Carbohydrate analyses at the Australian Proteome Analysis Facility (APAF)

Monosaccharide (and disaccharide) analyses are key techniques used in biochemistry and carbohydrate chemistry. They are used to identify and quantify the amount of simple sugars in a sample; typically, free sugars and those that are bound to glyco-conjugates such as glycoproteins, glycolipids, etc.

Our carbohydrate analysis services provide precise, comprehensive identification and quantification of common carbohydrates in various samples, including proteins, peptides, food products, and biological samples. Carbohydrate analysis is a versatile and targeted tool, providing valuable insights into the molecular composition of samples and for understanding protein structure, stability, function, and nutritional content.

Applications of Carbohydrate Analysis

- **Carbohydrate chemistry**: Determining the sugar composition of glycoconjugates (i.e., glycans attached to proteins and lipids).
- **Food Analysis**: Determining the nutritional content and quality of food products.
- **Quality Assurance**: Measuring the consistency of glycosylated monoclonal antibodies and biopharmaceuticals at monosaccharide level, thus helping to assess batch-to-batch variation and to understand their structure and function.
- **Biomedical Research**: Investigating glycosylation changes associated with diseases (i.e., cardiovascular disease, cancer) by measuring free and bound monosaccharide levels in biological fluids such as serum, plasma, saliva, urine, etc.
- **Environment and Ecological screening:** Analysis of carbohydrate concentrations from marine and freshwater samples, and soil extracts.

Challenges and Considerations

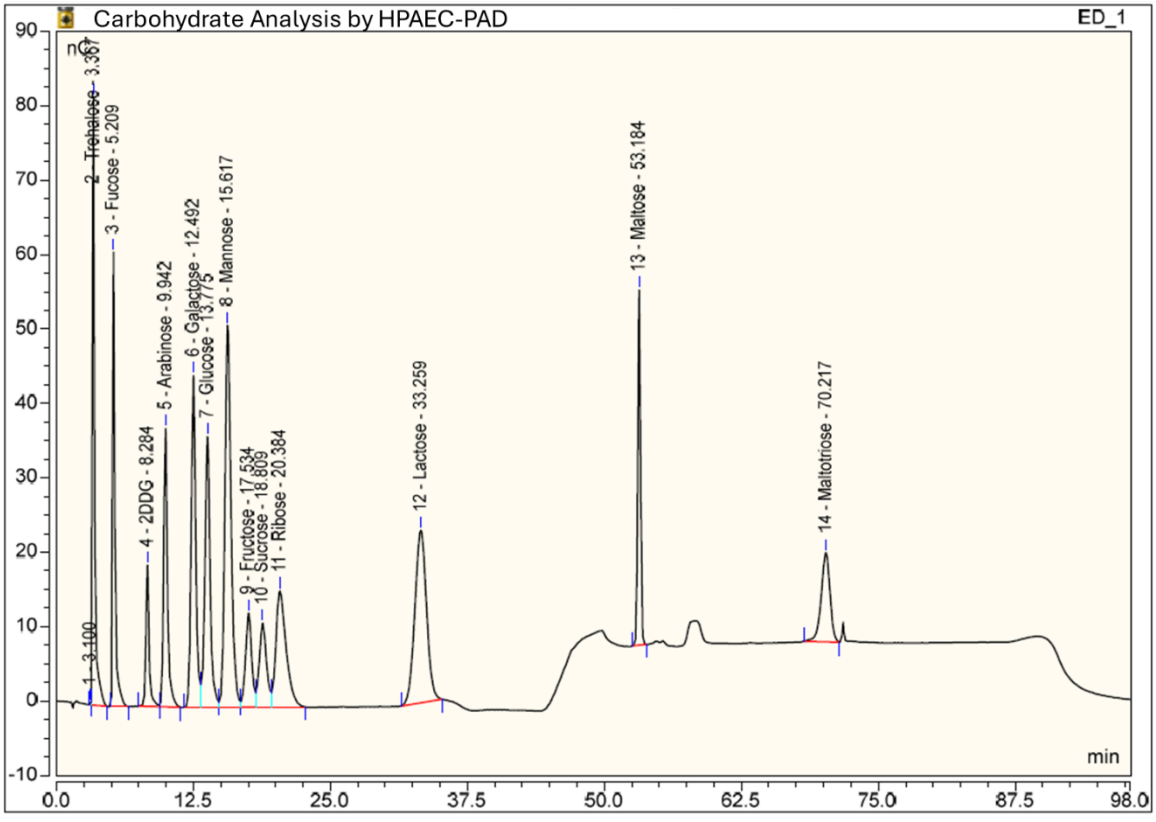
- **Sample Preparation**: Proper preparation is crucial to avoid contamination and ensure accurate results. We are happy to assist you with advice and assistance, but please note that buffer constituents such as elevated salts, lipids and detergents can interfere with the assay.
- **Sensitivity and Specificity**: Choosing the right detection method is pivotal to balance sensitivity and specificity in a way that is suitable for the sample type.

Method Summary

Carbohydrate analysis services are not yet ISO 17025-accredited, and involve the following:

1. **Hydrolysis**: Breaking down the carbohydrates that are bound to proteins into their constituent simple sugars. The sample is hydrolysed under acidic conditions.
    a. Hydrolysis is not performed for Free Monosaccharide analysis, wherein only 'free' or unbound carbohydrates (i.e., that are not attached to proteins, lipids or peptides, oligomeric carbohydrate structures such as starches or structural carbohydrates) are quantified.
2. **Separation**: Monosaccharides and/or disaccharides are separated using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD).
3. **Detection and Quantification**: Carbohydrate abundance is detected and quantified against a standard curve.

Example Chromatogram



Monosaccharide Analysis Services

Free Carbohydrate Analysis (no hydrolysis)	Amino Monosaccharide Analysis	Neutral Monosaccharide Analysis	Neutral Disaccharide Analysis	Acidic Monosaccharide Analysis	Sialic Monosaccharide Analysis	Additional Carbohydrates*
-	Glucosamine	-	-	-	-	Trehalose
-	Galactosamine	-	-	-	-	Maltotriose
Glucose	-	Glucose	-	-	-	Iduronic acid
Galactose	-	Galactose	-	-	-	Xylose*
Mannose	-	Mannose	-	-	-	* On request
Fucose	-	Fucose	-	-	-	
Ribose	-	Ribose	-	-	-	
Rhamnose	-	Rhamnose	-	-	-	
Arabinose	-	Arabinose	-	-	-	
Ribose	-	Ribose	-	-	-	
Fructose	-	-	Fructose	-	-	
Maltose	-	-	Maltose	-	-	
Lactose	-	-	Lactose	-	-	
Sucrose	-	-	Sucrose	-	-	
-	-	-	-	Glucuronic acid	-	
-	-	-	-	Galacturonic acid	-	
-	-	-	-	-	N-acetylneuraminic acid	
-	-	-	-	-	N-Glycolylneuraminic acid	
-	-	-	-	-	2-keto-3-deoxy-D-glycero-D-galacto-nononic acid (KDN)*	

Data reporting

APAF's standard procedure for monosaccharide and/or disaccharide analysis is to analyse a client sample in technical triplicate and then to report the averaged results.

Should you require the *results of individual replicates* to be reported, please notify the Technology Manager – Protein Analysis, Dr. David Cantor (david.cantor@mq.edu.au) at the time of quoting or *before* the report has been released. Once the report has been issued, any requests to report individual replicate data will incur an additional reporting charge.

Any modifications to the standard workflow should be identified and discussed with the Technology Manager prior to commencing any project.

Data Formatting

APAF offers two standard output formats for amino acid analysis results, at the discretion of APAF staff:

1. A PDF report, containing project details;
2. A PDF report coversheet, with a Microsoft Excel attachment.

Interpreting your results

Amount (µg/g, or as appropriate)

Calculation based on respective carbohydrate mass in the sample. These values express the concentration of respective carbohydrates that are observed, following sample preparation.

If no hydrolysis has been performed, the values correspond to the amount of freely available carbohydrates.

Following a procedure involving sample hydrolysis, this value corresponds to the amount of the carbohydrate in both the free- and -bound forms (i.e., incorporated into glycoprotein/s, glycolipids, etc).

Sample Submission and Shipment to APAF

For purified glycoprotein samples, we recommend sending 1-10mg of material in a sealed container under appropriate temperature conditions. This allows for representative sampling, and repeat analysis, should it be required. Where the available sample amount is lower than this, we recommend discussion with an APAF team member to determine whether a scaled-down assay is possible/appropriate.

For most complex sample types, we recommend sending 1-10g of material in a sealed container under appropriate temperature conditions. This allows for representative sampling, and repeat analysis, should it be required.

When possible, we recommend sending dry, homogenous samples, particularly for challenging material such as grains, whole plant/animal tissue, etc., to minimise sample heterogeneity, microsampling effects, and to reduce loss from additional processing.

When multiple analyses are requested, we generally recommend sending 5-10g of material for analysis.

When ready to despatch, please direct the parcel to the following address, with attention to the Protein Analysis team:

- Science Stores, Faculty of Science and Engineering, 14 Eastern Road room 186, Macquarie University, NSW, 2109, Australia

Further Enquiries

Please see our website for more information on the Australian Proteome Analysis Facility:

https://www.mq.edu.au/research/research-centres-groups-and-facilities/facilities/macquarie-analytical-and-fabrication-facility/australian-proteome-analysis-facility

Please direct enquiries or requests for further information to info.apaf@mq.edu.au, or to Dr. David Cantor (david.cantor@mq.edu.au).

Thank you,

The Protein Analysis Team