Methods For Quantitative Analysis of Uronic Acids in Biomass

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Occurrence
• Spermoasophyta
• Bryophyta Mosses
• Lichens
• Fungi
• Algae
• Archaea

Important
• Importance of Uronic Acids
  • Reaction/measurement timing critical
  • Different factors used depending on
  • Presence of lignin requires
  • Glucuronic acid reacts much more slowly than other uronic acids.

Total Uronic Acid Analysis

Analysis of Individual Uronic Acids

Anion HPLC
- Anion HPLC with pulsed amperometric detection can be used to analyze for neutral, and acidic sugars.
- Acidic mono- and oligosaccharides are strongly bound by anion-exchange resin requiring sodium acetate (0.3 M) as a mobile ion in the eluent (0.1 M NaOH). (Knaus, 1999)

Analysis of Individual Uronic Acids Cation HPLC
- There are very many GC and derivatization methods in literature.
- Most methods give multiple peaks for each uronic acid & sugar.
- None of the methods can analyze for the uronic acid – xylose.
- Complete hydrolysis of GX or AX is accomplished with trifluoroacetic acid or methanolic HCl. (Kardosova, 1988; Chaplin, 1982).
- Trimethylsilylated and peracetylated derivatives have been made coupled with oximation (Laine, 1971), and reduction (Lehrfield, 1982).
- Two methods give single peaks for each neutral and acidic sugar. TMS-diethyl dithioacetals (Knaus, 1988).

Analysis of Individual Uronic Acids Derivation and GC
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Carbon Acidification
- Biomass sample decarboxylated by boiling HCl (12%).
- CO2 absorbed in ascorbic acid and quantified by weight gain.
- Decarboxylation can take more than 4 hours. (Knaus, 1989).
- Decarboxylation by reflux in H3. Liberated CO2 absorbed in NaOH.
- CO2 quantified by change in conductance of NaOH.
- Suitable for 50 – 100 mg samples containing 1-10 mg uronic acids. (Tallmadge, 1981).
- Carbon dioxide method for uronic anhydride with a colorimetric method. (Scott, 1984).
- CO2 produced from non-uronic extracts and carbohydrates complicates analysis. Even after extraction of biomass decarboxylation gave 30 – 40% higher uronic anhydride contents than colorimetry method. (Scott, 1984).

Analysis of Individual Uronic Acids Cation HPLC
- 4-O-methylglucuronic acid (MeGA) and 4-O-methyl glucuronoxylose are not commercially available.
- MeGA has been synthesized from methyl glucoside. Biosynthesis is followed by methylation at the 4-position, deacetylation, and then oxidation with TEMPO. Overall yield (75%) (1992).
- GX and AX hemimellithecose have been extracted with alkali from biomass samples and holocellulose. (Tallmadge, 1984; Dritzeva, 1987).
- Partial acid hydrolysis should yield 4-O-methyl glucuronoxylose.
- Without good standards it is hard to believe that much progress will be made in developing a routine analysis.

Conclusions/Future Work
- A routine method for analysis of the individual uronic acids is needed if we are to track the fraction of xylose not released in acidic biomass pretreatment.
- So far either cationic or anionic HPLC appears the most promising, however, without good standards accurate analytical data will not be possible.
- A synthesis of MeGA is underway at NREL but has not yet yielded the desired compound.
- Isolation of the uronic acid – xylose from GX extracted from biomass will also be attempted.

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