

COMPLEX CARBOHYDRATE RESEARCH CENTER

ANALYTICAL SERVICE REPORT

Date: 12/17/17

Investigator: Cinda Wood

Subject: Glycosyl composition analysis of 1 sample.

Sample CW

CCRC Code: CW112817

Analyst: Ian Black

Methods:

Should any of these data be used in a publication, please include the following statement in the acknowledgment: This work was supported by the Chemical Sciences, Geosciences and Biosciences Division, Office of Basic Energy Sciences, U.S. Department of Energy grant (DE-SC0015662) to Parastoo Azadi " at the Complex Carbohydrate Research Center.

Glycosyl composition

Glycosyl composition analysis was performed by combined gas chromatography/mass spectrometry (GC/MS) of the per-O-trimethylsilyl (TMS) derivatives of the monosaccharide methyl glycosides produced from the sample by acidic methanolysis as described previously by Santander *et al.* (2013) *Microbiology* **159**:1471.

Briefly, the sample (5 ul) was heated with methanolic HCl in a sealed screw-top glass test tube for 18 h at 80 °C. After cooling and removal of the solvent under a stream of nitrogen, the sample was treated with a mixture of methanol, pyridine, and acetic anhydride for 20 min. After evaporation, the sample was derivatized with Tri-Sil® (Pierce) at 80 °C for 30 min. GC/MS analysis of the TMS methyl glycosides was performed on an Agilent 7890A GC interfaced to a 5975C MSD, using an Supelco Equity-1 fused silica capillary column (30 m × 0.25 mm I

Glycosyl Composition Analysis

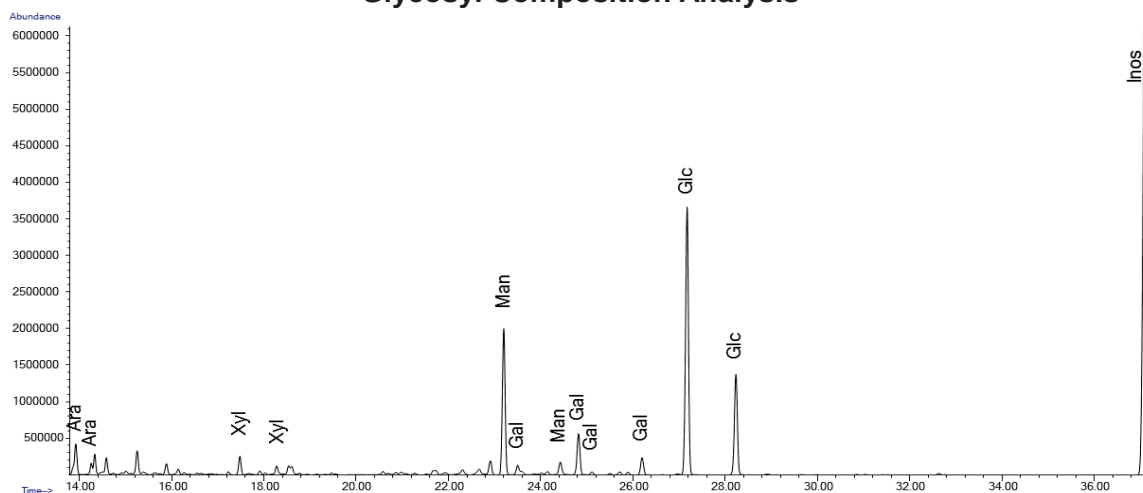


Figure 1: The GC chromatogram of the compositional analysis of the sample.

Sample	Glycosyl residue	Mass (μg)	Mol %
CW	Ribose (Rib)	n.d.	-
	Arabinose (Ara)	2.7	6.7
	Rhamnose (Rha)	n.d.	-
	Fucose (Fuc)	n.d.	-
	Xylose (Xyl)	1.4	3.3
	Glucuronic Acid (GlcA)	n.d.	-
	Galacturonic acid (GalA)	n.d.	-
	Mannose (Man)	11.9	24.4
	Galactose (Gal)	5.7	11.7
	Glucose (Glc)	26.3	53.8
	N-Acetyl Galactosamine (GalNAc)	n.d.	-
	N-Acetyl Glucosamine (GlcNAc)	n.d.	-
	N-Acetyl Glucosamine (GlcNAc)	n.d.	-
	$\Sigma=$	48.0	

Table 1: The estimated amounts and mole percentage of each detected monosaccharide in the sample.

Comments:

The sample chromatogram is shown above in Figure 1. The sample contained several monosaccharides, but the most abundant were mannose and glucose. Based on the chromatogram, it looks like the sample contains mainly glucans with small amounts of hemicellulose also present. 5ul of the sample was used for the analysis, and the estimated concentration of carbohydrate for the samples is 9.6mg/ml.

If you have any further questions, please contact Dr. Parastoo Azadi at 706-583-0629 or azadi@ccrc.uga.edu.

The glycosyl composition analysis reported above does NOT include the absolute configuration (D or L) of monosaccharides. The absolute configuration of monosaccharides can be determined with additional experiments at an additional cost.