

Analysis of Chondroitinsulphate

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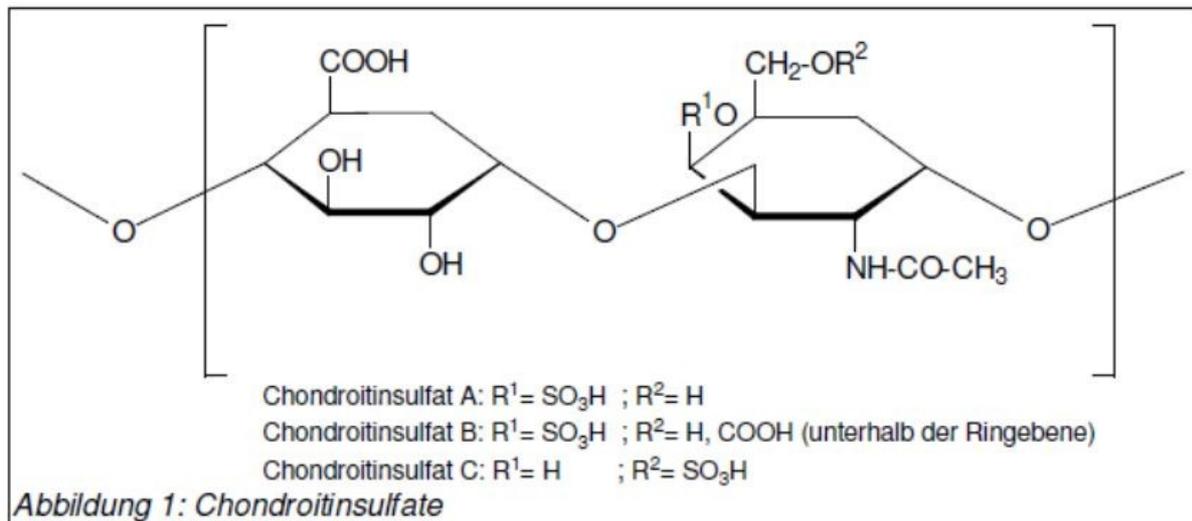
Introduction

This paper is not only a consumption of established methods for analysing chondroitinsulphate but also differentiates respectable methods in opposition to many inoperative methods.

Glycosaminoglucanes or „GAG“s (previously known as mucopolysaccharides) are negative charged polysaccharides (glucanes), consisting of 1,4-linked units of disaccharides. One mole of a uronic acid (D- glucuronic acid respectively L-iduronic acid) is linked in a setting of position 3 with one mole of a nitrogen-acetylated-amino sugar (NAG, glycosamine). In case of chondroitinsulphate regularly sulphuric acid is linked to a oxygen- or nitrogen molecule which results a broad acidic characteristic.

The chondroitinsulphates A and C are N-acetyl-D-galactosamine-4 (or -6)-sulphates..

Chondroitin B is a similarly build molecule, which is established as „dermatansulphate“ or better known as „β- heparin“ originated from L-iduronsäure und NAG-4-sulphate. [1]



Chondroitinsulphates are animal proteoglycans, principal constituents of cartilage, bones and connective tissues, but in mucus apparent too. These molecules are polymers disclosing a mole weight of 25-50 k Dalton, responding FALBE et al. [1995] more than 1000 k Dalton [1].

Traditionally the technical preparation is made from shark-cartilage, as well as from cattle- and porcine-trachea. Nowadays the chondroitinsulphate is produced only from porcine-trachea because of bovine spongiform encephalopathy (BSE) and increasingly from chickensternum.

The medicinal use of chondroitinsulphate is skin protection caused of burning, furthermore for prevention of fading connective tissues. Here it is applied orally as saggar. Founded on this

application it is requested by the pharmaceutical industry to determine the assay of chondroitinsulphate.

Whereas chondroitinsulphate is not mentioned in westwards pharmacopeias as an official monograph, then Japanese „Japonica“ (JSPI 1991) alludes „Sodium Chondroitin Sulphate“ (No. 04805) with a postulation of an „Assay“ of 2,5 -3,8% nitrogen and 5,5 - 7,0 % sulfur (siccum).

Since few years sodium chondroitin sulphate is mentioned as well in European Pharmacopeia EP V.05 [2064] as in the US Pharmacopeia (USP XXVIII [CAS 9082-07-9]) In the EP an assay of 95-105% siccum is assumed, the USP demands 90 – 105% siccum.

At this point it must be noted that no CRS (chemical reference substance) was available before these monographs came into effect. Nowadays CRS singly accredited exclusively for the mentioning monograph. Because of this fact no „real CRS“ is available.

We performed more than 3200 analysis of chondroitin with the ability to archive high-quality samples with high assay, high purity and special consistence. We stored the samples, covered by inert-gas, at a temperature of 4°C.

Especially for this analysis two chondroitinsulphates obtained from shark were preserved. Because of the fact, that no assay more than 100% is possible these two samples were archived as samples with a theoretical assay of 103 – 106% siccum but declared as „100% reference“. The assay was determined based on the „sulfuric-methode“.

Concerning the matter of facts the assay should be determined on the basis of parts of the molecule, (e.g. Na / N / S / SO₄ / glucuronic acid / NAG) or quantifying the whole molecule. Supposable the measurement of physical characteristics should be mentioned (e.g. optical rotation or spectrum-analysis).

For getting any relationship to the elemental formula, the amount and constitution of the fragments must be calculated.

We start with the perspective of a molecular-weight of 505,43 Dalton of chondroitinsodiumsulphate- monomers (siccum) [C₁₄H₁₉O₁₄ NSNa₂], (567,47 chondroitinsodiumsulphatemonomer x 3,5 Mol water) with

6,35% (5,65%) sulphur, 2,77% (2,47%) nitrogen and 9,10% (8,10%) sodium.

This assumption is hypothetical, because chondroitinsulphate is liable with natural variations.

The commercial quality of chondroitinsulphate contains 2 – 10% water, determined by desiccation at 105°C, the theoretical content of moisture is about 11%.

The calculation below is relating to the dried substance. The chart shows the particular components.

Amount of	CS x 3,5 aq	CS siccum	Catalog Merck	Catalog Fluka	Catalog Roth
Sulphur S	5,65 %	6,34 %	5,0 – 6,7 %	ca. 6,5 %	
Nitrogen N	2,47 %	2,77 %	2,3 – 2,9 %	ca. 2,5 %	2,4 – 3,2 %
Sodium Na	8,10 %	9,10 %			
Glucuronic acid	34,21 %	38,41 %		ca. 35 %	
Water	approx. 11,28%	n / a			

Here it has to be pointed out that all below mentioned methods not enclose the complete chondroitinsulphate- molecule. Some determinations of the assay describes the evaluation of component parts, they must be factorized to the whole molecule. Often the separated sulphate, which results from production, is not regarded.

Sometimes chondroitinsulphate is traded with enclosed certifications which are not suitable to the present formulation.

The determinations quoted below are well known and are used frequently in our laboratory.

In recent years chondroitinsulphate is actually traded with certificates based on unreliable methods.

Please note: In recent years we received about 30% of samples in our laboratory, especially from USA and Spain, with no content of sulfur, from manufacturing-hydrolysis of the raw material. This is chondroitinsulphate in no case !

The HPLC-analysis based on hexuronic-acid detection is not typical for the complete molecule, only the amino sugar fragment will be detected.

By definition: Chondroitinsulphate-sodium must have a content of sulfur within 5,0-6,5%!
This has to be manifested by separate analysis.

A. Chemical Determination

1. Analysis of Parts of the Molecule

1.1 Quantification of Sulphur

1.1.1 Quantification of Sulfur as sulphate (method according Ulex)

Acidolytic determination of chondroitin in oxidizing and boiling milieu with a coherent overage of barium.

Gravimetric determination of barium sulphate.

Average expenditure of time, not useful for some formulations, it might be manipulated with added sulphates.

1.1.2 Determination of Sulphur as sulphate using Parr bomb

High expenditure of time, Burn-up in oxygen, titration of sulphate with bariumperchlorat, mentioned in Japonica 1991, might be manipulated with additional sulfur.

1.1.3 Determination of sulfur as sulphate followed by ion exchange

Ion exchange with extreme cation exchanger, after wards titration with Na OH.

This Method is not specific, very susceptible to fake.

1.2 Determination of Nitrogen

1.2.1 Determination of nitrogen according Kjeldahl

Classical disintegration according to Kjeldahl, distillation, titration. Very exact, but easy to manipulate.

1.2.2 Photometric determination of nitrogen according to Folin-Ciocalteus

Chemical reaction of the sample with Folin-reagent, photometric determination at 500nm, more useful at 720nm. This method is frequently used, but not useful for chondroitin.

1.3 Determination of Sodium:

1.3.1 Incineration – AAS

Easy and quick method, but susceptible for manipulation.

1.3.2 Disintegration with high pressure – AAS

More time-consuming than 1.3.1 but equal.

1.4 Determination of glucuronic acid

1.4.1 Determination of hexuronic acid with Carbazol according to T. Bitter + H.M. Muir (1962)

Very complex reaction, with carbazole in presence of borate, photometric determination at 520nm.

A method with high diffusion.

This method is based on the determination of aminosugar; without coverage of the content of sulfur this conclusion is not significant.

1.4.2 item according to M. Kosakai + Z. Yosizawa (1979)

Modified method similar to 1.4.1, very time-consuming, but very exact with less risk of manipulation.

2. Analysis of the entire Molecule

2.1 Photometric determination using Dimethylmethylenblau (DMMB).

Permutation of the chondroitinsulphate in tris-buffered solution with DMMB in presence of iodine acetic acid.

Critical in terms of time, photometric determination at 525nm. This traditional method according to Farndale (1986) is very sensitive; the method according to de Jong (1992) is more complex, very exact but not specific for chondroitinsulphate.

2.2 Determination of the entire molecule using liquid chromatography (LC)

2.2.1 Determination with excluding-chromatography according to D.W. Choi et. al.

Recent method of excluding-chromatography, screening the real weight of the molecule. This method should better be used for clean-up than for quantification. Very time-consuming, expensive with high risk if manipulation.

2.2.2 Determination using ion-pair-liquid-chromatography

Very exact method for determination of chondroitinsulphate, even in compositions, but not even reproducible because of the appliance of the wavelength 195nm.

2.2.3 Determination using liquid-chromatography after enzymatic permutation

More complex in opposite to 2.2.2 and 2.2.3 because using special reagents (Chondroitinase ABC / Collagenase) but very specific. Expensive but very unforgeable.

2.3 Determination of the entire molecule using capillary-electrophoresis

Modern method funded in clinical analysis, validated for the determination of heparin.

Very expensive, needs complex attachment, unpractical validation of the method.

2.4 Determination of the entire molecule using titration

Titration with cetylpyridiniumchloride-solution coupled with turbidimetric detection.

Simple method, but not very clear detection.

(cf.: "Kommentar zum europäischen Arzneibuch" Bd.4 Nr. 6.3 [2064]).

Unspecific. Recommended INA-Method No. 120.002 in EP and USP. [9]

B. Physically determination

1. Optical rotation

Simple, but not common method, useful for onlinecontrolling. This method is not unforgeable.

2. Determination using infrared-spectroscopy

Specific, but practically useful for qualitative identification of chondroitinsulphate and heparin, useless for compositions.

Summary

Finally it can be assumed, that especially the determination of sulphate is the best choice for determination the assay of chondroitinsulphate. For commercial products an assay of 92-97% should be normally found.

For analysis in special cases with demand of high resolution and critical determination the liquid- chromatographic determination is the best choice because this method for determining the assay is based on a typical peak of chondroitin.

It is necessary to determine the exact content of sulfur to become manifest in the molecular composition.

The methods named 2.2.4 and 2.3 are very sensitive, but extreme time-consuming. They fit for forensic issues.

Zusammenfassung

Abschließend kann man zu der Betrachtung kommen, daß von den uns derzeitig bekannten o.g. Methoden die praxisnahe Schnellbestimmung des Chondroitingehaltes über den Schwefelgehalt die Methode der Wahl ist. Es werden bei handelsüblichen Produkten meistens Gehalte zwischen 92 und 97% i.T. ermittelt.

Bei Analysen für spezielle Zwecke mit Anspruch höchster Genauigkeit empfiehlt sich die Methode HPLC unter o.g. Bedingungen, da bei dieser Methode zur Gehaltsberechnung die Flächenbestimmung eines abgesicherten Chondroitinpeaks zugrunde gelegt wird. Es muß

aber in einer gesonderten Untersuchung der Sulfatgehalt überprüft werden. Die oben unter 2.2.4 und 2.3 genannten Methoden sind ebenso sehr genau, preislich und zeitlich jedoch außerordentlich aufwändig. Sie eignen sich besonders für forensische Schiedsanalysen etc.

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