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Validated High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection Method for the Determination of residual Keratan Sulfate and other glucosamine impurities in Sodium Chondroitin Sulfate

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Abbreviations: CS(s), sodium chondroitin sulfate(s); Eur, European; EP, European Pharmacopoeia; GAG(s), glycosaminoglycan(s); Gal, galactose; GalN, Galactosamine; GalNAc, N-acetylgalactosamine; GlcA, glucuronic acid; GlcN, Glucosamine; GlcNAc, N-acetylglucosamine; HPAEC-PAD, high-performance anion exchange chromatography with pulsed amperometric detection; KS, keratan sulfate; Mw, Molecular Weight; NSC, Chondroitin non sulfated; USP, United Stated Pharmacopoeia.

Abstract: An efficient and sensitive analytical method based on high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) was devised for the determination of glucosamine (GlcN) in Sodium Chondroitin Sulfate (CS). Glucosamine (GlcN) is intended as marker of residual Keratan Sulfate (KS) and other impurities generating glucosamine by acidic hydrolyzation. The latter brings CS and KS to their respective monomers. Since GlcN is present only in KS we developed a method that separates GlcN from GalN, the principal hydrolytic product of CS, and then we validated it in order to quantify GlcN. Method validation was performed by spiking CS raw material with known amounts of KS. Detection limits was 0.5% of KS in CS (corresponding to 0.1 µg/ml), and the linear range was 0.5% to 5% of KS in CS (corresponding to 0.1µg/ml-1 µg/ml). The optimized analysis was carried out on an ICS-5000 system (Dionex, Sunnyvale, CA, USA) equipped with a Dionex Amino Trap guard column (3 x 30 mm), Dionex CarboPac-PA20 (3 x 30 mm) and a Dionex CarboPac-PA20 analytical column (3 x 150 mm) using gradient elution at a 0.5 mL/min flow rate. Regression equations revealed good linear relationship (R^2 = 0.99, n = 5) within the test ranges. Quality parameters, including precision and accuracy, were fully validated and found to be satisfactory. The fully validated HPAEC-PAD method was readily applied for the quantification of residual KS in CS in several raw materials and USP/EP reference substance. Results confirmed that the HPAEC-PAD method is more specific than the electrophoretic method for related substance reported in EP and provides sensitive determination of KS in acid-hydrolyzed CS samples, enabling the quantitation of KS and other impurities (generating glucosamine) in CS.

Keywords: HPAEC-PAD; Method Validation; Keratan Sulfate; Chondroitin Sulfate

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1. Introduction

Sodium chondroitin sulfate (CS) is a glycosaminoglycan (GAG) composed of disaccharide units containing N-acetylgalactosamine (GalNAc) and a glucuronic acid joined by **(1•4)** [1]; [2]. Inadequate purification process can lead to residual presence of other GAGs. Keratan sulfate is one of the most common residual GAG likely to be found in large-scale production of chondroitin sulfates by raw purification procedures [3]. The KS can be selectively distinguished from CS owing to its disaccharide unit containing GlcNAc. The HPAEC-PAD method that we developed and validated, provides sensitive and accurate determination of glucosamine in acid-hydrolyzed sodium CS samples, enabling the identification of CS that has been contaminated with keratan sulfate (KS) and other glucosamine compounds. Furthermore, as already observed in literature [4]; [5], a more specific method for determination of residual KS is needed. We demonstrated that this method for related substance is more specific and accurate than the one reported in both USP [6] and EP [7] monographs. Briefly, after acidic hydrolyzation, CS is converted to its monomers Galactosamine and Glucuronic Acid, while KS is reduced to Galactose and Glucosamine. During method development we demonstrated (data not shown) that Galactose and Glucuronic Acid degradate during