JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

# A brief overview on Osteoarthritis and A new validated RP-HPLC method for the estimation of Glucosamine

**Roshani Uttamrao Suryavanshi [1], Rajasekaran S.[2]**

Research scholar, Bhagwant University, Ajmer, Rajasthan, 305004, India.

Department of Pharmacology, Bhagwant University, Ajmer, Rajasthan, 305004, India.

**ABSTRACT:**

The degenerative joint disease that results from breakdown of joint cartilage and lying beneath bone is called Osteoarthritis. It is the most common chronic joint condition. Osteoarthritis is called wear-and-tear arthritis, degenerative joint disease (DJD). Osteoarthritis is developed in old age people. The changes in osteoarthritis usually occurs slowly, though they are occasional anomaly. Inflammation or injury to joint causes bony changes, decline of tendons and ligaments and breakdown of cartilage. The first symptoms are joint pain and stiffness. The symptoms develop slowly over years.

**KEYWORDS –** Osteoarthritis, Degenerative joint disease, Glucosamine

## INTRODUCTION:

At start they occur only after exercise but can become continue over time. In other symptoms includes joint swelling, decreased range of motion. If back is affected, weakness, loss of sensation or feeling in a part of your body numbness of the arms and legs. In Osteoarthritis the most commonly involved joints are the two near the ends of the fingers and the joint at the base of thumbs, the knee and hip joints and the ends of the neck and lower back. Joints on one side of the body are more affected than those on the other. The manifestation can interfere with work and normal daily activities; not like sone other types of arthritis, only the joints, not internal organs are affected. Osteoarthritis can occur in any joints. However, the most commonly affected areas of the body include

Hands

Fingers

Shoulders

Spine typically at the neck or lower back

Hips

Knees

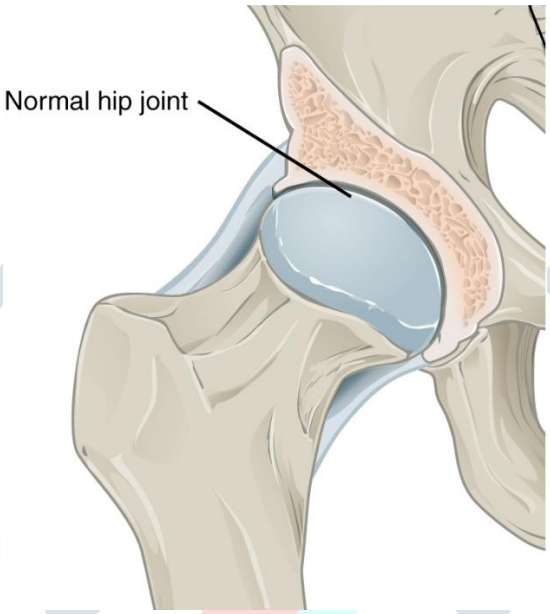



Fig. 1: Normal hip joint

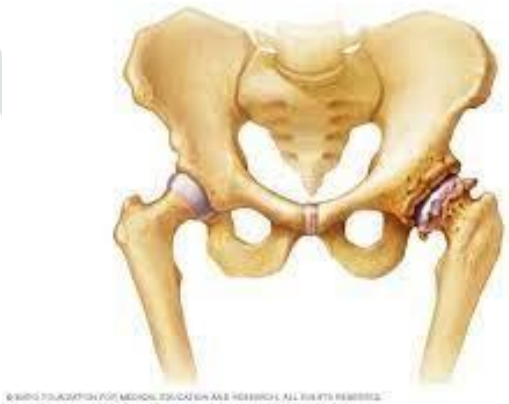


Fig 2: Affected hip joint

In so many causes some causes are previous joint injury or limb development and also inherited factor. Risk is greater in those who are overnight, have legs of different lengths, or have job that results in high levels of joint stress. It is also caused by mechanical stress on the joints and low-grade inflammatory processes. It develops as cartilage is lost and the underlying bone becomes affected. Also pain formation is occur during difficulties in exercise, in case of muscle loss. Diagnosis is typically based on signs and symptoms, with medical imaging and other tests used to support or rule out other problems. In contrast to rheumatoid arthritis. In Osteoarthritis the joints do not become hot or red.

Though Osteoarthritis is a degenerative joint disease that may causes gross cartilage loss and morphological damage to other joint tissues, more stable biochemical changes occur in the earliest stages of osteoarthritis progression. The water content of healthy cartilage is finally balanced by compressive force driving water out and hydrostatic and osmotic pressure drawing water. Collagen fibers bring to bear the compressive force, whereas the Gibbs-Donnan effect and cartilage proteoglycans create osmotic pressure which tends to draw water in.

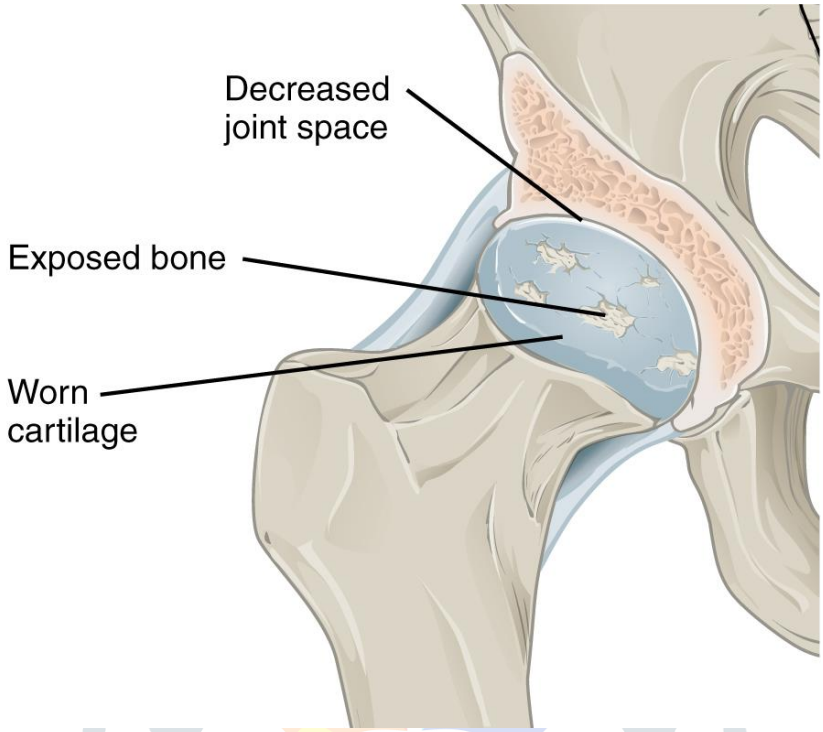



Fig 3: Detail picture of decreased joint space due to osteoarthritis

During beginning of osteoarthritis, the collagen matrix becomes more not properly planned and controlled and there is a decrease in proteoglycan content within cartilage. The breakdown of collagen fibers results in a net increase in water content. This increase occurs because there is an overall loss of proteoglycans and thus a decreased osmotic pull, it is greater than by a loss of collagen. Without the protective effects of the proteoglycans, the collagen fibers of the cartilage can become capable to degeneration and thus aggravate the degeneration. Inflammation of the synovium joint and in joint cavity lining. The surrounding joint capsule can also occur, though often mild compared to the synovial inflammation that occurs in rheumatoid arthritis. This can happen as breakdown products from the cartilage are released ingo the synovial space and the cells lining the joint attempts to remove them.

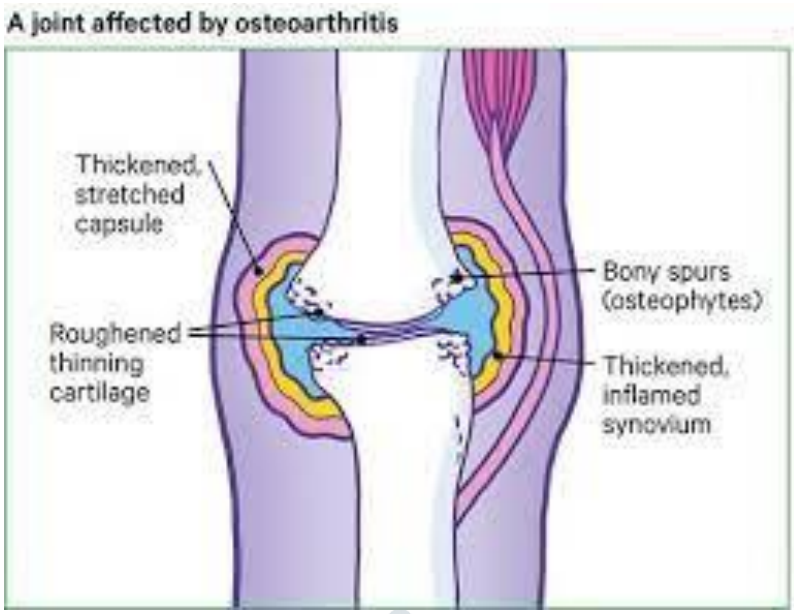



Fig 4: A joint affected by osteoarthritis

Other structures within the joint can also be affected. The ligaments within the joint become thickened and fibrotic and can become damaged and wear away. Menisci can be completely absent by the time a person undergoes a joint replacement. New bone outgrowths, called spurs or osteophytes, can form on the margins of the joints, possibly in an attempt to improve the congruence of the articular cartilage surfaces in the absence of the menisci. The subchondral bone volume increases and becomes less mineralized i.e., hypo mineralization. All these changes can cause problems functioning. The pain in an osteoarthritic joint has been related to thickened synovium and to subchondral bone lesions.

**There are two main types of osteoarthritis**

1) **Primary**: Most common, generalized, primarily affects the fingers, thumbs, spine, hips, knees and the big toes.
2) **Secondary**: Occurs with a pre- existing joint abnormality, including injury or trauma, such as repetitive or sports-related; inflammatory arthritis, such as rheumatoid, psoriatic, or gout, infectious arthritis; genetic joint disorder, such as Ehiers-Danlos also known as hypermobility or "double-jointed; congenital joint disorders or metabolic joint disorders.

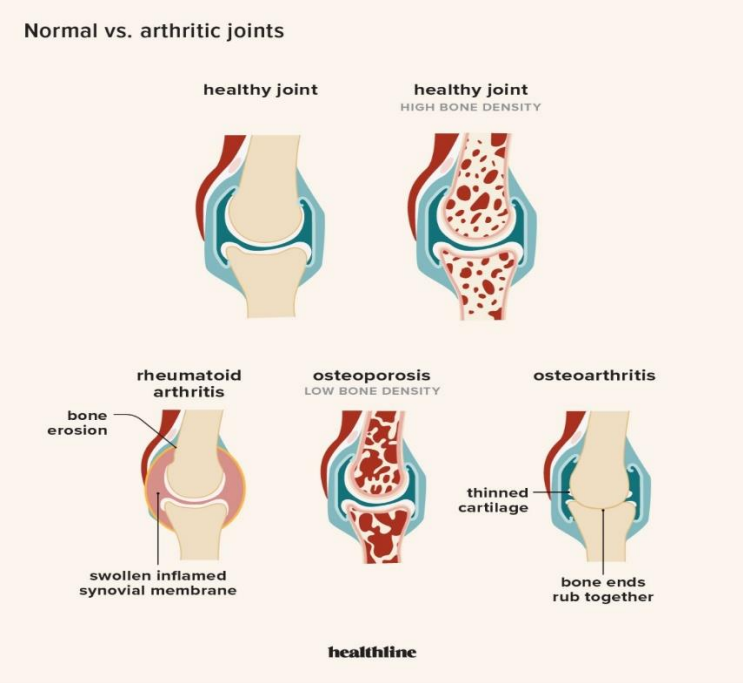



Fig 5: Normal and abnormal joint with Osteoarthritis

**The most common symptoms of Osteoarthritis include**

1) Joint pain
2) Stiffness in the joint
3) Loss of flexibility and reduced range of motion
4) Tenderness or discomfort when pressing on the affected areas with your fingers
5) Inflammation
6) Crepitus or grating, crackling, or popping sounds when you move your joints
7) Bone spurs or extra lumps of bone, which are typically painless.

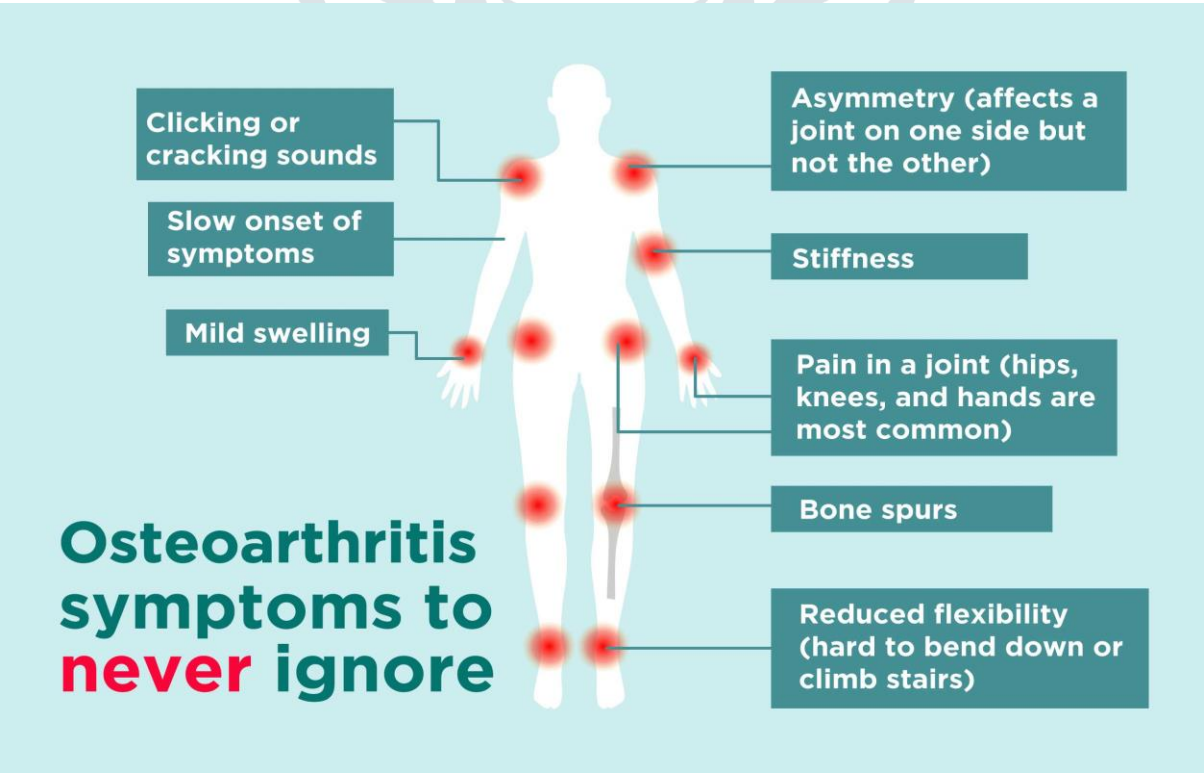



Fig 6: Osteoarthritis with its symptoms

As Osteoarthritis becomes more advanced, the pain associated with it may become more intense. Over time, swelling in the joint and surrounding area may also occur.

Osteoarthritis is caused by joint damage. This damage can have a cumulative effect overtime, which is why age is one of the main causes of the joint damage leading to osteoarthritis. The older you are, the more repetitive stress you have had on your joints.

**Other causes of joint damage include**

-past injury, such as torn cartilage, dislocated joints or ligaments injuries

-joint malformation

-obesity

-poor pressure

Certain risk factors increase your chances of developing osteoarthritis. They include

-having family with the condition, particularly parents or siblings

-gender, with women having higher rates of Osteoarthritis than men

-being at least 50 years old, according to the Arthritis Foundation

-having undergone menopause

-having an occupation that involves kneeling, climbing, heavy lifting or similar actions

-a history of injury

-being overweight or having obesity

-poor posture

-having another medical condition that affects your joint health, such and diabetes or a different types of arthritis

Having Osteoarthritis in one part of your body also increases your risk developing Osteoarthritis in other parts of your body.


Fig 7: Osteoarthritis affecting other parts of body

**Who is affected by osteoarthritis?**

Approximately 80% of older adults, ages 55 years and older, have evidence of osteoarthritis on X-ray. An estimated 60% experience symptoms. It is estimated that 240 million adults worldwide have symptomatic Osteoarthritis, including more than 30 million U. S. adults. Post- menopausal women have an increased incidence of knee osteoarthritis compared to men.

**How is Osteoarthritis treated?**

There is no cure for osteoarthritis. Mild to moderate symptoms are usually well managed by a combination of pharmacologic and non-pharmacologic treatments. Medical treatment and recommendations include;

-Medications (topical pain medicines and oral analgesics including non-steroidal anti-inflammatory medications, NSAIDs).

-Exercise (land-and water-based)

-Intermittent hot and cold packs (local modalities).

-Physical, occupational, and exercise therapy

-weight loss (if overweight)

-Healthy eating, managing diabetes and cholesterol.

-Supportive devises such as braces, orthotics, shoe inserts, cane, or walker.

-Intra-articular injection therapies (steroid, hyaluronic acid, gel)

-Complementary and alternative medicines strategies, including vitamins and supplements.

Surgery may be helpful to relieve pain and retore function when other medical treatments are ineffective or have been exhausted, especially with advanced osteoarthritis.

**The objectives of treatment are**

-Decrease joint pain and stiffness and delay further progression

-Improve mobility and function

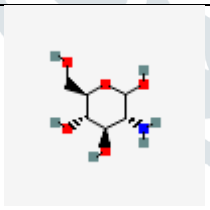
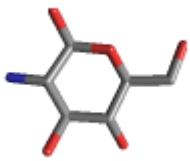
-increase patients' quality of life

The type of treatment regimen prescribed depends on many factors, including the patients age, overall health, activities, occupation and severity of the condition.

## GLUCOSAMINE

Glucosamine is commonly used as a treatment for osteoarthritis, but its acceptance as a medical therapy varies. It is an amino sugar and z prominent precursor in the biochemical synthesis of glycosylated proteins and lipids. Since glucosamine is a precursor for glycosaminoglycans, is major components of joint cartilage, supplemental glucosamine may help to rebuild cartilage and treat arthritis.

**Table 1: Drug Prefile**

| | | |
|---|---|---|
| Pub Chem CID | 439213 | |
| Structure |  |  |
| Molecular formula | C6H13NO5 | |
| Synonyms | 2-Amino-2-Deoxy-D-Glucopyranose<br>2-Amino-2-Deoxy-D-Glucopyranoside<br>2-Amino-2-Deoxy-D-Glucose<br>2-Amino-2-Deoxy-Glucopyranose<br>2-Amino-2-Deoxy-Glucopyranoside | |
| Molecular Weight | 179.17 | |

Glucosamine dietary supplements are commonly used for the management of osteoarthritis. However, clinical trials have outcomes with regard to joint function and disease progression. One of the possible reasons for variability in observed effects of glucosamine could be that, unlike prescription drugs, the quality of manufactured dietary supplements is not closely monitored in many countries. Therefore, there is the

possibility that actual amount of glucosamine present in a dietary supplement is different from that claimed on the label.

In previous studies, genetic predisposition, obesity, inflammation and excessive mechanical loading predispose to osteoarthritis occurrence and development. The structural changes cause joint pain and stiffness, swelling, tenderness which can eventually lead to incapacity and affect the quality of life of patients. Treatment strategies of Osteoarthritis include both pharmacological and non-pharmacological therapies. In those analgesics and non-steroidal anti-inflammatory drug (NSAIDs) are current treatment options for osteoarthritis due to their stronger efficacy. They act as symptomatic treatments without offering disease modification of osteoarthritis and they are accused for increased risk adverse events, including the gastrointestinal and cardiovascular system. Hence, attention is focused on ideal treatment, which can improve the clinical symptoms of osteoarthritis with better tolerability and safety profiles, such as symptomatic slow-acting drugs.

Glucosamine as important medicine are naturally occurring compounds in the body functioning as the principal substrates in the biosynthesis of proteoglycan. It is suggested that glucosamine is partially absorbed and then reaches the joints, exerting on relieving joint pain and showing the rate of joint destruction and cartilage loss. It is main category of agents potentially or theoretically acting as chondroprotective agents and disease-modifying osteoarthritis drugs. Many studies have shown a significant treatment effect, accompanied with remarkable safety, there is still controversy regarding the effectiveness of drug. Glucosamine is acylated form is natural constituent of some glycosaminoglycans i.e. hyaluronic acid and keratan sulfate in the proteoglycans found in articular cartilage, intervertebral disc and synovial fluid. Glucosamine can be extracted and stabilized by chemical modification and used as a drug or nutraceutical. It has been approved for the treatment of osteoarthritis in Europe to promote cartilage and joint health and is sold over the counter as dietary supplement in the United Status.

Various formulations of glucosamine have been tested, including glucosamine sulfate and glucosamine hydrochloride. In vitro and in vivo studies uncovered glucosamine's mechanism of action on articular tissues like cartilage, synovial membrane and subchondral bone and by testing its efficacy by demonstrating structure modifying and anti-inflammatory effects at high concentrations. Results from clinical trials have raised many concerns. Pharmacokinetic studies have shown that glucosamine is easily absorbed, but current treatment doses barely reach the required therapeutic concentration in plasma and tissue. The symptomatic effect size of glucosamine varies greatly depending on the formulations used and the quality of clinical trials. The effect size reduces when evidence for the structure modifying effects of glucosamine is sparse.

**Mode of Action of Glucosamine**-

Glucosamine (2-amino-2-deoxy-β-D-glucopyranose) is an endogenous aminomonosaccharide synthesized from glucose and utilized for biosynthesis of glycoproteins and glycosaminoglycans. Glucosamine is present in almost all human tissues, highly concentrated in connective tissues of the human body, and found at hightest concentrations in the cartilage. In humans, about 90 percent of glucosamine is absorbed when administered as an oral dose of glucosamine sulfate, and is rapidly incorporated into articular cartilage.

Glucosamine can be found in many forms, including sulphate, hydrochloride, N-acetyl-glucosamine, chlorohydreate salt, and as a dextrorotatory isomer. There is some dispute over which form is most effective. Pooled findings from studies using a specific commercial glucosamine sulphate product called Dona suggest that this formulation reduces osteoarthritis pain, whereas other formulations do not. Another study performrd in china provides some evidence that glucosamine hydrochloride and glucosamine sulphate are equally effective.

The sulphate salt of glucosamine forms one half of the disaccharide subunit of keratan sulphate, which decreases in patients with osteoarthritis. Hyaluronic acid found in articular cartilage and synovial fluid is composed of repeating dimeric units of glucuronic acid N-acetylglucosamine.

Possible mechanisms of action for the chondroprotective effect of glucosamine include direct stimulation of chondrocytes, incorporation of sulphur into cartilage, and protection against degradative processes within the body through altered gene expression. The exact mechanism of action for the possible effect of glucosamine is unknown.

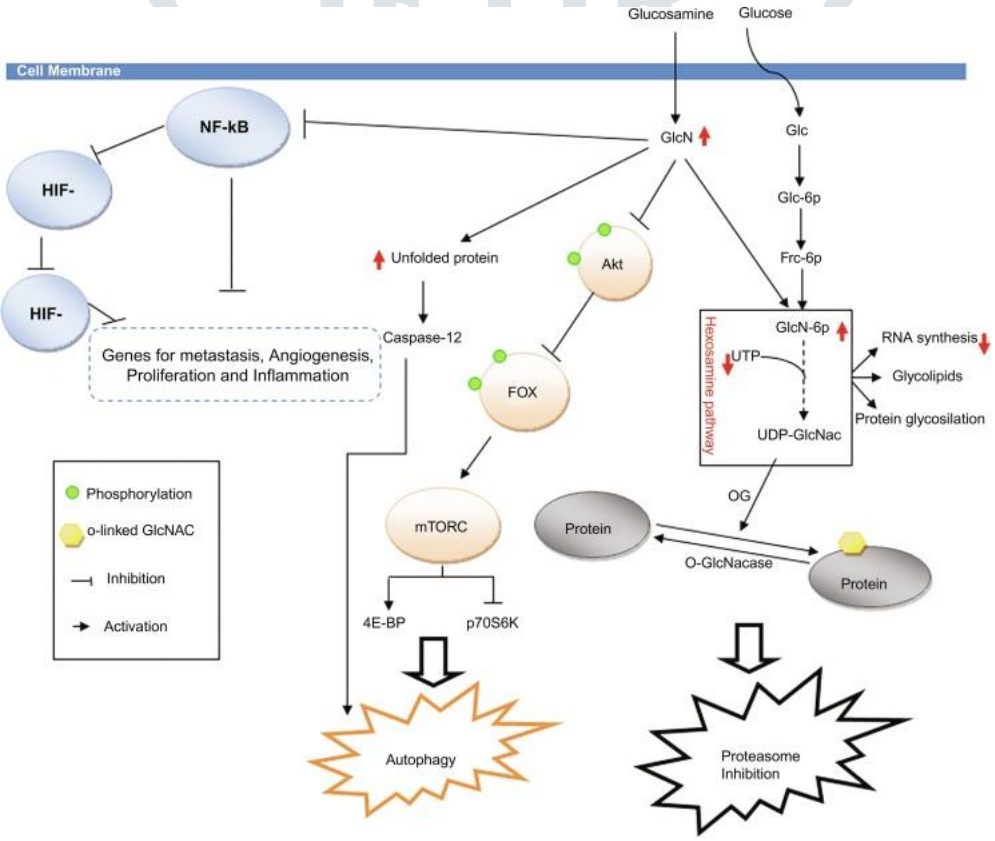



Fig 8: Mode of action of Glucosamine

**ANALYTICAL METHOD DEVELOPMENT:**

Strategies are created for new items when no official techniques are accessible. Exchange strategies for existing (Non-Pharmacopeias) items are produced to lessen the cost and time for better exactness and toughness. Trial runs are led, strategy is streamlined and approved. At the point when substitute technique proposed is expected to supplant the current system, relative research centre information including merits/bad marks ought to be made accessible.

**1)Steps involved in method development**

Documentation begins at the earliest reference point of the improvement procedure. A framework for full documentation of advancement ponders must be set up. All information identifying with these examinations must be recorded in research facility scratch pad or an electronic database.

**2)Analyte standard characterization**

a) All known data about the analyte and its structure is gathered i.e., physical and synthetic properties.

b) The standard analyte (100 % immaculateness) is gotten. Important course of action is made for the correct stockpiling (cooler, desiccators and cooler).

c) When various parts are to be investigated in the specimen grid, the quantity of segments is noted, information is collected and the accessibility of benchmarks for every one is resolved.

d) Only those strategies (spectroscopic, MS, GC, HPLC and so on.,) that are perfect with test solidness are considered.

**3)Method requirements**

The objectives or necessities of the diagnostic technique that should be created are viewed as and the expository figures of legitimacy are characterized. The required discovery limits, selectivity, linearity, range, exactness and accuracy are characterized.

**4)Choosing a method**

a) Using the data in the written works and prints, technique is adjusted. The strategies are altered wherever essential. Now and again it is important to gain extra instrumentation to repeat, change, enhance or approve existing techniques for in-house analytes and samples.If there are no earlier strategies for the analyte in the writing, from relationship, the aggravates that are comparable in structure and substance properties are examined and are worked out.

b) There is typically one compound for which explanatory strategy as of now exist that is like the analyte of intrigue.

**5)Instrumental setup and initial studies**

The required instrumentation is setup. Establishment, operational and execution capability of instrumentation utilizing research center standard working strategies (SOP's) are confirmed. Continuously new consumables (e.g. solvents, channels and gasses) are utilized. For instance, technique advancement is never begun on a HPLC segment that has been utilized before. The analyte standard in an appropriate infusion/presentation arrangement and in known fixations and solvents are readied. It is essential to begin with a real, known standard instead of with a perplexing specimen network. In the event that the specimen is greatly near the standard (e.g., mass medication), at that point it is conceivable to begin work with the real example

**6)Optimization**

Amid improvement one parameter is changed at once and set of conditions are secluded, as opposed to utilizing an experimentation approach. Work has been done from a sorted out deliberate arrangement, and each progression is recorded (in a lab journal) if there should be an occurrence of deadlocks.

## METHOD DEVELOPMENT PROCEDURE

The wide assortment of equipment's, segments, eluent and operation arrangements included elite fluid chromatography (HPLC) technique improvement appears to be mind boggling. The procedures impacted by the idea of analytes and for the most part take after the accompanying strides

**Steps:**

- Step 1 - Selection of the HPLC technique and beginning framework
- Step 2 - Selection of beginning conditions
- Step 3 - Selectivity enhancement
- Step 4 - System enhancement
- Step 5 - Method approval.

Contingent upon the general prerequisites and nature of the specimen and analytes, some of these means won't be fundamental amid HPLC investigation. For instance, a tasteful partition might be found amid step 2, subsequently steps 3 and 4 may not be required. The degree to which strategy approval (step 5) is explored will rely upon the utilization of the end investigation; for instance, a technique required for quality control will require more approval than one produced for an irregular examination. The accompanying must be considered when building up a **HPLC technique:**

**1)HPLC method development**

**Selection of the HPLC method and initial system.**

When building up a HPLC strategy, the initial step is dependably to counsel the writing to find out whether the detachment has been already performed and provided that this is true, under what conditions - this will spare time doing superfluous exploratory work. While choosing a HPLC framework, it must have a high likelihood of really having the capacity to dissect the specimen; for instance, if the example incorporates polar analytes at that point switch stage HPLC would offer both sufficient maintenance and determination, though ordinary stage HPLC would be considerably less plausible. Thought must be given to the accompanying:

**Sample preparation:**

- Does the example require disintegration, filtration, extraction, preconcentration or tidy up.

Is synthetic derivatization required to help identification affectability or selectivity

**2)Types of chromatography:**

1.Reverse stage is the decision for the lion's share of tests, yet in the event that acidic or essential analytes are available at that point turn around stage particle concealment (for powerless acids or bases) or switch stage particle matching (for solid acids or bases) ought to be utilized. The stationary stage ought to be C18 fortified.
2. For low/medium extremity analytes, typical stage HPLC is a potential hopeful, especially if the detachment of isomers is required. Carbon fortified stages are less demanding to work with than plain silica for ordinary stage divisions. For inorganic anion/cation investigation, particle trade chromatography is ideal. Measure prohibition chromatography would ordinarily be considered for breaking down high sub-atomic weight mixes

**3)Column dimensions:**

For most specimens (unless they are exceptionally intricate), long segments (25 cm) are prescribed to improve the segment effectiveness. A stream rate of 1-1.5 ml/min ought to be utilized at first. Pressing molecule size ought to be 3 or 5 μm.

**4)Detectors:**

**Thought must be given to the accompanying:**

- Do the analytes have chromophores to empower UV discovery
- Is more particular/delicate recognition required
- What discovery limits are important
- Will the example require compound derivatization to upgrade perceptibility as well as enhance the chromatography?

Fluorescence or electrochemical finders ought to be utilized for follow investigation. For preparative HPLC, refractive record is favored on the grounds that it can deal with high fixations without over stacking the locator. UV wavelength for the best affectability λmax ought to be utilized, which recognizes all example parts that contain chromophores. UV wavelengths beneath 200 nm ought to be maintained a strategic distance from on the grounds that locator clamor increments in this locale. Higher wavelengths give more noteworthy selectivity. The excitation wavelength finds the excitation most extreme; that is, the wavelength that gives the greatest emanation force. The excitation is set to the maximum value then the outflow is checked to find the discharge force. Determination of the underlying framework could, in this way, be founded on appraisal of the idea of test and analytes together with writing information, encounter, master framework programming and observational methodologies. 5**)Step 2 - selection of initial conditions**

This step determines the optimum conditions to adequately retain all analytes; that is,ensures no analyte has a capacity factor of less than 0.5 (poor retention could result in peak overlapping) and no analyte has a capacity factor greater than 10–15 (excessive retention leads to long analysis time and broad peaks with poor detectability). Selection of the following is then required.

**ANALYTICAL METHOD VALIDATION**

As per ICH Guidelines Method Validation can be characterized as "Establishing recorded proof, which gives a high level of affirmation that a particular movement will reliably create a coveted outcome or item meeting its foreordained details and quality attributes".

A measure for a noteworthy segment requires an alternate approach and acknowledgment criteria than a technique for a follow contamination. A last strategy might be performed at various destinations around the globe. Contrasts in HPLC instrumentation, lab gear and reagent sources and varieties in the abilities and foundation of faculty may require particular components in the HPLC technique. What's more, the improvement of various details of a similar medication with differing qualities or physical structures may require adaptability in strategy methods.

Technique approval think about incorporate framework appropriateness, linearity, exactness, precision, specificity, power, point of confinement of discovery, cutoff of evaluation and dependability of tests, reagents, instruments

**1)Framework Suitability**

Preceding the investigation of tests of every day, the administrator must set up that the HPLC framework and system are equipped for giving information of satisfactory quality. This is refined with framework appropriateness tests, which can be characterized as tests to guarantee that the technique can produce aftereffects of adequate exactness and Precision. The necessities for framework reasonableness are normally created after strategy improvement and approval have been finished

**Table 2: System Suitability Parameters and their recommended limits**

| **Parameter** | **Recommendation** |
|---|---|
| Capacity Factor (K') | The peak should be well-resolved from other peaks and the void volume generally K>2 |
| Repeatability | RSD ≤ 2% |
| Relative Retention | Not essential as the resolution is stated |
| Resolution($R_S$) | $R_S$ of > 2 between the peak of interest and the closest eluting |
| Tailing Factor(T) | T ≤ 2 |
| Theoretical Plates(N) | In general should be > 2000 |

**2)Linearity**

The linearity of a technique is a measure of how well an alignment plot of reaction versus focus approximates a straight line. Linearity can be evaluated by performing single estimations at a few analyte fixations. The information is then prepared utilizing a direct slightest squares relapse. The subsequent plot slant, catch and relationship coefficient give the coveted data on linearity.

**3)Precision:**

Exactness can be characterized as "The level of understanding among singular test outcomes when the methodology is connected over and over to different samplings of a homogenous specimen". A more far reaching definition proposed by the International Conference on Harmonization (ICH) separates exactness into three sorts:

- Repeatability
- Intermediate accuracy and
- Reproducibility

Repeatability is the accuracy of a technique under the same working conditions over a brief timeframe.

Middle exactness is the assention of finish estimations (counting guidelines) when a similar technique is connected commonly inside a similar research center.

Reproducibility inspects the accuracy amongst research centers and is frequently decided in communitarian studies or strategy exchange tests.

**4)Accuracy**

The exactness of estimation is characterized as the closeness of the deliberate an incentive to the genuine esteem. In a technique with high exactness, a specimen (whose "genuine esteem" is known) is broke down and the deliberate esteem is indistinguishable to the genuine esteem. Normally, exactness is spoken to and controlled by recuperation examines.

**There are three approaches to decide precision:**

1. Comparison to a reference standard
2. Recovery of the analyte spiked into clear lattice or
3. Standard expansion of the analyte

It ought to be clear how the individual or aggregate contaminations are to be resolved. e.g., Weight/weight or zone percent in all cases as for the major analyte.

**4)Specificity/selectivity**

The terms selectivity and specificity are frequently utilized conversely. As per ICH, the term particular by and large alludes to a strategy that creates a reaction for a solitary analyte just while the term specific alludes to a technique which gives reactions to various synthetic substances that might be recognized from each other. On the off chance that the reaction is recognized from all different reactions, the technique is said to be particular. Since there are not very many strategies that react to just a single analyte, the term selectivity is typically more proper. The analyte ought to have no impedance from different superfluous segments and be very much settled from them. A delegate chromatogram or profile ought to be created and submitted to demonstrate that the incidental pinnacles either by expansion of known mixes or tests from stretch testing are benchmark settled from the parent analyte.

**5)Robustness**

The idea of heartiness of a diagnostic system has been characterized by the ICH as "a measure of its ability to stay unaffected by little, however consider varieties in strategy parameters". A decent practice is to differ vital parameters in the technique efficiently and measure their impact on detachment. The variable strategy parameters in HPLC system may include stream rate, segment temperature, test temperature, pH and portable stage creation.

**6)Limit of detection**

Breaking point of discovery (LOD) is the most reduced centralization of analyte in a specimen that can be distinguished, yet not really quantitated, under the expressed test conditions. With UV finders, it is hard to guarantee the discovery accuracy of low level mixes because of potential slow loss of affectability of indicator lights with age or commotion level variety by identifier producer. At low levels, affirmation is required that the LOD and LOQ limits are achievable with the test technique each time. With no reference standard for a given contamination or intends to guarantee detectability, extraneous peak(s) could "vanish/show up." A rough technique to assess the possibility of the incidental pinnacle identification is to

utilize the rate asserted for LOD from the zone numbers of the analyte. A few methodologies for deciding the LOD are conceivable, contingent upon whether the methodology is a non-instrumental or instrumental·

Based on visual assessment

- Based on motion to-clamor
- Based on the standard deviation of the reaction and the slant

**The LOD might be communicated as:**

LOD = 3.3 σ/S

Where,

σ = Standard deviation of Intercepts of alignment bends

S = Mean of slants of the adjustment bends

The slant S might be evaluated from the adjustment bend of the analyte.

**Table 3: Characteristics to be validated in HPLC**

| **Characteristics** | **Acceptance Criteria** |
|---|---|
| Accuracy/trueness | Recovery 98-102% (individual) |
| Precision | RSD < 2% |
| Repeatability | RSD < 2% |
| Intermediate Precision | RSD < 2% |
| Specificity / Selectivity | No interference |
| Detection Limit | S/N > 2 or 3 |
| Quantitation Limit | S/N > 10 |
| Linearity | Correlation coefficient $R^2$ > 0.999 |

**METHODS OF QUANTITATIVE ANALYSIS:**

The sample or solute is examined quantitatively in HPLC by either peak height or peak area estimations. peak area is corresponding to the amount of material eluting from the section as long as the solvent flows at steady rate. Peak heights are relative to the measure of material just when top widths are consistent and are firmly influenced by the sample injection techniques. When the peak height or the peak areas are estimated, there are five guideline assessment strategies for evaluating the solute.

**1)Internal Standard Method:**

A known amount of the internal standard is chromatographer and area vs. concentration is resolved. At that point an amount of the inner standard is added to the crude example before any example pretreatment or partition activities.

The peak area of the norm in the sample run is contrasted and the peak area when the standard is run independently. This proportion fills in as correction factor for variety in test size, for misfortunes in any fundamental pretreatment tasks, or for fragmented elution of the example. The material chose for the interior

standard must be totally settled from contiguous sample components, must not meddle with the sample components and should never be available in samples.

$$\text{Area ratio} = \frac{\text{Area of sample}}{\text{Area of internal standard}}$$

$$\text{Sample concentration} = \frac{\text{Area of sample}}{\text{Area of internal standard}} \text{ x Concentration of standard}$$

**2)Calibration by Standards:**

Calibration curves for every part are set up from pure standards, utilizing indistinguishable infusion volumes of working conditions for standards and samples.

The centralization of solute is perused from its bend if the bend is straight.

X = K x Area

Where,

X = Concentration

K = Proportionality constant (slope of the curve)

In this evaluation method, just the area of the peaksof intrigue is estimated. Relative response factors must be viewed as while changing areas to volume and when the response of a given identifier varies for each molecular type of compounds.

3)**Standard Addition Method:**

This method is utilized when just hardly any samples are to be chromatographed. The chromatogram of the obscure is recorded, at that point a known amount of analyte(s) is included and the chromatogram is continued utilizing same reagents, instruments and other conditions. From the expansion in the peak area (or peak height), the original concentration can be figured by insertion.

The detector response must be a linear function of solute concentration and yield no sign at zero concentration of the analyte. Adequate time must pass between option of the standard and actual analysis to permit balance of included norm with any matrix interferant.

In the event that an instrumental reading (area/height) 'Rx' is acquired, from an sample of unknown 'x' and a perusing 'Rt' is gotten from the sample to which a known concentration 'a' of analyte has been included, at that point 'x' can be determined from:

A correction for dilution must be made if the measure of standard included changes the total sample volume essentially. It is consistently prudent to check the outcome by including at any rate one other norm

**4)External Standard Method**

It utilizes a different injection of a fixed volume of sample and standard solution. The peaks are coordinated and concentration is determined

## MATERIALS AND METHODSDRUGS AND CHEMICALS

- ✓ Glucosamine standards were obtained as gift sample from Spectrum Pharma Research Solution, Hyderabad, Telangana.
- ✓ Tablet formulation (Salbeto G Tablets 4mg of Glucosamine was purchased from local market.
- ✓ HPLC grade Acetonitrile, HPLC grade Water were purchased from Rankem. Mumbai, India.
- ✓ Potassium dihydrogen phosphate AR grade and Acetic acid was obtained from RANKEM, Mumbai, India.
- ✓ Orthophosphoric acid AR, Tetra Butyl Ammonium Hydrogen Sulphate (TBHS) hydrochloric acid, sodium hydroxide, and hydrogen peroxide were purchased from S.D. Fine Chemicals, Hyderabad and all solvents used in this work are HPLC grade.

**Instruments**

**Table 4: list of Instrument**

| Sl. No. | Instruments/Equipments/Apparatus |
|---|---|
| 1. | SHIMADZU with class-10vp Software with Isocratic Gradient with UV-Visible Detector (SPD-IOA), PUMP (LC-IOAT) and (LC-IOATvp). |
| 2. | UV-Visible Spectrophotometer (ELICO SL-159). |
| 3. | Electronic Balance (AFCOSET) |
| 4. | Ultra Sonicator(ENERTECH) |
| 5. | Thermal Oven |
| 6. | Hypersil Pack ODS ($C_{18}$) RP Column, 250 mm x 4.6 mm. |
| 7. | $P^H$ Analyzer (ELICO) |
| 8. | Triple Quartz Distillation Unit (BOROSIL) |
| 9. | HPLC Injecting Syringe (25 µl) (HAMILTON) |

## METHOD DEVELOPMENT OF GLUCOSAMINE

### 1)Preparation of Standard and Sample Solutions

### i)Preparation of Mobile Phase

The mobile phase was prepared by mixing 650 ml 0.1% $KH_2PO_4$ buffer and 350 ml of Acetonitrile. The mobile phase was degassed for 10 min by sonicating the solution before use.

Accurately Weighed and transferred 75mg of Glucosamine working Standards into a 50ml clean dry volumetric flask, add 3/4$^{th}$ volume of diluent, sonicated for 15 minutes and make up to the final volume with diluents. From the above stock solutions, 1 ml was pipetted out in to a 10ml volumetric flask and then made up to the final volume with diluents

### ii)Preparation of sample solutions for method validation

### 1) Accuracy

Accurately weighed and transferred 75mg of Glucosamine, 50 mL clean and dry volumetric flask, add 15ml of diluents (Water:ACN 50:50), then sonicated for 20min and make up the volume with diluent. From the above solution, 50%, 100% and 150% of analyte concentrations were prepared and 10µl of the solution

was injected into HPLC system.

**2)Linearity**

Six different concentration of Glucosamine was prepared separately and injected into HPLC system

**3)Precision**

Precision is the degree of repeatability of an analytical method under normal Operational conditions. Precision of the method was performed as intraday precision, Inter day precision.

**4)Intraday Precision**

To study the intraday precision, a known concentration of six replicates of Glucosamine standard solution was injected on the same and peak area was recorded.

**5) Robustness**

To validate the robustness of the developed method, a known concentration of freshly prepared Glucosamine (150µg/ml), standard solution was injected and record the peak tailing, USP plate count and peak area.

**2)Preparation of sample solutions for forced degradation studies**

**i)Preparation of Acid induced degradation product**

In acid hydrolytic degradation, to 1 ml of stock ssolution Glucosamine 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at $60^0$C. The resultant solution was diluted to obtain 150µg/ml,10 µg/ml, 50µg/ml solutions and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

**ii)Preparation of Base induced degradation product**

In acid hydrolytic degradation, to 1 ml of stock solution Glucosamine, 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at $60^0$C. The resultant solution was diluted to obtain 150µg/ml, 10 µg/ml, 50 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**iii)Preparation of Peroxide induced degradation product**

In peroxide degradation, 1 ml of 20% hydrogen peroxide ($H_2O_2$) was added to 1 ml of stock solution of Glucosamine, the solutions were kept for 30 min at $60^0$C. For HPLC study, the resultant solution was diluted to obtain 150µg/ml, 10 µg/ml, 50 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**iv)Preparation of Dry heat degradation product**

In thermal degradation studies, the standard drug solution was placed in oven at $105^0$c for 1 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 150µg/ml, 10 µg/ml, 50 µg/ml solutions and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

**v)Preparation of Photolytic degradation product**

The photochemical stability of the drug was also studied by exposing the 1500µg/ml, 100 µg/ml, 500 µg/ml solutions to UV Light by keeping the beaker in UV Chamber for 1hrs or 200-Watt hours/$m^2$ in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 150µg/ml, 10 µg/ml, 50 µg/ml

solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

**vi)Preparation of Neutral Degradation product**

Stress testing under neutral conditions was studied by refluxing the drug in water for 1hrs at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 150µg/ml, 10 µg/ml, 50 µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

## RESULT AND DISCUSSION

**Method development of glucosamine, its pharmaceutical dosage forms.**

**Optimized UV conditions**

UV – visible spectra from 400-200nm for Glucosamine standard solutions were taken. Glucosamine shows λmax at 280nm. By observing the UV spectra of standard solutions, it was taken for trials to develop HPLC method.

**Trial 1:**

**Chromatographic conditions:**

| | | |
|---|---|---|
| **Mobile phase** | **:** | 0.1% OPA:Acetonitrile(50:50) |
| **Flow rate** | **:** | 1 ml/min |
| **Column** | **:** | BDS C18 (4.6 x 150mm, 5µm) |
| **Detector wave length** | **:** | 280.0 nm |
| **Column temperature** | **:** | 30°C |
| **Injection volume** | **:** | 10µL |
| **Run time** | **:** | 10 min |
| **Diluent** | **:** | Water and Acetonitrile in the ratio 50:50. |
| **Results** | : | In this trial all peaks were eluted but but peaks shapes are not good and less USP plate count so, further trial is carried out. |

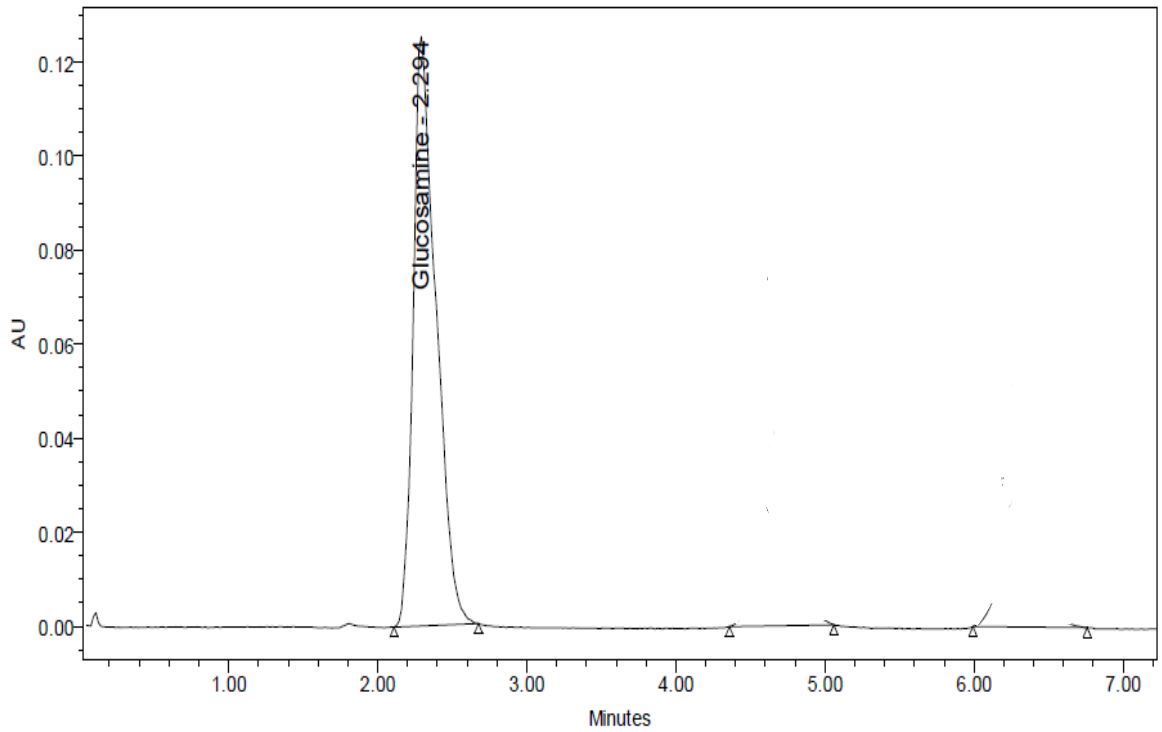



Fig 9: Trial chromatogram 1

**Trial 2:**

**Chromatographic conditions:**

| | | |
|---|---|---|
| **Mobile phase** | **:** | 0.1% OPA: Acetonitrile (55:45) |
| **Flow rate** | **:** | 1 ml/min |
| **Column** | **:** | BDS C18 (4.6 x 150mm, 5µm) |
| **Detector wave length** | **:** | 280.0 nm |
| **Column temperature** | **:** | 30°C |
| **Injection volume** | **:** | 10µL |
| **Run time** | **:** | 6 min |
| **Diluent** | **:** | Water and Acetonitrile in the ratio 50:50 |
| **Results** | **:** | All peaks were eluted but base line is not good and retention time is more so, further trials are conducted**.** |

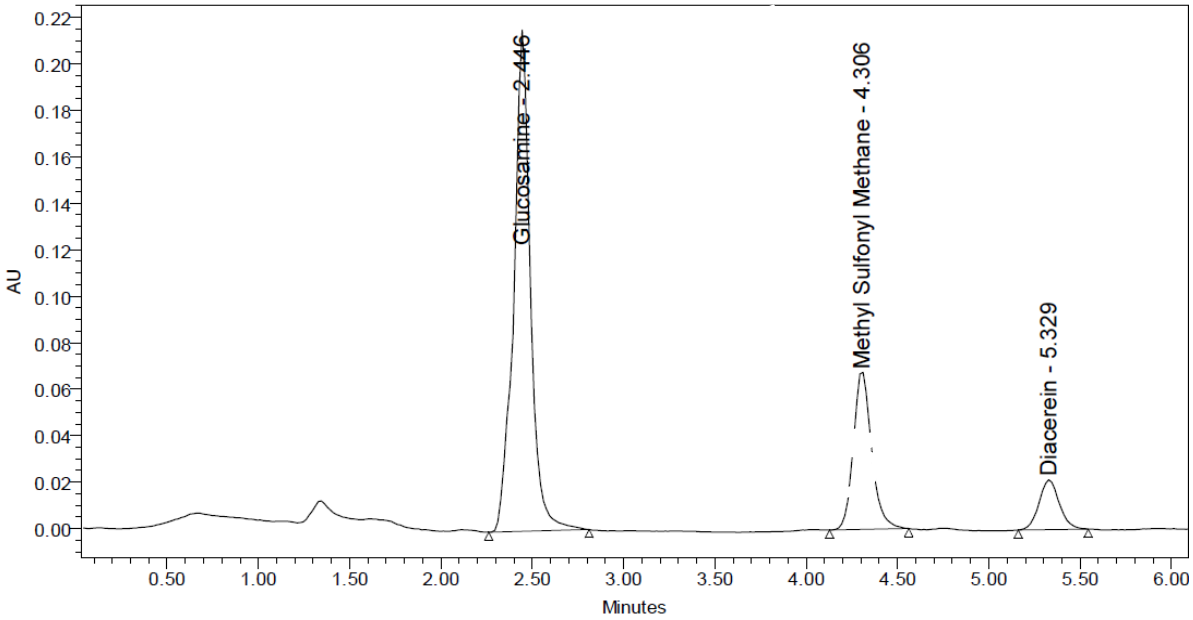



Fig10: Trial chromatogram 2

**Trial 3:**

**Chromatographic conditions:**

| | | |
|---|---|---|
| **Mobile phase** | : | 55% 0.1% OPA: 45% Acetonitrile (55:45) |
| **Flow rate** | : | 1 ml/min |
| **Column** | : | Altima C18 (4.6 x 150mm, 5µm) |
| **Detector wave length** | : | 280.0 nm |
| **Column temperature** | : | 30°C |
| **Injection volume** | : | 10µL |
| **Run time** | : | 10 min |
| **Diluent** | : | Water and Acetonitrile in the ratio 50:50 |
| **Results** | : | In this trial also, all peaks are eluted but diacerien peak shape was not good so; further trials are carried out. |

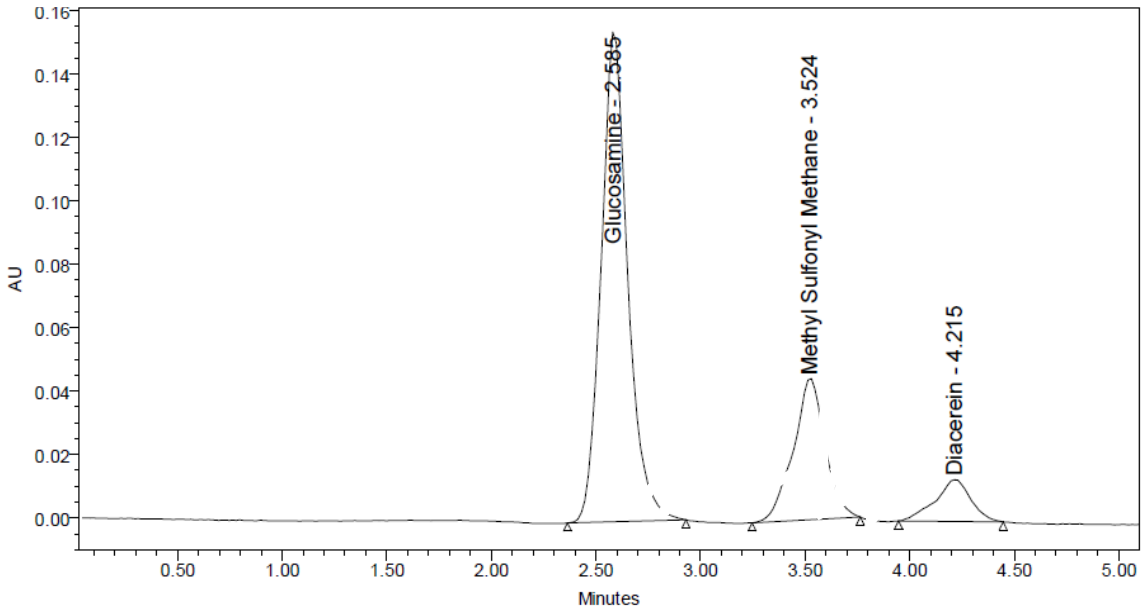



Fig 11: Trial chromatogram 3

**Optimized method:**

**Chromatographic conditions:**

**Mobile phase** : 65% 0.1N $KH_2PO_4$: 35% Acetonitrile

**Flow rate** : 1 ml/min

**Column** : Altima C18 (4.6 x 150mm, 5µm)

**Detector wave length** : 280.0 nm

**Column temperature** : 30°C

**Injection volume** : 10µL

**Run time** : 8 min

**Diluent** : Water and Acetonitrile in the ratio 50:50

**Results** : Both peaks have good resolution, tailing factor, theoretical plate count and resolution

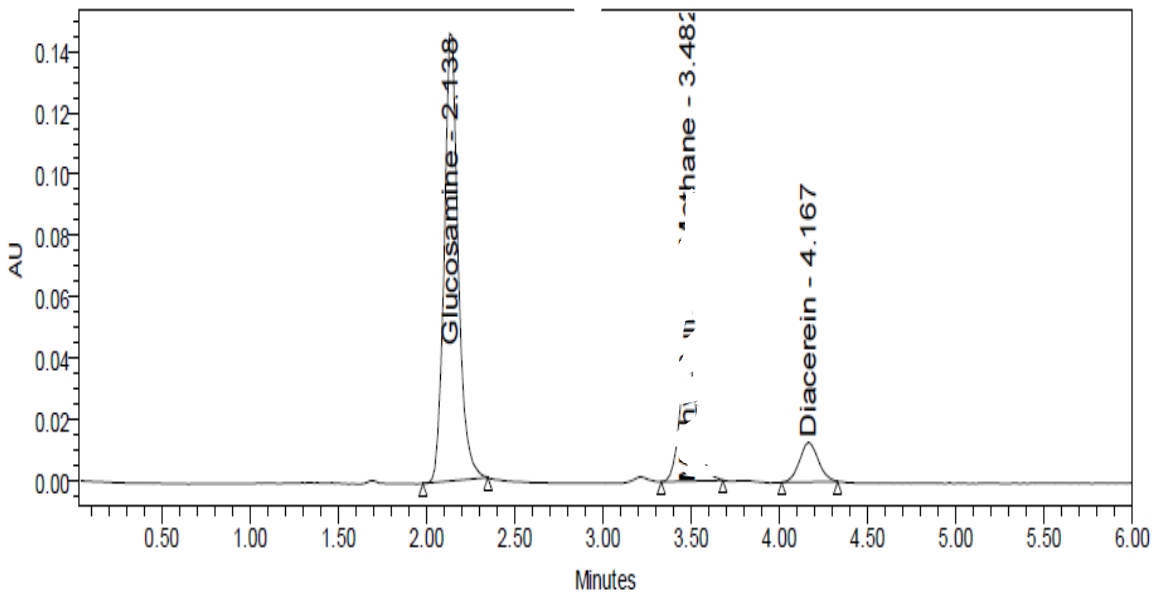



Fig 12: Optimized Chromatogram

**Observation:** Glucosamine was eluted at 2.138 min with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

**Method Validation**

The objective of validation of analytical method is to verify the characteristics of the proposed method suitability for its intended purpose. After developing a suitable method, it was validated for the following typical parameters such as accuracy, linearity, specificity, ruggedness, precision, robustness, LOD and LOQ according to ICH guidelines.

**Specificity**

The specificity of an analytical method is ability to measure the analytes accurately and specifically in the presence of unexpected components in sample matrix. It was evaluated by assessing interference from excipients in the pharmaceutical dosage form prepared as a placebo solution. The specificity of the method for the drug was also established by checking for interference with drug quantification from degradation products formed during the forced degradation study. The peak purity spectra of the Glucosamine, was found satisfactory under different stress condition. There was no interference of any peak of degradation product with drug peak.

**System suitability**

A system suitability test of the chromatographic system was performed before each validation run. Five replicate injections of freshly prepared standard solution containing 150µg/ml of Glucosamine, was injected. Parameters that were studied to evaluate the suitability of the system are retention time, peak area, number of theoretical plates and USP Peak tailing. The values of the system suitability parameters were given in table

**Table 5: System suitable parameters of Glucosamine**

| PARAMETERS | Glucosamine |
|---|---|
| Retention Time (min) | 2.138 |
| USP Plate count | 3457 |
| USP Tailing | 1.153 |

**Accuracy**

The percentage recoveries for Glucosamine was found to be 99.79%. The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method.

**Table 6: Recovery results of Glucosamine**

| % Level | Amount added (µg/ml) | Amount Recovered (µg/mL) | % Recovery | Mean % Recovery |
|---|---|---|---|---|
| 50% | 75 | 75.18414 | 98.14 | 99.79% |
| | 75 | 74.06255 | 100.25 | |
| | 75 | 74.91023 | 98.75 | |
| 100% | 150 | 149.293 | 99.88 | |
| | 150 | 150.1944 | 99.53 | |
| | 150 | 151.0254 | 100.13 | |
| 150% | 225 | 223.7669 | 100.68 | |
| | 225 | 224.7816 | 99.45 | |
| | 225 | 223.8734 | 99.90 | |

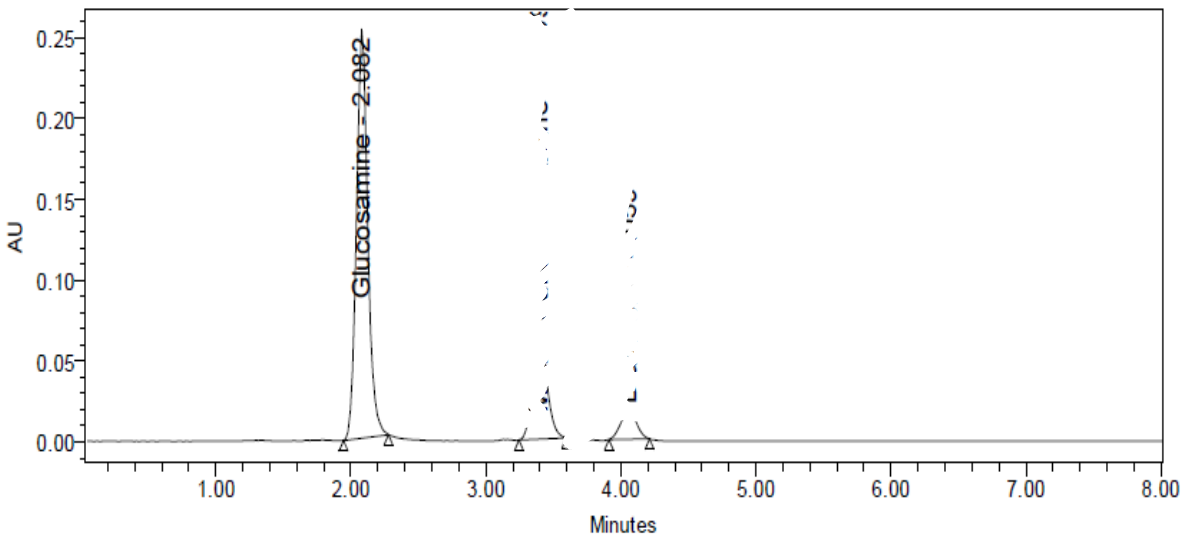



Fig 13: A typical standard chromatogram of Glucosamine at Accuracy 50 %

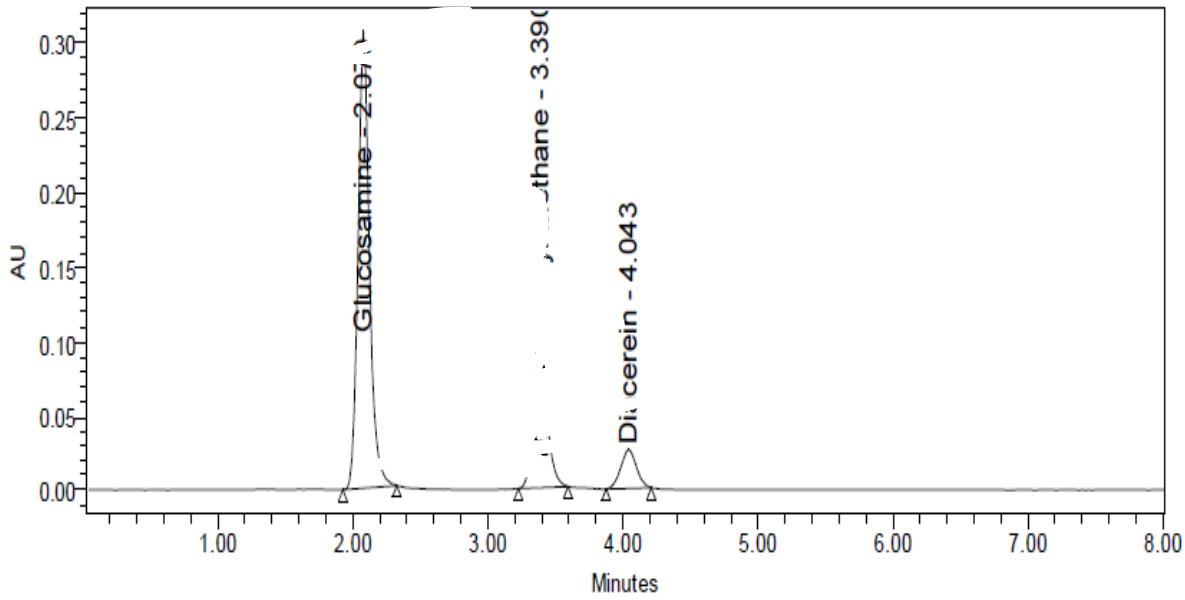



Fig 14: A typical standard chromatogram of Glucosamine at Accuracy 100 %

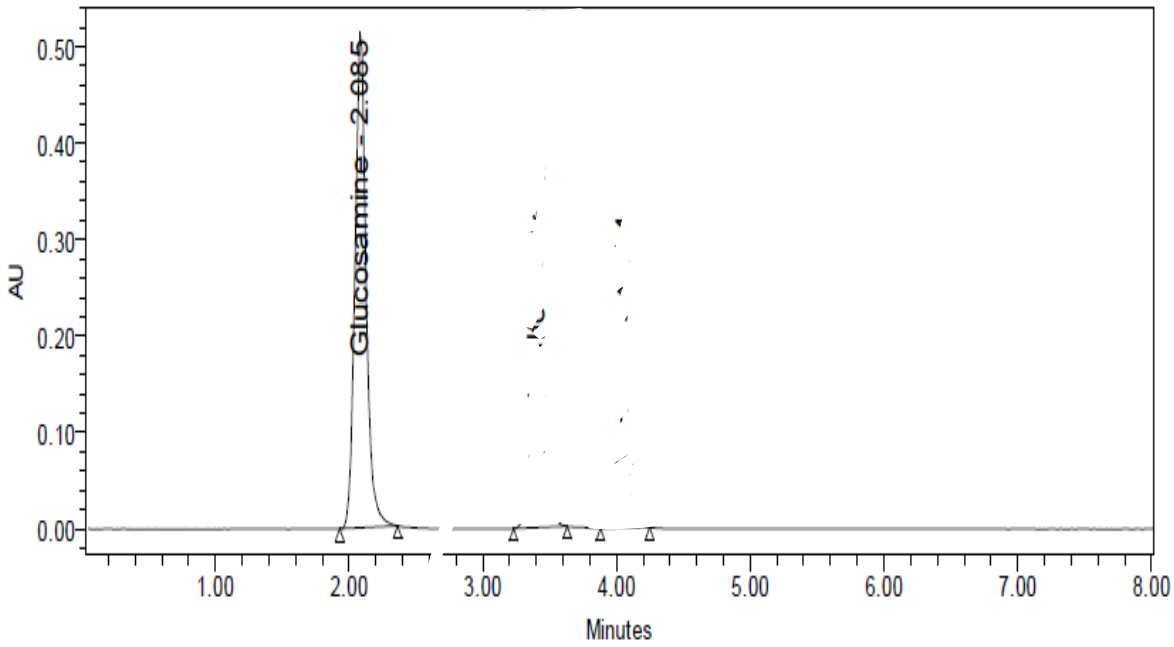



Fig 15: A typical standard chromatogram of Glucosamine at Accuracy 150 %

**Linearity**

The linearity range was in the interval of Glucosamine (37.5-225/ml), Diacerein (2.5-15µg/ml) and Methyl sulfonyl methane (12.5-75µg/ml), respectively. These were represented by a linear regression equation as follows: y (Glucosamine) = 9412x + 48133 ($r^2$=0.999). Regression line was established by least squares method and correlation coefficient ($r^2$) for Glucosamine found to be greater than 0.999. A calibration curve was plotted and the response of the drugs was found to be linear and the linearity results were given in table

Table 7: Linearity table for Glucosamine

| Glucosamine | |
|---|---|
| **Conc (µg/mL)** | **Peak area** |
| 0 | 0 |
| 37.5 | 204986 |
| 75 | 405264 |
| 112.5 | 618838 |
| 150 | 810513 |
| 187.5 | 1035654 |
| 225 | 1216150 |

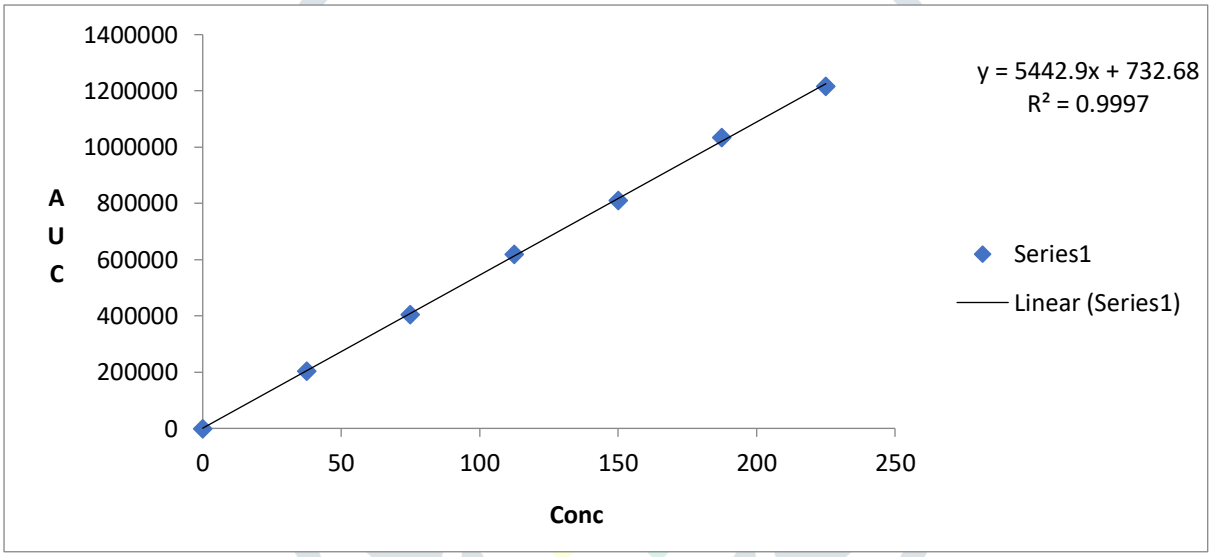



Fig 16: Linearity curve for Glucosamine

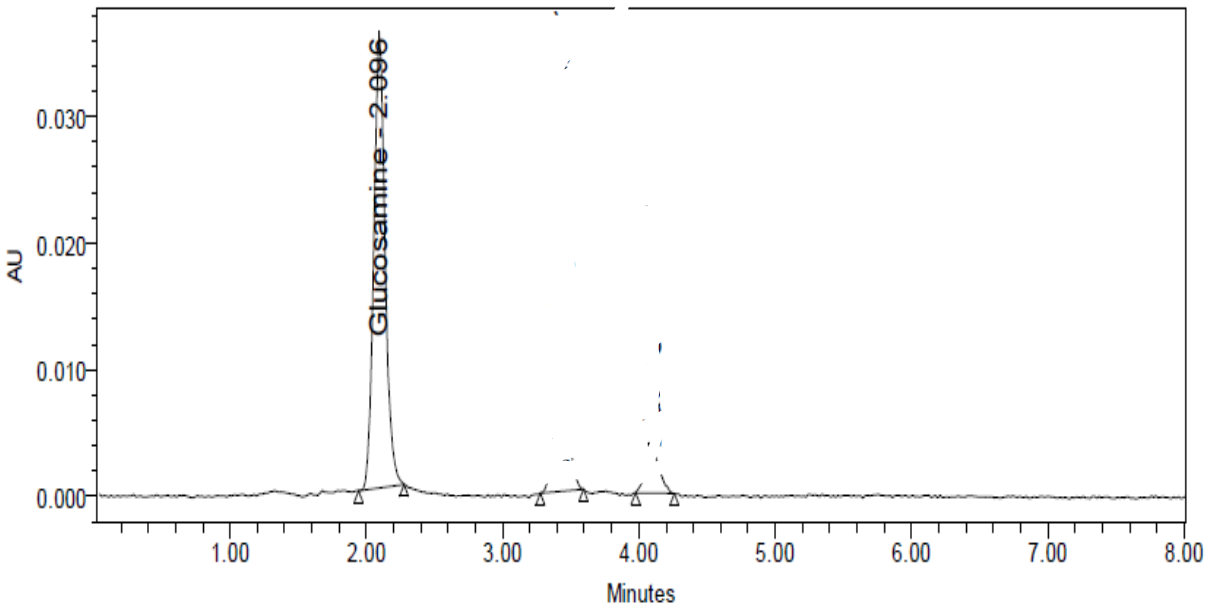



Fig 17: A typical standard chromatogram of Glucosamine at Linearity 25 %

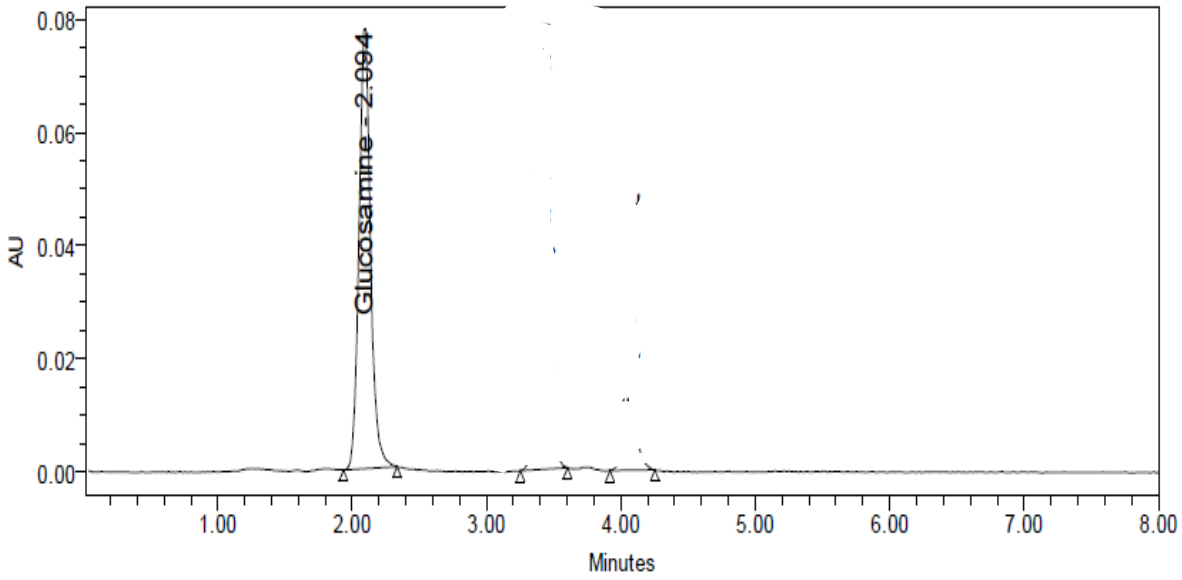



Fig 18: A typical standard chromatogram of Glucosamine, at Linearity 50 %

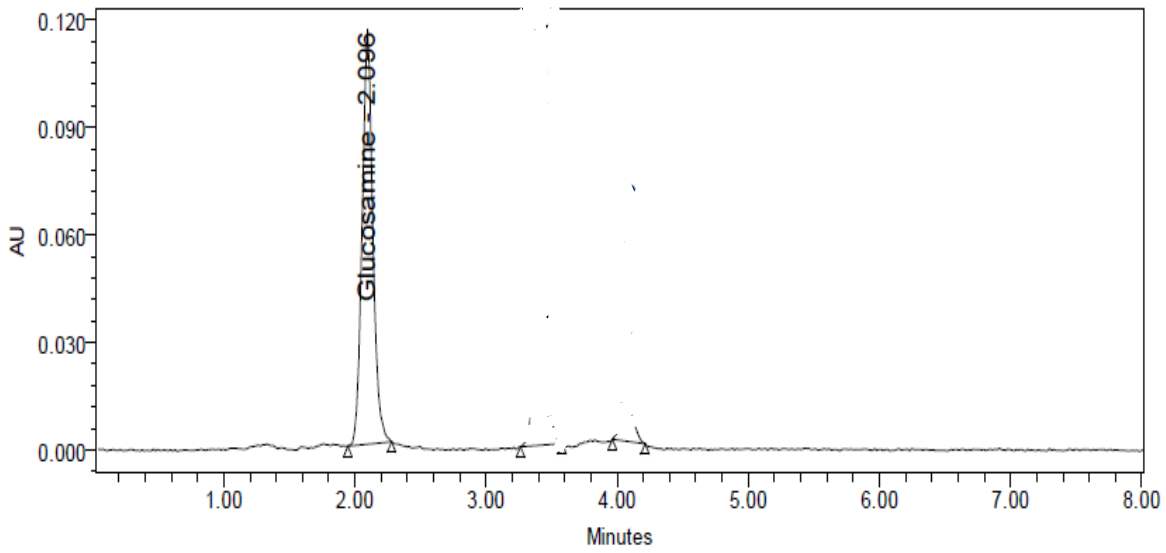



Fig 19: A typical standard chromatogram of Glucosamin at Linearity 75 %

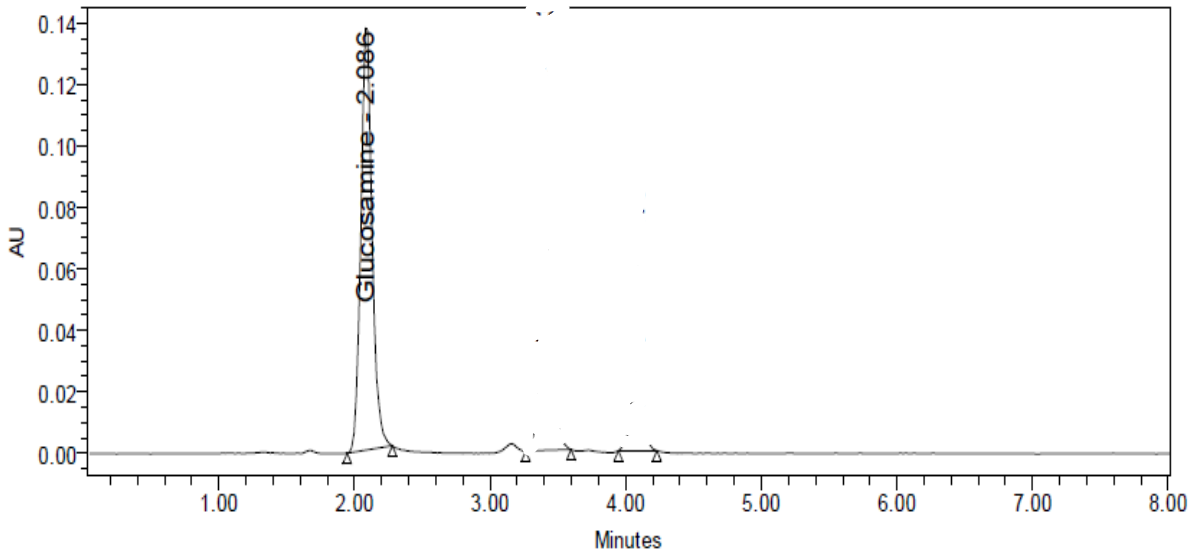



Fig 20: A typical standard chromatogram of Glucosamine, at Linearity 100 %

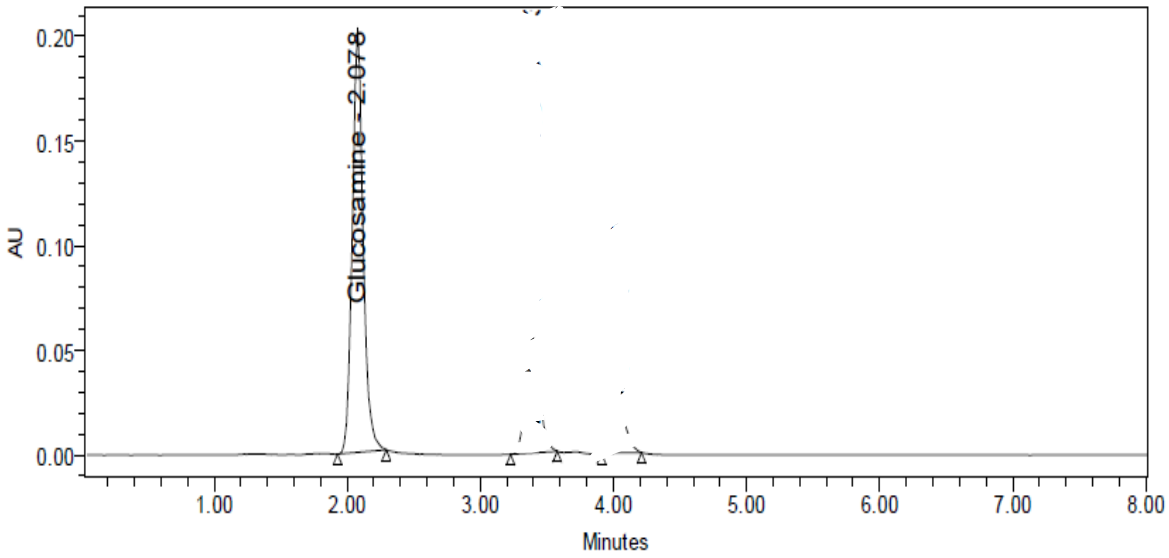



Fig 21: A typical standard chromatogram of Glucosamine at Linearity 125 %

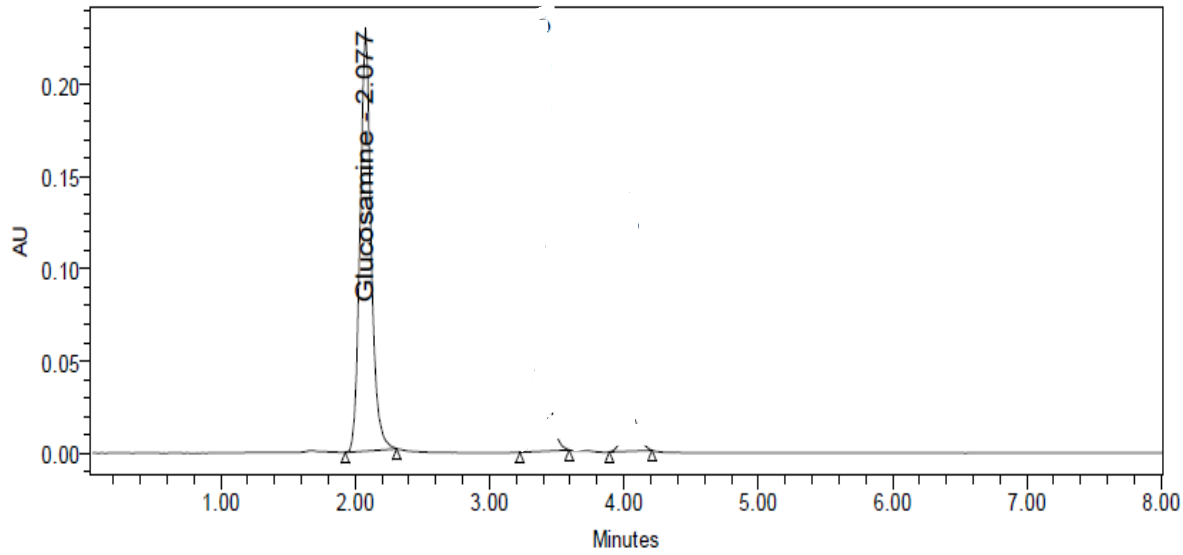



Fig 22: A typical standard chromatogram of Glucosamine at Linearity 150 %

**5 Precision**

The precision of the proposed method was investigated in terms of intraday and inter day precision. Intra-day repeatability was evaluated by performing six independent assays of the test sample preparation and calculating the % RSD and the interday precision (reproducibility) of the method was checked by performing same procedure on different days under the same experimental conditions. A known concentration of Glucosamine (150µg/ml) solution was prepared and analyzed. The % RSD was calculated for intraday and inter day precision and found to be below 2% which indicates the developed method was precise. The precision data of the drugs were shown in table

**Table 8: Intraday Precision data of Glucosamine**

| S.No | Glucosamine |
|---|---|
| | Intraday<br>Peak area |
| 1 | 816694 |
| 2 | 810225 |
| 3 | 814141 |
| 4 | 813558 |
| 5 | 801897 |
| 6 | 802742 |
| AVRG | 808513 |
| SD | 6211.5 |
| %RSD | 0.8 |

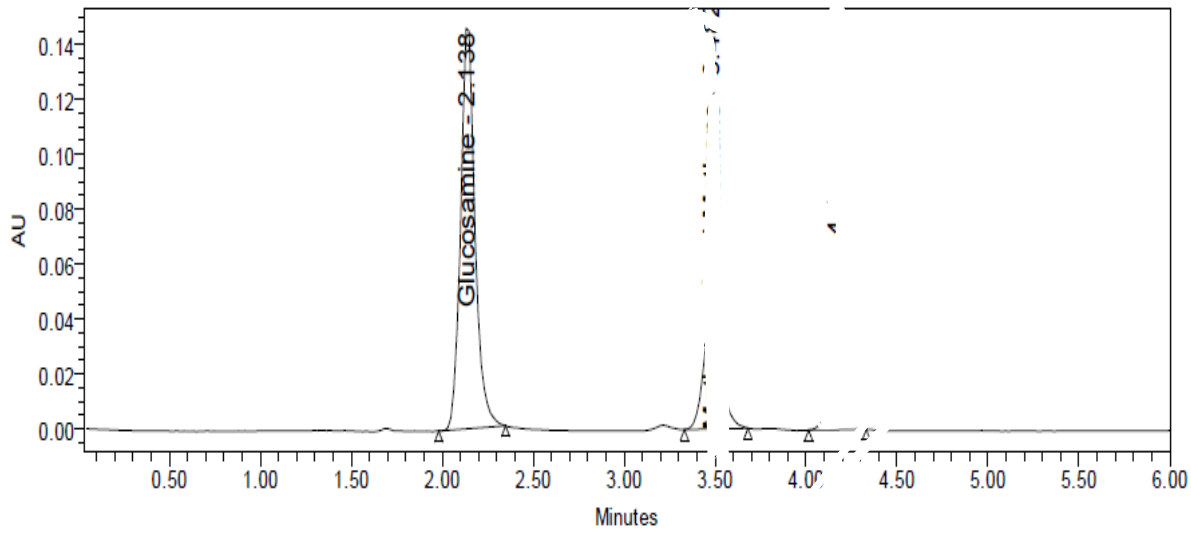



Fig 23: A typical intraday precision chromatogram of Glucosamine

## 6 Robustness

Robustness of the proposed method was studied by analyzing the samples of Glucosamine for some relevant factors which may influence the reliability of the developed method. Deliberate change in some of the factors including, flow rate (±0.1ml) was investigated for their degree of change with respect to proposed method by comparing the change in response with the original one. In all the conditions, good separation of the Glucosamine was achieved which indicate the developed analytical method remained selective and robust. The % RSD was determined in all the conditions and found to be within the limits of acceptance.

**Table 9: Actual conditions and proposed conditions of the method**

| Parameters | Actual conditions | Proposed variations |
|---|---|---|
| **Flow rate** | 1ml/min | 0.9, and 1.1ml/min |
| **Mobile phase ratio** | 66:35 % v/v | ±10% |
| **Temperature** | 30 $^0$C | 25 $^0$C, 35 $^0$C |

**Table 10: %RSD of Glucosamine**

| S.no | Condition | %RSD of Glucosamine |
|---|---|---|
| 1 | Flow rate (-) 0.8ml/min | 0.5 |
| 2 | Flow rate (+) 1.0ml/min | 0.5 |
| 3 | Mobile phase (-) 70B:30A | 0.3 |
| 4 | Mobile phase (+) 60B:40A | 0.5 |

| | | |
|---|---|---|
| 5 | Temperature (-) 25°C | 0.5 |
| 6 | Temperature (+) 35°C | 0.6 |

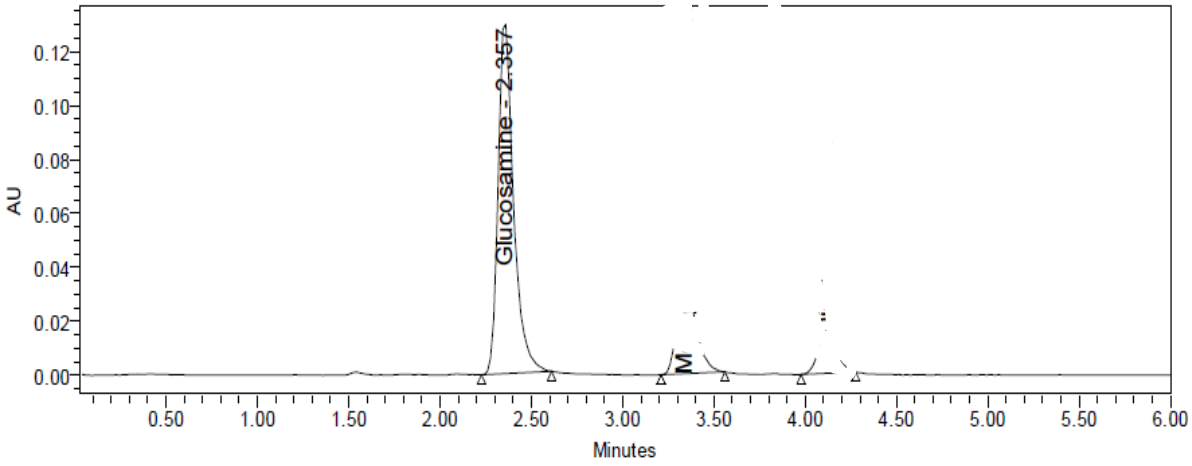



Fig 24: A typical robustness chromatogram of Glucosamine, Diacerein and Methyl sulfonyl methane at flow rate 0.9ml/min

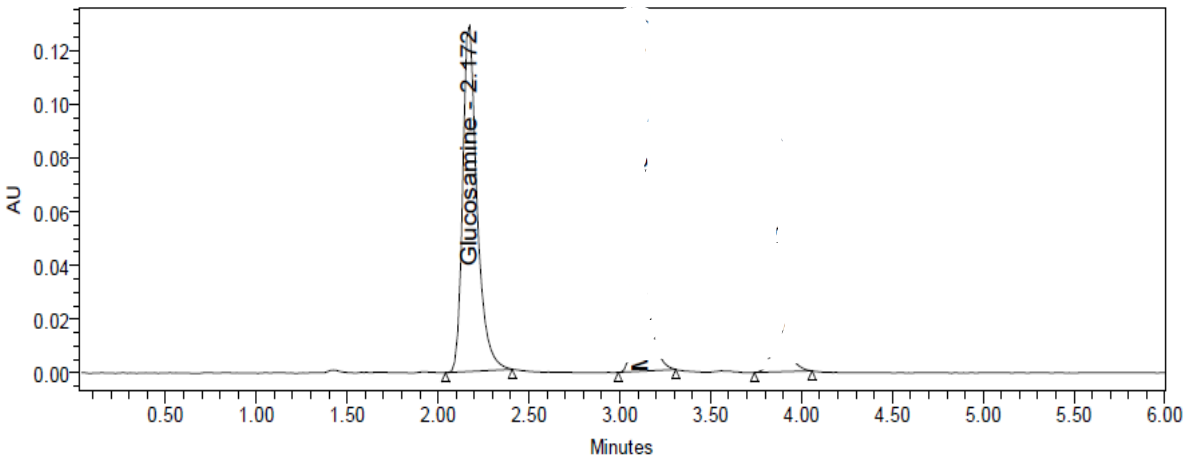



Fig 25: A typical robustness chromatogram of Glucosamine at flow rate 1.1ml/min

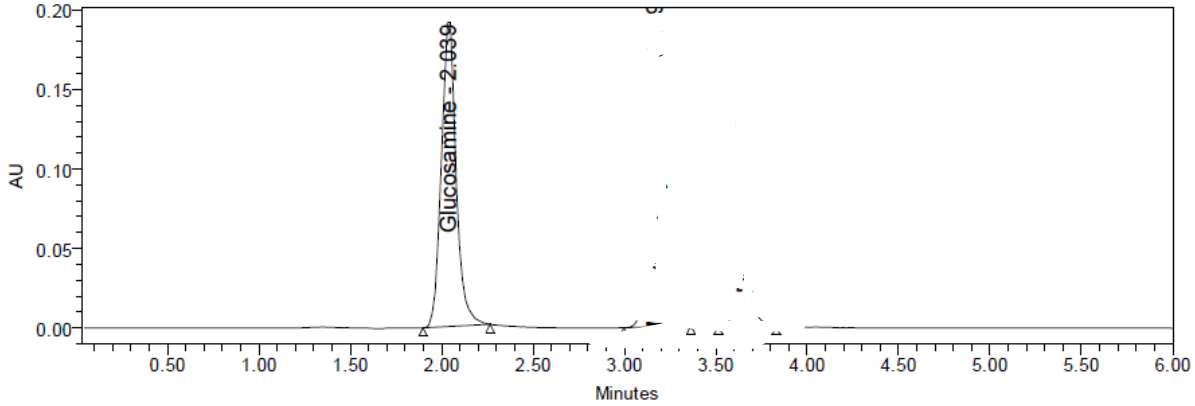



Fig 26: A typical robustness chromatogram of Glucosamine at temperature 25 $^{0}$C

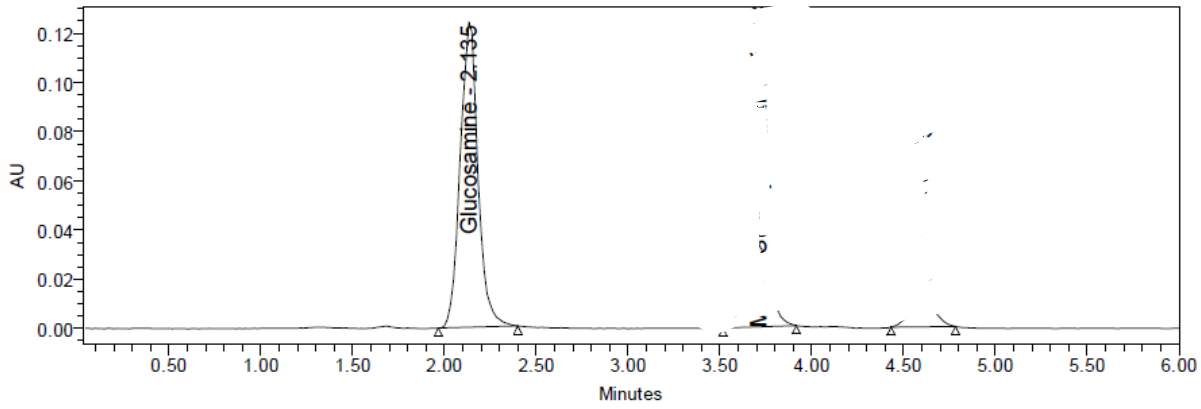
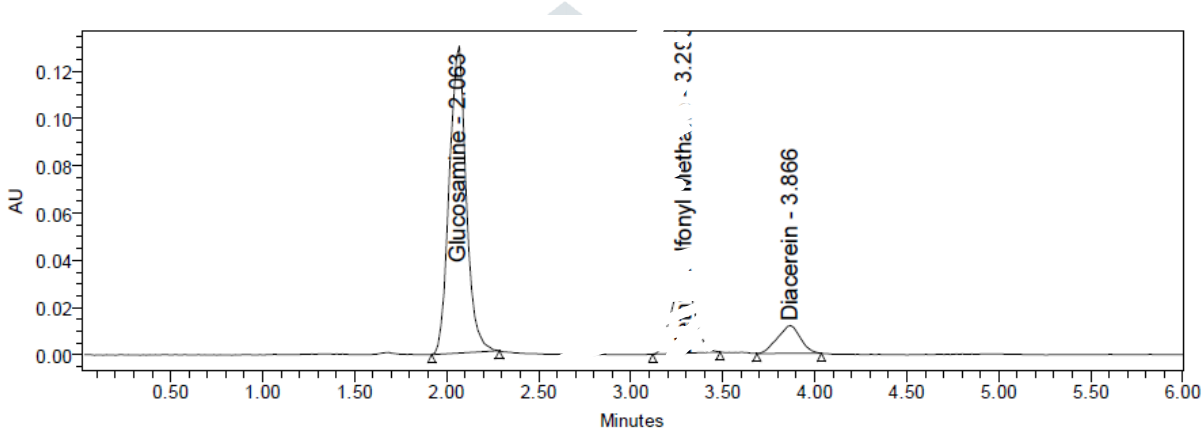



Fig 27: A typical robustness chromatogram of Glucosamin at mobile phase 55:45% v/v




**LOD and LOQ**

Limit of Detection (LOD) of an individual analytical procedure is the lowest amount of analyte is a sample, which can be detected but not necessarily quantitated and limit of quantitation (LOQ) of an analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were calculated from standard deviation of the response and the slope values of the three linearity curves using the formula 3.3 α/S for LOD and 10 α/S for LOQ, where α is standard deviation of response and S is mean of slope of three calibration curves. The LOD was calculated and found to be 0.47 µg/ml for Glucosamine. The LOQ was calculated and found to be 1.42 µg/ml for Glucosamin. The LOD and LOQ data was given in table 18.

**Table 11: LOD and LOQ results of Glucosamine**

| Sample | LOD | LOQ |
|---|---|---|
| Glucosamine | 0.47 µg/ml | 1.42 µg/ml |

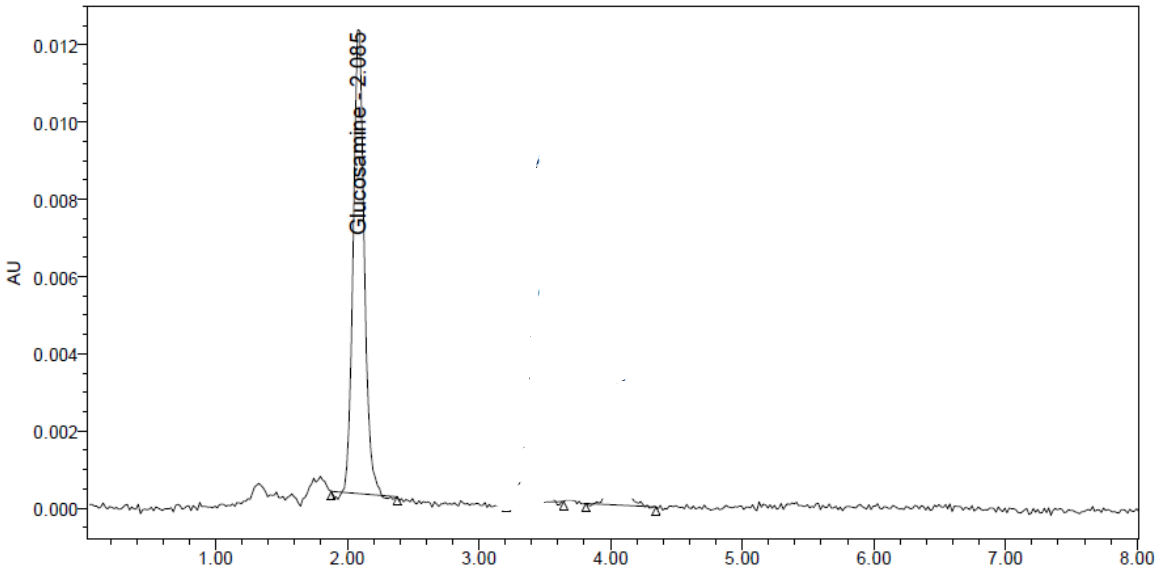



Fig 28: LOD chromatograms of Glucosamine

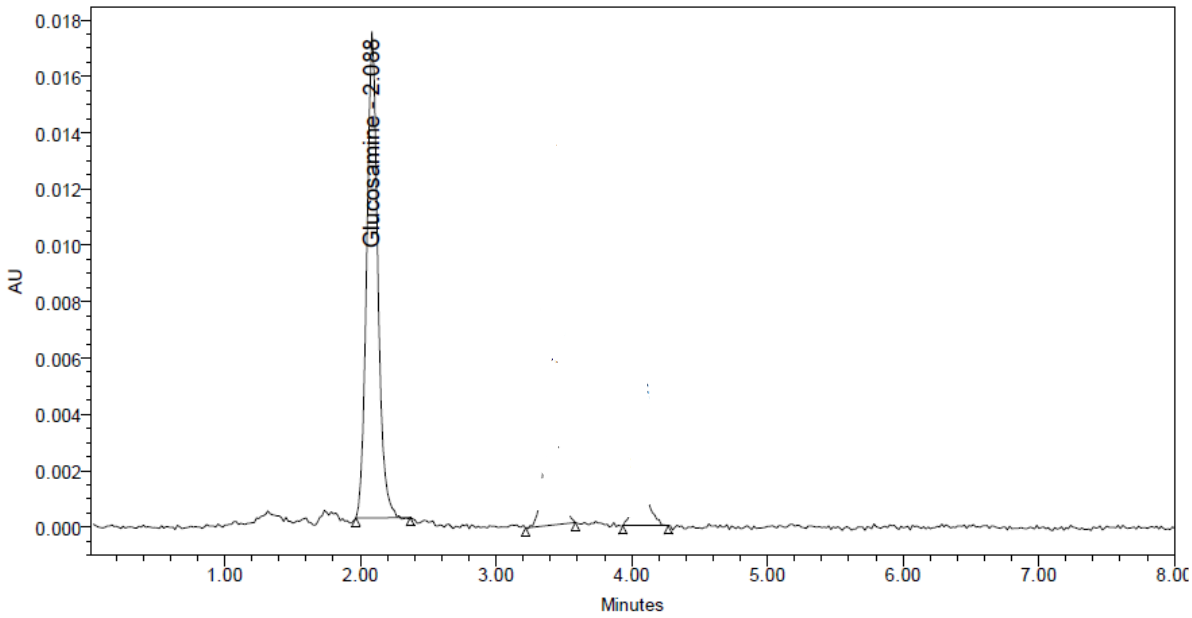



Fig 29 : LOQ chromatograms of Glucosamine

**Table 12: Assay Data of Glucosamine**

| S.no | Standard Area | Sample area | % Assay |
|---|---|---|---|
| 1 | 816694 | 812342 | 100.27 |
| 2 | 810225 | 815297 | 100.64 |
| 3 | 814141 | 809674 | 99.94 |
| 4 | 813558 | 810861 | 100.09 |
| 5 | 801897 | 809773 | 99.96 |
| 6 | 802742 | 809332 | 99.90 |
| Avg | 808513 | 811213 | 100.13 |
| Stdev | 6211.5 | 2282.9 | 0.282 |
| %RSD | 0.8 | 0.3 | 0.3 |

## Forced Degradation Studies

### Effect of Acid, Base hydrolysis and peroxide

In the acidic, alkali hydrolytic and **peroxide** degradation drugs has been shown minor degradation

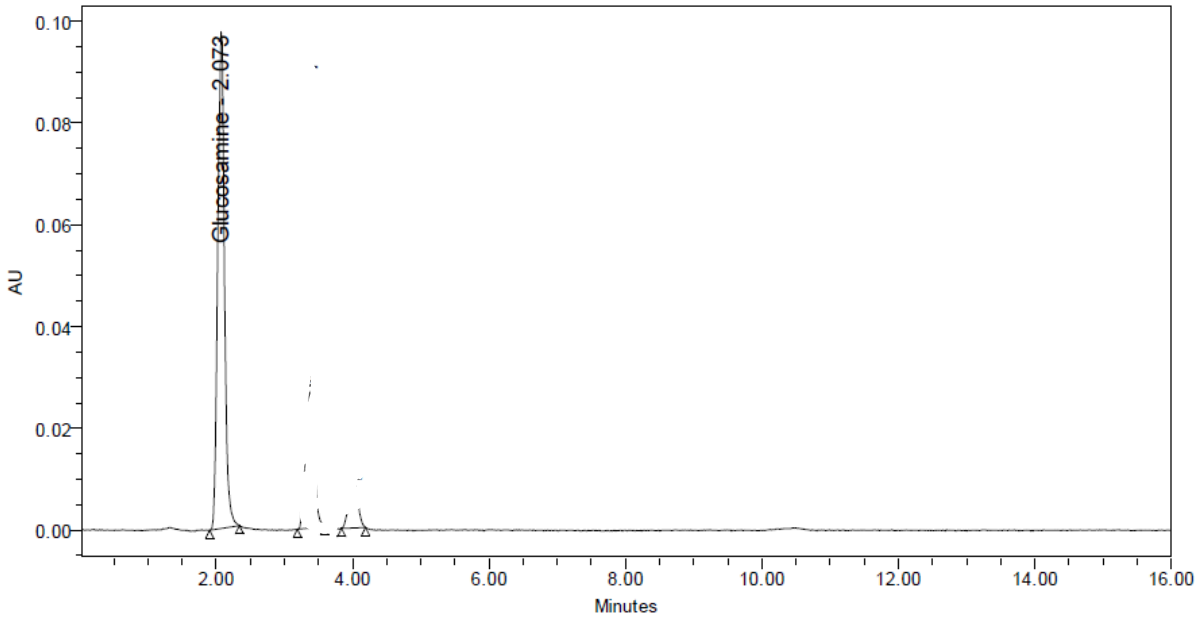



Fig 29: Acid degradation chromatogram of Glucosamine

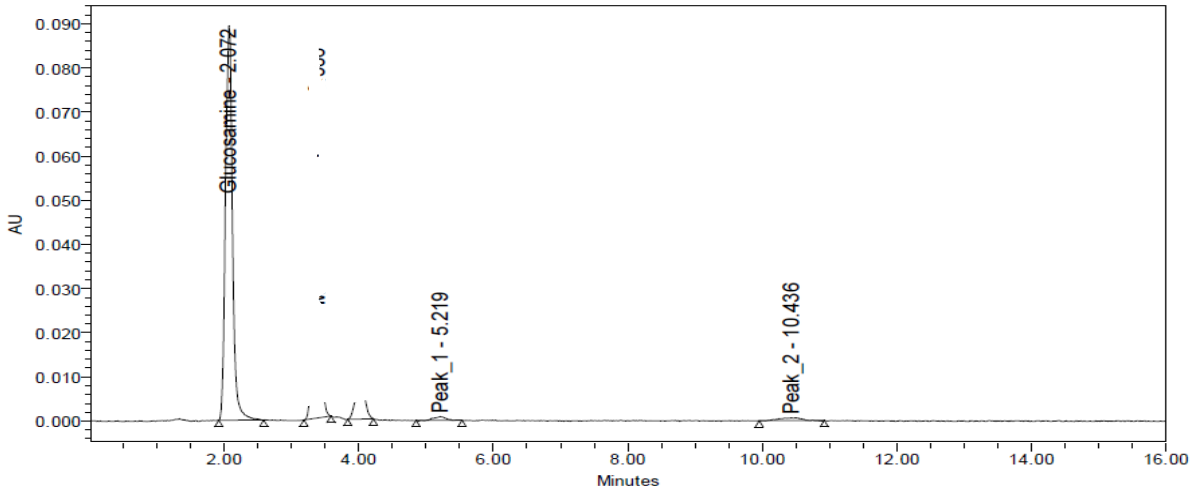



**Fig30: Base degradation chromatogram of Glucosamine**

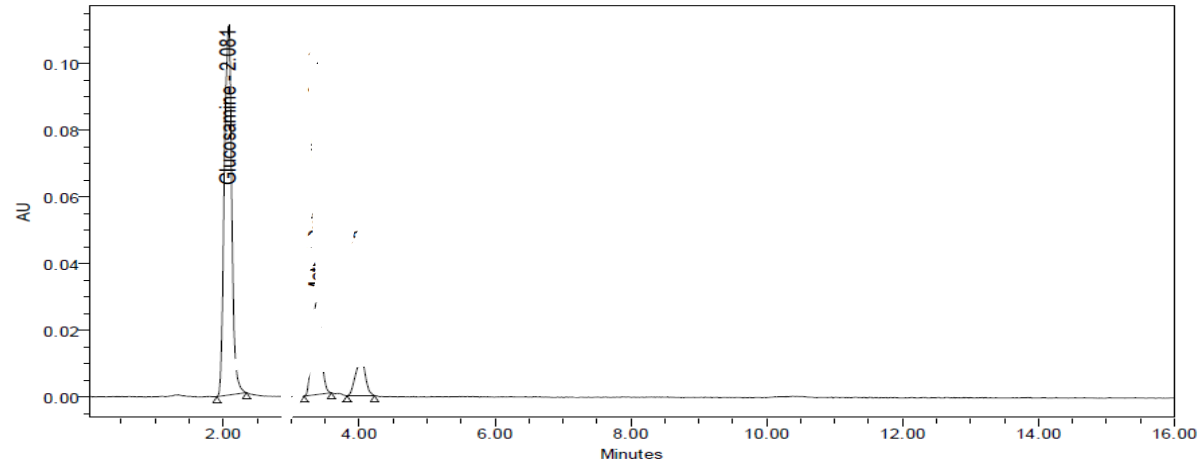



**Fig31: Oxidative degradation chromatogram of Glucosamine**

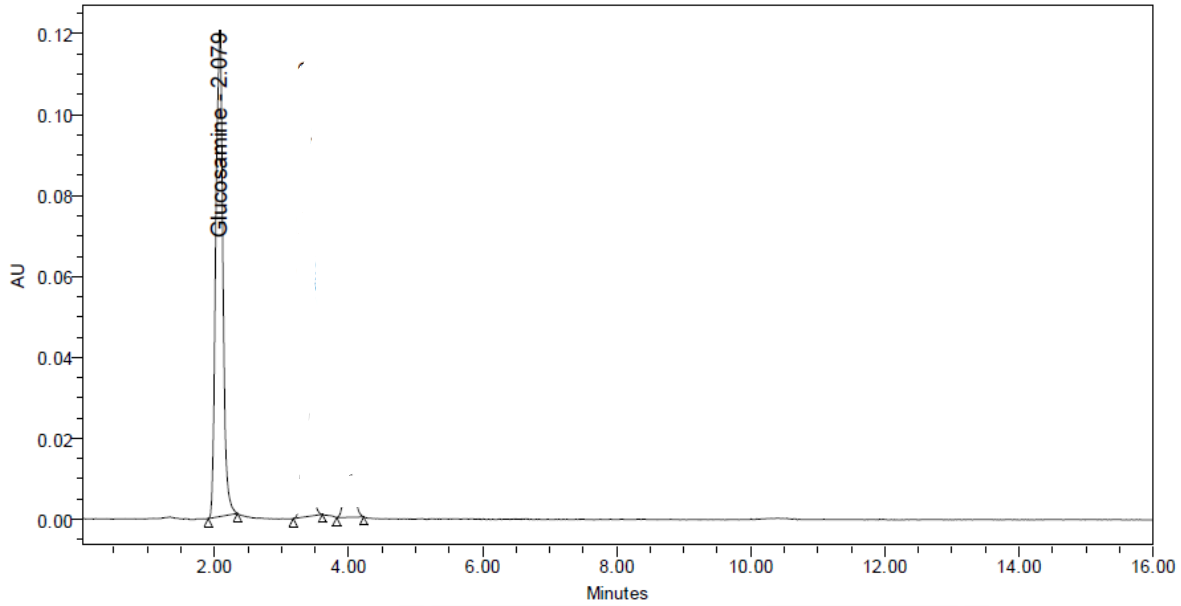



Fig 32: Thermal degradation chromatogram of Glucosamine, Diacerein and and Methyl sulfonyl methane

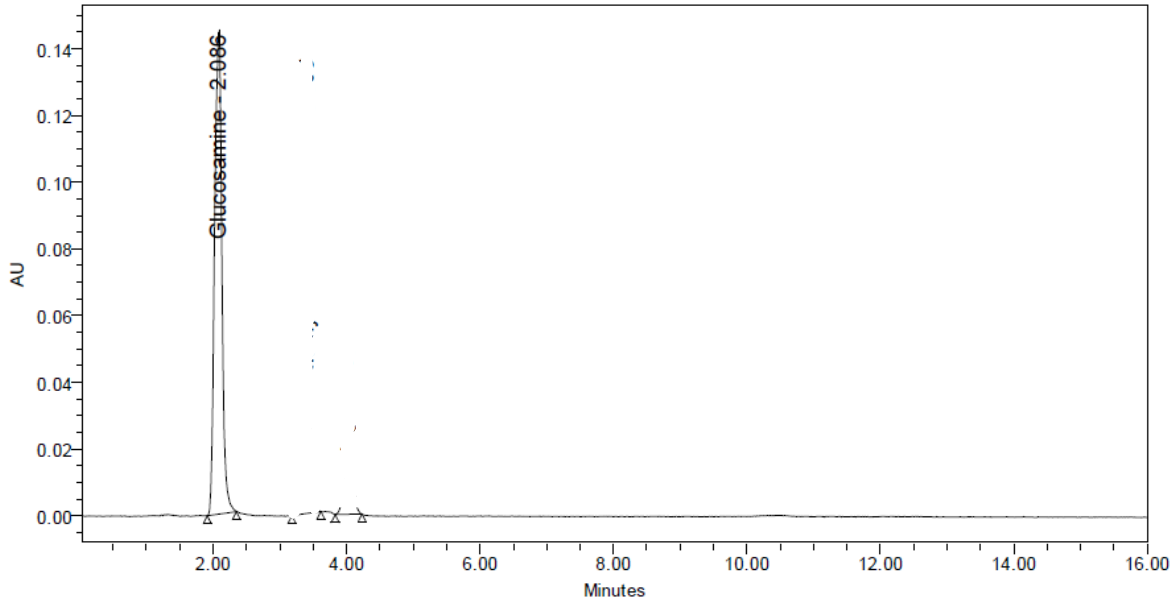



Fig 33: Photolytic degradation chromatogram of Glucosamine

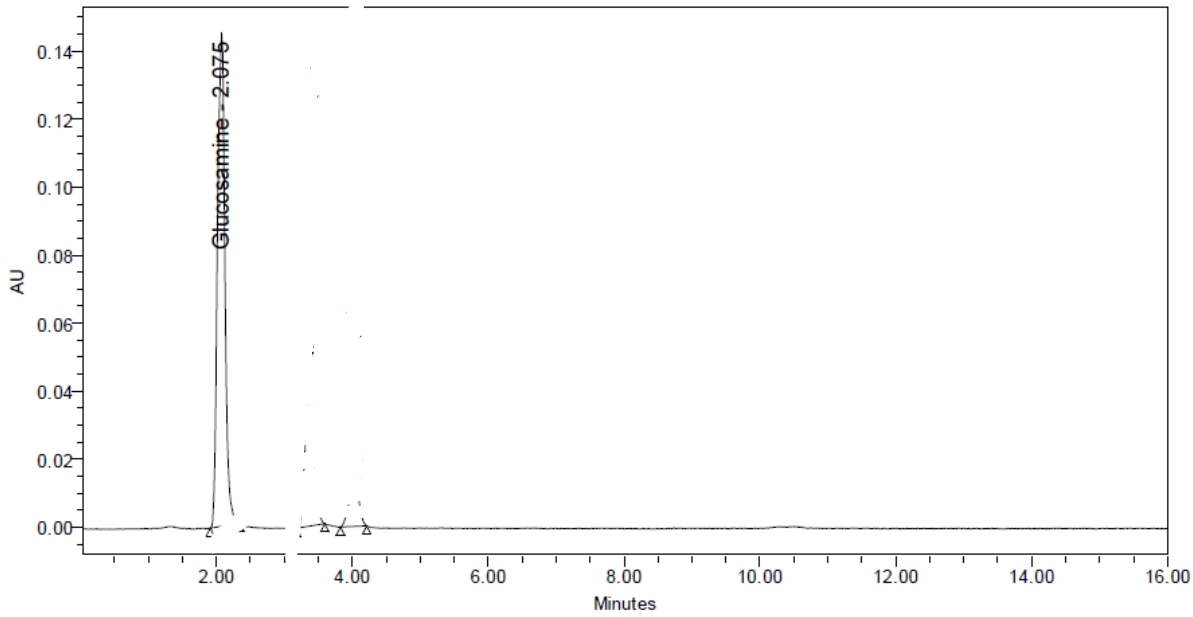



Fig 34: Neutral degradation chromatogram of Glucosamine

| Nature of degradation | Glucosamine | | |
|---|---|---|---|
| | AREA | %RECOVERED | % DEGRADED |
| Acid degradation | 775462 | 95.56 | 4.44 |
| Base degradation | 779954 | 96.11 | 3.89 |
| Peroxide degradation | 788321 | 97.14 | 2.86 |
| Dry heat degradation | 791754 | 97.57 | 2.43 |
| Photolytic degradation | 801414 | 98.76 | 1.24 |
| Neutral Degradation | 805369 | 99.24 | 0.76 |

Fig 35: Forced degradation studies of Glucosamine

**CONCLUSION:**

A new simple, rapid, sensitive, robust and economic RP-HPLC method was developed for simultaneous estimation of Glucosamine. The results of validation were found to be satisfactory and therefore the developed method can be used for the routine analysis of Glucosamine. Stability indicating studies of Glucosamine was also undertaken in the present study. The method was found to be very specific and there was no interference of any degraded compounds after forced degradation studies. The degraded products were successfully resolved and the method can be employed for determination of degraded products. The developed

stability indicating RP-HPLC method can be conveniently used for routine quality control analysis of Glucosamine industries for batch release.

The mobile phase is easy to prepare and economical. The sample recoveries in all formulations were in acceptable agreement with their respective label claims and they suggested non-interference of formulation excipients in the assessment. Subsequently, this technique can be effectively and advantageously received for routine investigation.

**SUMMARY:**

Pharmaceutical analysis just methods investigation of drugs. Today pharmaceutical analysis involves considerably more than the examination of active pharmaceutical ingredients or the formulated product. The pharmaceutical industry is under expanded examination from the administration and the public interested groups to contain costs and at reliably convey to market safe, efficacious item that satisfy neglected clinical needs. The pharmaceutical expert plays a significant guideline in guaranteeing character, security, adequacy, virtue, and nature of a medication item. The requirement for drug investigation is driven to a great extent by administrative prerequisites. The usually utilized trial of drug examination for the most part involve compendia testing strategy improvement, setting determinations, and technique approval. Analytical testing is one of the all the more fascinating ways for researchers to participate in quality cycle by giving real information on the the identity, content and purity of the medication items. New techniques are presently being improvement with a lot of thought to overall harmonization. Thus, new items can be guaranteed to have similar quality and can be brought to worldwide markets faster.

Pharmaceutical analysis possesses a vital function in statuary certification of drugs and their formulations either by the business or by the administrative specialists. In industry, the quality confirmation and quality control offices assume significant function in drawing out a protected and effective drug or dosage form. The current good manufacturing practices (CGMP)and the Food Drug Administration (FDA) rules demand for selection of sound techniques for examination with more prominent affectability and reproducibility. Accordingly, the unpredictability of issues experienced in drug investigation with the significance of accomplishing the selectivity, speed, low cost, simplicity, sensitivity, specificity, precision and accuracy in assessment of medications.

**Summary of Glucosamine**

| **Parameters** | **Glucosamine** |
|---|---|
| **Linearity** Range (µg/ml) | 37.5-225 µg/ml |
| Regressioncoefficient | 0.999 |
| Slope(m) | 5436 |
| Intercept(c) | 1280 |
| Regression equation (Y=mx+c) | y =5436.x + 1280 |
| **Assay (% mean assay)** | 100.13.% |

| | | |
|---|---|---|
| **Specificity** | | Specific |
| **System precision %RSD** | | 0.8 |
| **Method precision %RSD** | | 0.8 |
| **Accuracy %recovery** | | 99.79% |
| **LOD** | | 0.47 |
| **LOQ** | | 1.42 |
| **Robustness** | **FM** | 0.5 |
| | **FP** | 0.5 |
| | **MM** | 0.3 |
| | **MP** | 0.5 |
| | **TM** | 0.5 |
| | **TP** | 0.6 |

## REFERENCES


1. Taverniers I, De Loose M, Van Bockstaele E. Trends in quality in the analytical laboratory. II. Analytical method validation and quality assurance. Trends Analyt Chem. 2004; 23(7):480-490.
2. Araujo P. Key aspects of analytical method validation and linearity evaluation. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877 (23):2224–2234.
3. United States Pharmacopeia and National Formulary (USP 35 NF30), The United States Pharmacopeial Convention, Inc., Rockville, MD, 2012.
4. Rozet E, Ceccato A, Hubert C, et al. Analysis of recent pharmaceutical regulatory documents on analytical method validation. J Chromatogr A. 2007; 1158(1- 2):111–125.
5. International Conference on Harmonization, "Q2B: Validation of Analytical Procedures: Methodology; Availability," *Federal Register***62**(96), 27463–27467 (1997).
6. FDA, "Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls Documentation; Availability," *Federal Register (Notices)***65**(169), 52776–52777 (2000).
7. www.fda.gov/cder/guidance/cmc3.pdf
8. USP 25–NF 20, Validation of Compendial Methods Section (1225) (United States Pharmacopeal Convention, Rockville, Maryland, USA, 2002) p 2256.
9. G.A. Shabir, "Validation of HPLC Chromatography Methods for Pharmaceutical Analysis. Understanding the Differences and Similarities Between Validation Requirements of FDA, the US Pharmacopeia and the ICH," *J. Chromatogr. A.***987**(1-2), 57-66 (2003).
10. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics. 2nd. ed. B. Magnusson and U. Ornemark (eds.). 2014.