

## A simple HPLC method for identification of the origin of chondroitin sulfate in health foods

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### Abstract

Chondroitin sulfate (CS) is composed of repeating disaccharide units of the structure  $[-4)\text{GlcA}(\beta 1-3)\text{GalNAc}(\beta 1-)]_n$ , where GlcA is glucuronic acid and GalNAc is *N*-acetylgalactosamine. The disaccharide composition in CS varies among different animal tissues. For example, the “A-type unit” identified as  $[-4)\text{GlcA}(\beta 1-3)\text{GalNAc}4\text{S}(\beta 1-)]$  (where “S” designates a sulfonate residue) is a predominant disaccharide in mammalian or chicken tracheal cartilage CS, while the “C-type unit” identified as  $[-4)\text{GlcA}(\beta 1-3)\text{GalNAc}6\text{S}(\beta 1-)]$  is a major disaccharide found in shark or salmon nasal cartilage CS. In contrast, a significant amount of the “D-type unit” identified as  $[-4)\text{GlcA}2\text{S}(\beta 1-3)\text{GalNAc}6\text{S}(\beta 1-)]$  is characteristically found in CS isolated from shark cartilage, while the “E-type unit” identified as  $[-4)\text{GlcA}(\beta 1-3)\text{GalNAc}4\text{S},6\text{S}(\beta 1-)]$  is characteristic in squid cartilage. The unsaturated disaccharide analysis was required a gradient elution mode using reversed-phase ion-pairing chromatography or strong anion exchange chromatography. However, an easier and less expensive HPLC method for identification of the origin of CS in health foods is needed. In this study, we established a simple HPLC method with isocratic elution for reversed phase ion-pairing separation and UV detection. Disaccharide composition analysis of CS from 10 different health foods was performed to evaluate the utility of isocratic HPLC with UV detection. The results showed that shark cartilage was the origin of almost all the CS, while dermatan sulfate consisting of “B-type unit” as well as  $[-4)\text{IdoA}2\text{S}(\beta 1-3)\text{GalNAc}4\text{S}(\beta 1-)]$  was found in one of the health foods. Based on these observations, our simple HPLC method using isocratic elution for reversed phase ion-pairing separation is effective for regulation of the origin of CS in health foods according to the composition of disaccharide units.

Keywords : origin of chondroitin sulfate, regulatory science, isocratic elution, UV detection, HPLC

### I Introduction

Chondroitin sulfate (CS) is composed of repeating disaccharide units having the structure  $[-4)\text{GlcA}(\beta 1-3)\text{GalNAc}(\beta 1-)]_n$ , where GlcA is glucuronic acid and GalNAc is *N*-acetylgalactosamine. As shown in Figure 1, the disaccharide units in CS can be classified into five groups,  $[-4)\text{GlcA}(\beta 1-3)\text{GalNAc}4\text{S}(\beta 1-)]$  (A-type unit),  $[-4)\text{GlcA}(\beta 1-3)\text{GalNAc}6\text{S}(\beta 1-)]$  (C-type unit),  $[-4)\text{GlcA}2\text{S}(\beta 1-3)\text{GalNAc}6\text{S}(\beta 1-)]$  (D-type unit),  $[-4)\text{GlcA}4\text{S}(\beta 1-3)\text{GalNAc}6\text{S}(\beta 1-)]$  (E-type unit) and  $[-4)\text{IdoA}2\text{S}(\beta 1-3)\text{GalNAc}4\text{S}(\beta 1-)]$  (B-type unit). A disaccharide unit containing iduronic acid (IdoA) instead of GlcA is found in dermatan sulfate (DS), which is a

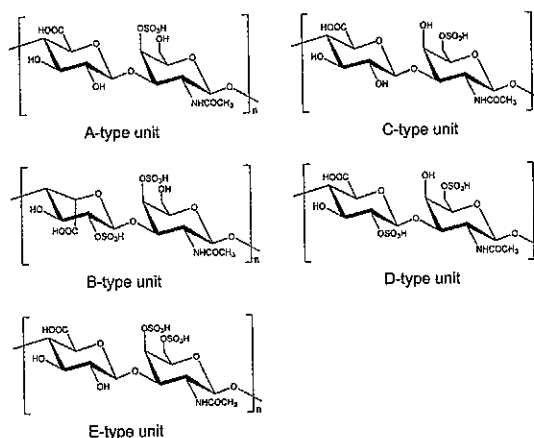


Fig. 1. Chemical structure of chondroitin sulfate