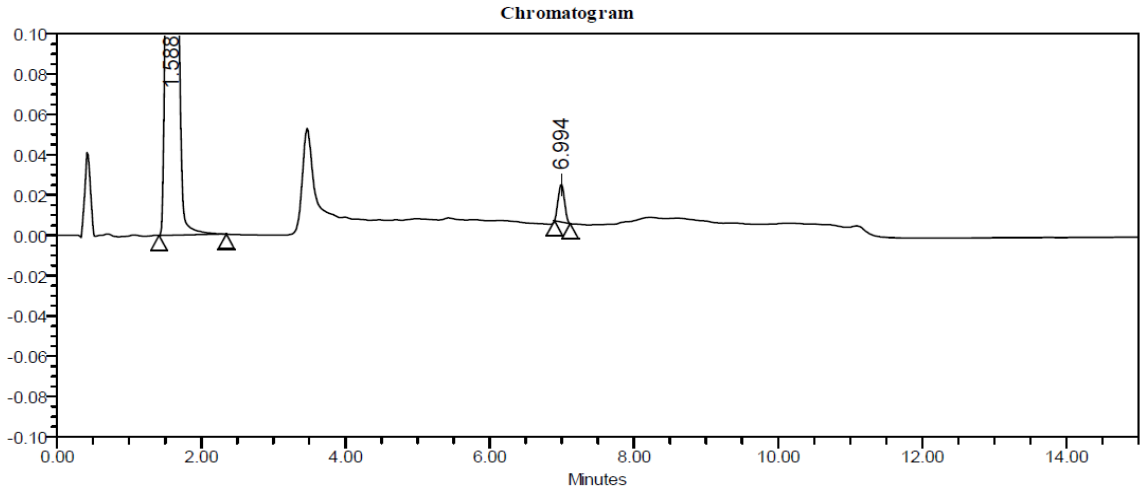
**Fig- 16 Acid hydrolysis A/F 6 hours**




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.59 | 4295853 | 97.41 | 1.1 | | 2.265 | 6.599 | Pass |
| 2 | LACTUM IMP | 6.99 | 114082 | 2.59 | 1.1 | 27.0 | 2.661 | 90.000 | Pass |

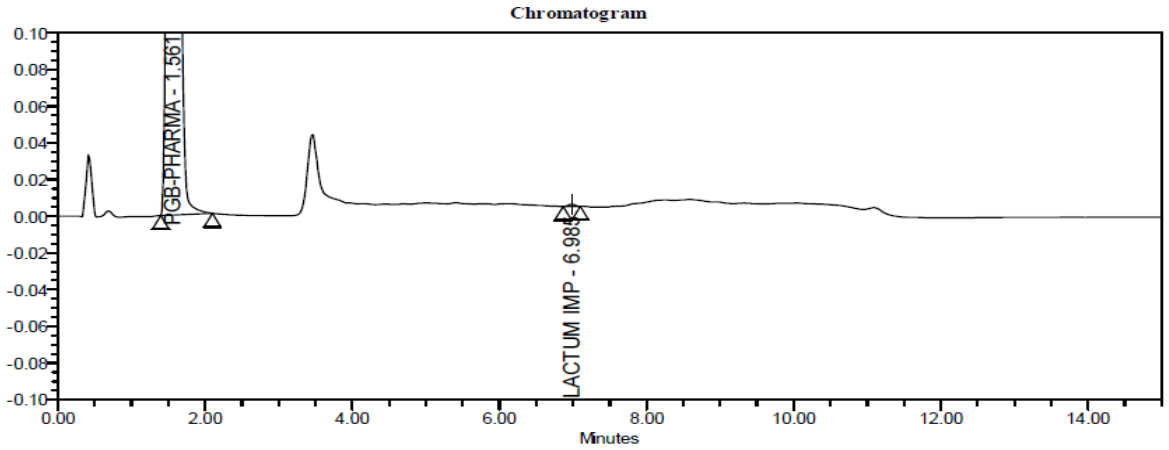
**Fig – 17 Base hydrolysis A/F 6 hours**




***Peak Results***

| | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.59 | 4357103 | 97.45 | 1.1 | | 2.316 | 9.262 | Pass |
| 2 | 6.99 | 114082 | 2.55 | 1.1 | 27.0 | 2.661 | 90.000 | Pass |

**Fig -18 Oxidation A/F 6 hours**



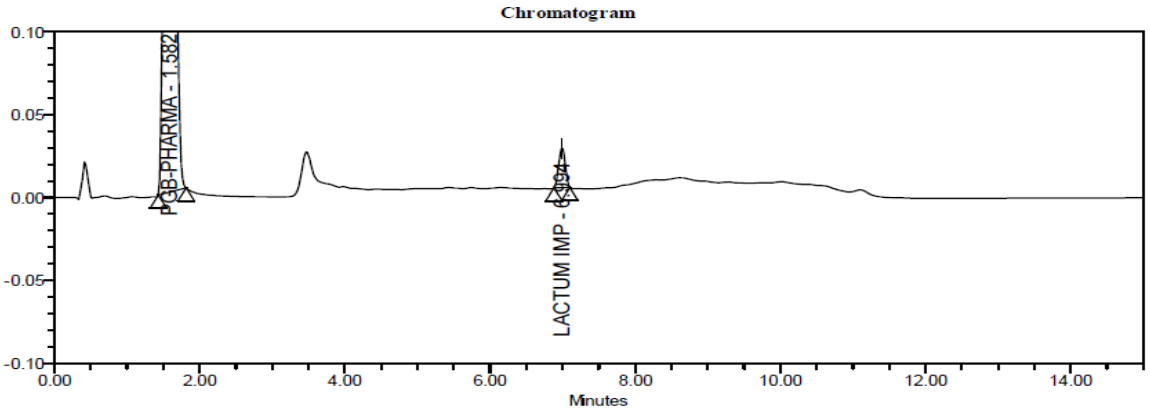

***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.56 | 5670231 | 99.86 | 1.2 | | 4.567 | 6.499 | Pass |
| 2 | LACTUM IMP | 6.98 | 7863 | 0.14 | 1.0 | 25.1 | 28.330 | 90.000 | Pass |

**Table- 4 Degradation Study: AFTER 12 HOURS**

| **Degradation condition** | **1N HCl** | | **1N NaOH** | | **5%$H_2O_2$** | | **purity test** |
|---|---|---|---|---|---|---|---|
| | **API (%area)** | **% Degradation** | **API (%area)** | **% Degradation** | **API (%area)** | **% Degradation** | |
| Acid hydrolysis at 60°C for 12 hrs | 97.02 | 2.98 | NA | NA | NA | NA | Pass |
| Base hydrolysis at 60 °C for 12Hrs . | NA | NA | 99.55 | 0.45 | NA | NA | Pass |
| Oxidation at RT 60°C for 12 Hrs | NA | NA | NA | N.A | 97.45 | 2.55 | Pass |

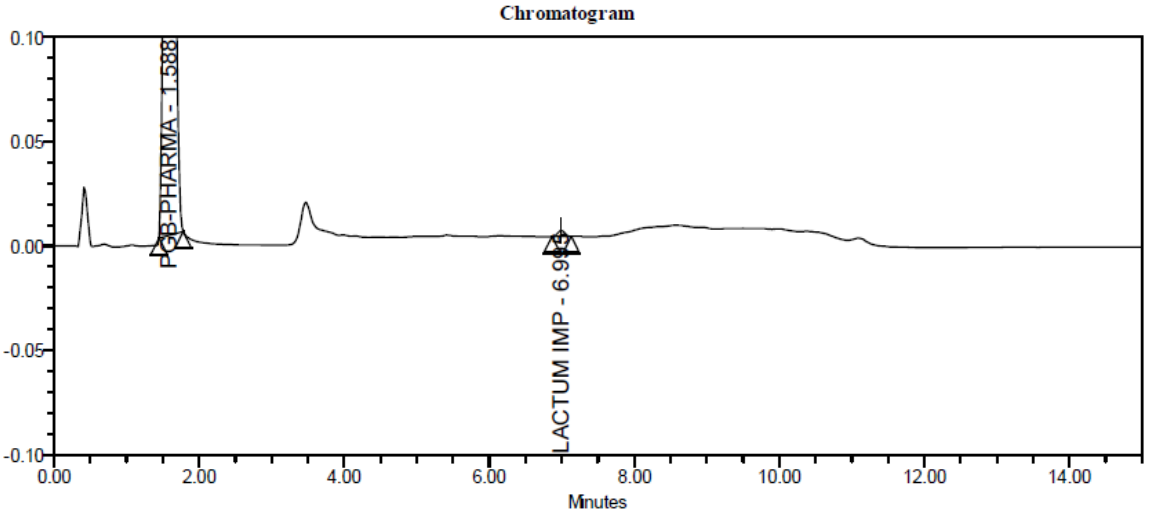
## Fig -19Acid hydrolysis A/F 12 hours




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.58 | 4898299 | 97.02 | 1.1 | | 1.424 | 3.146 | Pass |
| 2 | LACTUM IMP | 6.99 | 150692 | 2.98 | 1.0 | 25.9 | 1.055 | 47.314 | Pass |

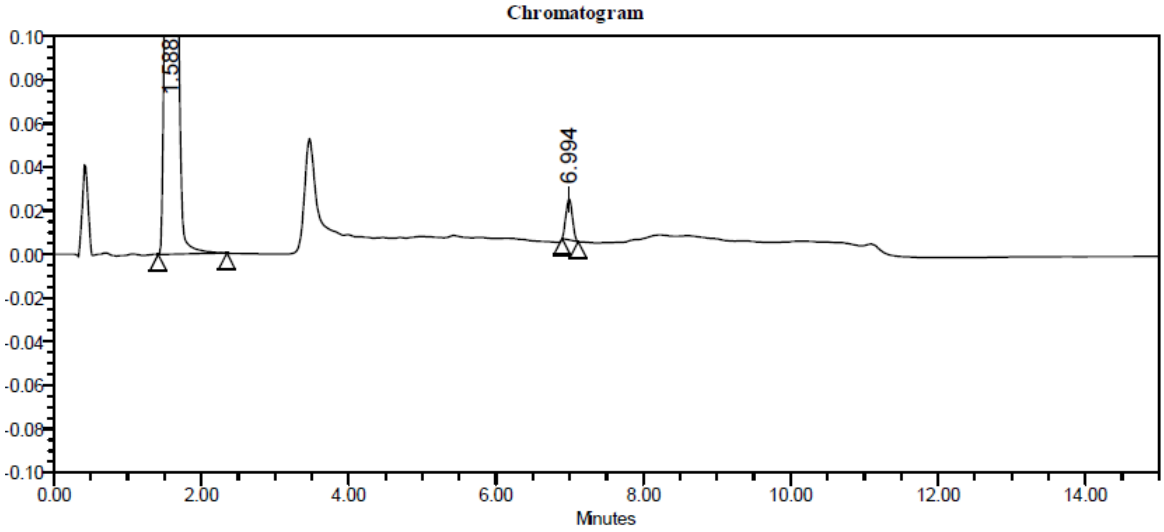
## Fig-20 Base hydrolysis A/F 12 hours




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.59 | 4066285 | 99.55 | 1.1 | | 2.975 | 4.809 | Pass |
| 2 | LACTUM IMP | 6.99 | 18392 | 0.45 | 1.0 | 26.4 | 19.214 | 90.000 | Pass |

**Fig – 21 Oxidation A/F 12 hours**



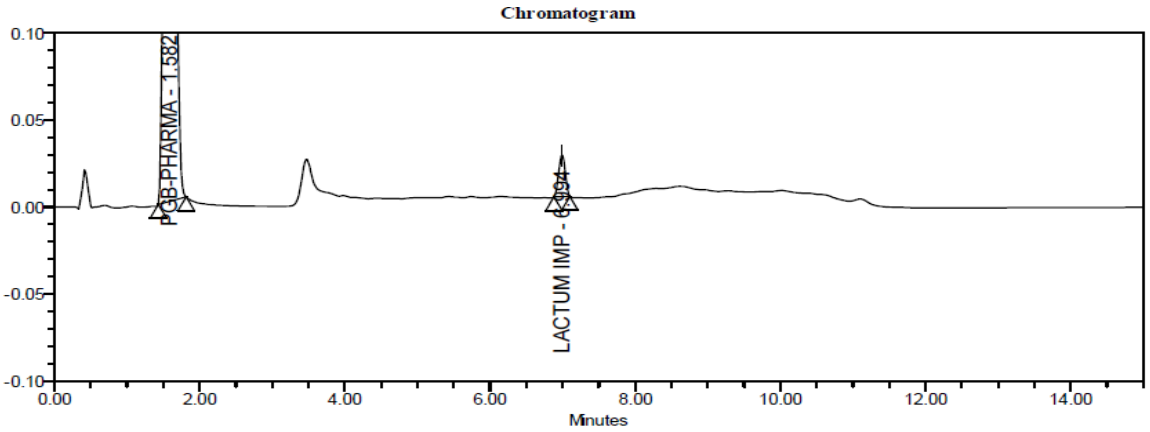

***Peak Results***

| | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.59 | 4357103 | 97.45 | 1.1 | | 2.316 | 9.262 | Pass |
| 2 | 6.99 | 114082 | 2.55 | 1.1 | 27.0 | 2.661 | 90.000 | Pass |

**Table-5 Degradation Study : AFTER 24 HOURS**

| **Degradation condition** | **1N HCl** | | **1N NaOH** | | **5% $H_20_2$** | | **purity test** |
|---|---|---|---|---|---|---|---|
| | **API (%area)** | **% Degradation** | **API (%area)** | **% Degradation** | **API (%area)** | **% Degradation** | |
| Acid hydrolysis 60 deg 24 hrs | 90.67 | 9.33 | NA | NA | NA | NA | Pass |
| Base hydrolysis at 60 deg for 24Hrs | NA | NA | 97.41 | 2.59 | NA | NA | Pass |
| Oxidation at RT 60 deg for 24 Hrs | NA | NA | NA | N.A | 89.63 | 10.37 | Pass |

**Fig-22 Acid hydrolysis A/F 24 hours**

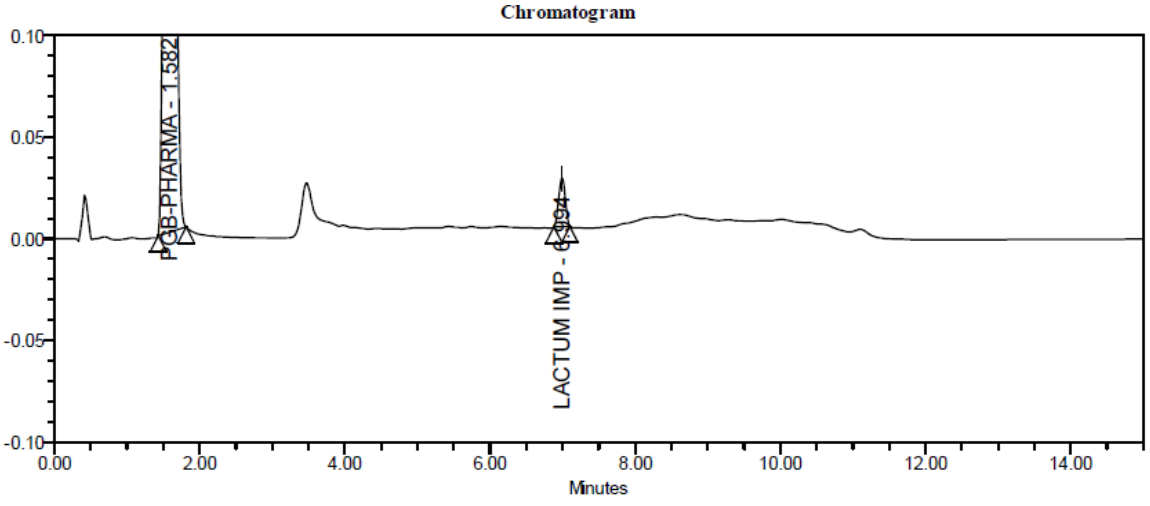



***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution |
|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.67 | 4386174 | 90.67 | 1.2 | |
| 2 | LACTUM IMP | 6.99 | 451561 | 9.33 | 1.0 | 25.9 |

| Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|
| 2.316 | 9.262 | Pass |
| 2.661 | 90.000 | Pass |

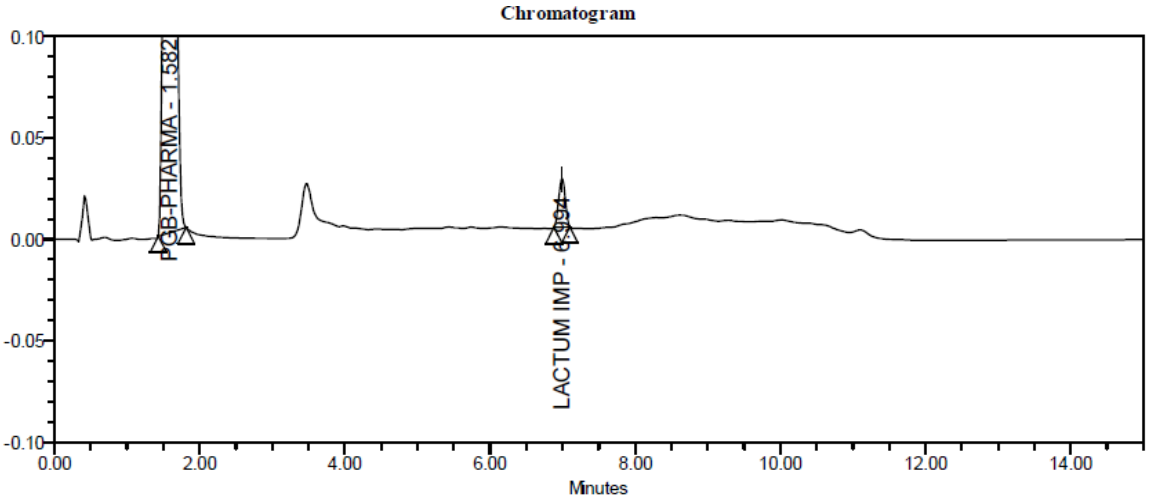
**Fig -23 Base Hydrolysis A/F 24 hrs**




***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.59 | 4295853 | 97.41 | 1.1 | | 2.265 | 6.599 | Pass |
| 2 | LACTUM IMP | 6.99 | 114082 | 2.59 | 1.1 | 27.0 | 2.661 | 90.000 | Pass |

**Fig -24 Oxidation A/F 24 hours**




***Peak Results***

| | Peak name | RT | Area | % Area |
|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.67 | 4386174 | 89.63 |
| 2 | LACTUM IMP | 6.99 | 507626 | 10.37 |

| USP Tailing | USP Resolution | Purity1 Angle | Purity1 Threshold | Purity_Test |
|---|---|---|---|---|
| 1.1 | | 2.265 | 6.599 | Pass |
| 1.1 | 27.0 | 2.661 | 90.000 | Pass |

**Conclusion:** Significant degradation was observed at room temperature and after 3,6,12, 24 Hrs Respectively

**Table-6 Solid state stability:**

| Characterization condition | Degradation report | Purity test |
|---|---|---|
| UV light | ND | Pass |
| White fluorescence light | ND | Pass |
| Thermal degradation | ND | Pass |

**Conclusion:-**

The results obtained in the solid state stability study indicate that Pregabalin is stable upon exposure to white fluorescent light.

a) The results obtained in the force degradation study Pregabalin was stable at different stress condition

b) The specificity of the method was confirmed and the method is stability indicating.

c) The specificity of the method was confirmed and the method is stability indicating.

**Establishment of LOQ by Signal-to-noise ratio method:** A Solution of Amide, Lactum, N-Methyl and Pregabalin of 0.1% is analyzed for Signal to noise ratio. Based on the S/N ratio of 0.1% blend solution LOQ Solution is prepared accordingly.

The S/N ratios of Amide, Lactum, N-Methyl Impurities and Pregabalin are as follows:

**Table- 7 Establishment of LOQ**

| Impurity Name | Amide Impurity | Lactum Impurity | N-Methyl Impurity | Pregabalin |
|---|---|---|---|---|
| LOQConcentration in mg/mL | 0.002 | 0.0005 | 0.005 | 0.006 |
| LOQ (%)Concentration with respect to test | 0.010 | 0.003 | 0.030 | 0.040 |
| S/N Ratio For LOQ solution | 10.51 | 9.91 | 9.95 | 9.93 |

**Fig- 25 chromatogram of LOQ**

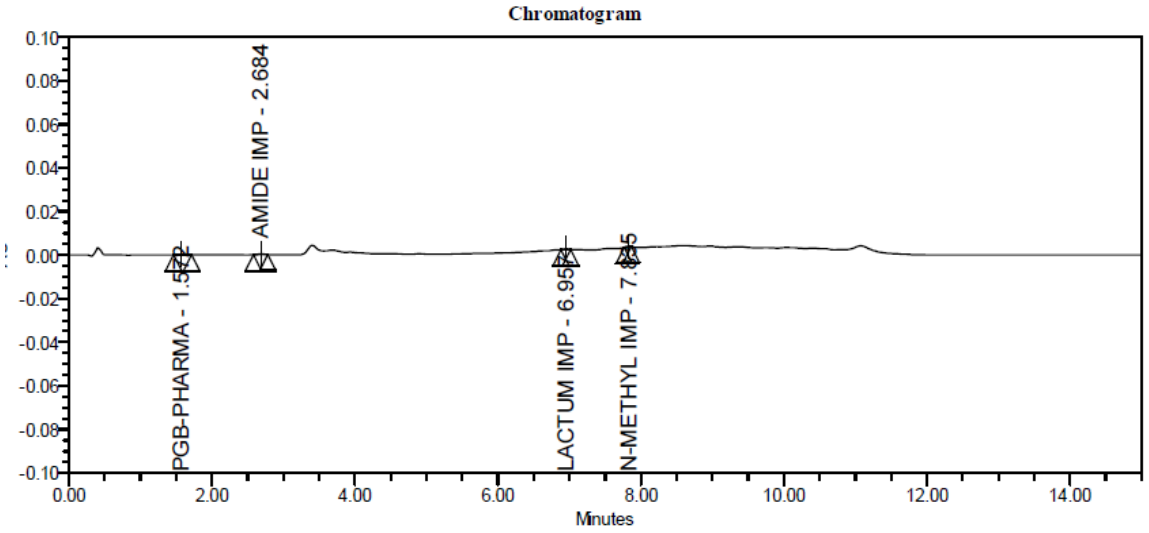



**Table : Establishment of LOQ results**

***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | S_N_Ratio |
|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.57 | 2088 | 30.85 | 1.1 | | 9.9 |
| 2 | AMIDE IMP | 2.68 | 2103 | 31.06 | 1.0 | 5.7 | 10.5 |
| 3 | LACTUM IMP | 6.96 | 1367 | 20.19 | 0.9 | 26.7 | 9.9 |
| 4 | N-METHYL IMP | 7.83 | 1211 | 17.90 | 0.7 | 7.6 | 10.0 |

LOD Solution of Amide, Lactum, N-Methyl and Pregabalin is diluted for 3.3 times and analysed for LOD.

The S/N ratios of Amide, Lactum, N-Methyl Impurities and Pregabalin are as follows:

**Table -8 Establishment of LOD by Signal-to-noise ratio method:**

| Impurity Name | Amide Impurity | Lactum Impurity | N-Methyl Impurity | Pregabalin |
|---|---|---|---|---|
| Concentration in mg/mL at LOD | 0.0006 | 0.00015 | 0.0015 | 0.0018 |
| Concentration(%) with respect to test at LOD | 0.004 | 0.001 | 0.010 | 0.012 |
| S/N Ratio at LOD | 3.76 | 3.44 | 3.10 | 3.59 |

**Fig -26 Chromatogram of LOD**

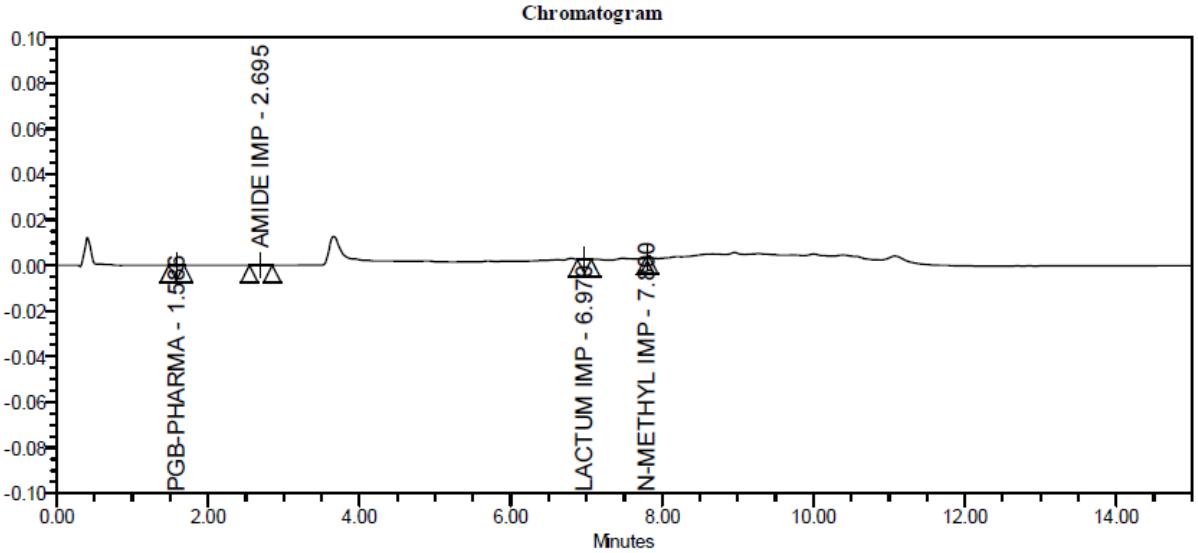



**Table 9 - Establishment of LOD**

***Peak Results***

| | Peak name | RT | Area | % Area | USP Tailing | USP Resolution | S_N_Ratio |
|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.59 | 786 | 29.79 | 1.0 | | 3.6 |
| 2 | AMIDE IMP | 2.69 | 1088 | 41.28 | 1.0 | 5.2 | 3.8 |
| 3 | LACTUM IMP | 6.97 | 563 | 21.35 | 0.9 | 22.6 | 3.4 |
| 4 | N-METHYL IMP | 7.80 | 200 | 7.58 | 0.6 | 2.8 | 3.1 |

**Conclusion:**

LOQ and LOD values of all the impurities were found to be within the acceptable criteria

**LINEARITY**

Linearity was conducted from LOQ to 150% with respect to sample solution.

**Fig-27 Chromatogram of blank**

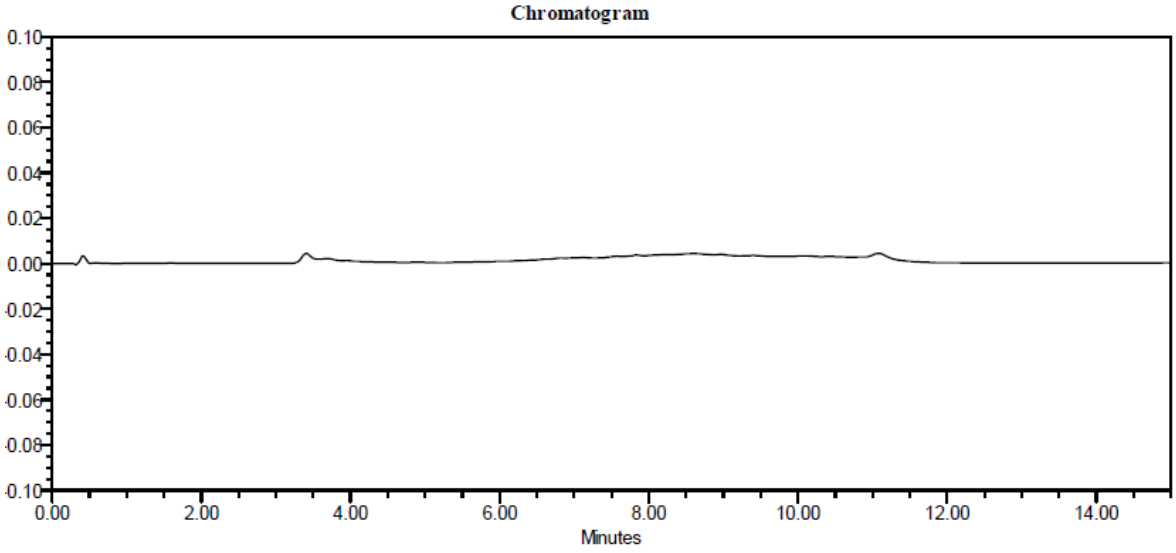



**Fig-28 Linearity at LOQ level:**

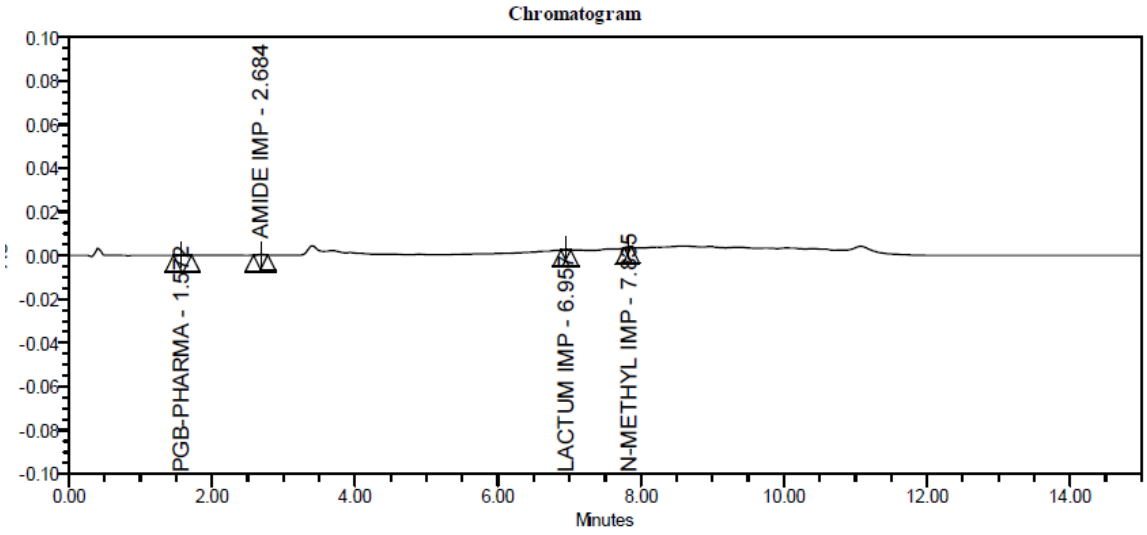



**Fig- 29 Linearity At 40 % Level :-**

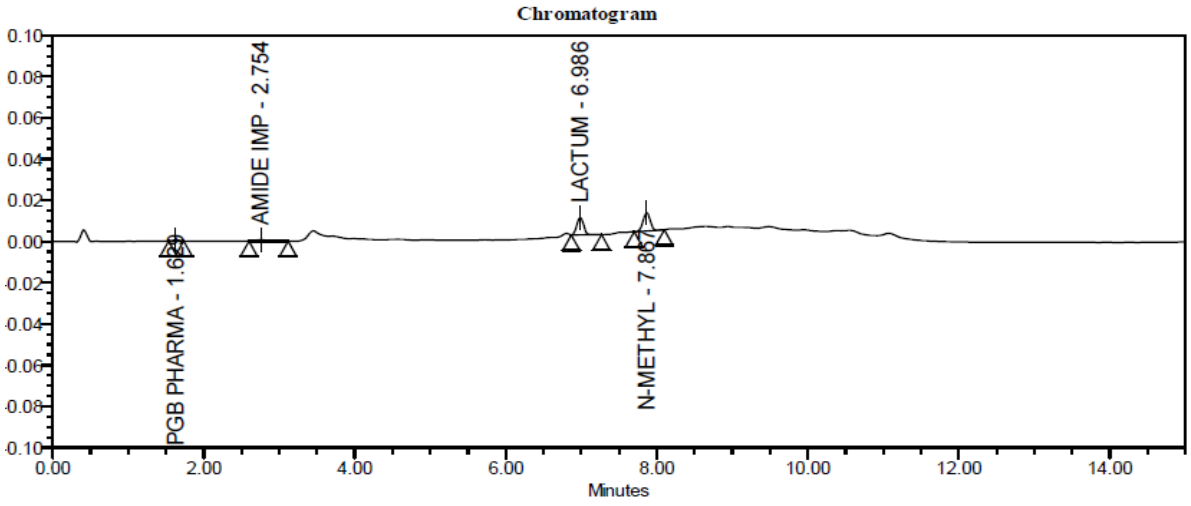


**Fig-30 Linearity at 60% level :-**

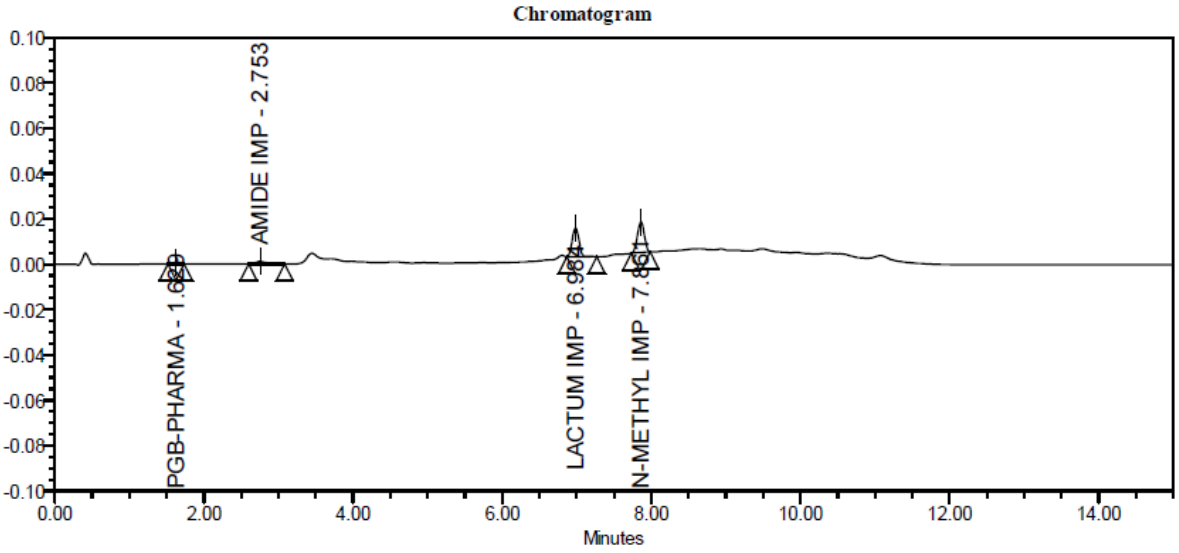



**Fig- 31 Linearity at 80% level**

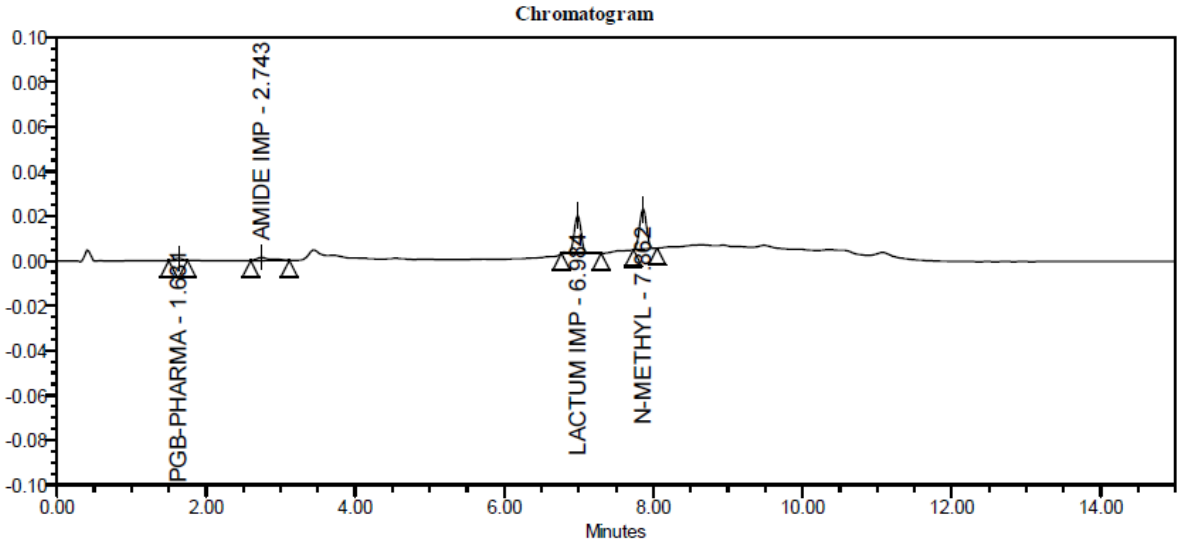



**Fig- 32 Linearity at 100 % level:**

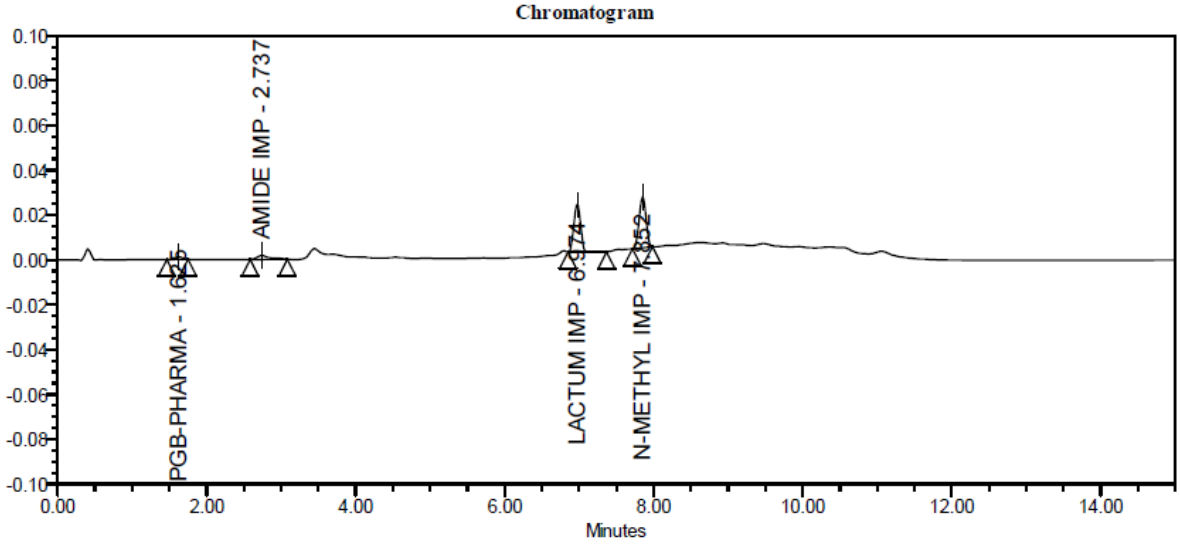


**Fig-33 Linearity at 120% level:**

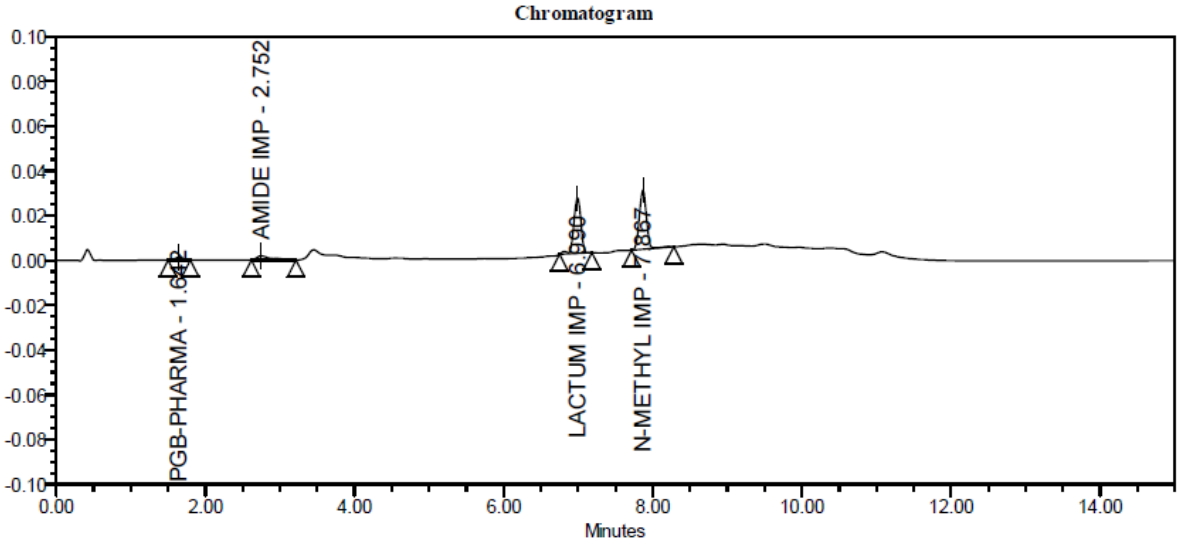



**Fig-34 Chromatogram at 150% level**

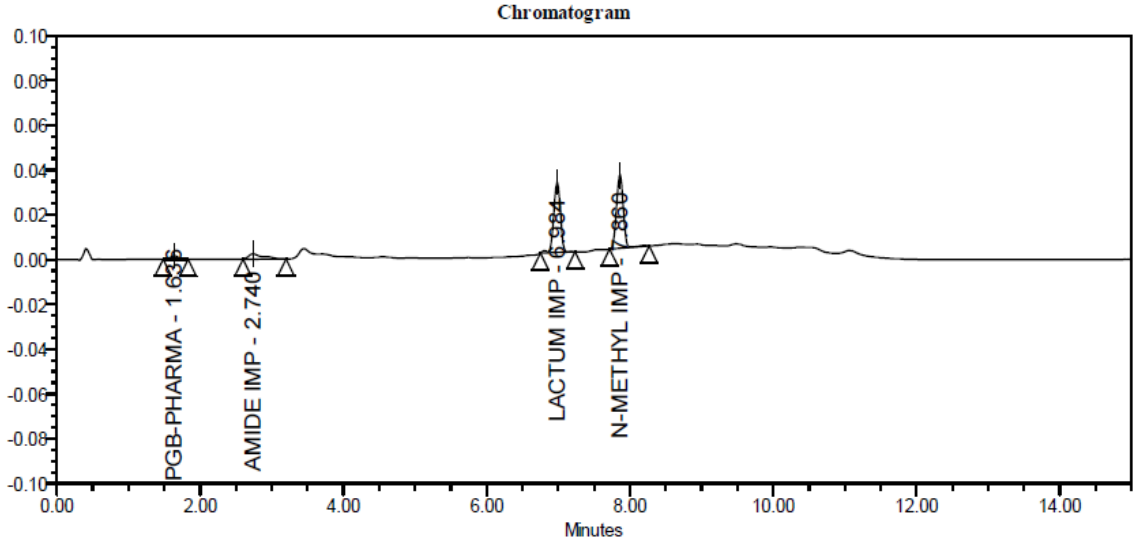



**Fig-35 Linearity graph of Amide impurity**

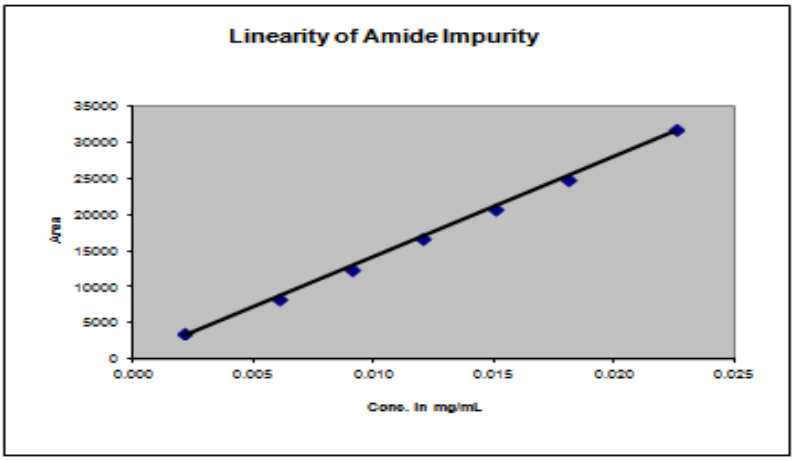


**Table 10-Linearity of AMIDE impurity result**

| Level | Conc.(mg/ml) | Peak area |
|---|---|---|
| LOQ | 0.002 | 3783 |
| 40% | 0.006 | 8498 |
| 60% | 0.009 | 12620 |
| 80% | 0.012 | 16888 |
| 100% | 0.015 | 21034 |
| 120% | 0.018 | 25075 |
| 150% | 0.023 | 31872 |
| **Slope** | 1380384 | |
| **Intercept** | 427.075 | |
| **Correlation coefficient** | **0.999** | |

**Fig-36 Linearity graph of Lactum impurity**

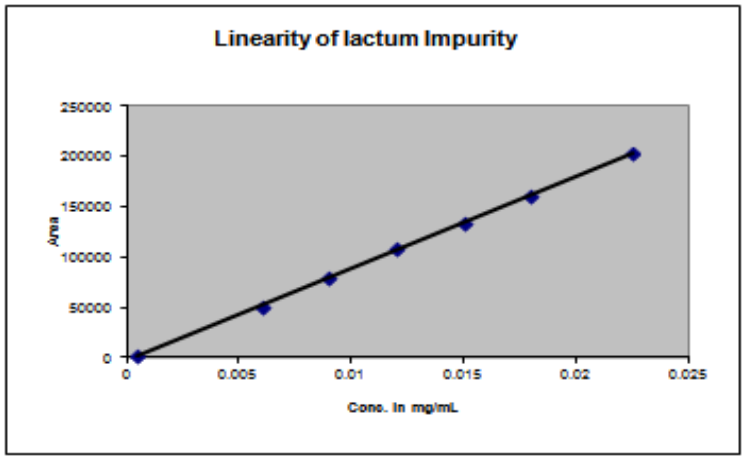



**Table 11: Linearity of LACTUM impurity Result**

| Level | Conc.(mg/ml) | Peak area |
|---|---|---|
| LOQ | 0.0005 | 3884 |
| 40% | 0.006 | 52267 |
| 60% | 0.009 | 81572 |
| 80% | 0.012 | 108837 |
| 100% | 0.015 | 134453 |
| 120% | 0.018 | 162945 |
| 150% | 0.0225 | 203784 |
| **Slope** | 9145379 | |
| **Intercept** | -1805.63 | |
| **Correlation coefficient** | **0.999** | |

**Fig- 37 Linearity graph of N-Methyl impurity**

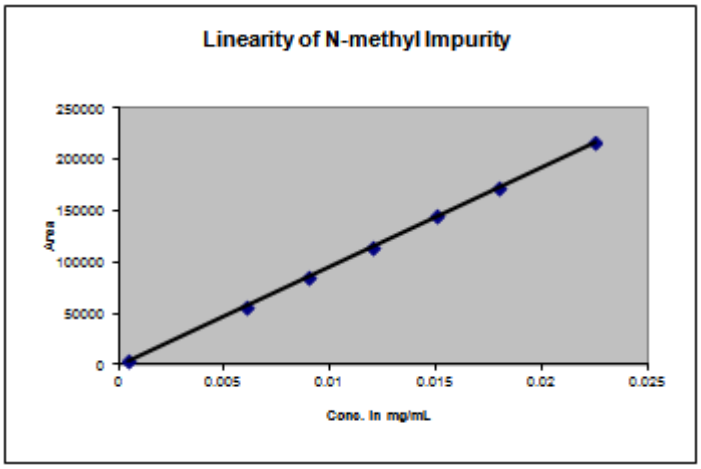



**Table12 - Linearity of N-Methyl impurity Result**

| Level | Conc.(mg/ml) | Peak area |
|---|---|---|
| LOQ | 0.005 | 3884 |
| 40% | 0.006 | 57536 |
| 60% | 0.009 | 86883 |
| 80% | 0.012 | 115671 |
| 100% | 0.015 | 145236 |
| 120% | 0.018 | 173715 |
| 150% | 0.0225 | 216832 |
| **Slope** | 9683721 | |
| **Intercept** | -570.269 | |
| **Correlation coefficient** | **0.990** | |

**Fig-38 Linearity graph of PGB -PHARMA**

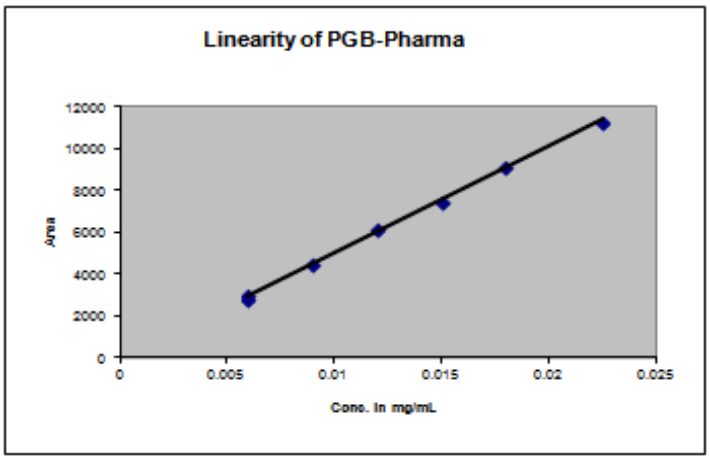


**Table- 13 Linearity of Pregabalin pharma results**

| Level | Conc.(mg/ml) | Peak area |
|---|---|---|
| LOQ | 0.006 | 2849 |
| 40% | 0.006 | 3029 |
| 60% | 0.009 | 4502 |
| 80% | 0.012 | 6187 |
| 100% | 0.015 | 7536 |
| 120% | 0.018 | 9132 |
| 150% | 0.0225 | 11363 |
| **Slope** | 511093.7 | |
| **Intercept** | -90.54 | |
| **Correlation coefficient** | **0.999** | |

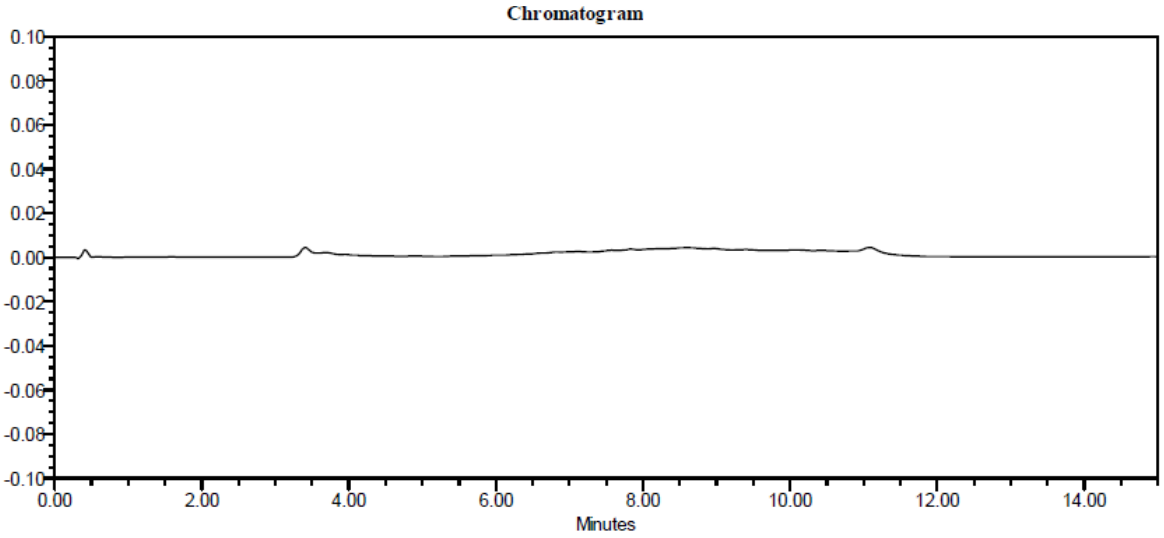
**Acceptance Criteria:**

The plot of concentration versus peak area for each impurity and Pregabalin should be linear with a correlation coefficient ($R^2$) not less than 0.990.

**Conclusion:** $R^2$ values were found to be 0.999, 0.999,0.990,0.999 for Amide ,Lactum N-Methyl and Pregabalin respectively

**PRECISION:-**

**Fig- 39 Chromatogram of blank**




**A. LOQ PRECISION**

A Solution of LOQ was prepared, injected six times and then analyzed

**Fig 40 : LOQ Precision,**

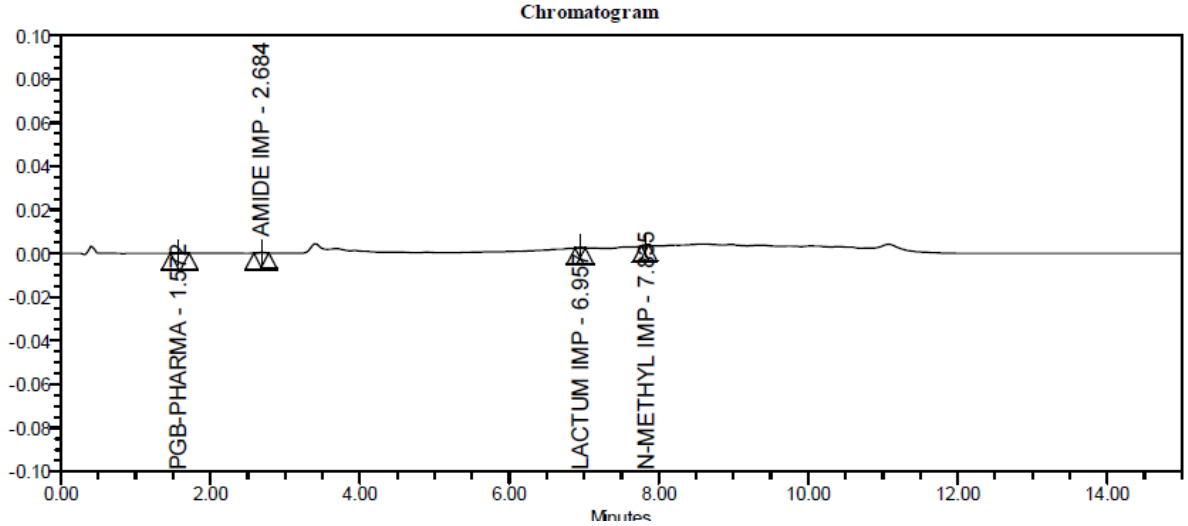



**Table 14: LOQ Precision result**

| Inj. | PGB-Pharma | Amide impurity | Lactum impurity | N-Methyl impurity |
|---|---|---|---|---|
| Inj 1 | 1869 | 3067 | 1852 | 5057 |
| Inj 2 | 1863 | 3162 | 1986 | 5168 |
| Inj 3 | 1879 | 3098 | 2012 | 5179 |
| Inj 4 | 1869 | 3058 | 1940 | 5128 |
| Inj 5 | 1843 | 3293 | 1879 | 4965 |
| Inj 6 | 1897 | 3193 | 1933 | 5099 |
| **Average** | 1870 | 3145 | 1933 | 5101 |
| **STDEV** | 16.27 | 82.01 | 55.63 | 71.60 |
| **%RSD** | **0.87** | **2.60** | **2.80** | **1.40** |

**Acceptance Criteria:** The % RSD of peak areas for each impurity should be≤ 5.0

**Conclusion:** The %RSD was found to be 0.87, 2.60, 2.80, 1.40 for Pregabalin, Amide ,Lactum ,N-Methyl Respectively

## METHOD PRECISION:

A Solution of, Pregabalin spiked with corresponding impurities at 100% level was prepared six times and then analyzed.

**Table 15 : method precision result**

| No. of Injections at 100% Level | AREA | | | % Recovery | | |
|---|---|---|---|---|---|---|
| | Amide impurity | Lactum impurity | N-Methyl Impurity | Amide | Lactum impurity | N-Methyl impurity |
| Inj 1 | 21113 | 134902 | 144531 | 99.62 | 99.66 | 100.48 |
| Inj 2 | 20962 | 134498 | 144653 | 100.34 | 99.96 | 100.4 |
| Inj 3 | 21142 | 134451 | 144730 | 99.48 | 100 | 100.34 |
| Inj 4 | 20824 | 134474 | 144920 | 101 | 99.98 | 100.21 |
| Inj 5 | 21628 | 134536 | 144479 | 97.25 | 99.93 | 100.52 |
| Inj 6 | 21079 | 134485 | 1445127 | 99.78 | 99.97 | 100.07 |
| **Average** | 21124 | 134557 | 144740 | 99.57 | 99.91 | 100.33 |
| **STDEV** | 249 | 156 | 224 | 1.16 | 0.11 | 0.15 |
| **%RSD** | 1.17 | 0.11 | 0.15 | 1.16 | 0.11 | 0.15 |

**Acceptance Criteria:** The % RSD for Recovery obtained for each impurity should be≤ 5.0.

**Conclusion:** The %RSD was found to be within the limits and results were satisfactory.

**SYSTEM PRECISION:**

Analysis was performed using a 100 % specification level of API and its impurities mix solution, (Reference Solution) injected 6 times & then analyzed.

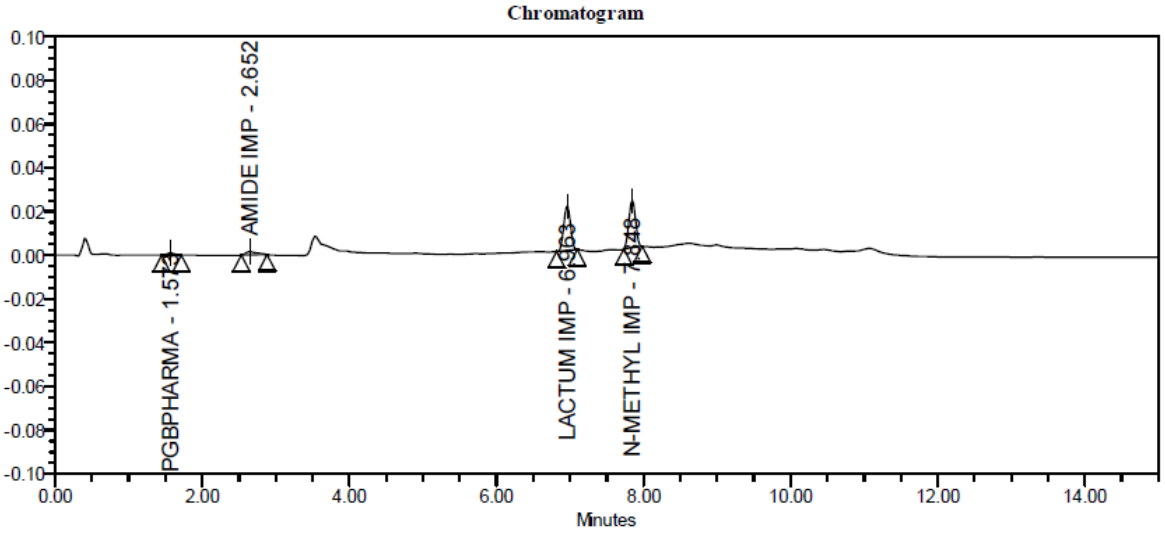



**Figure 41 : Chromatogram of System Precision**

**Table16 : System precision result**

| No of Injections at 100% level | Area | | | |
|---|---|---|---|---|
| | **PGB-Pharma** | **Amide Impurity** | **Lactum impurity** | **N-methy limpurty** |
| Inj 1 | 9222 | 14062 | 140328 | 145739 |
| Inj 2 | 9234 | 14088 | 140284 | 145525 |
| Inj3 | 9267 | 14103 | 140199 | 145520 |
| Inj 4 | 9275 | 14090 | 140322 | 145602 |
| Inj 5 | 9236 | 14086 | 140312 | 145643 |
| Inj 6 | 9249 | 14116 | 140234 | 145712 |
| **Average** | 9247 | 14090 | 140279 | 145623 |
| **STDEV** | 20.48 | 11.61 | 47.07 | 73.30 |
| **%RSD** | **0.22** | **0.08** | **0.03** | **0.05** |

System Precision, Peak result

**Acceptance Criteria:** The % RSD of peak areas of replicate injections for each impurity should be≤ 5.0

**Conclusion:** The %RSD was found to be within the limits and results were satisfactory.

**1.5.5 ACCURACY:**

Accuracy was conducted from LOQ to 150% with respect to sample solution

**Fig- 42 Chromatogram of blank**

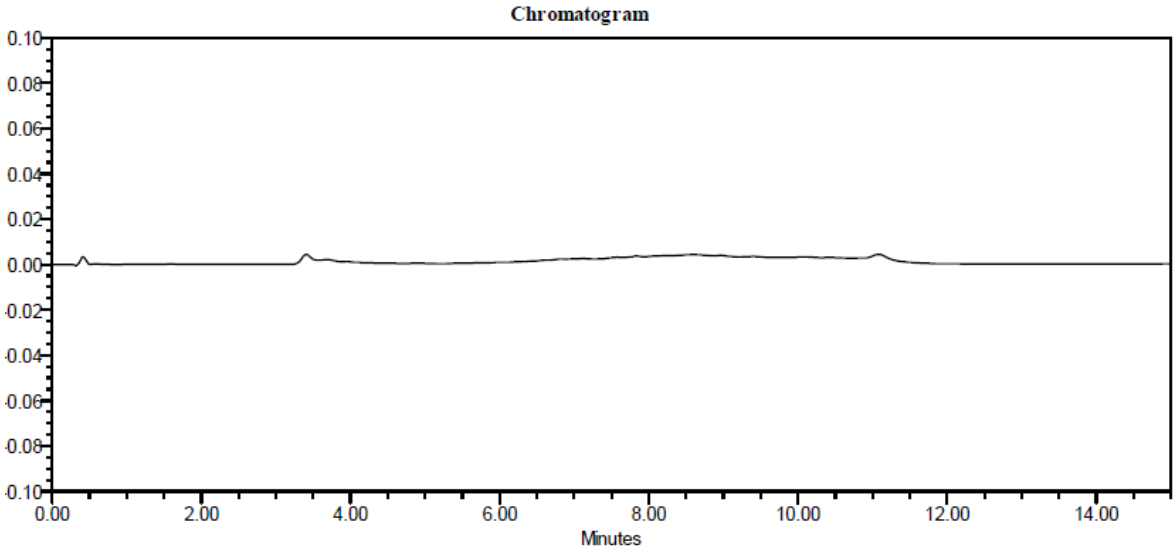


**Fig-43 0.1%Reference solution** :-

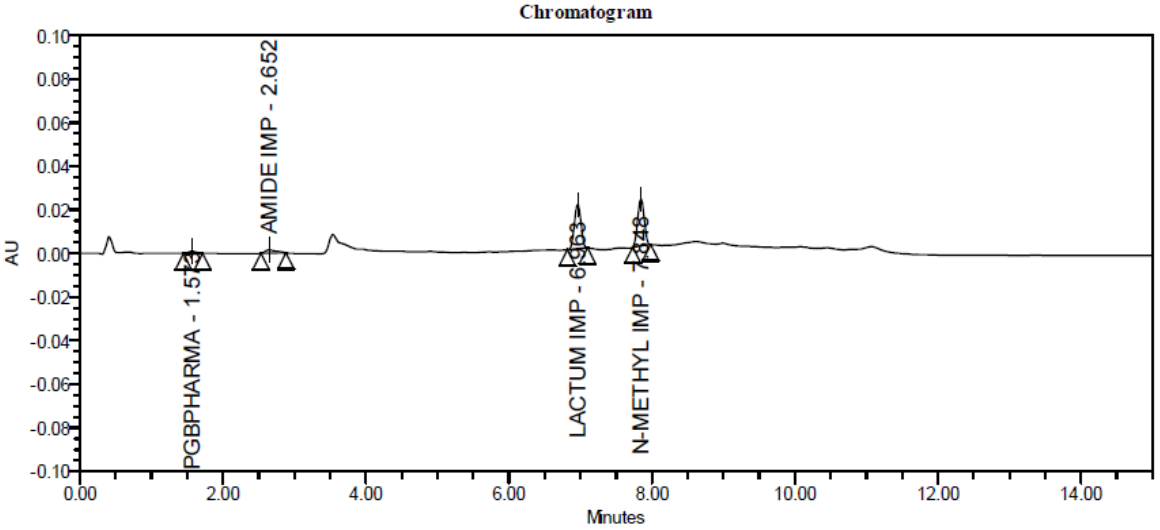



**Fig-44 At LOQ Level:**

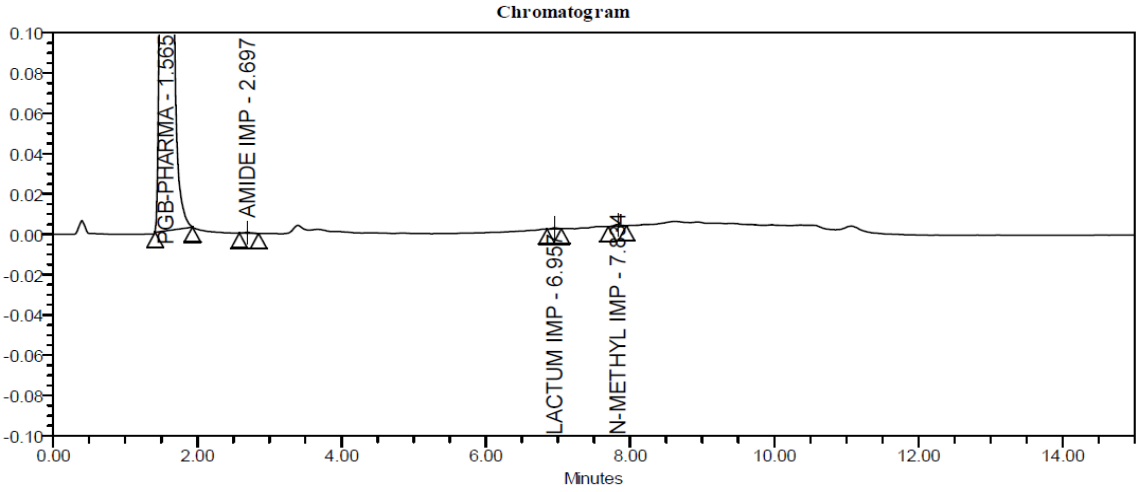



**Fig- 45 At 40% Level:**

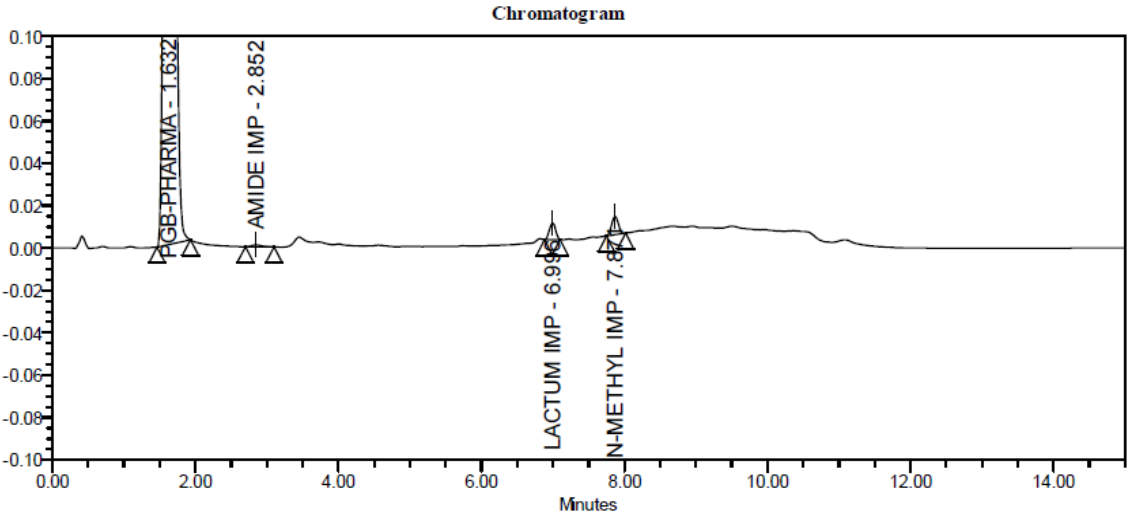


**Fig-46 At 100% Level:**

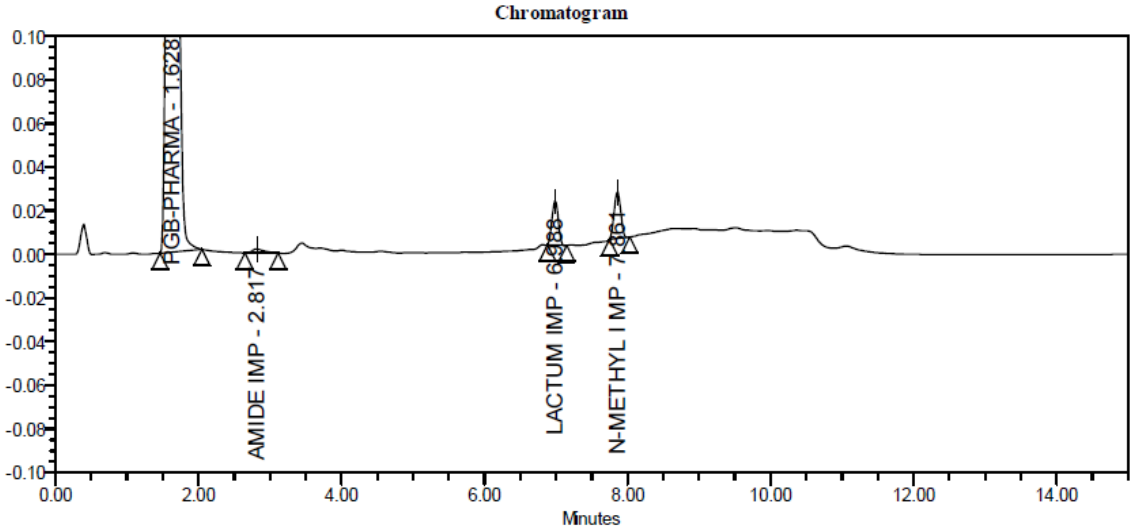



**Fig- 47 At 150% Level:**

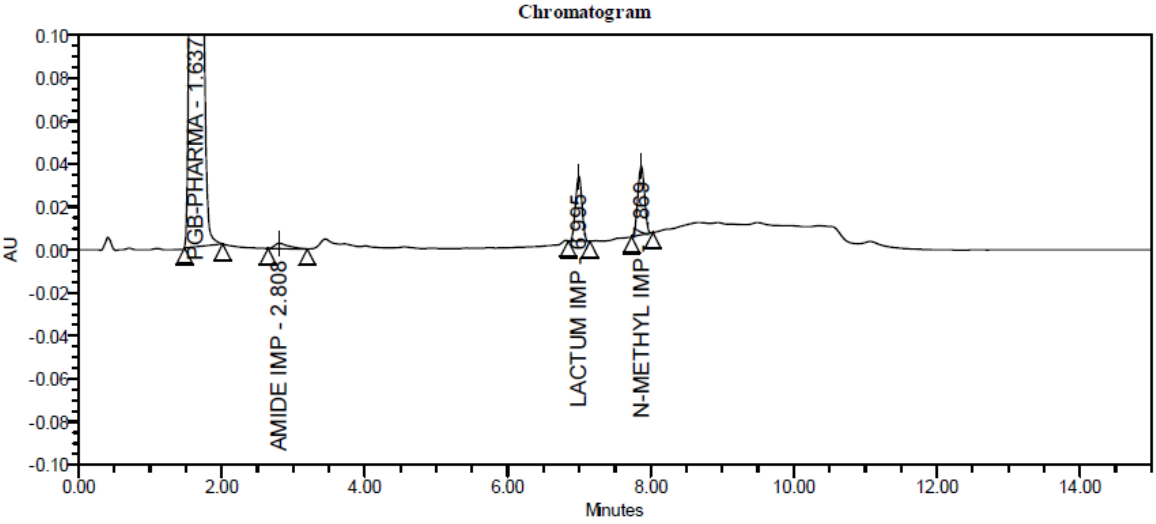



**Table 17:ACCURACY AT LOQ LEVEL**

| Level | Actual Area | | | Area Found | | |
|---|---|---|---|---|---|---|
| LOQ | Amide Impurity | Lactum Impurity | N-Methyl Impurity | Amide Impurity | Lactum Impurity | N-Methyl Impurity |
| Prep-1 | 3783 | 2569 | 2884 | 3790 | 2572 | 2889 |
| Prep-2 | 3785 | 2572 | 2889 | 3779 | 2576 | 2896 |
| Prep-3 | 3790 | 2576 | 2890 | 3795 | 2580 | 2896 |

**Table 18:ACCURACY AT 40 % LEVEL**

| **Level** | **Actual Area** | | | **Area Found** | | |
|---|---|---|---|---|---|---|
| 40% Level | Amide Impurity | Lactum Impurity | N-Methyl Impurity | Amide Impurity | Lactum Impurity | N-Methyl Impurity |
| Prep-1 | `8498 | 52267 | 57536 | 8513 | 52273 | 57542 |
| Prep-2 | 8501 | 52270 | 57540 | 8512 | 52282 | 57548 |

**Table 19 :ACCURACY AT 100% LEVEL**

| **Level** | **Actual Area** | | | **Area Found** | | |
|---|---|---|---|---|---|---|
| 100% Level | Amide Impurity | Lactum Impurity | N-Methyl Impurity | Amide Impurity | Lactum Impurity | N-Methyl Impurity |
| Prep-1 | 21034 | 134453 | 145236 | 21039 | 134462 | 145245 |
| Prep-2 | 21042 | 134459 | 145246 | 21056 | 134462 | 145261 |

**Table 20:ACCUARACY AT 150% LEVEL**

| **Level** | **Actual Area** | | | **Area Found** | | |
|---|---|---|---|---|---|---|
| **150% Level** | **Amide Impurity** | **Lactum Impurity** | **N-Methyl Impurity** | **Amide Impurity** | **Lactum Impurity** | **N-Methyl Impurity** |
| Prep-1 | 31872 | 162945 | 216832 | 31885 | 162956 | 216852 |
| Prep-2 | 31879 | 162952 | 216840 | 31890 | 162960 | 216854 |

**Table 21- Recovery at each level -**

| **NAME OF THE IMPURITY** | **% R.S.D & RECOVERY AT EACH LEVEL** | | | |
|---|---|---|---|---|
| | **AT LOQ LEVEL** | **AT 40 % LEVEL** | **AT 100% LEVEL** | **AT 150% LEVEL** |
| **AMIDE IMPURITY** | 100 | 100 | 99.9 | 99.9 |
| **% RSD** | 0.077 | 0.017 | 0.019 | 0.010 |
| **LACTUM IMPURITY** | 99.8 | 99.9 | 99.8 | 99.2 |

| | | | | |
|---|---|---|---|---|
| **%R.S.D** | 0.111 | 0.002 | 0.002 | 0.002 |
| **N-METHYL IMPURITY** | 99.8 | 99.9 | 99.7 | 99.9 |
| **%R.S.D** | 0.090 | 0.003 | 3.00 | 0.001 |

**Conclusion:** The %RSD, % recovery was found to be within the limits and the results were satisfactory.

**ROBUSTNESS**

**Table22 :Robustness**

| **Parameters** | **Change of conditions** | | **Tailing factor for PREGABALIN** | **Resolution between Pregabalin and Amide imp** |
|---|---|---|---|---|
| **Flow variation** (ml/min) | Actual | 0.30 | 1.0 | 3.6 |
| | Low | 0.28 | 1.1 | 3.4 |
| | High | 0.32 | 1.1 | 3.2 |
| **Column oven temp. variation** In **(**°C**)** | Actual | 40.0 | 1.0 | 4.2 |
| | Low | 38.0 | 1.1 | 3.9 |
| | High | 42.0 | 1.3 | 4.3 |

**Table 23 : Robustness at low tempararure (38°C )**

| **Inj.** | **PGB-Pharma** | **Amide impurity** | **Lactum impurity** | **N-Methyl impurity** |
|---|---|---|---|---|
| Inj 1 | 11425 | 21464 | 139214 | 141934 |
| Inj 2 | 11046 | 21780 | 139510 | 141570 |
| Inj 3 | 11272 | 21556 | 139265 | 141699 |
| Inj 4 | 11061 | 21772 | 139307 | 141563 |
| Inj 5 | 10981 | 21425 | 139582 | 141645 |
| Inj 6 | 10844 | 21327 | 139056 | 142701 |
| **Average** | 11101.83 | 21554 | 139322 | 141852 |
| **STDEV** | 191.13 | 170.72 | 177.40 | 399.33 |
| **%RSD** | **1.72** | **0.79** | **0.12** | **0.28** |

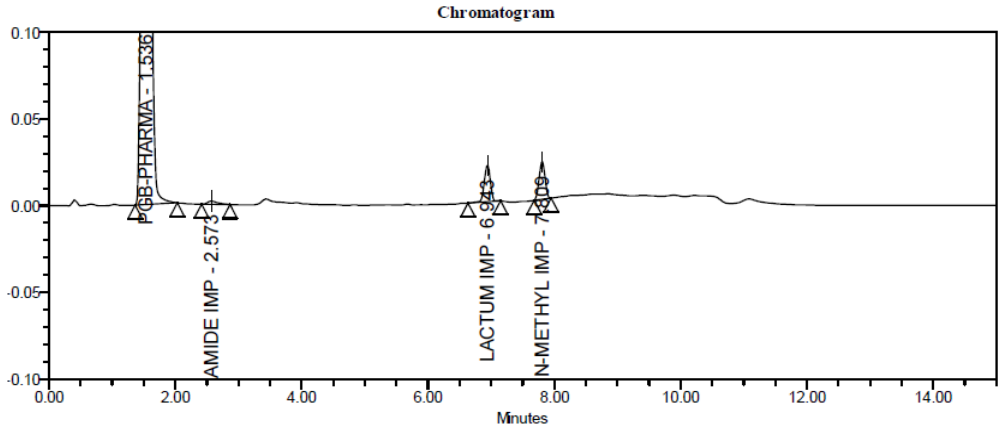



**Fig-58 At Low Temperature(38°C)**

***Peak Results***

| | Peak name | RT | Area | % Area | RT Ratio | USP Tailing | USP Resolution | Relative RT (min) |
|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.54 | 4391087 | 93.55 | | 1.1 | | |
| 2 | AMIDE IMP | 2.57 | 21771 | 0.46 | 1.68 | 1.5 | 3.7 | 1.037 |
| 3 | LACTUM IMP | 6.94 | 140524 | 2.99 | 4.52 | 0.9 | 17.0 | 5.407 |
| 4 | N-METHYL IMP | 7.81 | 140355 | 2.99 | 5.09 | 1.0 | 4.9 | 6.274 |

| | **PGB-Pharma** | **Amide impurity** | **Lactumimpurity** | **N-Methyl impurity** |
|---|---|---|---|---|
| Inj 1 | 11137 | 21595 | 139455 | 142996 |
| Inj 2 | 11153 | 21446 | 139208 | 143507 |
| Inj 3 | 11278 | 21735 | 139682 | 142632 |
| Inj 4 | 11054 | 21169 | 139223 | 142361 |
| Inj 5 | 11063 | 21727 | 139704 | 142975 |
| Inj 6 | 10814 | 21804 | 139412 | 142648 |
| **Average** | 11083.17 | 21579.33 | 139447 | 142853 |
| **STDEV** | 141.09 | 217.14 | 195.72 | 364.22 |
| **%RSD** | **1.27** | **1.00** | **0.14** | **0.25** |

**Table 24: Robustness at High temperature (42°C)**

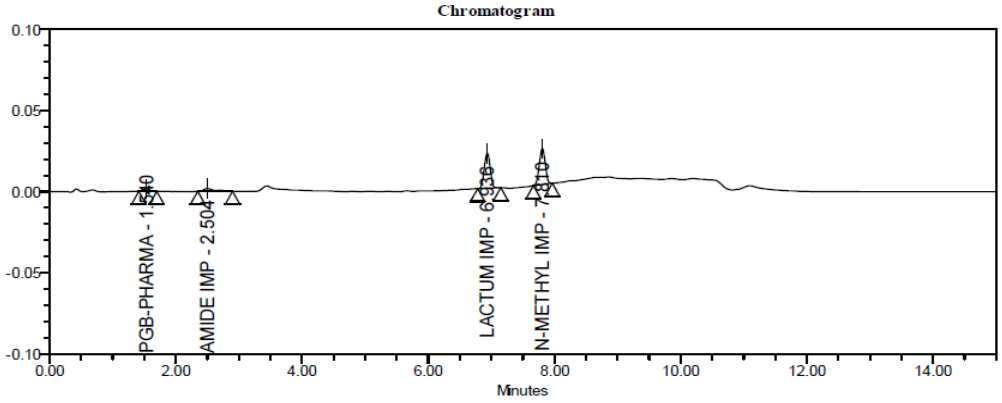



**Fig- 48 At High Temperature (42°C)**

***Peak Results***

| | Peak name | RT | Area | % Area | RT Ratio | USP Tailing | USP Resolution | Relative RT (min) |
|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.54 | 10672 | 3.35 | | 1.1 | | |
| 2 | AMIDE IMP | 2.50 | 22600 | 7.09 | 1.63 | 1.9 | 4.1 | 0.964 |
| 3 | LACTUM IMP | 6.94 | 139988 | 43.93 | 4.50 | 1.0 | 19.6 | 5.396 |
| 4 | N-METHYL IMP | 7.81 | 145388 | 45.63 | 5.07 | 1.0 | 4.9 | 6.270 |

| | **PGB-Pharma** | **Amide impurity** | **Lactum impurity** | **N-Methyl impurity** |
|---|---|---|---|---|
| Inj 1 | 11848 | 22602 | 154960 | 154424 |
| Inj 2 | 11678 | 22429 | 154887 | 155048 |
| Inj 3 | 11630 | 22427 | 154840 | 155038 |
| Inj 4 | 11902 | 22989 | 154991 | 155844 |
| Inj 5 | 11604 | 22461 | 154955 | 154170 |
| Inj 6 | 11817 | 22083 | 154268 | 155739 |
| **Average** | 11746. | 22498 | 154816 | 155043 |
| **STDEV** | 114.03 | 269.34 | 250.53 | 615.27 |
| **%RSD** | **0.97** | **0.19** | **0.16** | **0.39** |

**Table 25 : Robustness at Low flow (0.28ml/min)**

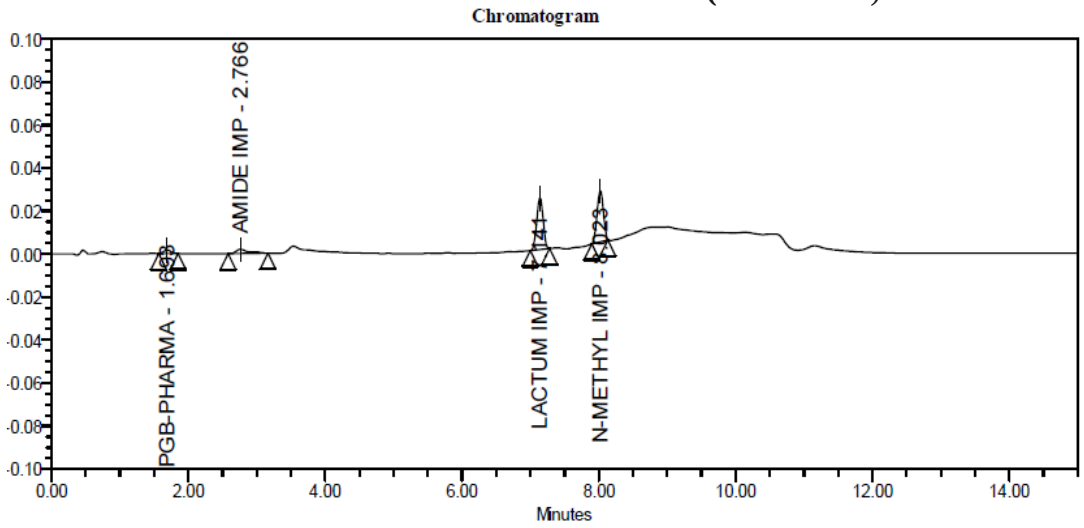



**Fig-49 At Low Flow(0.28ml/min)**

***Peak Results***

| | Peak name | RT | Area | % Area | RT Ratio | USP Tailing | USP Resolution | Relative RT (min) |
|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.69 | 11615 | 3.37 | | 1.1 | | |
| 2 | AMIDE IMP | 2.77 | 24955 | 7.25 | 1.63 | 1.8 | 4.3 | 1.073 |
| 3 | LACTUM IMP | 7.14 | 155342 | 45.10 | 4.22 | 1.0 | 18.6 | 5.448 |
| 4 | N-METHYL IMP | 8.02 | 152496 | 44.28 | 4.74 | 1.0 | 5.0 | 6.330 |

**Table 26:Robustness at High flow (0.32ml/min)**

| | **PGB-Pharma** | **Amide impurity** | **Lactumim purity** | **N-Methyl impurity** |
|---|---|---|---|---|
| Inj 1 | 10161 | 23022 | 125318 | 130208 |
| Inj 2 | 10111 | 20375 | 125567 | 130437 |
| Inj 3 | 10037 | 20281 | 125856 | 129980 |
| Inj 4 | 10027 | 20075 | 125112 | 131021 |
| Inj 5 | 9882 | 20710 | 125896 | 130189 |
| Inj 6 | 10055 | 20433 | 125166 | 130296 |
| **Average** | 10045 | 20366 | 125485 | 130355 |
| **STDEV** | 86.47 | 190.06 | 311.46 | 327.50 |
| **%RSD** | **0.86** | **0.93** | **0.24** | **0.25** |

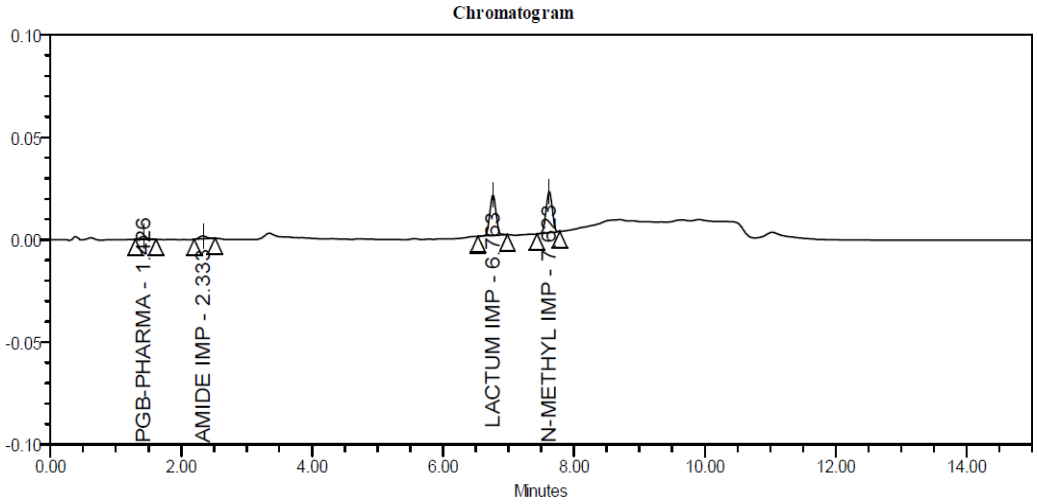



**Fig -50 At High flow (0.32ml/min)**

***Peak Results***

| | Peak name | RT | Area | % Area | RT Ratio | USP Tailing | USP Resolution | Relative RT (min) |
|---|---|---|---|---|---|---|---|---|
| 1 | PGB-PHARMA | 1.43 | 9726 | 3.42 | | 1.1 | | |
| 2 | AMIDE IMP | 2.33 | 10969 | 3.86 | 1.64 | 0.9 | 4.6 | 0.908 |
| 3 | LACTUM IMP | 6.76 | 131213 | 46.12 | 4.74 | 1.0 | 23.1 | 5.337 |
| 4 | N-METHYL IMP | 7.62 | 132572 | 46.60 | 5.35 | 1.0 | 4.8 | 6.197 |

**Acceptance criteria:**

The resolution between Pregabalin and Amide impurity should be not < 2.

Peak tailing of Pregabalin should be not more than 2

**Conclusion:**The resolution between Amide and Pregabalin was found to be >2 , peak tailing of Pregabalin was found to be 1.0.

In flow variation and temperature variation the RRT remains same

## 4. DISCUSSION and CONCLUSION

A Robust method for Pregabalin was developed using ACQUITY UPLC H-Class system running Empower Software. From the obtained results it can be concluded that the above method is quite precise, accurate, simple, and repeatable method with a shorter run time of 15 minutes. After multiple trials, by carrying out the changes of mobile phase composition, pH, temperature, flow rate the method was found to be optimised at total run time of 15minutes, using HALO C18,(50x2.1 mm, 2.7µm) column, flow rate at 0.3ml/min, injection volume 4µl .sodium dihydrogen salt is used as buffer (PH 7 ) with NaOH Buffer and ACN (98:2 v/v) as mobile phase-A and Acetonitrile (100%) as mobile phase-B, detection wavelength of 210nm. Stress studies were performed at a concentration of 15mg/ml of drug. The linearity studies were performed at LOQ, 40%, 60%, 80%, 100%, 120%,150% of the specification level with respect to sample.

The developed method was successfully validated for its linearity, range, precision, accuracy and specificity in accordance with the requirements of ICH guidelines.The results of the study showed that, the proposed UPLC method was simple, rapid,

precise, accurate and stability indicating, which can be used for the routine analysis for the determination of Pregabalin and its related impurities.

## 7. FUNDING

This research does not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## 8. REFERENCE

1. Lena Ohannesian, Anthony J Streeter. 2008. Hand Book of Pharmaceutical Analysis.In Drugs and Pharmaceutical Sciences.Vol 117. James Swarbrick (Ed.).Marcel Dekker, Inc., New York; 87- 88,104.
2. ICH guideline, Q3A (R2) Impurities in New Drug Substances, Food and Drug Administration, USA, February 2006
3. ICH guideline, Q3B (R2) Impurities in New Drug Products, Food and Drug Administration, USA, February 2006
4. Kavitapilaniya and ,harish.k: Recent trends in impurity profile of pharmaceuticals j Adrpharm Techno Res,2010,1(3),302-310
5. ICH guideline, Q3A (R2) Impurities in New Drug Substances, Food and Drug Administration, USA, February 2006.
6. Swartz,M E.;Murphy,B.J: Ultra performance liquid chromatography, tomorrow's Hplc technology today. *Lab Plus Int* 2004**,** *18* (3): 6-9.
7. UPLC -An introduction and review ,Michel E,Swartz ;waters corporation ,Milford ,Massachusetts ,USA ;Journal of Liquid Chromatography & Related technologies ,28: 1253 -1263 ,2005
8. Satinder Ahuja Henrik Rasmussen Hplc Method Development For Pharmaceuticals 2007 1st Edition 452-455.
9. Lioyd R.Snyder: Practical Hplc Method Development**,** 3rd Edition:751-780
10. Satinder Ahuja Henrik Rasmussen ;Hplc Method Development For Pharmaceuticals 2007 1st Edition:452-455.
11. Lena Ohannesian, Anthony J Streeter. 2008. Hand Book of Pharmaceutical Analysis.In Drugs and Pharmaceutical Sciences. Vol 117.James Swarbrick (Ed.).Marcel Dekker, Inc., New York; 87- 88,104
12. ICH guideline, Validation of analytical procedures, text and methodology Q2 (R1)
13. Khopkar SM. 2004. Basic Concepts of Analytical Chemistry.New Age International (P) Ltd., : New Delhi; 1-5, 20-24.
14. James W Munson. 2001. Pharmaceutical Analysis Modern Methods. Part – B. Marcel Dekkerss, Inc., New York; 15-38.
15. Hobart H Willard, Lynne L Merritt, Jr., John A Dean, Frank A Settle, Jr. 1986. Instrumental Methods of Analysis. 7th edn., CBS Publishers and Distributors: New Delhi; 1, 592, 622-628.
16. Mendham J, Denney RC, Barnes JD, Thomas MJK. 2008. Vogel's Text book of Quantitative Chemical Analysis. 6th edn., Dorling Kindersley(India) Pvt. Ltd., New Delhi; 29,36,289-295.
17. Sethi PD. 1997. Quantitative Analysis of Drugs in Pharmaceutical Formulations. 3rd edn., CBS Publishers and Distributors: New Delhi; 6-9.
18. Douglas A Skoog, Donald M West, James F Holler, Stanley R Crouch. 2007.Fundamentals of Analytical Chemistry. 8th edn., Thomson Asia Pvt. Ltd: Singapore; 4, 921, 975.
19. Ashu M, Parmar S, Nagarajan K. Development and validation of rapid HPLC method for determination of Pregabalin in bulk drug and capsule dosage forms Der Pharma Chemica 3(1): 482-489 2011
20. Prashant P , Tanmay S. Development and validation of HPLC method for the determination of pregabalin in bulk and

in pharmaceutical formulations .Research Journal of Pharmacy and Technology 5(6) : 2012

21. Kasawar G. B, Farooqui M. N. Development and Validation of HPLC Method for the Determination of Pregabalin in Capsules. Indian Journal of Pharmaceutical Sciences 72(4): 517–519. 2010.
22. Kannapan N Nayak S.P.,Venkatachalam T., Prabhakaran V. Analytical RP-HPLC Method for Development and Validation of Pregabalin and Methylcobalamine in Combined Capsule Formulation Journal of Applied Chemical Research, 13, 85-89 (2010)
23. Sarvesh Kumar Mishra, B.M.gurupadhyya and Surajpal Verma. Stability Indicating RP-HPLC method for determination of pregabalin using ICH guidelines. International Journal of Natural Product Science 1: 130 2012
24. Narmada P, Vijaya lakshmi G. RP-HPLC method development and validation for determination of methylcobalamin and pregabalin in combined capsule dosage form.International journal of pharmaceutical sciences 4(1) 25-29. 2013
25. Naresh Chandra Reddy M and Chandra Sekhar. RP-HPLC Determination of Related substances of Pregabalin in bulk and pharmaceutical dosage form, International Journal of Chemical and Pharmaceutical Sciences. 3 (2). 2012.