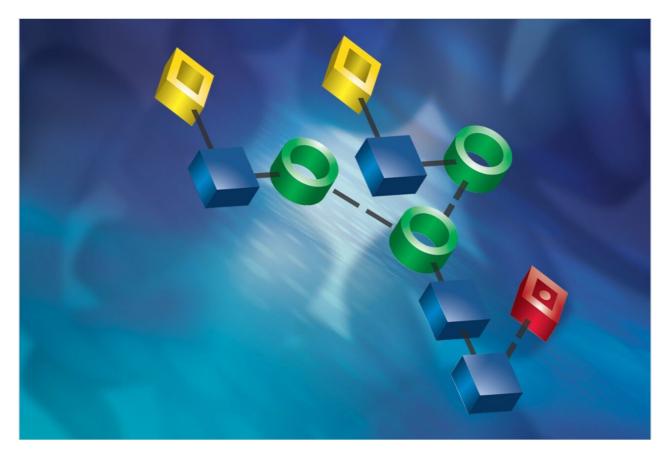
Waters[™]

A Systematic Approach to Glycan Analysis using HILIC-UPLC and an Online Database of Standardized Values

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief enables the systematic analysis of fluorescently labeled N-glycan pools released from recombinant biotherapeutics and separated by HILIC-UPLC chromatography. Data are interpreted with the aid of a robust online glycan database generated from standardized retention values.

Benefits

NIBRT's GlycoBase 3+ is a web-enabled proprietary resource that contains normalized retention data, expressed as Glucose Units, or GU values, for more than 600 2AB-labeled N-linked glycan structures.

Introduction

Glycosylation is the most complex posttranslational protein modification and it is estimated that more than half of all eukaryotic proteins are glycosylated. In order to elucidate the functional outcomes of glycosylation and to characterize the glycoprotein more fully, it is essential to define the monosaccharide sequence, linkage, and anomericity of the covalently attached oligosaccharides.

Glycosylation of biotherapeutics is a critical product attribute and adverse changes in glycosylation can significantly alter product efficacy and safety. Furthermore glycosylation of biotherapeutics can be influenced by dissolved oxygen, pH, carbon source, temperature during manufacture, as well as by the choice of expression system. These variations can put product integrity at risk and therefore the ability to monitor glycosylation accurately and rapidly is essential at all stages of the process.

The U.S. FDA, the EMA, and other regulatory bodies have started to increase pressure on manufacturers to analyze the glycosylation of therapeutics more fully, and also to demonstrate how their process can affect glycan composition. However, there are no robust workflows for systematic detailed, quantitative, sensitive, glycan analysis.

Results and Discussion

The National Institute for Bioprocessing Research and Training (NIBRT) in Ireland is a world-class institute that provides training and research in the biopharmaceutical industry. NIBRT's mission is to provide a unique experience for trainees in an environment that replicates a modern bioprocessing facility. In parallel, NIBRT also undertakes leading bioprocessing research in collaboration with industry partners and provides contract analytical services.

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Sample Name		Number of Glycans				Sala actas data sente concessa Salacitatikan pelakatikan Salacitatikan pelak
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Glycan Name	Structure	Mass	HILIC-UPLC	HUIC-HPLC	RP-UPLC	
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		1949. 1979.22P				41
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	0-8-8	avg.: 1438.298	6.31 ±0.035			and the second second second
	are al	mana: 1437.513				Human Serum
ARTONIA			6.32 ±0.088			100 Inventase
	B 0-8-8	avg.; 1479.350				Mil Acces go Mil Coters
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EISIAIB		avg.: 1869.737	6.36 ±0.000			218 358

Figure 1. Overview of GlycoBase 3+, NIBRT's UPLC N-glycan database.

Waters Corporation and NIBRT, led by Prof. Pauline Rudd, have developed a comprehensive solution to glycan analysis that combines NIBRT's novel bioinformatics database solution, GlycoBase 3.0+, with the unique capabilities of the Waters ACQUITY UPLC System and Glycan Separation Technology chemistries for HILIC-UPLC separations. GlycoBase 3.0+ is a web-enabled proprietary resource that contains normalized retention data (expressed as Glucose Units, or GU values) for more than 600 2-AB labeled N-linked glycan structures. These values were obtained by systematic analysis of released glycans from a diverse set of glycoproteins using Waters HPLC and UPLC technologies and the NIBRT glycan analytical platform.

Two orthogonal technologies, exoglycosidase sequencing and mass spectrometry, were used to confirm every structure, generating a high-quality glycan library that can be used as a general tool or interrogated in various ways, e.g., for each of the biotherapeutic glycoproteins analyzed. Data stored in a web-accessible database is accessed through a customized software application with a simple intuitive interface.

Each entry/glycan is comprehensively annotated and includes:

- A pictorial representation of the structure depicting monosaccharide sequence, stereochemistry, and glycosidic linkages in a variety of color or black-and-white formats (Oxford notation that includes embedded linkage information, CFG notation)
- · GU retention time values and descriptive quality statistics
- · Molecular mass (average and monoisotopic) for each glycan
- · Monosaccharide composition
- · Graphical representations of relative glycan composition and antennary structure
- · Related reference information (biological context, taxonomy, diseases, enzyme specificities)
- · Links from raw data to experimental acquisition conditions

	Data						
q	Summa	y Raw Dat	ta NIBR	T Records	Public Records		
-	Run	RP-HPLC**	NP-HPLC*	UPLC*	Sample	Profile	Taxonomy
Download h-def	1	1			Human Serum	see conditions	Homo sapiens
Physical-Chemical Properties	2				Human Serum	see conditions	Homo sapiens
Monoisotopic 1.665.624	3	3			Human Serum	see conditions	Homo sapiens
Isotopic Average 1,666.544	4	4			Human Serum	see conditions	Homo sapiens
Residues 9	5	5			Human Serum	see conditions	Homo sapiens
Monosaccharide Composition	6	6			Human Serum	see conditions	Homo sapiens
Hex: 3	7	7			Human Serum	see conditions	Homo sapiens
HexNAc: 5	8	8			Human Serum	see conditions	Homo sapiens
NeuNAc: 0 Fucose: 1	1	9			Human Serum	see conditions	Homo sapiens

Figure 2. Detailed experimental and supporting data for the selected glycan, F(6)A2B.



Figure 3. A bank of Waters ACQUITY UPLC Systems in NIBRT's analytical laboratory.

Conclusion

A glycan database, GlycoBase 3.0+, of protein N-linked oligosaccharides released from a range of biotherapeutic proteins has been developed for the biopharmaceutical industry using a combination of Waters and NIBRT technologies. Reproducible, sensitive, standardized and high-throughput glycan HILIC separations, in combination with an online glycan bioinformatics resource, GlycoBase 3.0+, allows the identification of glycans based on three orthogonal data attributes.

An additional feature of 2-AB fluorescently labeled N-linked glycans is that the data generated by chromatography can be used for relative quantitation, which when combined with Glycobase 3.0+ is a powerful tool for the biopharmaceutical industry. The dynamic range allows glycans present at <1% of the glycan pool to be quantified.

These novel capabilities will facilitate the more rigorous characterization of biopharmaceutical protein products required by today's regulatory environment.

Waters would like to acknowledge Mark Hilliard, Weston Struwe, Giorgio Carta, John O'Rourke, Niaobh McLoughlin, and Pauline Rudd from NIBRT for this work.

Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

720004203, December 2012

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