IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736. Volume 18, Issue 6 Ser. 1 (June 2025), PP 41-51
www.iosrjournals.org

Analytical Method Development and Validation of Glucosamine Sulphate and Its Impurities by Using AQbD Approach

Ms. Kanchan T. Sawarkar
Priyadarshini J. L. College of Pharmacy Nagpur

Abstract:
Background*: Pharmaceutical firms are confronting issues with pharmaceutical quality control. The classic OFAT approach required testing final products. It is time-consuming and expensive. Instead, the new AQbD strategy seeks to instill quality in the product throughout the manufacturing process, reducing the likelihood of product failure. A high-performance liquid chromatography (HPLC) method was devised to detect glucosamine sulphate impurities in bulk and solid dose formulations. There are many methods developed for the same but by using new DoE tools we can develop more robust method.*
Materials and Methods*: The HPLC separation was achieved on Phenomenex 100-5 C-18 column (5 µm 100Å, 250 mm X 4.6 mm) using a mobile phase Acetonitrile: Potassium dihydrogen ortho phosphate buffer (80:20 v/v pH 3.0) at a flow rate of 1.0 ml/min and UV detection at 210 nm. The method was validated for specificity, linearity, solution stability, accuracy, precision, limit of detection, and limit of quantitation. CPP were selected by risk assessment Programme.*
Results*: The detector response for glucosamine sulphate was linear over the selected concentration range from 100 to 500 ug/ml with a correlation coefficient 0.9997. The accuracy was between 97.40 – 100.37%. The precision (R.S.D.) amongst five sample preparations was 0.029(intraday) & 0.879(interday). The limit of detection and the limit of quantitation are 3.307 and 10.023 ug/ml, respectively.*
Conclusion: *Forced degradation study of glucosamine was carried out under acidic, alkaline, oxidative, thermal, and neural conditions. It was found that glucosamine was stable in all conditions except in oxidative degradation. The amount estimated in oxidative degradation by HPLC and HPTLC was found to be 95.28 and 95.33 respectively.*
Key Word*: Quality by design (QbD, Design of experiments (DoE), High performance liquid chromatography (HPLC), CPP (Critical process parameters).*

Date of Submission: 09-06-2025 Date of Acceptance: 20-06-2025

I. Introduction

Glucosamine is a natural amine sugar derived from the chitin found in sea prawns and crab shells. Studies have revealed that glucosamine when combined with chondroitin sulphate, it can strengthen cartilage, increase chondrocyte activity, and minimize cartilage breakdown. It is used as dietary supplement in osteoarthritis or degenerative joint disease. According to the ICH recommendations Q3A(R2) impurity is defined as "any component of the new drug substance that is not the chemical entity defined as the new drug substance". Chromatography is one of the preferable methods of impurity detection[1,2,3,4,5]. The International Conference on Harmonisation (ICH) Q8 (R1) guideline describes QbD as "a systematic approach to development that begins with predefined objectives and emphasises product and process understanding and control, based on sound science and quality risk management." When the concept of QbD is applied to the development of analytical methods, it is called Analytical Quality by Design. In conventional practice, analytical procedures were based on one factor at a time (OFAT), which optimises one parameter at a time while keeping others constant while waiting for the desired outcomes. This strategy consistently led in constrained robust behaviour, failure to comply with outcomes, and the need for a revalidation methodology. The AQbD examines scientific understanding in method implementation sequences, starting with product quality[6,7,8,9,10].

II. Material And Methods

Materials

Following marketed preparations Glucosamine sulphate U.S.P. (500 mg) were used for comparative study:
Lubrijoint 500 (WALLACE PHARMACEUTICALS PVT LTD),
Cartigen 500 PHARMED LIMITED

Instruments

- Precision Balance: A Precision Balance model Citizen Cy220 having sensitivity 0.1 mg.
- Spectrophotometer: Double beam UV-Visible spectrophotometer model shimadzu UV 1800- and 10-mm matched quartzes cell was used for measurement of sample solutions.
- HPLC System: HPLC model- SHIMADZU with pump- SPD-20AD (LC-20A) having variable wavelength, UV-Visible detector, and Rheodyne sample injector (20 µl) was used.
- Column: The analytical column was Phenomenex 100-5 C-18, 5 µm 100Å, 250 mm X 4.6 mm.
- Assay of drug by suitable official / reported method: 0.25 g GS was dissolved in 50 ml of water and 1 ml of 0.1 M HCl was added and titrated with 0.1 M NaOH using methyl orange indicator. Blank titration was carried out. 1 ml of 0.1 M NaOH is equivalent to 0.02867 g of C6H13NO8S. The percentage purity of Glucosamine sulphate was found to be 99.82 %.

Reagents and Solutions:

The diluent was optimized as mixture of Water and Acetonitrile (50: 50 v/v).

- Preparation of potassium dihydrogen ortho phosphate buffer (pH:3.0): Accurately weighed 1.36 gm of potassium dihydrogen ortho phosphate in a 1000 ml of volumetric flask and add about 900 ml of milli-Q water and degas to sonicate and finally make up to the volume with water. Then added 1ml of triethyl amine and pH was adjusted to 3.0 with dilute orthophosphoric acid solution.
- Preparation of the drug solution:
  Stock standard solution [A]: Weighed 10 mg of active drug glucosamine sulphate sodium chloride was transferred to 10 ml of volumetric flask and volume make up with diluent. This made 1000 µg/ml concentration of stock standard solution.
  Working standard solution [B]: Appropriate dilutions were made from the above resulting solution with optimized mobile phase so as get a concentration of 100 µg/ml. This solution was used further in the experiment.

Chromatographic Column

Column : Phenomenex 100-5 C-18, 5 µm 100Å, 250 mm X 4.6 mm.
Detection Wavelength : 210 nm
Injection Volume : 20 µl
Flow Rate : 1.0 ml/min
Temperature : Ambient
Mobile phase : Acetonitrile: Potassium dihydrogen ortho phosphate buffer (80:20) pH 3.0
The chromatographic conditions were set as per established parameters and mobile phase was allowed to equilibrate with stationary phase as indicated by the steady baseline.

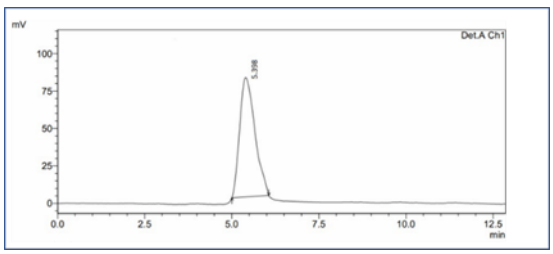


Fig1: HPLC chromatogram of glucosamine sulphate

Quality by Design approach for optimization of HPLC method:

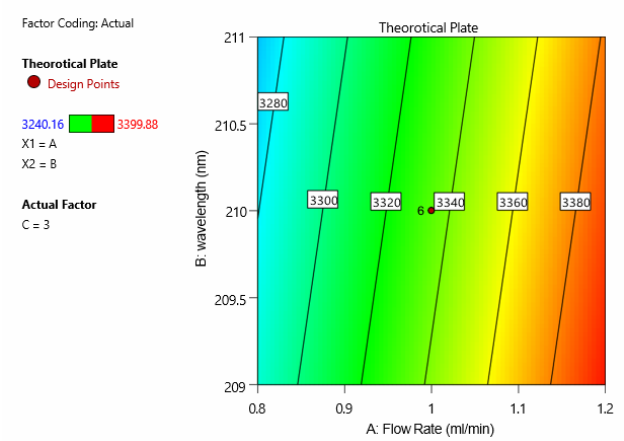
Critical quality attributes were identified for this method. Central composite drug design was used for research methodology. A 33 factorial design was applied for the optimization of the processes. Design Expert ® software version 13 was used for the QbD studies. The critical quality attributes (CQA) identified for this method were flow rate of mobile phase, wavelength, and pH of mobile phase. The critical analytical attribute (CAA) for this method were number of theoretical plates, tailing factor and retention time of drugs to be analyzed.

III. Result

Experimental data for AQbD study of GS (HPLC)

1. ANOVA for Linear model (Response 1: Theorotical Plate)

Final Equation: Sqrt (Theorotical Plate) = +71.24166-0.068393wavelength-0.504316 pH



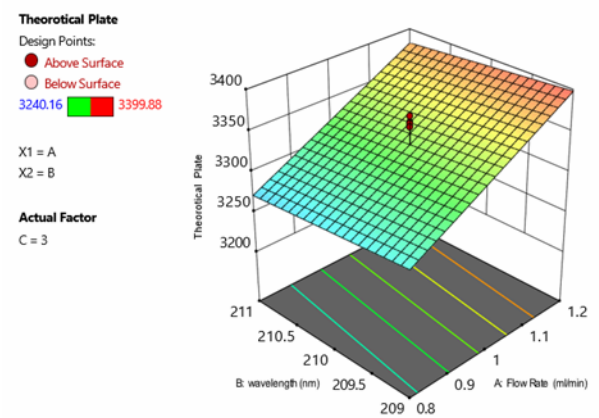
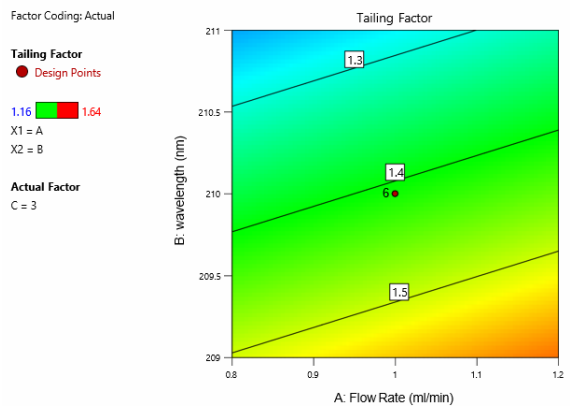


Fig. 2: Contour plot of response (Theoretical plate) **Fig. 3: 3D plot of response (Theoretical Plate)**

2. ANOVA for Linear model (Response 2: Tailing Factor)

Final Equation: Sqrt (Tailing Factor) = +12.97475+0.087302 Flow Rate-0.056197 wavelength-0.024639 pH



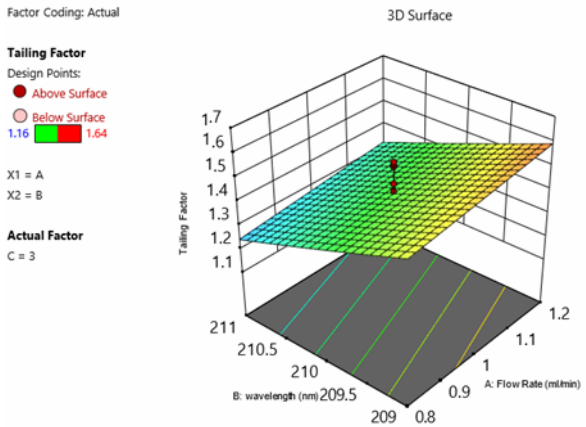


Fig. 4: Contour plot of response (Tailing Factor) **Fig. 5: 3D plot of response (Tailing Factor) for**

3. ANOVA for Quadratic model (Response 3: Retention Time)

Final Equation: Retention Time = +6.27688+4.43327 Flow Rate-0.025878 wavelength+0.159176 pH

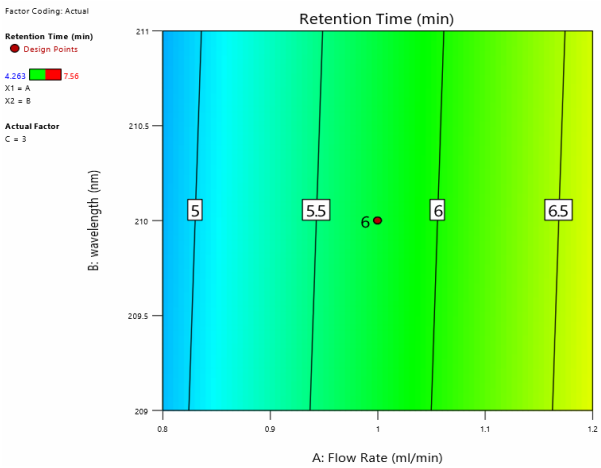


Fig. 6: Contour plot of response (Retention time)

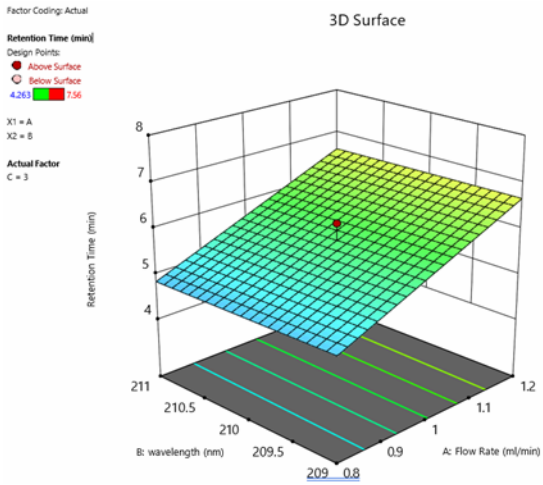


Fig. 7: 3D plot of response (Retention time)

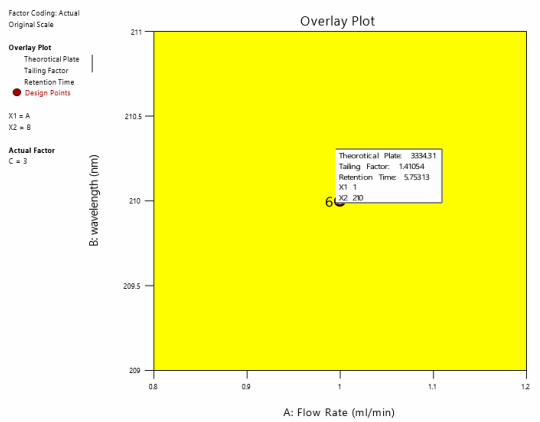


Fig. 8: Overlay plot of response

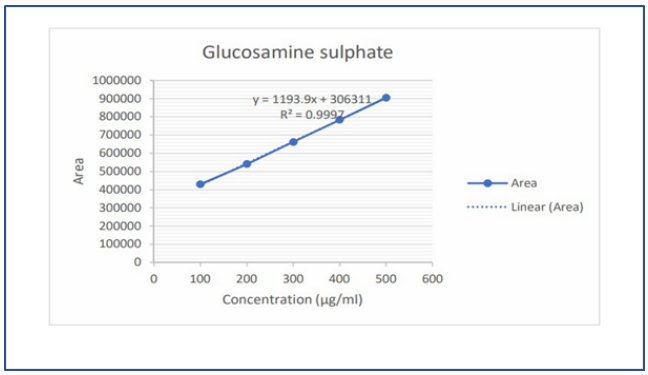


Fig. 9: Calibration curve of Glucosamine Sulphate

The percentage RSD values are less than 2% indicate that proposed method is falls within acceptable limits. The critical parameters for quality control study were selected and run on design expert version 13 software. Model was found significant in given range of concentration.

Analysis of marketed formulation by proposed method

Weigh accurately powder equivalent to 10mg of GS, dissolved in diluent, shake properly and make up the volume. The solution was sonicated for 5min. The solution was filtered through Whatman filter paper and aliquot portion of the filter was diluted with mobile phase to get a conc. of 100 µg/ml of the same. The chromatogram was recorded. The contents of drugs were calculated. The results are shown table:

Cartigen (Hard Gelatin Capsule)

Average weight of Capsule content = 515 mg (each capsule contains 500 mg GS). Weight of the capsule content equivalent to 10 mg of glucosamine sulphate was 10.3 mg.

Lubrijoint (Tablet)

Average weight of tablet = 835 mg (each tablet contains 500 mg GS). Weight of the tablet powder equivalent to 10 mg of glucosamine sulphate was 16.7 mg.

$$\% \text{ Label claim} = \frac{AUC_{test} \times Conc_{std} \times D.F. \times \text{Avg. wt. of tablet}}{AUC_{std} \times Wt_{test} \times \text{Label claim of tablet}} \times 100$$

Fig. 10: Formula for percentage label claim

Table 1: Data for assay of Cartigen (HPLC)

Sr. No.	Amount of tablet powder taken (gram)	Amount of drug estimated (gram)	% Label claim
1	10.4	10.36	99.68
2	10.3	10.20	99.12
3	10.3	10.20	99.03
4	10.6	10.58	99.87
5	10.5	10.44	99.50
Statistics			
Sr. No.	**Mean**	**±SD**	**% RSD**
1	99.44	0.3593	0.3613

Table 2: Data for assay of Lubrijoint

Sr. No.	Amount of tablet powder taken (gram)	Amount of drug estimated (gram)	% Label claim
1	16.4	16.09	98.15
2	16.6	16.33	98.43
3	16.5	16.21	98.25
4	16.7	16.46	98.62
5	16.7	16.49	98.76
Statistics			
Sr. No.	**Mean**	**±SD**	**% RSD**
1	98.44	0.2525	0.2565

Validation of HPLC method

The proposed method was validated as per ICH guidelines Q2(R1) for linearity and range, precision, accuracy, ruggedness, robustness, LOD and LOQ.

1. **Precision:** It is expressed as ±SD and % RSD of any measurements & was ascertained by replicate analysis of homogenous sample

Table 3: Precision data of GS

Sr. No.	Drug concentration (µg/ml)					
	100		300		500	
	Intraday	Interday	Intraday	Interday	Intraday	Interday
1	429777	438601	622402	667534	805407	813306
2	429635	436402	662450	647833	805764	823305
3	429775	438320	662860	666031	805914	813354
Statistics						
Mean	429729	437774.3	662570.7	660466	805695	816655
% RSD	0.018	0.273	0.037	1.660	0.032	0.705

2. **linearity and range:** The series of solutions of GS were analyzed in the range of 100 to 500 µg/ml.
3. **Accuracy:** It was determined based on recovery study performed by standard addition method.

4. **Ruggedness**

Ruggedness should be used as a parameter evaluating constancy of the results when external factors such as analyst, laboratory, instrument, reagents and days are varied. The studies of ruggedness were carried out under different conditions i.e., intraday, interday and different analyst. The results are shown in table;

Table 4: Linearity performance parameters for GS

Sr. No.	Parameters	Results
1	Linear dynamic range (µg/ml)	100 – 500
2	Slope	1193.9

3	Y-intercept	306311
4	Correlation coefficient	0.9997

5.

LOD and LOQ

The theoretically determined values of detection and quantification limits were crossed by actual analysis of these conc. using propose methods. The LOD and LOQ were calculated using the equation given below.

LOD = 3.3σ/s,

LOQ = 10σ/s where;

σ = the standard deviation of the response

S = the slope of the calibration curve

Table 5: LOD and LOQ data of GS (HPLC)

Sr. No.	Parameters	Results
1	LOD	3.30 µg/ml
2	LOQ	10.2 µg/ml

Table 6: Data for accuracy of Cartigen

Sr. No.	Relative concentration (µg/ml)				
	Unspiked sample	Added sample	Total amount estimated	Recovered sample	% Recovery
80 %					
1	100	80	179.59	79.60	99.48
2			180.12	80.12	100.16
3			179.39	79.39	99.24
100 %					
1	100	100	198.12	98.12	98.12
2			199.72	99.72	99.72
3			200.16	100.16	100.16
120 %					
1	100	120	220.804	120.804	100.37
2			219.196	119.196	99.33
3			220.880	120.880	100.37

Statistics

Sr. No.	Recovery level	Mean	±SD	% RSD
1	80 %	99.62	0.477214	0.479002
2	100 %	99.33	1.073561	1.080766
3	120 %	100.02	0.600444	0.600304

Table 7: Data for accuracy of Lubrijoint

Sr. No.	Relative concentration (µg/ml)				
	Unspiked sample	Added sample	Total amount estimated	Recovered sample	% Recovery
80 %					
1	100	80	178.59	78.59	98.23
2			177.92	77.92	97.40
3			179.04	79.04	98.80
100 %					

1	100	100	198.62	98.62	98.62
2			199.63	99.03	99.03
3			199.13	99.13	99.13
120 %					
1	100	120	218.15	118.15	98.45
2			217.96	117.96	98.30
3			219.01	119.01	99.17

Statistics				
Sr. No.	**Recovery level**	**Mean**	**±SD**	**% RSD**
1	80 %	98.14	0.704012	0.717331
2	100 %	98.92	0.270247	0.273179
3	120 %	98.64	0.465081	0.471493

Table 8: Data for Ruggedness of Cartigen (HPLC)

Level	AUC of Standard	AUC of Sample	% Assay	Mean	%RSD	Retention time
Intraday	429777	428761	99.76	99.66	0.116	5.31
		427635	99.50			
		428618	99.73			
Interday	429777	425221	98.93	98.87	0.142	5.34
		425565	99.01			
		424146	98.68			
Different analyst	429777	425909	99.09	98.87	0.160	5.35
		424275	98.71			
		424791	98.83			

Table 9: Data for Ruggedness of Lubrijoint (HPLC)

Level	AUC of Standard	AUC of Sample	% Assay	Mean	%RSD	Retention time
Intraday	429777	424490	98.76	98.46	0.370	5.36
		424146	98.68			
		421009	97.95			
Interday	429777	420321	97.79	97.76	0.230	5.33
		421946	97.47			
		421310	98.02			
Different analyst	429777	420880	97.92	97.76	0.534	5.35
		417184	97.06			
		422556	98.31			

STRESS DEGRADATION STUDY OF GS BY HPLC

The stress degradation study of GS was done as per ICH Q1A (R2) and photostability as per ICH Q1B guidelines.

Hydrolysis in acidic conditions

It was performed in 0.1 N HCl conditions. 2 ml of working solution was transferred to 10 ml volumetric flask, to it 2 ml of degradant 0.1 N HCl was added and allowed to stand for 2 hours at room temperature. The samples were neutralized and diluted up to 10 ml

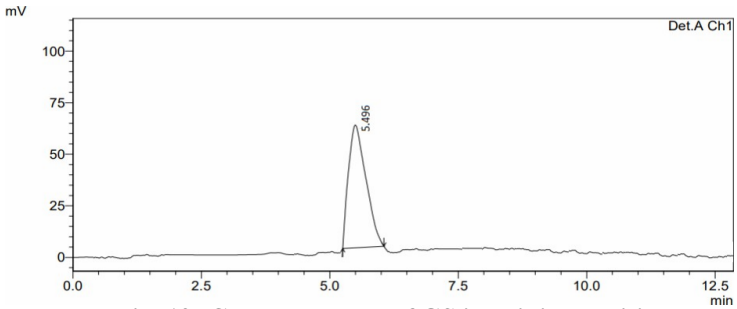


Fig. 12: Chromatogram of GS in acidic conditions

Table 11: Results of acidic hydrolysis study

Sr. No.	Sampling time interval (hour)	% Label claim
1	0	99.34
2	2	99.30
3	4	99.15
4	6	99.07
5	8	98.89
6	24	98.83
7	3 days	98.77
8	5 days	98.69

1. Hydrolysis in basic conditions

It was performed in 0.1 N NaOH. 2 ml of working solution was transferred to 10 ml volumetric flask, to it 2 ml of degradant 0.1 N NaOH was added and allowed to stand for 2 hours at room temperature and diluted up to 10 ml. The probable mechanism of alkaline hydrolysis is it opens the carbohydrate ring, exposes either the ketone or aldehyde group which is rearranged to form an enediol. It is a reaction of drug with water under basic medium

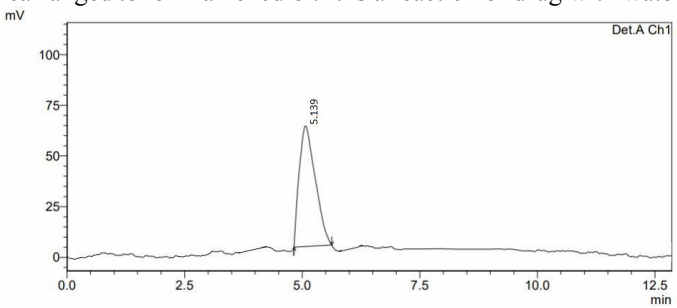


Fig. 13: Chromatogram of GS in alkaline hydrolysis

Table 12: Results of alkaline hydrolysis study

Sr. No.	Sampling time interval (hour)	% Label claim
1	0	99.88
2	2	99.81
3	4	99.75
4	6	99.66
5	8	99.08
6	24	98.98
7	3 days	98.92
8	5 days	98.81

2. Oxidative degradation

To 2 ml of each working standard solution and 2 ml of 3 %v/v of hydrogen peroxide was added into volumetric flask.

It was allowed to stand for 2 hours at room temperature. The resultant solution of glucosamine was suitably diluted to 10 ml using diluent and chromatogram was recorded. Samples are withdrawn after a specific time interval.

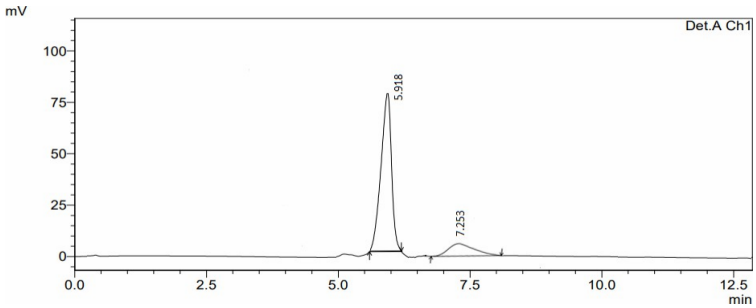


Fig. 14: Chromatogram of GS in oxidative degradation (Impurity A)

Table 13: Results of oxidative degradation

Sr. No.	Sampling time interval (hour)	% Label claim
1	0	99.78
2	1	97.05
3	2	95.28

3. Thermal degradation

For dry heat degradation 50 mg of drug was transferred to petri plate and was exposed to dry heat at 60 °C for 24 hours. The resultant solution was suitably diluted and chromatogram was recorded.

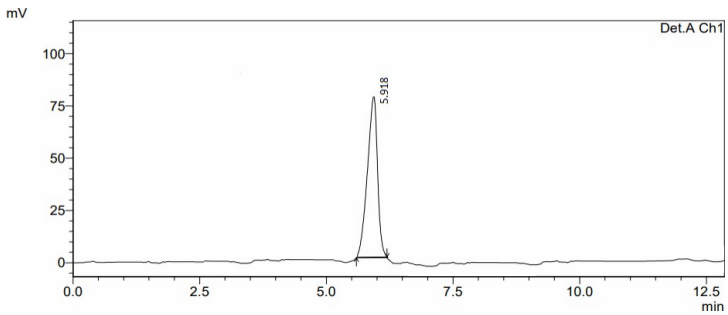


Fig. 15: Chromatogram of GS in thermal degradation

Table 14: Results of thermal degradation

Sr. No.	Sampling time interval (hour)	% Label claim
1	0	99. 93
2	2	99.82
3	4	99.75
4	6	99.72
5	8	99.50
6	24	99.44
7	3 days	99.22
8	5 days	99.16

4. Neutral degradation at room temperature

For neutral degradation 50 mg of drug was transferred to petri plate and was exposed to room temperature for 24 hours. The resultant solution was suitably diluted and chromatogram was recorded. When a drug is stored in temperatures that are too high or too low, the drug's chemical stability will likely be impacted. That means that the drug may degrade and form impurities.

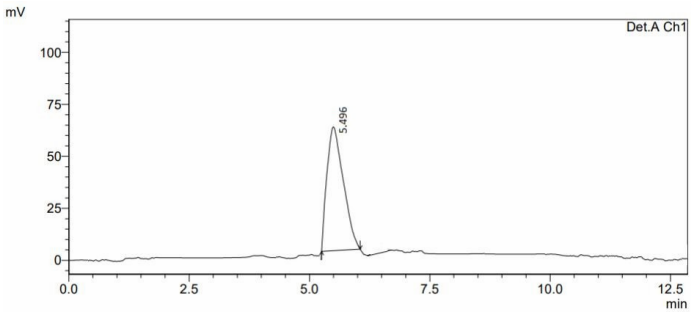


Fig. 16: Chromatogram of GS in neutral degradation

Table 15: Results of neutral degradation

Sr. No.	Sampling time interval (hour)	% Label claim
1	0	99. 89
2	2	99.80
3	4	99.67
4	6	99.65
5	8	99.58
6	24	99.43
7	3 days	99.32
8	5 days	99.20

IV. Conclusion

Glucosamine was shown to be stable under all situations except oxidative destruction. The amount estimated in oxidative degradation by HPLC was found to be 95.28. The impurities found was named as Impurity A. The drug can be estimated by analytical methods like GC, GC- MS, LC-MS which may be economical, specific and precise. The new AQbD approach instead aims to build a quality in the product during the process, so that the chances of product failure are minimized.

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