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Production and characterization of glucosamine hydrochloride from crustacean shell wastes

R Sumithraa[1], T Vijaykumar[1], C Sheeba Anitha Nesakumari[2], R Muthezhilan[3], Thirunavukkarasu N[4*]
[1] Department of Advanced Zoology and Biotechnology, Dr. Ambedkar Government Arts College (Autonomous), Vyasarpadi, Chennai, Tamil Nadu, India
[2] Department of Zoology, Madras Christian College (Autonomous), Tambaram, Chennai, Tamil Nadu, India
[3] Department of Marine Biotechnology, AMET University, Chennai, Tamil Nadu, India
[4] Department of Advanced Zoology and Biotechnology, Dr. Ambedkar Government Arts College (Autonomous), Vyasarpadi, Chennai, Tamil Nadu, India

Abstract
The bycatch resources of crustaceans were taken from Royapuram Fish Landing Centre, Kasimedu, Chennai. The stomatopod - *Harpiosquilla gonyptes* and shrimps - *Panapenaeopsis maxillipedo* and *Penaeus indicus* were collected and chitin was extracted from the shells of the crustacean samples. The chitin further used for the production of glucasomaine hydrochloride. The glucasoamine hydrochloride was derived from the chitin by acid hydrolysis. The samples were confirmed through FT-IR spectrum against standard glucosamine hydrochloride. The stomatopod *H. gonyptes* shows the peaks at 3676 cm^{-1}, 3404cm^{-1}, 2848 cm^{-1}, 1629 cm^{-1} and 1012 cm^{1}. The *P. maxillipedo* shows the peaks at 3712 cm^{-1}, 3408 cm^{-1}, 2370 cm^{-1}, 1629 cm^{-1}, 1095 cm^{-1} and 1033 cm^{-1}. Whereas the peaks through FT-IR of the shrimp *P. indicus* shows 3676 cm^{-1}, 3404 cm^{-1}, 2372 cm^{-1}, 1641 cm^{-1} and 1186 cm^{-1}. In the present study the intense bands were observed 3400 cm^{-1} (*H. gonyptes*), 3408 cm^{-1} (*P. maxillipedo*) and 3404 cm^{-1} (*P. indicus*) which reveals that association of O-H and N-H stretching. The peaks at 1629 cm^{-1} (*H. gonyptes*), 1629 cm^{-1} (*P. maxillipedo*) and 1641 cm^{-1} (*P indicus*) which reveals that association of NH_2. Whereas the peaks at 1012 cm^{-1} (*H. gonyptes*), 1095 cm^{-1} & 1033 cm^{-1} (*P. maxillipedo*) and 1186 cm^{-1} (*P. indicus*) which reveals that association of secondary alcohol -OH. All the above peaks levels were confirm that samples were glucosamine hydrochloride. The present study was found as simple, efficient and suitable for the preparation of glucasoamine from crustacean shells thereby recycling crustacean wastes.

Keywords: crustacean shell waste, chitin, glucosamine hydrochloride

Introduction

The major economically important group of crustaceans include lobster, shrimps, and crabs about 40-50 % of total weight of crustaceans goes as waste while processing for human food and the slower degradation of crustacean shell waste has become the major concern in sea food processing industries. Proper use of crustacean wastes allows recovery of value added by products which are having potential applications in the field of food and medicine (Santos *et al.*, 2012; Khor and Lim, 2003; Cira *et al.*, 2002; Healy *et al.*, 1994; Acosta *et al.*, 1993) [9, 4, 2, 3, 1]. Glucosamine has been prepared from various crustaceans. Glucosamine is an amino monosaccharide acting as a preferred substrate for the constitution of glycosaminoglycan chains. Glucosamine has anti-Cancer (Jung *et al.*, 2012; Oh *et al.*, 2007) [5, 8]. Anti-inflammatory (Nagaoka *et al.*, 2011) [7] and antibacterial (Kim, 2011) [6] effects. Glucosamine and its derivative like N-acetylglucosamine (GlcNAc) and D-glucosamine hydrochloride (GlcNHCl) have attracted much attention owing to their therapeutic activity in arthritis and been approved as a food supplement by FDA. Glucosamine is a poly-hydroxylatedprimary amine, used in the body as a molecular element for special macromolecules, the proteoglycans, and important constituents of the articular cartilage. Glucosamine hydrochloride, Glucosamine sulphate and N-acetylglucosamine are the commonly used glucosamine derivatives.

System line, which contains glucosamine, is promoted for use among young athletes. Elation, another fruit-flavoured beverage containing glucosamine, is produced by Coca-Cola and Procter & Gamble. Glucosamine hydrochloride can be considered as a nutraceutical by virtue of its properties. As a pharmaceutical product its preparation has only begun now in India. A number of products have been launched in the Indian market with glucosamine hydrochloride and glucosamine sulphate as the major ingredient. Glucosamine is a highly valued commercial product and hence the details of its production technology and its chemical properties are not widely available in literature. In this context it is felt that the technologies for the production of glucosamine hydrochloride in the purest form with maximum yield and least investment will help the industry significantly.

Adverse effects of glucosamine by any route are minimal with gastrointestinal symptoms, drowsiness, headache, and skin rash being reported. Although no known interactions exist between glucosamine and foods or other herbal supplements, there are two well-documented interactions between glucosamine and antidiabetic and cancer chemotherapy drugs. Glucosamine might increase insulin resistance or decrease insulin production resulting in elevated blood glucose levels. This increase in blood glucose may require increases in administered insulin and/or hypoglycemic agents such as sulfonylureas and metformin.

Therefore, glucosamine should be used only in diabetic patients who exhibit tight blood glucose control. The present study, has been aimed to produce Glucosamine Hydrochloride from crustacean shells of stomatopod *Harpiosquilla gonyptes* and shrimps - *Panapenaeopsis maxillipedo* and *Penaeus indicus.*

Materials and Methods

Collection of Shell Sample

The shells of shrimps and stomatopod were collected from Royapuram fish landing centre, Kasimedu, Chennai. The chitin is obtained from the shells of stomatopod *Harpiosquilla gonyptes* (Fig.1) and shrimps - *Panapenaeopsis maxillipedo* (Fig.2) and *Penaeus indicus* (Fig.3). These are the richest source of chitin and the major sources of glucasoamine hydrochloride.

Fig 1: *Harpiosquilla gonypte*

Fig 2: *Panapenaeopsis maxillipedo*

Fig 3: Penaeus indicus

Preparation of chitin

This process involves deprotenisation and demineralization. The procedure was adopted by Central Institute of Fishery Technology, Kochi has been followed in the present study.

Deprotenisation

The shells were deprotenised with 5% NaOH solution and boiled for 30 min with 70 – 80^{0}C. Till the neutral pH attained the shells washed thoroughly.

Demineralisation

Deprotenised shells were demineralised with hydrochloric acid. At room temperature the shells were demineralised with 1.25N HCl. After 24 hours, the shells were quite squashy and were rinsed with water to remove acid and calcium chloride and dried.

Preparation Glucosamine Hydrochloride

Glucosamine hydrochloride is prepared by hydrolysis of chitin in the presence of concentrated hydrochloric acid. The temperature and time of the reaction mixture is generally maintained around 95^{o}C for 2 hours. The hydrolysate contains the residual chitin, glucosamine and compounds formed due to charring. The reaction mixture can be processed further in two ways. In one method, it is cooled and filtered to remove acid and the residue containing glucosamine is dissolved in water, decolourised with charcoal, and filtrate is then crystalized by rotary evaporation, the crystals are washed with methanol and collected by filtration, dried in an oven to obtain the final product.

Fourier Transform – Infra Red (FT-IR) Spectrum

The quality parameters of chitin & chitosan was analysed by Fourier Transform Infrared (FT-IR) Spectrometry (Shigemasa *et al.*, 1996). FT-IR spectroscopy of solid samples of chitin and chitosan relied on a Bio-Rad FTIS-40 model, USA. Sample (10 µg) was mixed with 100 µg of dried Potassium Bromide (KBr) and compressed to prepare a salt disc (10mm diameter).

Results and Discussion

The bycatch resources of crustaceans were taken from Royapuram Fish Landing Centre, Kasimedu, Chennai. The species were stomatopod - *Harpiosquilla gonyptes* and shrimps - *Panapenaeopsis maxillipedo* and *Penaeus indicus.* The chitin was extracted from the shells of the crustacean samples. The chitin further used for the production of glucasomaine hydrochloride.

Glucosamine is glycoprotein derived from marine exoskeletons or produced synthetically as the sulfated or the hydrochloride salt. It is an endogenous substance required for the synthesis of glycoproteins, glycolipids, and glycoaminoglycans that are found in tendons, ligaments, cartilage, and synovial fluid and are required to maintain healthy articular cartilage. Glucosamine is part of the structure of chitosan and chitin which compose the exoskeletons of crustaceans, arthropods and fungi. The hydrolysis of chitosan results in monomers of β-(1-4)-linked-D-glucosamine (GlcN), an amino monosaccharide with physiological importance to the human body. The extracted glucosamine hydrochloride from shrimp and stomatopods were compared with standard glucosamine hydrochloride through FT-

IR spectrum (Figs 4 – 7). The standard glucosamine hydrochloride were shows the major peaks at 3600 cm^{-1}, 3400 cm^{-1}, 2800 cm^{-1}, 1600 cm^{-1} and 1000 cm^{-1}.

The stomatopod *Harpiosquilla gonyptes* shows the peaks at 3676 cm^{-1}, 3404cm^{-1}, 2848 cm^{-1}, 1629 cm^{-1} and 1012 cm^{-1}. The *Panapenaeopsis maxillipedo* shows the peaks at 3712 cm^{-1}, 3408 cm^{-1}, 2370 cm^{-1}, 1629 cm^{-1}, 1095 cm^{-1} and 1033 cm^{-1}. Whereas the peaks through FT-IR of the shrimp *Penaeus indicus* shows 3676 cm^{-1}, 3404 cm^{-1}, 2372 cm^{-1}, 1641 cm^{-1} and 1186 cm^{-1} (Table.1).

Table 1: Showing the FT-IR spectral values (cm^{-1}) of standard Glucosamine Hydrochloride and different Glucosamine Hydrochloride samples from *H. gonyptes, P. maxillipedo and P. indicus*

S. No.	Standard Glucosamine Hydrochloride (cm^{-1})	*Harpiosquilla gonyptes* (cm^{-1})	*Panapenaeopsis maxillipedo* (cm^{-1})	*Penaeus indicus* (cm^{-1})
1	3600	3676	3712	3676
2	3400	3404	3408	3404
3	2800	2848	2370	2372
4	1600	1629	1629	1641
5	1000	1012	1095 1033	1186

The FT-IR spectrum of G-HCl produced exhibits an intense band at 3470-3300 cm^{-1} associated with the O-H and N-H stretching, a NH_2 scissoring band at 1615 cm^{-1} and at 1094 cm^{-1} due to secondary alcohol –OH (Fig.4). In the present study the intense bands were observed 3400 cm^{-1} (*Harpiosquilla gonyptes*), 3408 cm^{-1} (*Panapenaeopsis maxillipedo*) and 3404 cm^{-1} (*Penaeus indicus*) which reveals that association of O-H and N-H stretching (Fig.5-7). The peaks at 1629 cm^{-1} (*Harpiosquilla gonyptes*), 1629 cm^{-1} (*Panapenaeopsis maxillipedo*) and 1641 cm^{-1} (*Penaeus indicus*) which reveals that association of NH_2. Whereas the peaks at 1012 cm^{-1} (*Harpiosquilla gonyptes*), 1095 cm^{-1} & 1033 cm^{-1} (*Panapenaeopsis maxillipedo*) and 1186 cm^{-1} (*Penaeus indicus*) which reveals that association of secondary alcohol -OH. All the above peaks levels were confirm that samples were Glucosamine Hydrochloride. Based on the experimental results, it is concluded that the preparation of Glucosamine hydrochloride by acid hydrolysis method is effective. The method is convenient and simple. The preparation of glucosamine hydrochloride from crustacean shell waste via chitin would successfully minimize the environmental pollution by the shrimp shell processing.

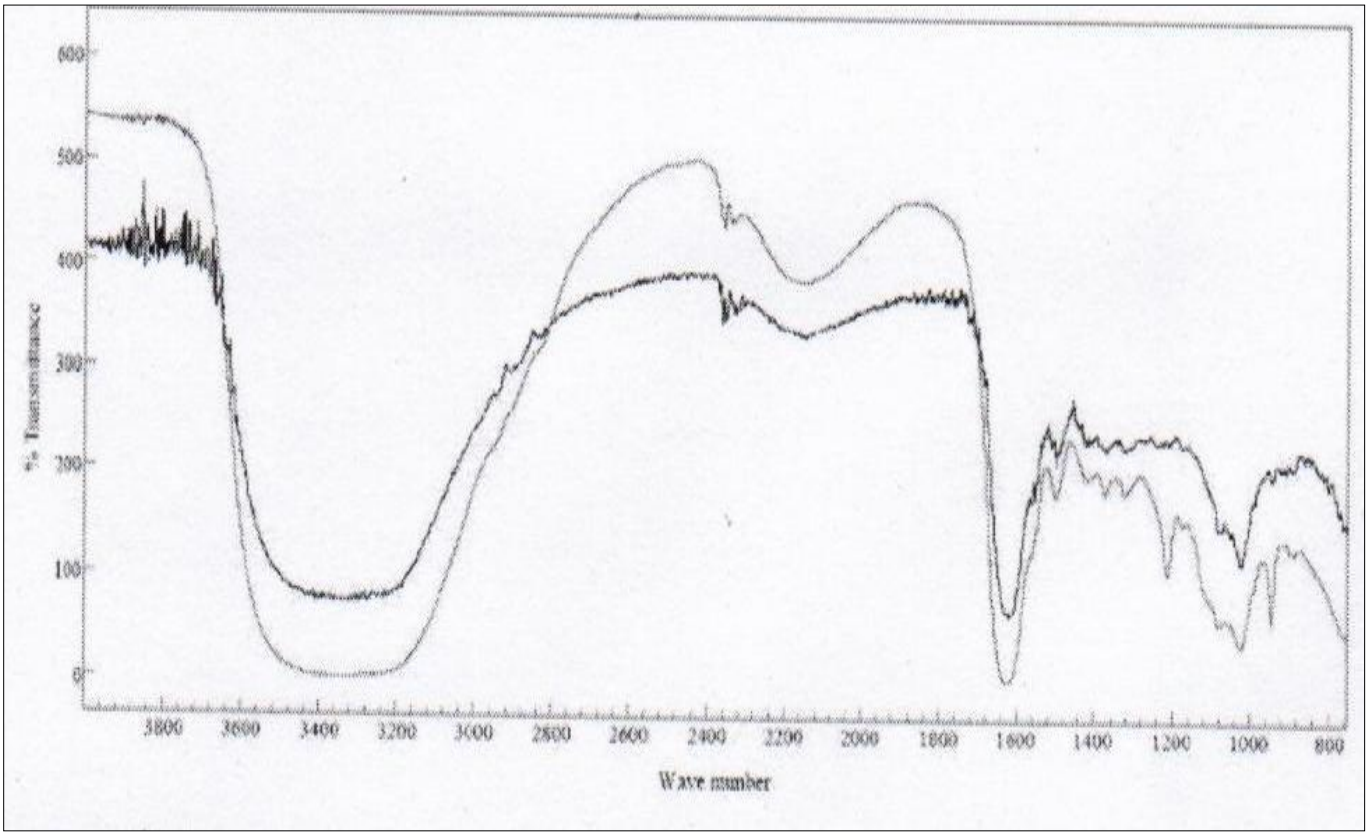

Fig 4: Standard Glucosamine Hydrochloride

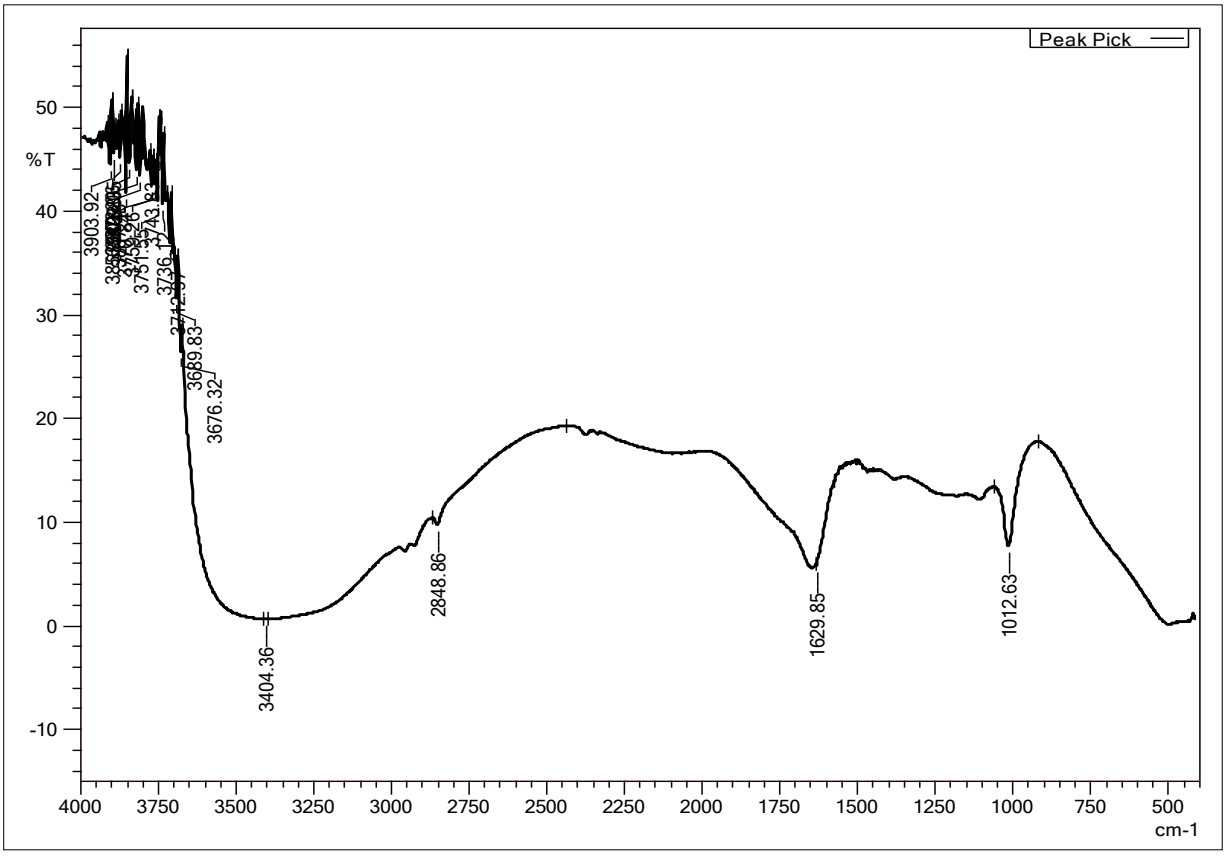

Fig 5: Glucosamine Hydrochloride from stomatopod *Harpiosquilla gonyptes*

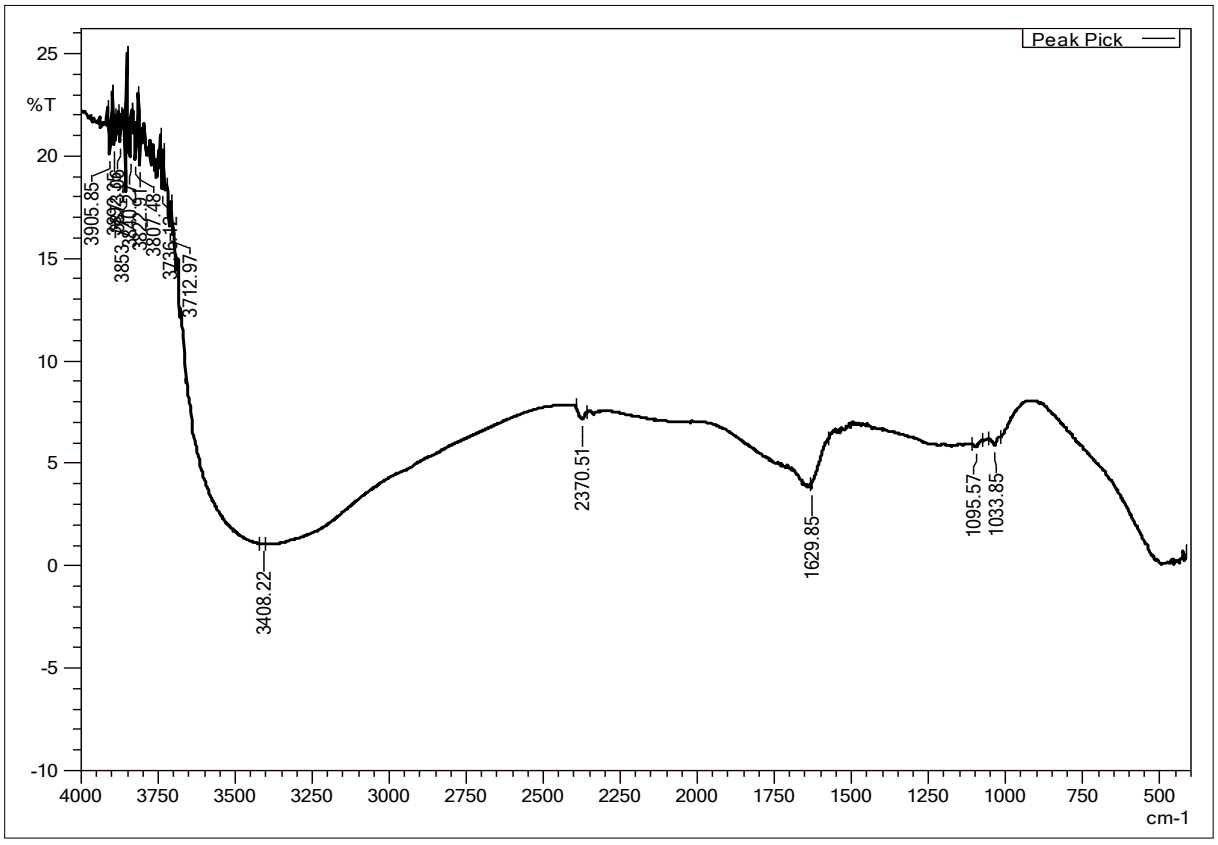

Fig 6: Glucosamine Hydrochloride of *Panapenaeopsis maxillipedo*

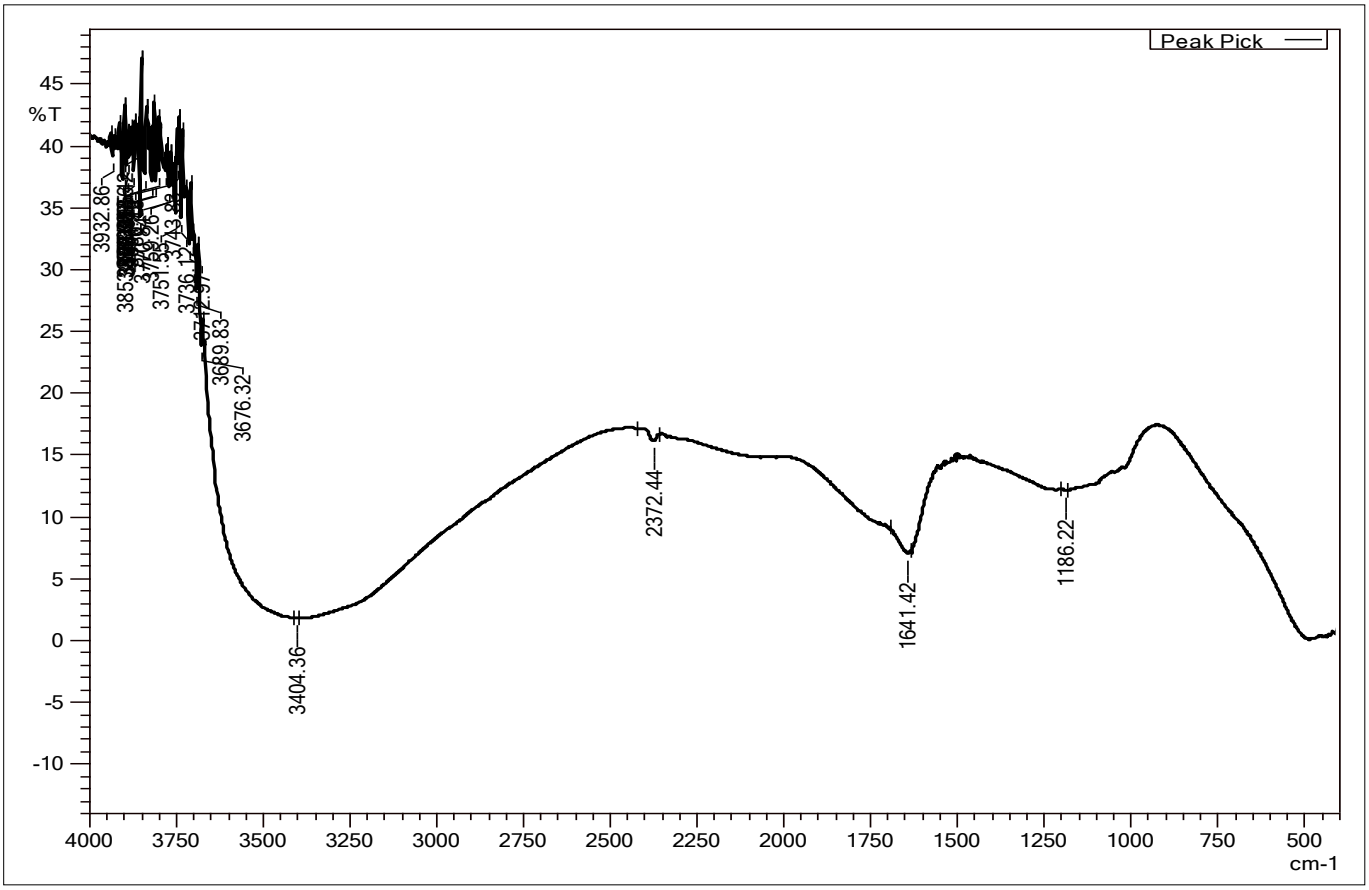

Fig 7: Glucosamine Hydrochloride of *Penaeus indicus*

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