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# Quick characterization of uronic acid-containing polysaccharides in 5 shellfishes by oligosaccharide analysis upon acid hydrolysis

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ABSTRACT

Uronic acid-containing polysaccharides (UACPs) including well-known glycosaminoglycans (GAGs) and some non-GAGs exist widely in animal kingdom. Although numerous methods have been established to analyze GAGs, few methods are available for non-GAG UACPs. In the present study, a protocol to identify all kinds of UACPs with repeating disaccharide units of hexosamine and uronic acid was demonstrated, and UACP components in five shellfishes, namely *Turritella fortilirata* Sowerby (GTF), *Batillaria zonalis* (GBZ), *Nassarius variciferus* (GNV), *Monodonta labio* Linnaeus (GML), and *Argopecten irradians* Lamarck (BAI) were primarily revealed. After a simple isolation procedure, crude polysaccharides were depolymerized by controlled acid hydrolysis, and then the resulting oligosaccharides were detected by HPLC coupled with mass spectrometer after 1-phenyl-3-methyl-5-pyrazolone (PMP) labeling. According to chromatograms using the triple quadrupole mass spectrometer in the multiple reaction monitoring (MRM) mode, chondroitin sulfate (CS) was found in GNV and GML, a non-GAG named abalone gonad sulfated polysaccharide (AGSP) with a backbone of →4)-β-GlcA-(1 → 2)-α-Man-(1→ repeating units in GBZ, and both of AGSP and CS in BAI and GTF. Further characterization of tetrasaccharides and sulfated/acetylated disaccharides by HPLC combined with an ion trap mass spectrometer confirmed the structural identification of CS and AGSP, and indicated CS in GTF and BAI was Type C. These results suggest the 5 mollusks as potential resources for CS and AGSP. And the analysis protocol presented in this study was powerful and effective for quick characterization of UACPs including GAGs as well as non-GAGs in complicated matrix.



## 1. Introduction

Uronic acid-containing polysaccharides (UACPs) with repeated disaccharide units exist widely in animal kingdom. Most of them have been identified as glycosaminoglycans (GAGs) [1–5], while a few have been characterized as non-GAG UACPs [6,7].

GAGs are linear polysaccharides and generally composed of repeating disaccharide units of hexosamine and uronic acid [8], and they possess a variety of favorable biological and therapeutic activities, such as anticoagulation and antithrombotiction [9], anti-inflammation [10], anti-oxidation [11], cartilage regeneration [10,12], and so on [13]. According to repeating disaccharide units and the connection of glycosidic bond, GAGs are grouped into heparin (HP)/heparan sulfate (HS), chondroitin sulfate (CS)/dermatan sulfate (DS), keratan sulfate (KS) and hyaluronic acid (HA) [14,15]. The analysis of disaccharide fragments preserves the most frequently applied analytical assessment for GAG populations [16]. GAG disaccharides for analysis are commonly released through the use of bacterial polysaccharide lyases [17,18] or deamination cleavage by nitrous acid [19].

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**Table 1**
The shellfishes investigated in the present study.

| Class | Order | Family | Species | Abbreviations |
|---|---|---|---|---|
| Gastropoda | Mesogastropoda | Turritellidae | *Turritella fortilirata* Sowerby | GTF |
| | | Batillariidae | *Batillaria zonalis* | GBZ |
| | Neogastropoda | Nassariidae | *Nassarius variciferus* | GNV |
| | Archaeogastropoda | Trochidae | *Monodonta labio* Linnaeus | GML |
| Bivalvia | Anisomyaria | Pectinidae | *Argopecten irradians* Lamarck | BAI |

Non-GAG UACPs seem also widely distributed in mollusks as indicated by our previous investigation [6,7]. However, unlike GAGs, studies on non-GAG UACPs have just begun, and limited information is available on their possible presence by now [6]. Nevertheless, the investigation on non-GAG UACPs demands novel analysis methods other than these general methods based on enzymatic digestion [17,18] or deamination cleavage [19] which could only depolymerize GAGs. Because the glycosidic linkages of uronic acids are more stable towards acids than those of neutral sugars, acid hydrolysis could release disaccharides and tetrasaccharides with a uronic acid at the non-reducing end in relatively high yield [6,20]. Therefore, after the pretreatment of acid hydrolysis, both GAGs and non-GAG UACPs could be analyzed by detecting these oligosaccharides. A method was initially set by us previously to identify UACPs by analyzing disaccharides in their acid hydrolysates using HPLC coupled with an ion trap mass spectrometer, which could be applied to analyze crude polysaccharide extracts without further purification and identify the UACPs in biological organisms within a short time [7,21]. Since conventional polysaccharide identification process is typically time-consuming and labor intensive, requiring isolation and multistep purification followed by NMR spectra and other analyses, it is meaningful to develop a method to characterize polysaccharides quickly. Then, the previously reported method using HPLC-MS after acid hydrolysis showed a great potential in quick identification of UACPs. However, its detection performance was not satisfied due to the low sensitivity and precision. Moreover, disaccharide analysis is too limited to characterize the UACPs because their glycosidic linkages and substituted groups may also differ. Therefore, efforts are needed for improvement of the previous method to analyze GAGs as well as non-GAG UACPs.

Marine mollusks have attracted much attention because of their bioactive UACPs, such as CS and HP [1,22,23]. To speed up the exploration of these bioresources, it is necessary to find a quick protocol for the UACP analysis.

The present study demonstrated a novel protocol to characterize UACPs by analyzing disaccharides and other oligosaccharides released through acid hydrolysis with different acid concentration, which could provide more information on the UACP composition and structures. And the novel analysis protocol was applied to profile UACPs in 5 marine shellfishes, namely *Turritella fortilirata* Sowerby (GTF), *Batillaria zonalis* (GBZ), *Nassarius variciferus* (GNV), *Monodonta labio* Linnaeus (GML), and *Argopecten irradians* Lamarck (BAI).

**Table 2**
Parameters for MRM analysis of PMP-labeled disaccharides on the triple quadrupole mass spectrometer.

| No. | $Q_1$ Mass (Da) | Q3 Mass (Da) | CE (volts) | DP (volts) |
|---|---|---|---|---|
| 1 | 687.30 | 511.20 | 40 | 100 |
| 2 | 686.30 | 492.10 | 40 | 100 |
| 3 | 686.30 | 525.20 | 30 | 90 |
| 4 | 686.30 | 510.12 | 40 | 80 |
| 5 | 687.30 | 373.00 | 50 | 100 |
| 6 | 687.30 | 187.00 | 70 | 90 |
| 7 | 686.30 | 175.00 | 70 | 100 |
| 8 | 686.30 | 187.00 | 70 | 100 |
| 9 | 687.30 | 175.00 | 70 | 90 |
| 10 | 686.30 | 373.00 | 50 | 100 |

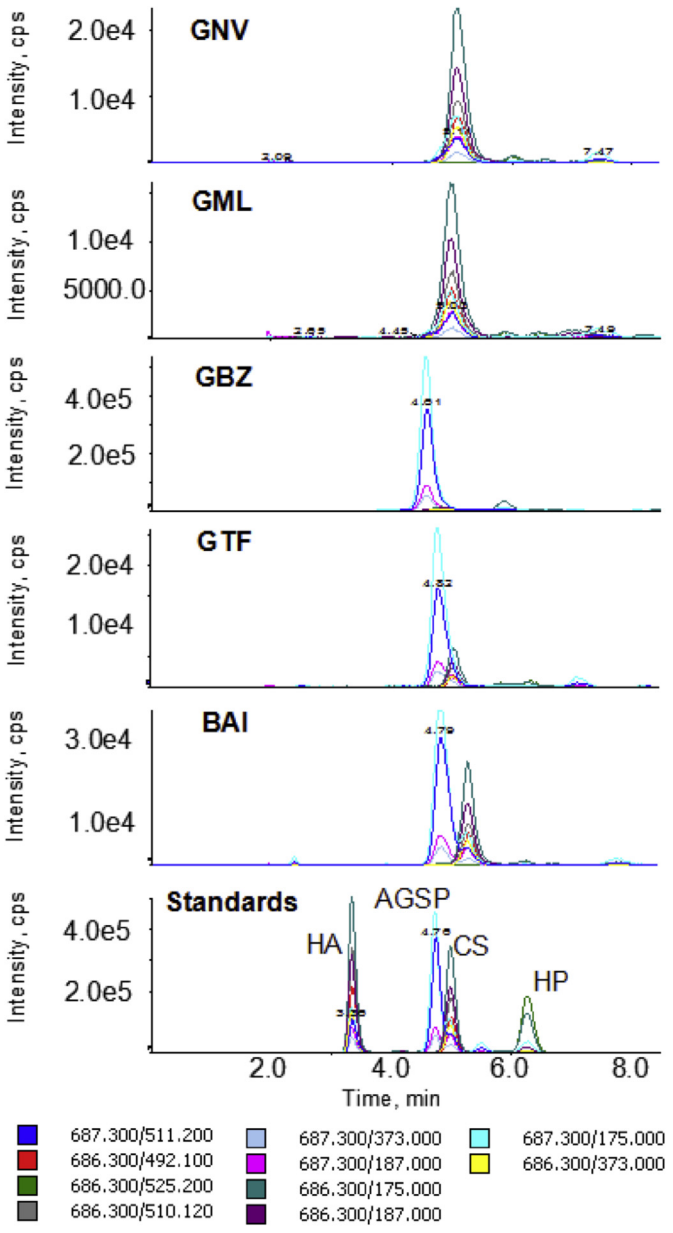


**Fig. 1.** MRM chromatograms of PMP-labeled disaccharides derived from uronic acid-containing polysaccharides (UACPs) of 5 shellfishes and mixed standard UACPs (CS, HP, HA, and AGSP).