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RESEARCH ARTICLE

DEVELOPMENT AND PHYSICOCHEMICAL CHARACTERIZATION OF GLUCOSAMINE AND CHONDROITIN GRANULES

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ABSTRACT

Osteoarthritis and arthrosis are commonly and severe diseasesin modern society. The life expectancy has increased and, in consequence, the morbility of these pathologies. Glucosamine chlorhydrate and chondroitin sulfate have become alternatives for the treatment of the mentioned diseases. They have few interactions with other drugs and encourage endogenous processes of pain relieve. The objective of the present study was to develop a pharmaceutical product formulate as a granulated of glucosamine/ chondroitin 1500 mg//1200 mg. The product indication is the osteoarthritis treatment. The development processes included the next steps: pre-formulation, analytical method development, and quality control specifications. Three pilot batches were developed and its potencies, pH value, granulometry, appearance, dosage weight and residual moisture were evaluated.
It was found that the analyzed products were according with USP 38 specifications. The labeled amount was between 90% -110%, the residual moisture had a value inferior to 2,50 ± 1,3 %. The particle average size was 50mm with a normal distribution of granules sizes, which demonstrated that manufacturing process was reproducible and according with established quality standards.

INTRODUCTION

Osteoarthritis or arthrosis is a common disease and is the most important cause of disability among elderly patients. The prevalence of asymptomatic arthrosis in Spanish population is around 43 %, having a higher percentage in women (52 %) than men (29 %). (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

In Spanish population the prevalence of knee asymptomatic arthrosis is closed to 10 % (6 % in men and 14 % in women) and hand asymptomatic arthrosis is approximately 6% (2% in men and 10% in women). However, these numbers may vary significantly according to diagnostic criteria (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011). Knee arthrosis is the biggest cause of pain and dysfunction in patients older than 65 years, having an important socioeconomical impact.

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About 10% of consults in primary attention are related with knee arthrosis. The disabilities attributable to knee osteoarthritis are equivalent to that caused by heart diseases and higher than any other elderly-related pathologies (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).Until recently, treatment for arthrosis was based on administration of NSAID, which quickly improve the painful symptoms. However, NSAID are not capable of modify the disease's evolution, therefore, after suppression of treatment the symptoms reappear. Additionally, these treatments have relevant interactions with other drugs and are related with important adverse effects, such gastrointestinal, cardiovascular, hepatic and renal problems (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

It has been demonstrated that some compounds can produce beneficial effects on articular cartilage. The SYSSADOA have an efficacy similar to NSAID, with the difference that its effect takes longer to bereach and persists after the treatment suppression. (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

This pharmaceutical group includes drugs such as intraarticular hyaluronic acid, chondroitin sulfate and glucosamine sulfate orally. All of them are part of the cartilage matrix, and have a better security than NSAID as an advantage. (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

Additionally, several recent studies have indicated that these compounds can delay or stop the course of the arthrosis disease. Because of these reason, they have been nominated generically as condroprotective agents or structure disease modifying osteoarthritis drugs (SDMOAD) (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

Physicochemical properties of chondroitinsulfate and glucosamine hydrochloride Chondroitin sulfate is a glycosaminoglycan. These are important constituents of the extracellular matrix of the cartilage. Glycosaminoglycans are organized in packs of high molecular weight, known as proteoglycans. (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011)

The proteoglycans provide to cartilage its mechanical and elastical properties. They have an important capacity to retain water allowing the articular cartilage to stretch when a mechanical force is applied, which providea large capacity to withstand significant loads (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

Glucosamine sulfate is an active compound present in the human organism, synthesized from chitin and extracted from shells and/or crustaceans' shells. It is a natural aminomonosacarid that is substrate for the biosynthesis of the proteoglycans. (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

The chondroitin sodium sulfate is a natural copolymer conformed mainly by two disacarids obtained from cartilage of terrestrial and marine species. 4-sulfate and 6-sulfate proportion depends on the animal species (Sweetman, 2009).

This compound is an hygroscopic white powder, totally water-soluble and practically insoluble in alcohol and acetone. It is extremely hygroscopic once dried and must be stored in hermetically sealed containers protected from light. A 5% aqueous solution has a pH valueof 5.5 to 7.5 (Sweetman, 2009).

The sodium salt obtained from lineal sulfated glycoaminoglycans of bovine, porcine and aviary origin is usedby humans for feeding. It consists in the sodium salt of the esther sulfateN- acetylchondrosamine and the copolymer of D-glucuronic acid. These hexoses are linked by β-1,4 y β-1,3 bounds to the polymer. On the other hand, the chondrosamine molecules are sulfated, in position 6 and mainly in position 4 (Sweetman, 2009).

The glucosamine is found inmucoproteins and mucopolysacarids. It is implied in the synthesis of glicosaminoglycans, which in turn are important components of cartilage, tendons and ligaments. (Sweetman, 2009).

The glucosamine must be synthesized by the body, but the synthesis decrease in older people. Thus, glucosamine and its salts have been recommended as treatment for rheumatic diseases, including osteoarthritis. (Sweetman, 2009).

Mechanism of Action

At least four mechanism can contribute to delay the progression of arthrosis: the synthesis inhibition of inflammatory agents (prostagladines and NO) mediated by interleuqina-1, the synthesis inhibition of the catabolic enzymes, like metaloproteases of the matrix, the synthesis stimulation of hyaluronate, proteoglycans and components that conform the extracellular matrix, and the apoptosis reduction of articular condrocytes. (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

The therapeutic efficacy of chondroitin sulfate can be due to the anti-inflammatory activity and the synthesis stimulation of proteoglycans, as well as the cut back in catabolic activity ofchondrocytes. The chondroitin sulfate also act by the inhibition of proteolytic enzymes (such as metaloproteases, colegnases and elastases) andpro-inflammatory agents (TNF-α, IL-1α, COX-2, PGE2, NFκB) (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

It has been observed that chondroitin sulfate acts in different articular compartments, as the cartilage, the subcondral bone and the synovial membrane. It reduce the osseous imbalance produced by arthrosis and decrease the swell and articular spillage characteristic of the inflammatory process related with the disease (Abad Santos et al, 2011).

Also, the chondroitin sulfate reduce the synthesis of nitrous oxide (NO) in joints. It is a mediator implied in the cartilage degradation (Abad Santos, Ochoa Mazarro, & Garcia Garcia, 2011).

The glucosamine is a substrate in proteoglycans biosynthesis. It has been demonstrated the relationship between this substrate and the synthesis of proteoglycans in articular cartilages (Abad Santos et al, 2011).

Additionally, it possesses an anti-inflammatory activity independent from COX. Therefore, it is well tolerate in gastrointestinal and general terms. Glucosamine also inhibit the cartilage-destructing enzymes, such as collagenase, aggrecanase, phospholipase A2 and lysosomal enzymes. On the other hand, it reduces the formation of compounds that cause tissue damage, like the radicals produced by macrophages (Abad Santos et al, 2011).

The effects of glucosamine over NO synthesis are not completely clear. Some scientists suggests that glucosamine diminish the synthesis of NO, a biochemical mediator that increase cartilage degradation, while others proposes it do not have effect at all (Abad Santos, *et al* 2011).

Development of a Pharmaceutical Granulate

The development of pharmaceutical products can be divided in two stages: design and production. The design period finalize with the production of a defined batch that is according with the quality specifications. In this stage, it is necessary to consider all technical and scientific knowledge to produce the best medication. On the other hand, the production stage consists in reproduce, at industrial scale and according with quality specifications, the product designed initially.

There are many factors that determine the quality product. Based in these factors the development process of a product can be established. Additionally, the pharmaceutical form, excipients, conditioning material, fabrication method, controls in processes and the storage conditions, are also involved in product quality. Moreover, the knowledge of the active ingredients properties is essential (Le Hir, 1995).

Before de formulation process, a preliminary investigation and characterization of active ingredients is necessary. Information

about its therapeutic activity, physicochemical properties, and pharmacokinetic and biopharmaceutic aspects must be known (Le Hir, 1995; Velazquez, 2010; Brunton, 2012).

The aqueous solubility of active ingredients is an important physical property related to the selection of the administration route and, in consequence, with its bioavailability, a parameter that defined the quantity and rate of drug absortion. It is necessary to consider that solubility of some active ingredients is pH-dependent, and it is related with its distribution between two phases (Le Hir, 1995; Velazquez, 2010; Brunton, 2012).

In pharmaceutical industry, the pre-formulation phase takes place in the investigation and galenic development areas. This work is donein collaboration of quality control and stability laboratories.

It is fundamental to characterize the properties of the active ingredients because they determine the formulation process. Certain important variables must be taken in consideration during manufacturing process of pharmaceutical products, some of them are mentioned below:

Active Ingredient

The active ingredient can be presented in salts, hydrates and different crystallized forms. To choose one form or the other it is necessary to consider the administration route, the stability and bioavailability of the drug (Le Hir, 1995; Velazquez, 2010; Brunton, 2012).

Routeof Administration

Bioavailability of the active ingredient defined the administration route. It is also defined by the treatment duration, the frequency and the patient characteristics (Le Hir, 1995; Velazquez, 2010;Brunton, 2012).

Pharmaceutical Form

The pharmaceutical from is relate with the administration route. Usually, oral route is the first option (Le Hir, 1995).

Auxiliary Substances or excipients

Excipients must be chemically inert or innocuous to avoid reactions with the active ingredients (Le Hir, 1995). The auxiliary substance spurpose is to allow an adequate presentation of the product. Usually, it is necessary to use a mixture of excipients, from natural or synthetic origin, to reach a specific presentation (Le Hir, 1995; Velazquez, 2010; Brunton, 2012).

The principal excipients functions are listed below:

- Facilitate the administration of active ingredients.
- Improve the efficacy of the active ingredient.
- Guarantee the stability and conservation of the medication until its expiry date (Le Hir, 1995).

Pharmaceutical forms: powders and granules

The powders are solid preparations that consist in a mix between the active ingredients and excipients. The function of excipients is dependent of the previewed use for the product. The granules are solid preparations that consist in dry aggregates of powder particles (Aulton, 2004). The products that are commercialized as powders and granules are often dispensed as: powders for internal use, unidosepowders or granulates or fine powders for external uses (Aulton, 2004).

Advantages and disadvantages of powders and granules:
Advantages:

1. Solid preparations are more chemically stable than liquid ones.
2. Powders and granules are useful to dispense drugs in high doses.
3. Powders and granules for oral administration have a faster dissolution rate than tablets or capsules, as these must disintegrate before the drug dissolves (Aulton, 2004).

Disadvantages

1. Powders and granules where doses are not pre-divided into individual quantities are less convenient for every-day transport. Some packaging methods for divided preparation, such as heat-sealable laminated sachets, are convenient to carry individual doses.
2. The masking for unpleasant flavors in these preparations could be difficult. Formulating an effervescent product is the most common alternative for taste-masking.
3. Powders and granules in high quantities are not suitable for the administration of potent drugs with low doses.
4. Powders and granules are not an election method for the administration of drugs which are inactivated in, or cause damage to, the stomach. These drugs must be administered with enteric coated (Aulton, 2004).

Granulation

Granulation consists in a process in which primary powder particles are stocked up and form bigger structures with other particles, called granules. Generally, the granules sizes are between 0,2 and 4 mm (Aulton, 2004).

Reasons to granulate

The main reason consists in preventing the segregation of components that conformed the mix of powders. (Aulton, 2004).

The segregation can be attributed to differences in density or size between the powder mixture. The smaller particles tend to migrate to the base and the bigger ones are above them (Aulton, 2004).

Granules size distribution must also be controlled. This is because granules also could segregate in function with their sizes (Aulton, 2004).

Another reason to granulate is to improve the flow properties of the mixtures. Many powders with a small size are cohesive and have an irregular shape and surface, in consequence, they have a poor flow. Granules with a homogenous diameter and a regular surface have better flow properties (Aulton, 2004).

Finally granulation also improves the compaction characteristics of mixtures. The solute migration, produced during the drying stage because of pulverization, create a rich layer of agglutinant over the granules, improving the compaction properties (Aulton, 2004).

Additionally, the granulation of toxic materials reduces the risk of generating toxic powders. Also, granules have a higher density than powders, which facilitates the transportation and storage of these materials (Aulton, 2004).

Granulation Methods

Granulation methods can be divided in two types: wet methods and dry methods. In both of them is necessary to use a number of different excipients to obtain a suitable formulation, diluents are used to reach an appropriate weight and size, disintegrating agents are added to aid fragmentation of granules and the binders facilitate de granules formation (Aulton, 2004).

The wet granulation method will be employed for the manufacturing of the glucosamine and chondroitin product. This method involve the massing of a mixture of dry powders using a granulating fluid. It contains a volatile and non-toxic solvent that must be removed by drying techniques (Aulton, 2004).

The most common liquids used are water, ethanol and isopropanol either alone or in combination. The granulation liquid could be used alone, or as a solvent that contain a binder agent to ensure the particle adhesion once the granule is dry (Aulton, 2004).

The most ecological and economical option is water, but it can affect the drug stability causing hydrolysis in sensitive products. It also needs more drying time than organic solvents, extending the exposure to heat and possibly affecting its stability (Aulton, 2004).

In the wet granulation method, it is necessary that wet mass pass through a sieve to produce granules. Then, these granules are sieving again to break up the agglomerated granules and separate the fine material (Aulton, 2004).

Granulation Mechanisms

In wet granulation methods, the granulation fluid must be distributed in the powders mix by mechanical agitation. The particles are adhered to each other by films of liquid and due to the agitation they adhere to even more particles (Aulton, 2004).

There are three main mechanism of granule formation involved in wet granulation process:

Nucleation

Nucleation is produced by contact and adhesion between particles due to the liquid bridges. In this process the particles are linked to form a pendular state.

Agitation increase the density of pendular bodies to form the capillar state. These bodies act as growth nucleus to enhance the formation of granules. (Aulton, 2004).

Transition

This stage is characterized by the presence of a large number of small size granules with a large size distribution.

The nucleus can grow in two ways: isolated particles can link to the nucleus by pendular bridge formation, or well two or more nucleus can link to form a bigger structure (Aulton, 2004).

Granulate Growth

If the granule continues growing, it is possible to obtain structures more spherical and larger and the average size of the particles in the granulation system will increase over time. On the other hand, if the agitation continued, an over-massed system will be produced and it will not be used. This over-massed system is dependent on the amount of liquid added and the properties of the materials forming the granules (Aulton, 2004).

The four possible mechanisms involved in granule growth are mentioned below:

- Coalescence: it occurs when two or more granules are joined together to form a larger one.
- Breakage: in this mechanism the granules are broken in fragments which adhere to more granules.
- Abrasion transfer: it happened when agitation produce the attrition the granules. This eroded material adheres to other granules, increasing their size.
- Layering: it is when a secondary powder mixture is added to the granules. In this case, the secondary powders are adhered to granules and form a layer over their surface, increasing their size (Aulton, 2004).

MATERIALS AND METHODS

The product was developed by wet granulation method. Dry granulation was not a choice because of the large percentage of active ingredients in the formulation. It becomes difficult to obtain a granule by dry mix due to the difference in densities between the particles that conform the powder mix, which is related with difficulties in the adherence of the particles.

When using the wet granulation method, the granulating fluid enhances de particles adhesion. Absolute ethanol was used as humectant agent, and as the solvent of polivinilpirrolidone (PVP), the binder agent. This agent ensures the adhesion between powder particles once the granulate is dried.

Table 1 Laboratory equipment used for the fabrication of products

Equipment	Brand	Model
Granulator	KitchenAid	KSMC50SPL
Scale	Mettler Toledo	PB3002
Platter Oven	Fisher Scientific	630F
V Mixer	Llealprocess	151301
Manual Sealant	TEW Electric HeatingEquipment	THS206

The componentsused in the formulation of the productare indicatedin the following table:

Table 2 Materials used in the product fabrication.

Material	Use or Function	Recommended Percentage (%)
1. Glucosaminechlorhydrate	Active Principle	40
2. CondroitinSufate	Active Principle	30
3. Polivinilpirrolidone (PVP) K12	BinderAgent	0,5-5
4. Coloidal SiliciumDioxide	DehumydifyingAgent	0,1-1
5. Coloidal SiliciumDioxide	LubricantAgent	c.s
6. Tangerine-Lime Flavor	FlavouringAgent	c.s
7. Sucralose	Edulcorant	c.s
8. Yellow color dye	Colorant	c.s
9. AbsoluteEthanol	Solvent	Csp 100

Manufacturing Process

The following process was designed using the wet granulation method:

1. The PVP K12was dissolved in absolute ethanol.
2. The colloidal silicium dioxide was added slowly to the mixture in step1. It was agitated until complete homogenization. This mixture corresponds to the granulating solution.

3. The glucosamine chlorhydrate was mixed with the chondroitin sulfate for 3 minutes. The granulating solution mentioned in step 2 was added to this mixture.
4. Then, the granulate was dried in the platter oven. Cold airwas used to eliminate the alcohol smell. Then, the temperature was increased to 40-45 ºC to evaporate the ethanoland reached a2- 2, 5%moisture content.
5. The colloidal siliciumdioxide, tangerine-lime flavor, sucralose and yellow color dye was mixed in a different recipient.
6. The mixture was sieved through a 25 sieve.
7. Excipients from step 5 and 6 were mixed together for 10 minutes.
8. Finally, the product was subdivided in envelopes with an average weight of 3,37 g ±5%.

Once the qualitative and quantitative formula of the product and its respective manufacturing method was established; the test batches was produced. In this phase, was necessary the control of physical and chemical parameters with the objective of standardizing the fabrication process.

The objective was to determine if the formula and its manufacturing method allowed the fabrication of a product according with the critical parameters to be evaluated.

The first test batch was 300g of product (Test Batch N.1). It corresponded to 100 packets of the product, with a unitary average weight of 3000 mg ±5%. The following control parameters were evaluated:

- Appearance
- Flavor
- Envelope weight
- Residual moisture
- pH in solution
- Glucosamine and chondroitinassay

The values obtained in the aforementioned parameters were adopted as a reference to establish the control parameters in the product fabrication process.

Consequently another test batch was produced (Test Batch N.2) without any variation to the quantitative and qualitative formula. The only difference with Test Batch N.1 was the batch size. In this case, 600g was produced which corresponds to 200 product packets. The objective was to determine if larger batches was according with the control parameters.

A new test batch was prepared (Test Batch N.3). The batch size was 682,85g, corresponding to 200 product packets. The average unitary envelope weight was 3370mg±5% and presented a weight variation respect Test Batch N.1 and N.2.

The weight variation was attributed to the modification in the qualitative and quantitative formula. Initially, the 8 % moisture indicated in the certification analysis of chondroitin sulfate was not considered. Also, initially the calculations were done considering the equivalent of glucosamine as sulfate salt and not as a chlorhydrate salt. In consequence, was necessary to modify the excipients proportion, always maintaining the relationship between active ingredients and excipients established in the original batched.

Physical and Chemical characterization for the final product

- Shape and appearance: shape and appearance were evaluated by simple observation and were established as parameters for the granulate aspect evaluation.
- Granulometry: the sieves (10, 20, 30, 40, 50, 60, 80 mm) were selected and weighted. They were placed in a vibration apparatus with a lowering gradient according to the sieve opening, including a collection flask. The powder mixture was placed in the uppermost sieve and subjected to vibration during 10 minutes. Passing this time, the sieves were weighed again and the fraction of powder retained in each sieve was determined.

The equipment used was a sieve vibrator Sieve Shaker Model RX-86.

- Envelope weight: one packet content was weighed in an analytic scale and toke as reference for each batch of the developed product.
- pH in dissolution: the content of a product pack was dissolved by mechanical agitation in a beaker containing 250 mL of water. The dissolution pH was measured using a pH-meter.
- Residual moisture: an amount bigger than 500 mg of granulate was placed in a moisture scale during 15 minutes at 70ºC, the equipment report the moisture content. The dried granulated was weighed to determine the final weight.

Quantification of the active ingredients in the product

Chromatographic Conditions
Mobile Phase: Acetonitrile (ACN)/ Distilled water (H20)/ Phosphoric Acid (H_3PO_4) (10/90/0,1), Fluid Velocity: 1,0 mL/min, Injection Volume: 10 uL, Column: C18, Detector: UV, 195nm.

Standard purity

Chlorhydrate Glucosamine 98,4%
Chondroitin Sulfate 96,7%
Assay

An internal analytical method was developed to analyze the product by high pressure liquid chromatography (HPLC). This method allows the quantification of both active ingredients in the same assay. The procedure is explained in the following steps:

Mobile phase preparation

Acetonitrile 10%, PhosphoricAcid 0,08%, octanesulfonic acid sodium salt 0,12%, water 90%.
Process

1. In 1L beaker, 0,8mL of $H_3PO_4$and 1,2g of octanesulfonic acid was added and dissolved in 900 mL of water.
2. 100mL of acetonitrile was added to the mixture of step 1.
3. The mobile phase was filtered through a 0,45um filter.

Sample preparation

Process

1. 3 pulverized samples from the same batch are used in the product analysis.

2. The equivalent of 40,00 mg of glucosamine, according to the average of three packets of the product, were put in a 100,00 mL volumetric flask to obtained 440 ppm glucosamine concentration and 350 ppm chondroitin concentration.
3. Water was added until 80mL and the mixture was sonicated during 20 minutes.
4. The volumetric flask was cooled. Then, it was filled with distilled water.
5. The sample was filtered through a 0,45um filter and placed in a HPLC vial.

Standard preparation

Reference standard

The reference standards were the raw materials used as the active ingredients in the product manufacturing.

Process

The reference standards were prepared by triplicate using three different concentrations, which corresponds to 80%, 100% and 120%. The preparation is described below:

Glucosamine Standard (440 ppm)

1. 100,00 mg of the standard were weighed, and placed in a volumetric flask of 50,00 mL.
2. The volumetric flask was filled with 25,00mL of distilled water and was sonicated during 20 minutes.
3. The volumetric flask was left cool and filled with distilled water.

Chondroitin standard (350 ppm)

1. 87,50 mg of the standard were weighed and placed ina volumetric flask of 50,00 mL.
2. 2,50 mL of acetonitrile was added to the volumetric flask. It was gently agitated.
3. The volumetric flask was filled with 25,00mL of distilled water and was sonicated during 20 minutes.
4. The volumetric flask was left cool and filled with distilled water.

Standard mix

1. 10,00mL of the chondroitin and glucosamine standards solutions were placed in a volumetric flask of 50,00 mL.
2. The volumetric flask was filled with distilled water.
3. The mixture were filtered through a 0,45um filter and placed in a HPLC vial.
4. Measurements were made with the available HPLC equipment.

Sample readings and HPLC measurements

1. Three standards were injected (80%, 100%, 120%).
2. Each sample was injected only once.
3. Each standard was injected again by triplicate (80%, 100%, 120%).

The sample and standard injection were performed under the chromatographic conditions stipulated above.

Table 3 Equipment used

Equipement	Brand	Model	InternalCode
High-pressureliquidchromatograph	Agilent	1200	06CL06
Analyticalscale	OHAUS	Discovery	01BA13
Analyticalscale	OHAUS	Explorer Pro	08BA23
Ultrasonicshaker	Branson	3510	06US02

RESULTS

Each of the manufactured batches were evaluated under a series of quality parameters; the results shown in the following data:

Table 4 Quality parameters evaluation for Test Batch N.1

Trial	Test	Specification	Result
Test Batch Nº1	Appearance	Homogeneousgranulated powder	Homogeneousgranulatedpowder
	Flavor	Pleasant tangerine-lime taste	Pleasant tangerine-lime taste
	Envelopeweight	3 g ± 5%	3,003g
	Solution pH	6-May	5,56
	Residual moisture	2-3%	2,5%
	Glucosaminecontent	90-110%	101,5%
	Chondroitincontent	90-110%	95,5%

Table 5 Quality parameters evaluation for Test Batch N.2

Trial	Test	Specification	Result
Test Batch N.2	Appearance	Homogeneousgranulated powder	Appropriate
	Flavor	Pleasant tangerine-lime taste	Appropriate
	Envelopeweight	3 g ± 5%	3,005g
	Solution pH	5-6	5,56
	Residual Moisture	2-3%	2,65%
	Glucosamincontent	90-110%	102,6%
	Chondroitincontent	90-110%	100,5%

Table 6 Quality parameters evaluation for Test Batch N.3

Ensayo	Prueba	Especificación	Resultado
Test Batch N.3	Appearance	Homogeneous granulated powder	Appropriate, granulates in homogeneous powder
	Flavor	Pleasant tangerine-lime taste	Appropriate, pleasanttangerine-lime
	Envelope weight	3 g ± 5%	3,005g
	Solution pH	6-May	5,28
	Residual Moisture	2-3%	2,34%
	Glucosamine percentage	90-110%	104,6%
	Chondroitinpercentage	90-110%	104,6%

Granulometry

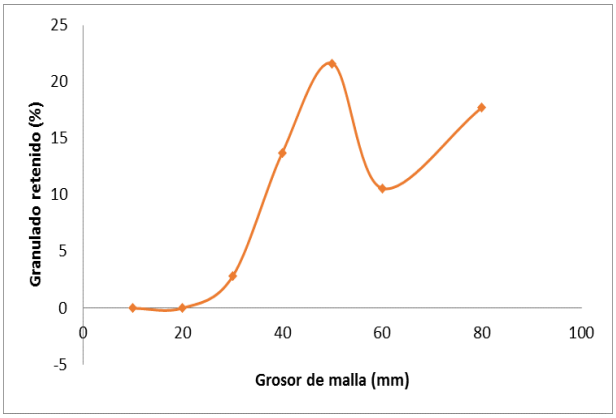

Figure 1 Particle size distribution in the final product.

Active ingredients quantification by HPLC

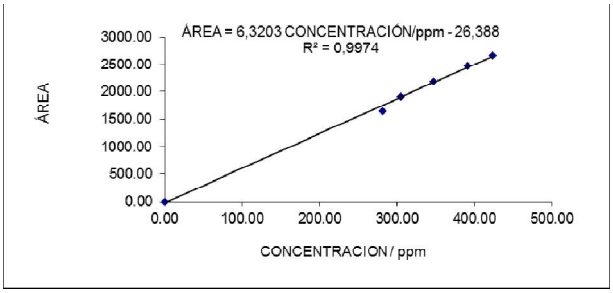

Figure 2 Calibration curve obtained to chondroitin quantification in final product.

Table 7 Areas and concentration values of chondroitin standard

Concentration Interval (%)	Concentration (ppm)	Areas
0	0,00	0,00
80	281,44	1652,40
90	305,44	1910,40
100	347,04	2186,30
110	391,20	2471,10
120	423,36	2672,40

Table 8 Calibration curve statistics for chondroitin quantification

Estadísticas de la regresión		
Multiplecorrelationcoefficient	0,9986	
Coefficient of determinationR^2	0,9973	
Adjusted R^2	0,9966	
Typical Error	55,367	
Observations	6	
	Coefficients	*Typical Error*
Interception	-26,388	52,54
Pending	6,320	0,16

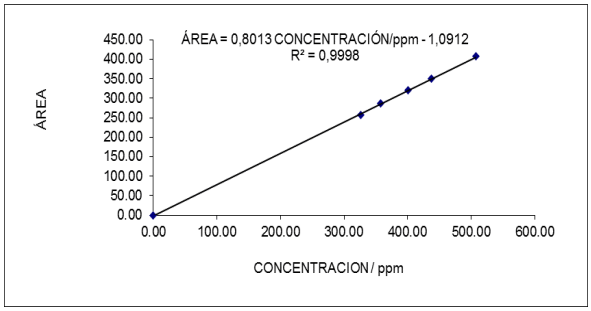

Figure 3 Calibration curve obtained to glucosamine quantification in final product

Table 9 Areas and concentration values of glucosamine standard

Concentration Interval (%)	Conccentration (ppm)	Area readings
0	0,00	0,00
80	326,24	256,70
90	357,44	286,10
100	400,48	319,50
110	436,96	349,40
120	507,20	407,10

DISCUSSION

The product formulated with glucosamine and chondroitin as granulate have a quantity equivalent to 1,500mg of glucosamine and 1,200mg of chondroitin.

Table 10 Calibration curve statistics for glucosamine quantification

Regressionstatistics		
Multiplecorrelationcoefficient	0,9999	
Determination Coefficient R^2	0,9998	
Adjusted R^2	0,9997	
Typical Error	2,1385	
Observations	6	
	Coeficcients	*Typical Error*
Interception	-1,091	2,022
Pending	0,801	0,005

According Table 4, Table 5 and Table 6the product is a clear yellow powder with a pleasant tangerine-lime sweet taste. The final product average weight is 3 ± 0,2grams and its pH in dissolution is 5,48 ± 0,20, considering the average of the three batches.

The labeled amount in the final product is 100.2 ±3.72 of chondroitin and 102,9 ± 1,28 of glucosamine. Both of them are according with the specifications established in the Pharmacopeia of United States No 38 (Pharmacopeia United States of America Convention, 2016). These specifications establish a labeled amount between 90 – 110 %.

The particle size distribution in the formulation was quasi-normal, with an average of 50 mm. It was notice a trend of particle size inclination to 60 mm, but always keeping the normal size and range of distribution. From these results is important to mention that there is not bimodal distribution (normal distribution), which would implies a failure and a problem in the granulation method. The granulometric distribution obtained in the three batches demonstrates that the wet granulation method is homogeneous and reproducible.

About the chromatographic analysis, it is necessary to consider that the product have two active ingredients with similar physicochemical characteristics. Thus, is important to demonstrate the analytical method linearity and specificity, since it must guarantee a proper quantification of active ingredients, which is necessary to determine the products potency and realized stability studies.

Given the highly hygroscopic characteristics of the active ingredients, especially chondroitin, the residual moisture inthe granulate is a critical parameter. The residual moisture in the formulation was 2,50% ± 1,3, which is according with the literature specifications. This parameter is critical in the manufacturing process and in product stability due to the possibility of chondroitin hydrolysis.

The linearity of 5 levels of concentration is shown in Table 3 and Table 4. There is a good separation between the chromatographic peaks. The glucosamine retention time was at 1,5 minutes, the chondroitin retention time was at 2 minutes and the excipients started to appear at 3,6 minutes. These retention times indicates a good system specificity. About the linearity of the analytical method, it shows a determination coefficient of 0,9974 for chondroitin, and a value of 0,9998 for glucosamine.

The results in the linearity analysis are according with the acceptance criteria, which established that linearity is validate in the assay interval. Also, working with a concentration between 80-120 %, the system maintain the linearity and guarantee true data.

All of the analyzed batches showcase a proper formulation and manufacturing process, because of the reproducibility of physical and chemical parameters between different batches and the similar labeling percentage determined.

CONCLUSIONS

A relevant conclusion is the fact that wet granulation method for glucosamine chlorhydrate and chondroitin sulfate (dosed as 1500mg of glucosamine and 1200mg of chondroitin) is homogeneous in terms of particle size, residual moisture, labeled amount, unitary dosage weight, appearance, flavor and smell.

The granulate potency for the three batches was according with the USP 38 specifications, having a value between 90% and 110% of the labeled amount. The analytical system linearity in concentrations between 80% and 120% was acceptable; the correlation coefficients were superior to 0,995, which is the established parameter for the lineal correlation between two variables. Also, the chromatographic method is specific to the determination of both active ingredients in the same granulate.

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