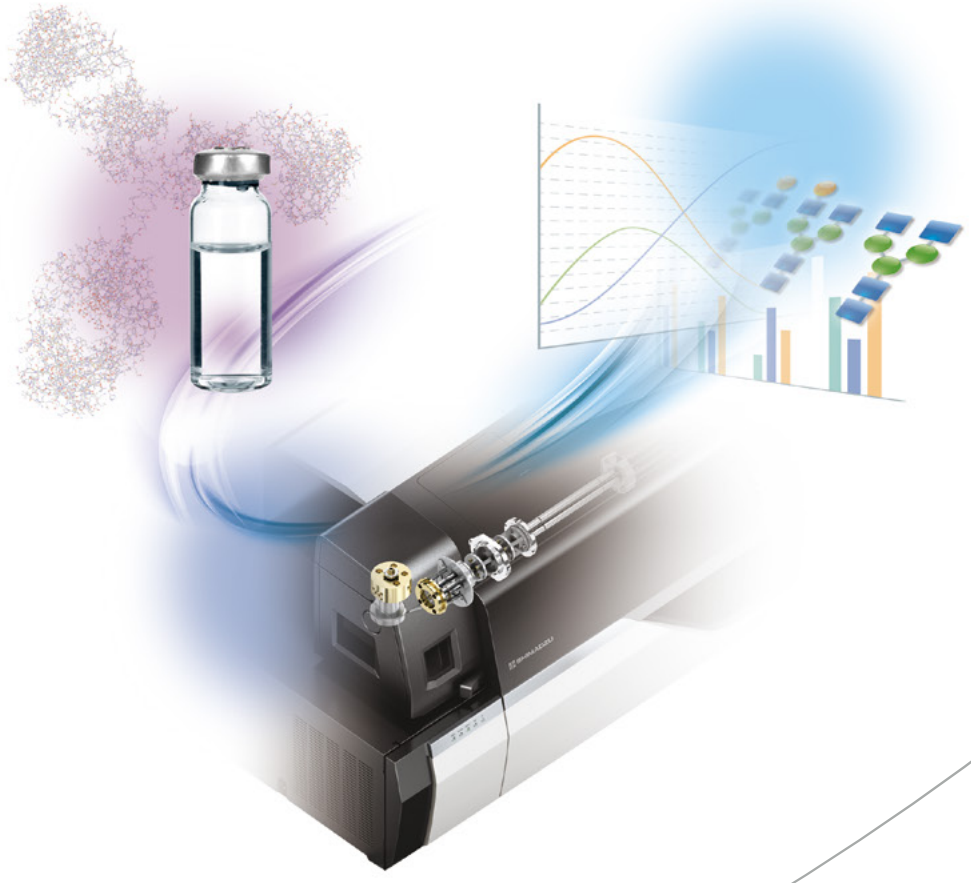
C146-E294

SHIMADZU
Excellence in Science

Software Platform for Glycan Quantification and Qualification by LCMS-8060/8050

Erexim Application Suite



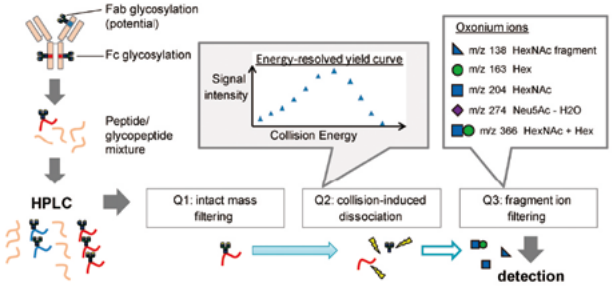
Simplifies Glycan Heterogeneity Analysis at Individual Glycosylation Sites

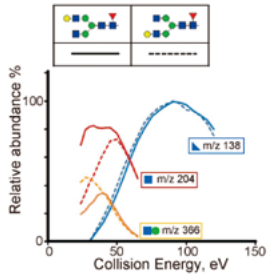
Analysis of N-linked glycans are most frequently performed by first detaching the glycan from the protein. Although this approach is accurate in both quantitative and qualitative respects, the result given is an averaged picture of glycans derived from all possible glycoproteins and glycosylation sites. In order to focus on the glycan heterogeneity occurring at a specific glycosylation site of interest, analysis needs to be performed at the glycopeptide level using enzymatic protein digests. However, since glycopeptides have unique masses, data analysis requires labor-intensive informatics and manual data manipulation. Erexim Application Suite is designed to facilitate the analysis of site-specific glycan heterogeneity by providing customizable ready-to-use methods and automated data analysis.

- Supports all glycan structures with a customizable database
- MRM method file generated with minimal user input
- Visualization of quantitative and qualitative results

What is Erexim™ (Energy-resolved oxonium ion monitoring)?

When analyzing glycans or glycan-containing molecules by MS/MS, the product ions generated by fragmentation include a high abundance of glycan-derived low *m/z* ions called the oxonium ions. Although the species and relative abundance of oxonium ions reflect the glycan structure of origin, conventional MS/MS provides insufficient features to differentiate between glycan structures. Energy-resolved oxonium ion monitoring, abbreviated as Erexim, adds another dimension to MS/MS data by acquiring data at a series of collision energies (CE) of fragmentation. A plot of the change in oxonium ion abundances with respect to CE, the Erexim profile, now contains the resolving power to differentiate between similar glycan structures. Erexim requires triple quadrupole mass spectrometry for its ultrafast scan speed to acquire a multitude of data points and for its quantitative ability to acquire reproducible profiles. Moreover, one of the product ions targeted in Erexim is specific to the N-glycan core structure and is an ideal reporter ion for relative quantitation of glycan structures.

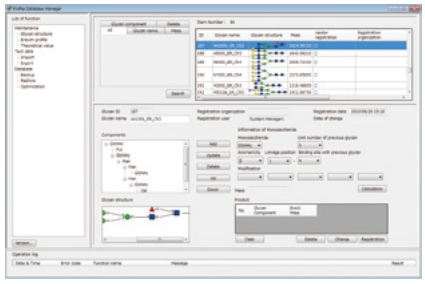




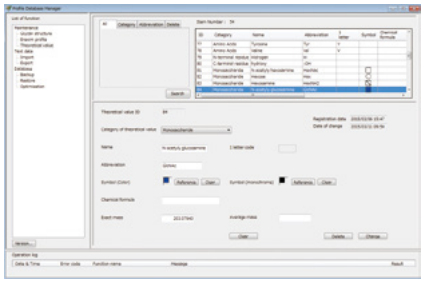
Reference: A. Toyama *et al.*, *Anal. Chem.* 2012, 84, 9655–9662

Customizable glycan structure database **Profile Database Manager**

The database of Erexim Application Suite contains 45 entries of N-glycan structures. Each entry contains monosaccharide composition, linkage information, amino acid sequence (if glycopeptide) and the reference Erexim profile. Entries may be added by customers to keep updated with research progress.



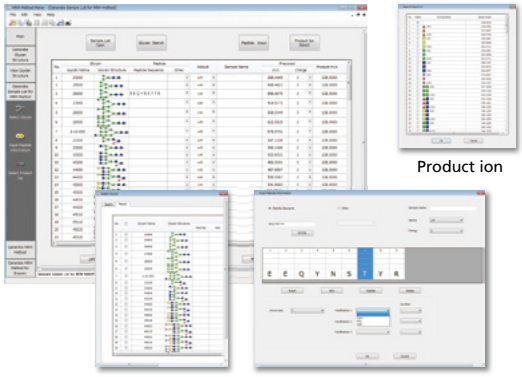
Glycan structure manipulation



Detailed definition of components

Compile input into a "ready-to-use" method **MRM Method Maker**

For detailed quantitative analysis, as well as for Erexim profile data acquisition, the number of MRM transitions may be hundreds and it is extremely labor-intensive to design them correctly. MRM Method Maker automatically produces MRM transitions according to the selection of glycan structures of interest, peptide sequence, ion adduct type and other inputs. MS acquisition parameters such as CE, dwell time, etc. can also be assigned collectively.

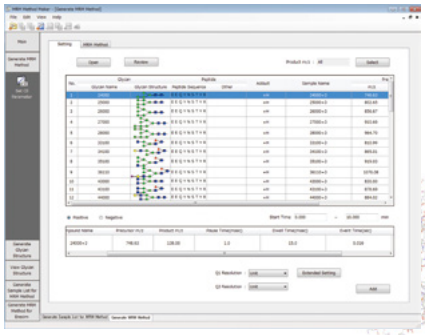


Product ion

Glycan structure

Peptide (label) and adducts

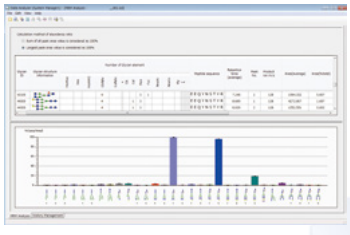
Selection of analysis targets



Setting MS Acqusition Parameters

Automated graphical representation of data **Data Analyzer**

Because glycans and glycopeptides are detected at multiple charge states in LC/MS, their quantitation requires complex data manipulation to correctly combine all ions derived from each molecular species. This process is automated by the Data Analyzer, and the result will be presented graphically, either as a bar chart of relative abundance or as an Erexim profile plot.

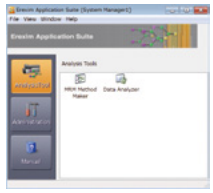


Bar chart of relative abundance

Erexim profile plot

Erexim™ Application Suite — from glycan analysis to data presentation

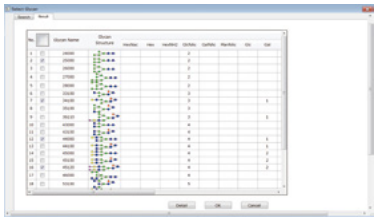
Each application is executed from the main page of LabSolutions.



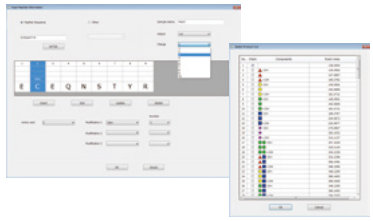
1st Step: Analysis of glycan heterogeneity (Quantitative Analysis)

MRM analysis workflow

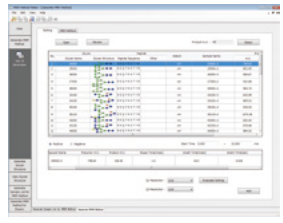
Launch MRM Method Maker



Select glycan structures of interest

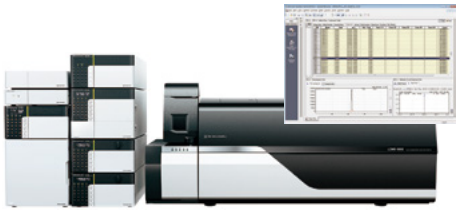


Enter peptide information, charge states, adducts, product ion species



Select parameters for MS Acquisition

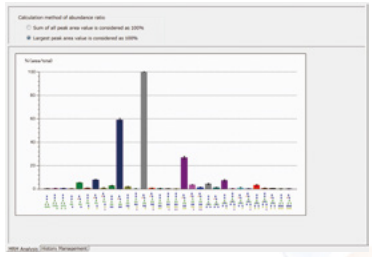
Method File generated



LCMS-8060/8050
Analysis and peak detection

Launch Data Analyzer

Load raw data

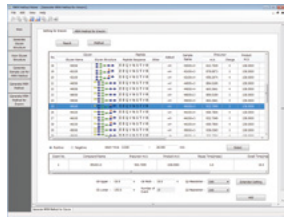


Graph of relative abundance of glycans

Reporting

2nd Step: Erexim Profile Acquisition (Qualitative Analysis)

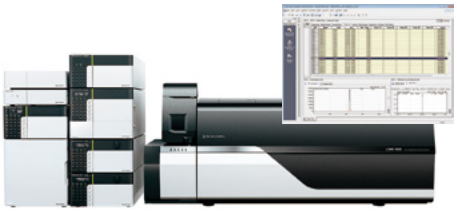
Erexim profiling workflow



Launch MRM Method Maker

Load MRM analysis data; Select glycan structure for Erexim profiling

Parameter setting for Erexim

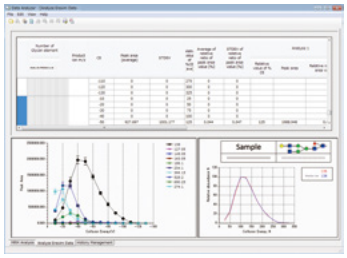


LCMS-8060/8050
Analysis and peak detection

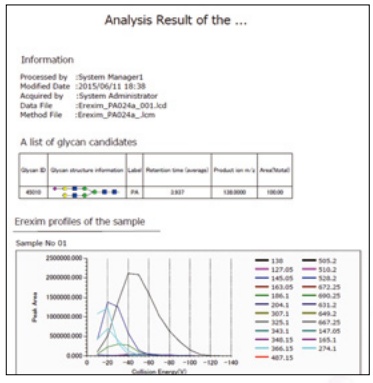
Method File generated

Launch Data Analyzer

Load raw data



Erexim profile plot
(register to database)



Reporting

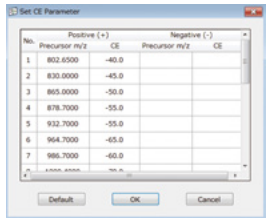
Analysis of a commercially available IgG glycopeptide

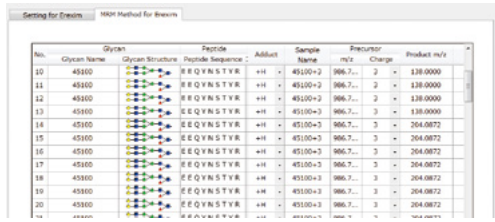
Here we show an example of N-glycan heterogeneity analysis by Erexim Application Suite, targeted specifically for the glycosylation site in the Fc region of a commercially available monoclonal antibody. The sample was prepared by digesting the antibody solution (50 µg) with trypsin for 2 hours, then removing hydrophobic peptides and residual trypsin by passing the reaction mixture through a Supel-Select HLB SPE cartridge. The flow-through fraction is rich in Fc region glycopeptides.

1 Using the MRM Method Maker, glycan structures of interest were selected to generate the list of target glycopeptides.

Glycan ID	Structure	Glycan ID	Structure	Glycan ID	Structure	Glycan ID	Structure	Glycan ID	Structure
26000		44000		45110		53000		54110	
27000		44100		45020		53100		55010	
28000		45000		45120		54000		55110	
23100		45100		34000		54100		56000	
33000		44010		33100		55000		56100	
43000		44110		34100		55100			
43100		45010		34110		54010			

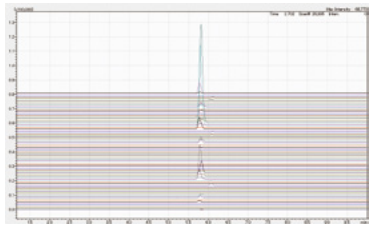
2 MS acquisition parameters such as Dwell Time, Pause Time, CE were entered, which converts the compound list to MRM transitions. CE values can be filled automatically with empirically derived optimum values.





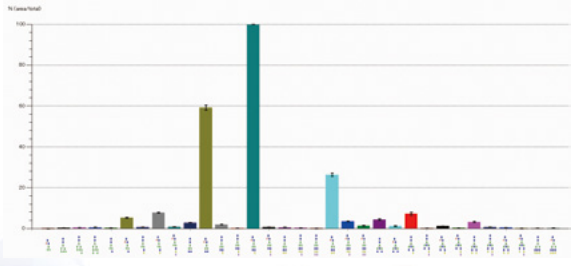
3 The MRM transition list generated in step (2) was saved as a method file, which was downloaded to the LCMS-8060 to perform the analysis. Three replicate measurements were performed.

Column: Aeris Peptide XB-C18 2.1 × 150 mm (Phenomenex)
Mobile Phase A: 0.1% Formic Acid
Mobile Phase B: 90% Acetonitrile / 0.1% Formic Acid
Gradient: 2%B (0–2 min) – 30%B (10 min) – 98%B (11–12 min) – 2%B (12–15 min)
Flow Rate: 0.3 mL/min
Injection Volume: 10 µL



MRM chromatogram

4 After performing peak integration with LabSolutions, the saved data was loaded into Data Analyzer. The results shown below were automatically generated.



Ratio graph of N-linked glycans binding to the IgG Fc region
(Amino acid sequence of the Fc region: EEQYNSTYR)

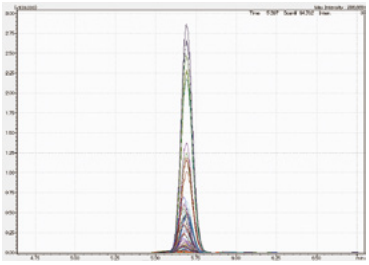
Glycan ID	%Area	STDEV	Glycan ID	%Area	STDEV	Glycan ID	%Area	STDEV
23100	0.009	0.003	44010	0.075	0.019	54010	0.079	0.007
26000	0.111	0.036	44100	43.191	0.62	54100	0.482	0.056
27000	0.19	0.052	44110	0.281	0.021	54110	0.114	0.053
28000	0.241	0.037	45000	0.229	0.061	55000	1.382	0.251
33000	0.113	0.007	45010	0.12	0.05	55010	0.264	0.035
33100	2.278	0.104	45020	0.027	0.025	55100	0.17	0.055
34000	0.291	0.039	45100	11.383	0.585	55110	0.014	0.012
34100	3.339	0.049	45110	1.511	0.142	56000	0.034	0.022
34110	0.37	0.043	45120	0.571	0.135	56100	0.084	0.046
43000	1.228	0.127	53000	1.871	0.321			
43100	25.506	1.055	53100	0.521	0.129	Total	100	
44000	0.837	0.102	54000	3.084	0.601			

Ratio of N-linked glycans

5 Using MRM Method Maker, Glycan Structure ID 45100 and 44100 was selected the target for Erexim profile acquisition. A Collision Energy (CE) range of –10 ~ –130 V at 10 V intervals was selected.

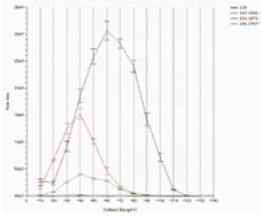
6 The information generated in Step (5) was saved as the LabSolutions Method File, which was downloaded to the LCMS-8060 to perform analysis. Three replicate measurements were performed.

Column: Aeris Peptide XB-C18 2.1 × 150 mm (Phenomenex)
Mobile Phase A: 0.1% Formic Acid
Mobile Phase B: 90% Acetonitrile / 0.1% Formic Acid
Gradient: 2%B (0–2 min) – 30%B (10 min) – 98%B (11–12 min) – 2%B (12–15 min)
Flow Rate: 0.3 mL/min
Injection Volume: 10 µL

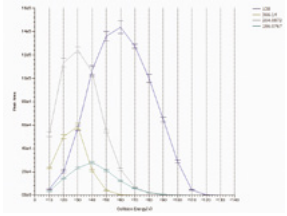


MRM Chromatogram

7 After performing peak integration in LabSolutions, the saved data was loaded into Data Analyzer. The results shown on the right are the Erexim profile plots generated.

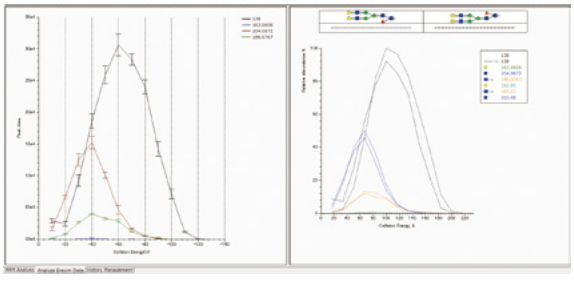


Erexim profile plot for Glycan ID 45100

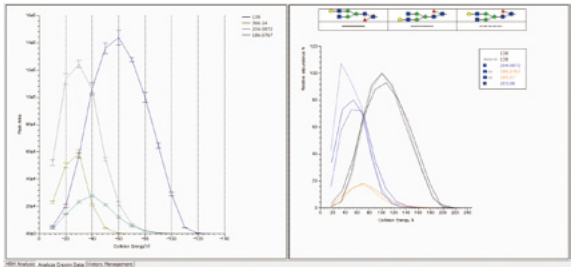


Erexim profile plot for Glycan ID 44100

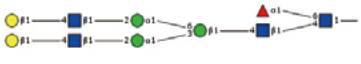
8 Referring to the Erexim plots registered in the database revealed that the newly acquired data appeared similar to the reference data of the same glycan mass, giving an indication that what was detected from the sample had the same structure as Glycan ID 45100_ER_Ch3. In contrast, the acquired data for Glycan ID 44100 was different from two of the reference profiles having the same glycan masses, 44100a_ER_Ch3 and 44100b_ER_Ch3. The curve for product ion *m/z* 204 in the acquired data fell in between the two reference profiles, providing the researchers important information regarding the composition of the sample.



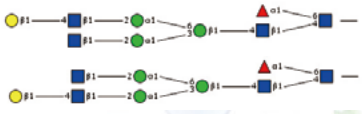
Comparison of Erexim profile plots of Glycan ID 45100 (Left panel: acquired data, Right panel: overlay of acquired data onto reference plot)



Similarly, acquired data (left) and overlay of two reference plots (right)



Structure of 45100_ER_Ch3



Structures of 44100a_ER_Ch3 (top) and 44100b_ER_Ch3 (bottom)

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Printed in Japan 3655-08507-30ANS