RESEARCH ARTICLE

Formulation, Development, Characterization of Tablet Containing Glucosamine Sulphate from *Agaricus bisporus*

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ABSTRACT

The present study aimed to synthesize glucosamine sulphate from a natural vegan source, formulate it into tablets, and evaluate its physicochemical and pharmaceutical properties. Glucosamine hydrochloride was extracted from the cell wall of *Agaricus bisporus* (*A. bisporus*) mushrooms by converting chitin into chitosan, followed by enzymatic hydrolysis with α-amylase and glucoamylase. The hydrolyzed product was subsequently converted into glucosamine sulphate and characterized using FTIR, PXRD, thermal analysis, microscopic examination, and micromeritic studies. The compound was further formulated into tablets and evaluated for pharmaceutical performance. Glucosamine sulphate was successfully synthesized from *A. bisporus,* with an overall yield of 2% from wet biomass. FTIR confirmed the structural identity, while PXRD established its crystalline nature. The product exhibited low hygroscopicity (0.2% mo555isture content) and good thermal stability, as indicated by DSC. Micromeritic studies showed acceptable flow and compressibility properties, supporting suitability for tableting. Coated tablets demonstrated efficient drug release, achieving \~90% dissolution within 60 minutes. Glucosamine sulphate synthesized from *A. bisporus* mushrooms showed satisfactory yield, desirable physicochemical properties, and rapid dissolution, highlighting its potential as a sustainable natural source for the prevention and management of osteoarthritis.

Keywords: Glucosamine hydrochloride, Glucosamine sulphate, Tissue-regeneration, Osteoarthritis, Chitosan, Cartilages

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INTRODUCTION

The skeletal system in the human body confers a unique structure that supports bipedal locomotion and an upright posture. Bones, joints, cartilages, and ligaments work together in harmony to carry out this function. Joints function optimally when the cartilage remains strong, intact and, firm in place. Cartilage consists of a complex extracellular matrix composed of glycosaminoglycans and proteoglycans[1]. Osteoarthritis may develop as a result of interactions between biological and mechanical factors that affect this matrix[2]. Apart from rheumatoid arthritis the most prevalent form of autoimmune arthritis - osteoarthritis (OA) arises primarily from wear and tear rather than autoimmune dysfunction[3,4].

The causes of primary OA include the action of inflammatory mediators such as TNF-α and interleukins, lack of physical activity, depletion of sex hormones, and aging[5,6]. Secondary osteoporosis, on the other hand, may result from imbalances in calcium, vitamin D, and sex hormones, as well as conditions such as Cushing's syndrome, inflammatory disorders like rheumatoid arthritis, long-term glucocorticoid use, excessive alcohol consumption, and hypogonadism[7,8]. Treatment options include lifestyle modifications such as weight loss, yoga, or Tai Chi, as well as pharmacological approaches such as NSAIDs, glucocorticoids, and opioids. In severe cases, surgical joint replacement may be necessary[9-11].

Cartilage is the primary tissue affected in OA, and glycosaminoglycans represent a critical structural component of its extracellular matrix. Glucosamine sulphate functions as a cartilage-restorative agent by promoting the repair of degenerated cartilage tissue[12]. Glucosamine, an amino sugar, serves as a key precursor in the biosynthesis of glycosylated proteins and lipids. In association with N-acetylglucosamine, it contributes to the formation of the polymer chitosan, which is derived from chitin, the principal structural component of fungal cell walls[13]. Chitosan possesses wide-ranging biomedical applications, particularly in advanced drug delivery systems, due to its superior mucoadhesive capacity and inherent antimicrobial and antioxidant properties[14,15]. Furthermore, its biocompatibility and biodegradability render it an attractive material for pharmaceutical and biomedical use[16].

Natural sources of glucosamine include fungi (e.g., mushrooms), marine exoskeletons such as crab carapace, as well as plant-based sources including seeds of *Phaseolus vulgaris* (kidney bean) and the cotyledons of *Vigna radiata*

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(mung bean)[17,18]. Glucosamine supports the collagen and protein matrix of connective tissue, which stabilizes joints and provides shock-absorbing capacity. It also plays a critical role in the synthesis of hyaluronic acid, a key component of synovial fluid[19,20]. With advancing age, articular cartilage progressively loses its ability to sustain healthy cellular growth, while synovial fluid production and quality decline, particularly in individuals with increased body weight[21]. These changes contribute to the development of OA, a condition characterized by articular surface degeneration and painful joint motion.

Although OA was initially regarded as a disease confined to articular cartilage, recent evidence demonstrates that it involves the entire joint as an organ. Secondary changes associated with OA include subchondral bone remodelling, meniscal tears and extrusion, osteophyte formation, bone marrow lesions, and pathological alterations of the joint capsule, synovium, ligaments, and periarticular muscles[22-24].

Glucosamine has been widely recognized as a nutraceutical compound with significant potential in maintaining joint health and function. It plays a critical role in cartilage regeneration and exerts protective effects against arthritis by inhibiting pro-inflammatory cytokines, suppressing protease activity, modulating nuclear factor kappa B (NF-κB) signalling, and reducing prostaglandin E_2 production[25,26]. Collectively, these multifaceted mechanisms highlight glucosamine's therapeutic potential as a disease-modifying agent for degenerative joint disorders.

In the present investigation, glucosamine was produced from a natural, vegetarian source, *Agaricus bisporus* (button mushroom), which represents an alternative to conventional marine-derived sources such as shellfish. Chitin, a structural polysaccharide abundantly present in the fungal cell wall, was isolated and subsequently deacetylated to yield chitosan. This chitosan was then subjected to controlled enzymatic hydrolysis using α-amylase and glucoamylase, resulting in the release of glucosamine monomers. To enhance chemical stability and improve handling characteristics, the obtained glucosamine was converted into its salt form, most commonly glucosamine sulphate or hydrochloride. The stabilized compound was further processed into tablet dosage forms, which were evaluated for physicochemical characteristics, stability, and potential applicability in therapeutic use. This approach not only provides a sustainable vegetarian source of glucosamine but also underscores the feasibility of fungal biomass as a renewable raw material for nutraceutical development.

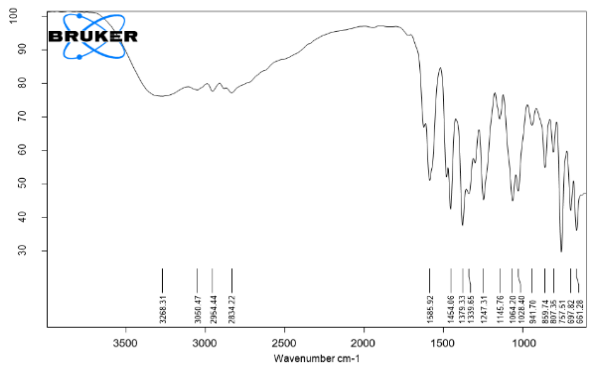

Figure 1: FTIR of Glucosamine sulfate powder

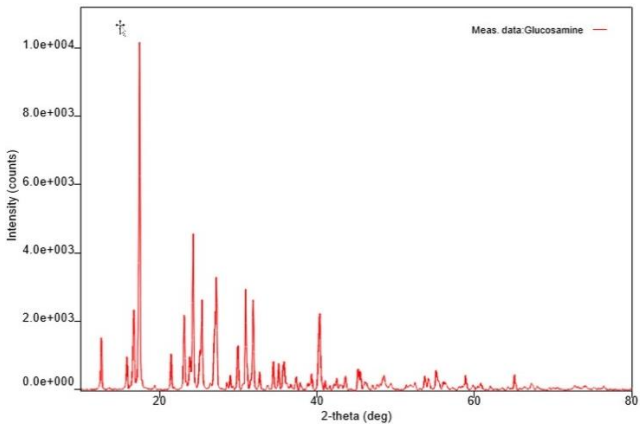

Figure 2: PXRD of Glucosamine sulfate

MATERIALS AND METHODS

Chemicals

Button mushroom (*Agaricus bisporus)* was purchased locally from Harem Biotech Agro Farming, Saale, and Nagpur. The enzymes glucoamylase and alpha-amylase were the generous gift by Anthem Bioscience Pvt. Ltd, Bangalore, and Karnataka. Koll coat IR® was also gifted by BASF Pharma. All other chemical used were purchased locally and were of AR grade.

Preparation and Purification of Chitosan from Mushroom

Collected button mushroom was chopped in smaller pieces and was micronized using mixer grinder. Exactly 250 g. of this paste was taken and mixed with 800 ml of 1M NaOH. The mixture was kept for 24 hours. Next day it was mixed well using magnetic stirrer for 2 hours and filtered. To the residue obtained, 500 ml of 1M HCl was added, allowed to stand for 2 hours and filtered. To filtrate 75 ml of sodium hypochlorite was added followed by addition of 100 ml of 40% NaOH. The mixture was heated on water bath for 3 hours and was filtered to obtain chitosan.

In order to purify the chitosan, it was added to 400 ml of 3% aqueous acetic acid solution, kept overnight and filtered. The filtrate was neutralized by dropwise addition of 1 M NaOH with continuous stirring on magnetic stirrer (300-350 rpm). The chitosan gets precipitated out in this which was then filtered.

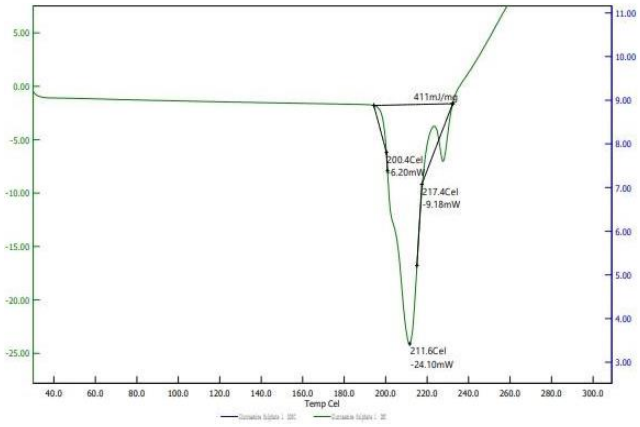

Figure 3: DSC thermogram of Glucosamine sulfate

Table 1: Organoleptic and physicochemical properties of Glucosamine sulphate

S. No.	Parameters	Results
1	Appearance	Off white powder
2	Taste	Saline-salty taste
3	Texture	Smooth texture
4	Moisture content	0.2%
5	Particle size (µm)	1.7641 ± 0.1979
6	pH	4.40
7	Melting Point (˚C)	190 - 200

(Mean±SD, n=3)

Preparation of Glucosamine HCL from Chitosan

Enzymatic hydrolysis of the chitosan obtained was carried out by using enzyme α-amylase and glucoamylase[27]. In this 20 g of chitosan was weighed and mixed with 1 L of 1% acetic acid. To this 2.5 g of α-amylase was added and pH was adjusted to 5. This was kept overnight and filtered. Filtrate was kept in oven for 2 days at 55℃. to get glucosamine HCl to be precipitated out, ethanol to the tune of five times of volume of filtrate was added and precipitate was filtered.

Synthesis of Glucosamine Sulphate from Glucosamine HCl

Using prepared glucosamine HCl 3% w/v of it was prepared and mixed with 3 % w/v sodium sulphate solution. The reaction mixture was stirred for 2 hours maintaining at 50℃. reaction mixture was then filtered and filtrate was dried to obtain glucosamine sulphate. Crude glucosamine was further decolorized using 0.5g. activated charcoal in 10% w/v solution and heating at 55℃ for 1 hour[28].

Evaluation of Glucosamine Sulphate Powder

The prepared glucosamine sulphate was evaluated for various parameters. The results of the tests performed were as follows.

Organoleptic Properties

The color, odor, taste, and physical appearance of glucosamine sulphate were evaluated. Additionally, the pH of a 1% w/v solution was determined using a digital pH meter.

Moisture Content

Pre-weighed 2 g. Glucosamine sulphate kept at ambient temperature and humidity, it was heated at 60℃ to the constant weight to determine the equilibrium moisture content.

Particle Size Analysis

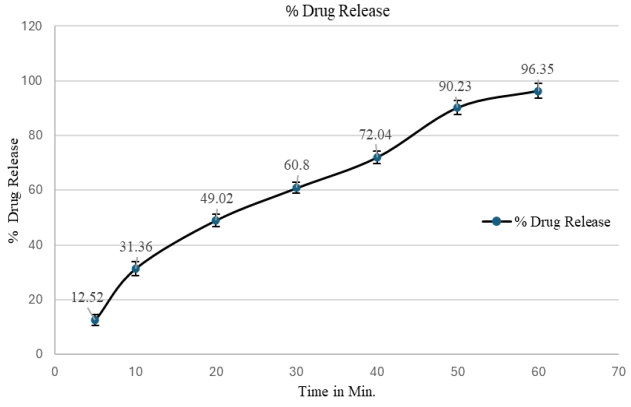

Figure 4: % Drug release of glucosamine sulphate tablet in *in-vitro* dissolution studies

Table 2: Micromeritic Properties of Granules (Mean±SD, n=3)

S. No.	Parameters	Results
1	Bulk density(g/ml)	0.55 ± 0.0016
2	Tapped density(g/ml)	0.71 ± 0.0025
3	Angle of repose (°)	41.50 ± 1.35
4	Flow property	Passable
5	Hausner's ratio	1.27 ± 0.027
6	Carr's compressibility Index (%)	9.33 ± 1.38

Table 3: Evaluation parameters of Glucosamine Sulphate Tablet (Mean±SD, n=3)

S. No.	Parameters	Observed values
1	Hardness (KG/cm^2)	5.1 ± 0.4 KG/cm^2
2	Thickness (mm)	4.3 ± 0.3 mm
3	Diameter (mm)	7.7 ± 0.3 mm
4	Friability (%)	0.85 %
5	Weight Variation (mg)	552 ± 12 mg
6	Wt. of Uncoated Tablet (mg)	552 ± 2 mg
7	Wt. of film-coated Tablet (mg)	560 ± 5 mg
8	Disintegration time (min) Uncoated Tablet	5.83 ± 0.8 min
9	Disintegration time (min) Film-Coated Tablet	8.5 ± 0.5 min

Particle size analysis was performed employing the Dynamic Light Scattering (DLS) technique (Bitesize DLS 500). The sample was dispersed in a suitable solvent and DLS technique measures the hydrodynamic diameter of particles based on the scattering of light as they undergo Brownian motion. This also gives polydispersity index (PDI) which provides insights into the uniformity or heterogeneity in particle size of powder sample. Lower PDI values is for narrower particle size distribution, indicates more homogeneous sample and higher PDI values comes for broader distribution of particle sizes indicates heterogeneous sample[29,30].

FTIR Studies

FTIR gives an idea about the functional group present in the testing sample. The FTIR spectra of prepared glucosamine sulphate were analysed from Shimadzu, FTIR spectrophotometer in the range 400 cm^{-1} to 4000 cm^{-1}.

X-Ray Diffraction (XRD) Studies

The crystallinity of the prepared glucosamine sulphate was analysed by powder X-ray diffraction study using Bruker AXS D8 Advance. The study was performed using Cu radiation generated at 25 mA intensity and 35 kV voltage and at wavelength 1.5406 Å. The PXRD pattern was obtained between 3° to 90° (2θ).

Differential Scanning Calorimetry (DSC)

DSC pattern shows exothermic and endothermic crests which are helpful in determining polymorphic nature, melting point, co-crystal formation, heat of fusion, glass transition temperature and endothermic and exothermic behaviour of a compound. The thermograms were obtained for Glucosamine sulphate by increasing the temperature at the rate 10ºC per minute up to 200ºC by using Differential scanning calorimeter[31].

Preparation and Evaluation of Glucosamine Sulphate Granules

Granulation for preparation of tablets was done by wet granulation technique using 2% polyvinyl pyrrolidone PVP K-30 in isopropyl alcohol. Granules were dried at 60ºC for 2 hours. For uniform granules this was then passed through mesh No. 22. These were then evaluated for particle size analysis, micromeritics studies and Kawakita analysis. In micromeritic studies angle of repose was determined using funnel method[32]. Tapped density, bulk density, Carr's compressibility index, Hausner's ratio and Kawakita analysis was done using bulk density testing apparatus (Sharma Scientific Industries, Ambala Cantt.). Angle of repose gives an idea about flow properties of the material, while tapped density, bulk density, Carr's compressibility index and Hausner's ratio explains about flow properties as well as compaction properties. Kawakita plot gives us two values of constant 'a' and 'b'. The 'a' value indicates a total reduction in the volume of consolidating material, and 'b' value is inversely proportional to yield strength[33].

Formulation of Glucosamine Sulphate Tablet

Dried and lubricated granules were then compressed using 16 station rotary tablet compression machines (Pharma N Pack, Thane) at 2-ton pressure with about 550 mg filling capacity. The compressed tablets were then coated with the polymer Koll coat IR (BASF Pharma) by the dip coating technique[34].

Evaluation of Glucosamine Sulphate Tablet

To evaluate the prepared glucosamine sulphate tablets, following tests were performed.

Organoleptic Properties, Dimensions and Weight Variation

Prepared tablets were checked for appearance, defects, size and shape and weight variation.

Hardness and Friability

Tablet hardness was determined by using Monsanto hardness tester, keeping single tablet in between anvils and applying pressure to break the tablet. To ensure mechanical strength, friability test of tablet was performed by using Roche friabilator by taking 20 tablets and allowing them to fall from 6 inches height for 100 times with speed of 25 RPM.

Disintegration Study

Disintegration time for glucosamine tablet was determined by using USP digital tablet disintegration test apparatus (VEEGO Instrument Corporation, VTD-AVP). In this, 6 tablets of glucosamine sulphate formulation were used taking 900 ml of 0.1 M HCl as a disintegration medium. The test was carried out at temperature 37°C ± 0.5°C. Disintegration time for uncoated and coated tablets was studied separately.

In-vitro Drug Release Studies

Tablet dissolution and drug release study was performed using a USP dissolution type II apparatus (Electro lab, TDT-06L) at 37 ± 0.5°C with a rotation speed of 50 rpm/ min and 900 ml of dissolution medium (simulated gastric fluid) per vessel used. 5 ml aliquot was taken after suitable time interval and was replenished with fresh dissolution media. Aliquots taken was analysed by UV-visible spectrophotometer at 240 nm[35].

Assay for Active Content using High-Performance Liquid Chromatography (HPLC)

The quantification of the active pharmaceutical ingredient (API) was carried out using a validated High-Performance Liquid Chromatography (HPLC) method. The chromatographic separation was achieved on a C18 Poroshell column (typically 4.6 × 100 mm, 2.7 µm particle size), which offers high resolution and efficiency for small molecule analysis. The mobile phase consisted of acetonitrile and phosphate buffer (pH adjusted to 3.0–3.5) in the ratio of 3:1 (v/v), optimized for appropriate retention and peak symmetry. The phosphate buffer was prepared using potassium dihydrogen phosphate (KH_2PO_4) and pH was adjusted using orthophosphoric acid. A flow rate of 1.0 mL/min was maintained throughout the analysis. The injection volume was set at 20 µL for all samples and standards. The detection wavelength was selected based on the UV absorbance maxima (λ_{max}) of the compound of interest, ensuring optimal sensitivity and specificity. All samples were filtered through a 0.45 µm membrane filter prior to injection to remove any particulate matter. The assay was performed under isocratic conditions at ambient temperature, and the retention time of the analytes was noted. Quantification was done by comparing the peak area of the sample with that of the standard using an external calibration method.

RESULTS AND DISCUSSION

Preparation of Glucosamine Sulphate from Chitin Present in Cell Wall of Mushroom Agaricus bisporus

Cell wall of fungi is made up of chitin which is chemically polysaccharide containing long chain polymer of N-acetylglucosamine. This chitin when reacted with strong alkali causes deacetylation producing chitosan which is a copolymer of N-acetyl-d-glucosamine and d-glucosamine. Chitosan can be converted to glucosamine either by acid hydrolysis or by enzymatic hydrolysis[36]. In this work, we selected the method of enzymatic hydrolysis. In this chitosan is first treated with α-amylase hydrolyse β-1,4 linkages in chitosan yielding water-soluble chito-oligosaccharides which is further treated with glucoamylase causing hydrolysis producing glucosamine.

Evaluation of Glucosamine Sulphate

Organoleptic Properties

Prepared glucosamine sulphate was of off-white colour after decolorization with activated charcoal. Particle size distribution was of polydisperse type and pH of prepared 15 w/v solution was acidic i.e. 4.4. Melting point obtained was 190-200 ˚C which is analogues with the reported melting point.

Moisture Content

The sample after keeping at ambient temperature was found to have very less moisture content.

Particle Size Analysis

Hydrodynamic diameter of the product obtained indicated fine powder while value of PDI shows the polydisperse nature of the sample.

FTIR of Glucosamine Sulphate Powder

The observed peaks of the respective functional group are in the reference ranges (Fig. 1). The observed O-H stretching at 3268 cm^{-1}, N-H stretching at 3050 cm^{-1}, C-H stretching at 2954 cm^{-1}, C-N stretching at 1247 cm^{-1}, C-O

stretching at 1145 $cm^{-1,}$ and C-C stretching at 1064 cm^{-1}. The data obtained indicate that the observed peaks are in the reference range given for glucosamine sulphate[37].

PXRD of Prepared Glucosamine Sulphate Powder

The powder X ray diffractogram (Fig. 2) shows multiple intense peaks. This shows the crystalline nature of the prepared compound, and the highest peak obtained is at 17.423(2Ø), followed by various less intense peaks. This reflects the crystalline nature of glucosamine sulphate.

Thermal Analysis of Glucosamine Sulphate

Differential scanning colorimetric studies (Fig. 3) shows sharp endotherm obtained at 211 ˚C declining from195 ˚C which supports actual melting point obtained at 195-210˚C. This also indicates that the prepared Glucosamine sulphate is stable enough up to a very high temperature, i.e. 195˚C.

Evaluation of Glucosamine Sulphate Granules

Micromeritic Properties

Angle of repose of the glucosamine sulphate indicates poor flow properties, which might be due to irregular crystalline shape and good hardness of the particles. Values of Hausner's ratio and Carr's compressibility index also indicates the poor flow but better compressibility properties[38].

Consolidation Studies (Kawakita Plot)

Kawakita plot gives idea about degree of cohesion and compactibility of powders and granules. Value of constant 'a' obtained was 0.2504±0.0225, that of 'b' was 0.1715±0.0352 with coefficient of corelation 0.9925±0.0025. As the value of 'a' is higher than the value of 'b' the granules shows good compactibility with good yield strength.

Evaluation of Glucosamine Sulphate Tablets

Organoleptic Properties, Dimensions and Weight Variation

Prepared glucosamine sulphate tablets found to have good appearance and uniformly coated. The tablets were of uniform dimensions and weight variation was within acceptable range (Table 3).

Hardness and Friability

Hardness of the tablets found to have within standard range of 4-6 kg/cm^2 and friability also less than 1% of standard value. This implies that tablets were of sufficient strength and can bear wear and tear during transit and handling (Table 3).

Disintegration Properties

Film coated tablet of glucosamine sulphate was found appropriate within the standard value. Less disintegration time might be because of freely water-soluble polymers used in the coating of tablets (Table 3)

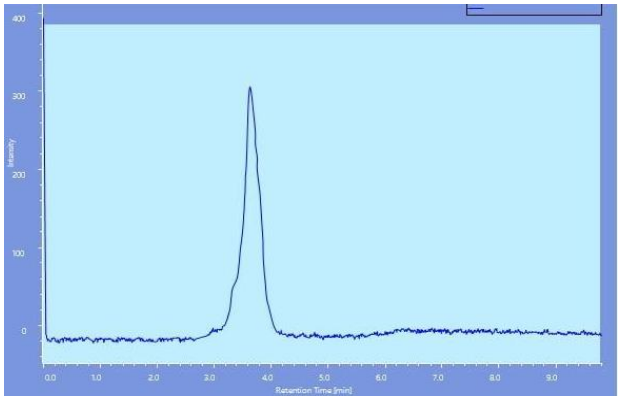

Figure 5: Retention Time of Glucosamine sulfate

In-vitro Drug Release Studies

In-vitro drug release study of prepared glucosamine sulphate tablets was determined in 0.1 N HCl as dissolution medium. Tablet Formulation shows 96.35% drug release in first 60 minutes itself (Fig. 4). This is due to good water solubility of the active ingredient.

Assay (Percentage Purity) of Glucosamine Sulphate Tablet

The High-Performance Liquid Chromatography (HPLC) analysis of the test sample showed a distinct and sharp peak at a retention time of approximately 3.9 minutes. The peak displayed good symmetry and high intensity, indicating a strong and specific response from the detector for the analyte of interest. No significant interfering peaks were observed near the retention time, suggesting adequate specificity of the method. The baseline was stable throughout the run, confirming the absence of matrix interference.

The retention time of the standard compound was also recorded around 3.9 minutes, (Fig. 5) confirming the identity of the analyte in the sample. The area under the peak was used for quantification against the calibration curve, and the active content was found to be within the acceptable range as per the assay specifications.

The HPLC method developed for the assay of the active compound demonstrated excellent chromatographic performance. The use of a C18 Poroshell column provided sharp peak resolution within a short runtime, facilitating efficient analysis. The chosen mobile phase composition (acetonitrile and phosphate buffer in 3:1 ratio) was effective in achieving good separation and peak shape.

CONCLUSION

From the present study, it is evident that *Agaricus bisporus* serves as a promising natural source for the production of glucosamine sulphate, an important nutraceutical for cartilage rejuvenation. The yield of glucosamine from 250 g of raw wet **A. bisporus** was 5 g (2%). Enzymatic hydrolysis of chitosan proved to be an efficient and sustainable method for obtaining glucosamine hydrochloride, comparable to the conventional acid hydrolysis technique. FTIR and spectroscopic analyses confirmed the presence of glucosamine sulphate in the final product. The compound exhibited thermal stability over a wide temperature range and demonstrated low hygroscopicity. Tablets prepared from the synthesized glucosamine sulphate could be easily granulated by conventional methods, showing good strength, compressibility, and acceptable flow properties. Compression yielded tablets with adequate hardness and low friability. *In vitro* dissolution studies indicated rapid drug release, with maximum release achieved within the first 60 minutes. Overall, *A. bisporus* can be considered a reliable vegan source of glucosamine sulphate for nutraceutical applications.

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