1986 Glycan Oligomers in Mild and Strong Acid Extracts of Developing and Mature Cotton (G. hirsutum) Fibers and Textile

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Abstract

The cellulose polymer in cotton fibers may comprise of as many as 20,000 glucose units. The model of cellulose biosynthesis postulates that glucose units are added to the polymer one at a time. Since little protein is found in raw cotton fiber, such a biosynthetic model would require a very high enzyme reaction rate. Moreover, if there are no subunits in cellulose, differences in fiber properties would be determined solely by physical arrangement of linear cellulose strands in the cotton microfibril. Sequential extraction of developing cotton fibers with distilled water at 0^0 C, 0.1 N HCl at 100°C for 30 min, followed by treatment with a mixture of boiling 80% acetic, 1.8N nitric acid (Updegraff reagent) at 100°C for 30 min extracts a series of glucose-rich oligomers. Concentrations of readily soluble sugars, and oligomers extractable in 0.1 N HCl decrease as fibers mature; while those extractable with strong acid increase. Oligomers that co-chromatograph with those found in developing fibers are also found in mature fiber, processed textiles, and in a commercial cellulose standard. Presence of the oligomers in all the cellulose sources examined, and the apparent increase in difficulty to extract them as fiber matures, suggest that the oligomers are naturally occurring subunits of cellulose

Keywords: glycan, oligomer, cellulose, fiber

Introduction

Cotton fibers are single cells. and are considered to be a particularly pure form of cellulose, generally described as 88-96% cellulose and only 1.1-1.9% protein (Wakelyn et al. 2007). The degree of polymerization of cellulose in cotton has been estimated at approximately 20,000 D-glucopyranosyl units (Wakelyn et al. 2007). Considering the estimated size of the polymer, the diversity of forms of cellulose found in nature, and the varying properties of different types of cellulose, including those differences commonly found among cotton fiber from different cultivars and locations, it is notable that the most complete biosynthetic model of cellulose assembly only accounts for the roles of monosaccharides, sucrose and UDP-glucose as precursors (Delmer and Haigler, 2002). The model does not describe the synthesis of cellulose beyond the linkage of single glucose units unto a presumably

accreting polymer. Such a model does not account for the presence of any compounds other than glucose as components of cellulose. Moreover, if the cellulose units are consistently uniform, all differences in properties of pure cellulose, including difference in cotton fiber properties would depend on the physical arrangement of linear cellulose units in micofibrils. However, if compounds more complex than a monomeric glucose residue can be consistently extracted from cotton fibers, such compounds might correlate with fiber properties and be used for selection (Murray et. al, 2006;Murray, 2006).

Cold water extraction of developing fibers yields several monosaccharides, disaccharides, sugar alcohols, and larger sugars including inositol, arabinose, glucose, fructose, melibiose, sucrose, manninotriose, verbascotetraose, raffinose, stachyose, verbascose and, tentatively, ajugose (Murray, 2000). Other sugars and sugar alcohols extracted include galactitol, galactinol, arabitol, mannitiol and ribose (A.K. Murray, unpublished results). The relative abundance of these sugars can be used as node and age-specific (days post anthesis) indicators of fiber development, and consequently of plant stress (Murray, 1998, 2000). Dilute acid extractions of developing and mature cotton fibers have revealed a series of glycan oligomers, with putative degrees of polymerization from at least four to possibly as many as thirty (Murray and Nichols, 2001). The oligosaccharides extracted by the 0.1 N HCl procedure may also be used as indicators of fiber development, and therefore relate to cell wall synthesis (Murray, 2000). The chemical identity of the oligomers does not appear to differ among fibers of different maturities; however the quantities and relative proportions of the oligomers vary with developmental, physiological, and genotypic variables (Murray and Nichols, 2004). Such a series of oligomers series has also been found in other cellulose sources (Murray, 2003). The work reported here illustrates that the oligomers and/or closely related compounds are incorporated into multiple levels of the cotton fiber in association with cellulose. We hypothesize that it may be possible to further elucidate cotton fiber biosynthesis by determining the structures of this series of oligomers and characterizing their possible role in cellulose and cotton microfibril assembly. Materials and Methods

Extractions

Immature and mature fibers were sampled from cotton cultivar 'Delta and Pine Land (D&PL) 50' grown at Starkville, Mississippi. Fibers were first extracted with water at 0°C to remove monosaccharides and soluble oligosaccharides (Murray, 1998) (Fig.1.). Typically, a 5-10 mg sample was diced with a razor blade and placed in a 1.7 ml screw cap plastic tube to which 0.5 ml water was added, the tube shaken, then placed in a Branson 85 W sonicator filled with ice water. Following removal of the cold water extract with a Pasteur pipette, 0.5 ml of 0.1 N HCl was added and the tube was placed in a boiling water bath for 30 min to extract the glucose containing oliogmers (Murray, 2000). The HCl extracts were neutralized with an equivalent amount of 1 N NaOH prior to high pH anion exchange chromatography with pulsed amperometric detection HPAEC-PAD. In the case of fabric, typically 20-30 mg of material was extracted in a volume of 1.0 ml. Next, 1.0 ml of acetic nitric reagent (Updegraff, 1969) was added and the samples were boiled for 30 min. The samples were then taken to dryness on a Speed-Vac and dissolved in water for HPAEC-PAD.

Chromatography

HPAEC-PAD was performed using a CarboPac PA-1 column. The eluent was 150 mM sodium hydroxide, isocratic from 0 to 5 min then a linear sodium acetate gradient from 5 to 40 min going from 0 to 500 mM in 150 mM NaOH at a flow rate of 1 $mlmin^{-1}$. The detector wave form was the following: 0–0.20 s, 0.1 V; 0.20–0.40 s, 0.1 V; 0.41–0.42 s, _-2.0 V,0.43-0.44s, -0.10V, 0.44s-0.50s, -0.10V; integration 0.20–0.40 sec.

Results

Earlier work on the glycan oligomers chiefly involved extracting products with 0.1N HCl, a relatively weak acid treatment (Murray *et al*., 2001). The major peaks observed following 0.1 N HCl extraction include several soluble sugars and sugar alcohols (Figure 2A). As expected, the most prominent monosaccharide peak is glucose at a retention time of about 4.5 min. The major peak preceding glucose is arabinose at a retention time of about 3.9 min. Earlier eluting peaks and their approximate retention times are inositiol at 1.8, arabitol at 2.1, galactitiol at 2.5 and mannitiol at 2.6 min respectively. The peak immediately following glucose is fructose, which is not always resolved from ribose under the conditions used here. The first of the oligomers elutes at a retention time of approximately 12.3 min.

By use of a much stronger acid extraction, the acetic nitric reagent of Updegraff (1969), several oligomers are extracted that co-chromatograph with major and minor peaks observed in the dilute acid extraction, including certain minor peaks between those more clearly observed when samples are initially extracted with 0.1N HCl (Figures 2A and 2B). These observations suggest that the compounds in the minor peaks may not be extracted fully by the weaker acid treatment, but require more aggressive treatment to liberate them. The highly labile monosaccharide, fructose, is not found in the acetic nitric extract when such extraction follows extraction with 0.1N HCl; nor is arabinose, present in the acetic nitric extracts. Quantitatively, much more oligomeric material is extracted with the acetic nitric reagent than with the 0.1N HCl. (Note that the detector scale in Figure 2B is 2.5X that of Figure 2A.)

Progressive Incorporation of Oligomeric Units into Developing Cotton Fibers.

Comparison of the chromatograms of fibers of different age suggests that the relative abundance of oligomers extracted with 0.1N HCl decreases with the age of the fibers. Since all extractions are done on a dry weight basis, the relative proportion of new cell wall material being synthesized should decrease with fiber age; however, we suggest that the magnitude of the effect strongly suggests that the relative concentrations of the oligomers extractable in mild acid is decreasing relative to those obtained by the same reagents at earlier developmental stages. In contrast, the oligomers extracted with acetic nitric reagent increase in abundance with increasing developmental age of the fibers (Figures 2A and 2B). This result suggests that as the fiber matures, the oligomers are incorporated into the cellulose polymer in some modified form.

Oligomers Are Found In Processed Cotton Textiles.

Due to the apparent relationship between the oligomers extracted with weak acid compared with those extracted by the stronger acetic nitric reagent, samples of a finished fabric were analyzed. The fabric was made from the high quality Acala cultivar, 'Ultima.' Oligomers with the same retention times are found in the textile as are found in fibers (Figure 3.). As with the fibers, more material is extracted with the acetic nitric reagent compared to that extracted with dilute acid. Since the fabric had been wet-processed, the presence of the soluble sugars is much reduced. (Detector scales differ by a factor of 30X between the 0.1 N HCl and acetic nitric extractions, respectively.)

Oligomers Are Found in Chemical Standards for Cellulose

Since glycan oligomers were found in fibers and textiles, samples of a commercial, cellulose powder commercially sold by Sigma-Aldrich Company as a chemical standard for cellulose

determination was also extracted (Figure 4.). The cellulose was obtained by Sigma-Aldrich from FMC Corporation. Details of the extraction and purification procedure are not available other than the description, "an α-cellulose preparation is treated with a mineral acid." The same oligomers were found in the commercial cellulose standard with a similar relationship between the products extracted by the dilute acid and Updegraff reagents.

Discussion

Discovery of the acid labile oligomers and the diagnostic value of their relative distribution in fibers of differing ages suggested a role for the oligosaccharides as structural and possibly as biosynthetic intermediates (Murray, et. al., 2001). The additional observation that the same oligosaccharides are extracted by the acetic nitric reagent and the dilute acid, and that the distribution of the acetic nitric extracted oligomers shifts towards larger oligomers in progressively older fiber samples reinforces the hypothesis that the oligomers are diagnostic of fiber maturity. The apparent reciprocal distribution of the HCl extractable oligomers and the acetic nitric extractable oligomers (Fig. 2A vs. 2B) suggests that the oligomers may be related, or be released from related structures. Also chemically more rigorous extraction conditions are required to extract the oligomers from older tissue.

Extraction of the same oligomers by both HCl and acetic nitric reagent from different sources suggests that their presence may be universal in cellulose. An important point is that the retention times of the oligomers reported in this work do not coincide with those of any known cello-oligosaccharides. The presence of the oligomers in cotton fibers and other cellulose sources, the changes in distribution in the oligomers in extracts of fibers of different ages, and their different relative concentrations in mild and strong extractions of samples of different ages strongly suggests that the biosynthesis of cell wall polysaccharides is more complex than presently described. We hypothesize that the dilute and strong acid extracted oligomers are diagnostic of fiber development and may correlate with fiber properties, and be developed as a means to assist with selection for fiber properties.

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Figures.

Figure 1. Extraction procedures for cotton fibers and other samples.

Figure 2. Distribution of glycan oligomers in developing and mature fibers in mild and strong acid extracts. A. HCl extract, B. Acetic nitric extract.

Figure 3. Comparison of glycan oligomers in HCl extract and acetic nitric extract of cotton fabric made from the cultivar, Ultima.

Figure 4. Comparison of glycan oligomers in HCl extract and acetic nitric extract of cellulose powder.

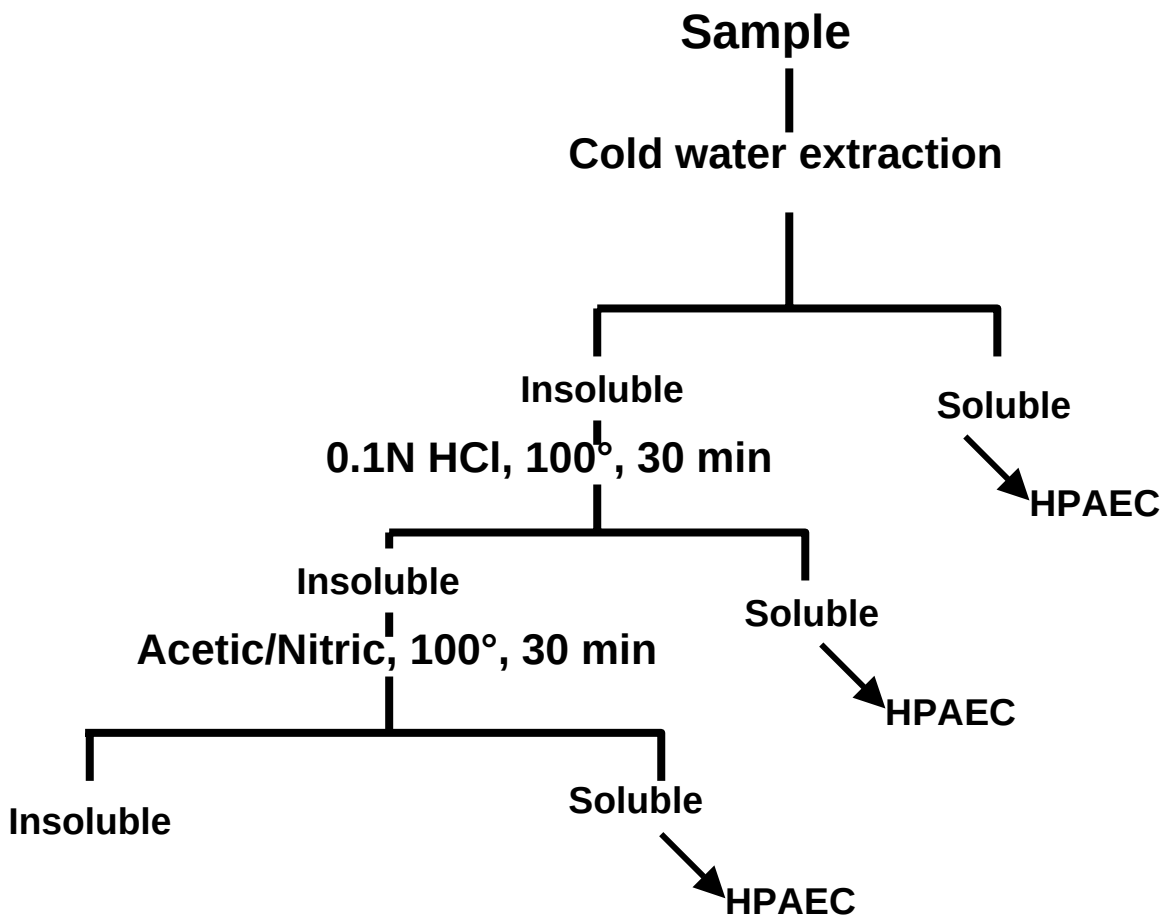

Sample
Cold water extraction
Insoluble
Soluble
0.1N HCl, 100°, 30 min
HPAEC
Insoluble
Soluble
Acetic/Nitric, 100°, 30 min
HPAEC
Insoluble
Soluble
HPAEC

Figure 1.

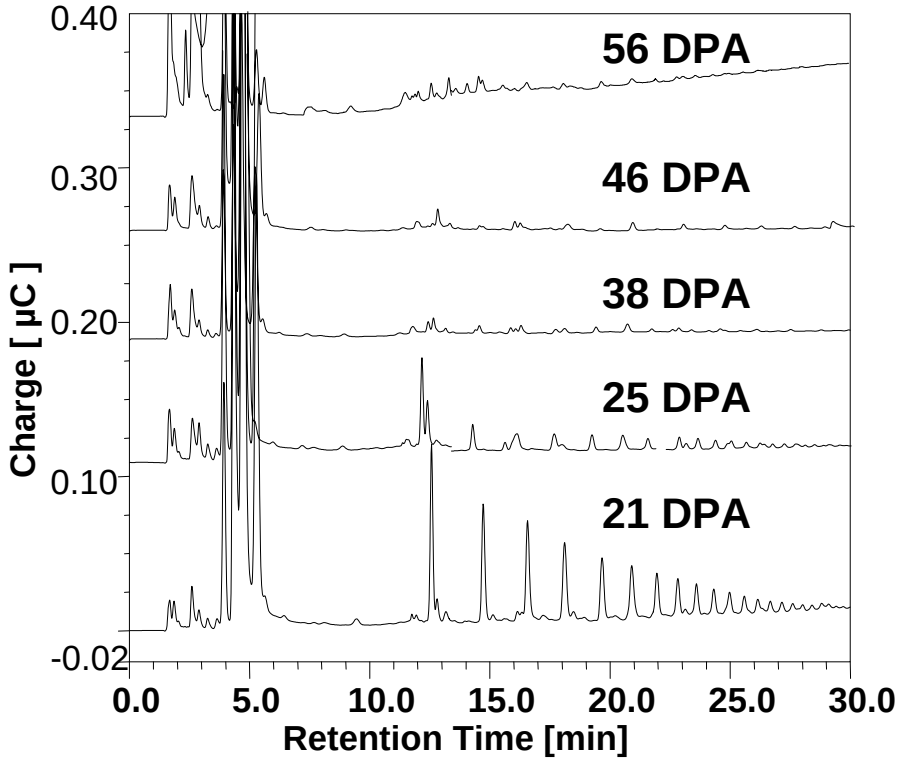


Figure 2A.

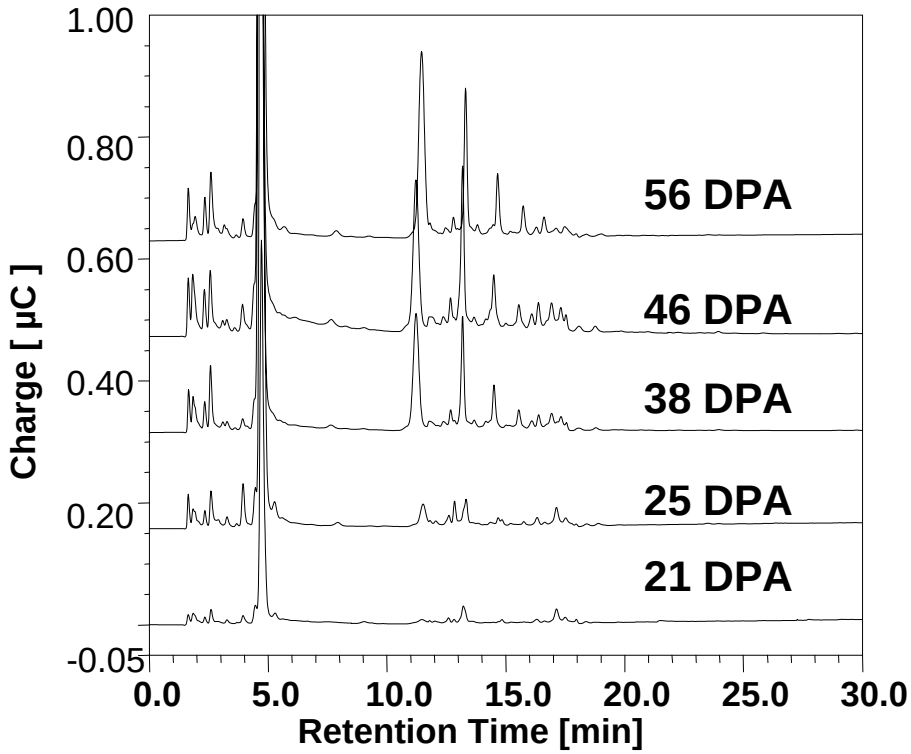
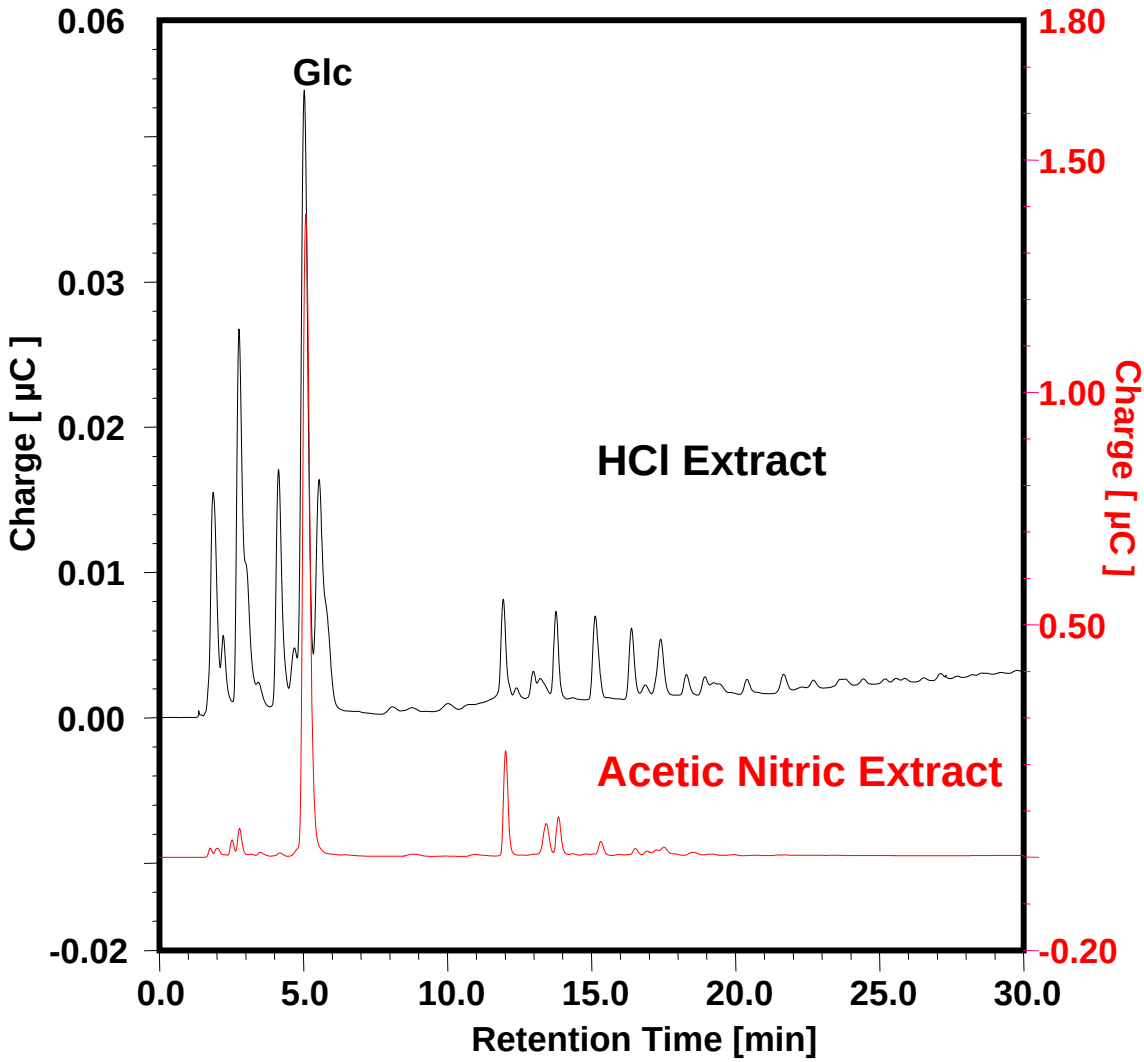


Figure 2B.


Glc
HCl Extract
Acetic Nitric Extract
Charge [µC]
Charge [µC]
Retention Time [min]
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0.03
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5.0
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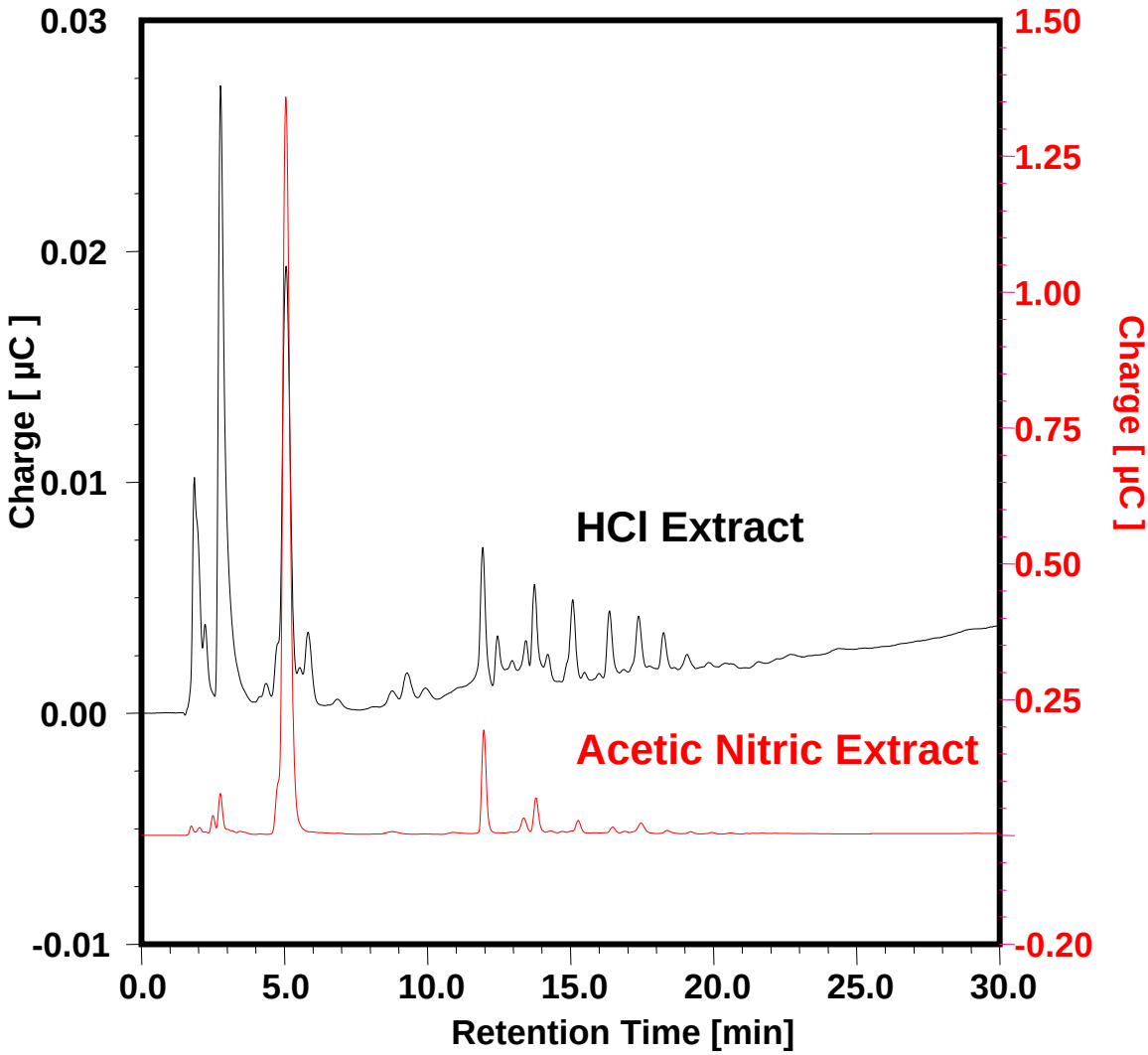
Figure 3.


0.03
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Charge [µC]
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0.50
0.25
-0.20
Charge [µC]
HCl Extract
Acetic Nitric Extract
0.0
5.0
10.0
15.0
20.0
25.0
30.0
Retention Time [min]

Figure 4.