Rapid CE-MS analysis of released N-glycan: optimized workflow for direct CE compatibility

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Introduction

N-glycan profiling is a critical step of biopharmaceuticals analysis as N-glycans play an important part in both activity and safety of monoclonal antibodies and other glycosylated biotherapeutics [1]. The gold standard methodology for N-glycan analysis involves LC-FLR-MS analysis after N-glycan release from the protein backbone and derivatization with fluorophores that allow both fluorescence and mass spectrometry detection [1].

N-glycan profiling is essential for both drug and process development, to select optimum clones and to optimize process conditions that can influence the final relative abundance of individual glycoforms. Moreover, profiling of N-glycans is required at batch release and is critical for analytical assessment of biosimilars.

Significant effort to improve the analytical workflow have been made in the last decade, both to speed up and standardize sample preparation as well as LC-MS analysis and data processing.

The use of sample preparation kits like Rapifluor (Waters) has simplified sample preparation, while efforts are still required to speed up separation and MS analysis. Another approach for N-glycans analysis is usually based on CE-MS [2].

In this study we coupled rapid glycan release and labelling provided by the RapiFluor-MS kit with the fast microfluidic electrophoretic separation on the ZipChip interface with Orbitrap mass spectrometry as detection tool for accurate mass analysis and tested the workflow on an in-house produced IgG1. In the last part of the work, we evaluated the coupling of the workflow with DMT-MM derivatization [3] for discrimination of sialic acid linkage position and were able to visualize low levels of sialylated N-glycans present in the same sample.

Methodology

100 µg of IgG1 were used to perform N-glycan release and labelling according to RapiFluor kit instructions. Clean-up step was performed with 5% AcCN in H_2O. After sample preparation, labelled and clean N-glycans were dried down and then resuspended in 10 uL of Peptide Diluent (included in the Peptide kit, 908 Devices) and loaded on an HR chip. For DMT-MM derivatization, 50 µL of 0.1 M DMT-MM in MeOH were added to the released and labelled N-glycans, incubated at 80 °C for 1 hour and dried down using a SpeedVac. CE separation was performed injecting 3 nL of the sample and applying a voltage of 500 V/cm. ZipChip Ti interface was hyphenated to an Orbitrap Exploris 240 (Thermo Scientific). MS acquisition was performed using the following settings; ion transfer tube 320 °C, Orbitrap resolution 60,000 (at 200 m/z), RF lens 55 %, scan range 600 – 2,000 m/z, normalized acquisition gain control 100% and maximum injection time 50 ms.

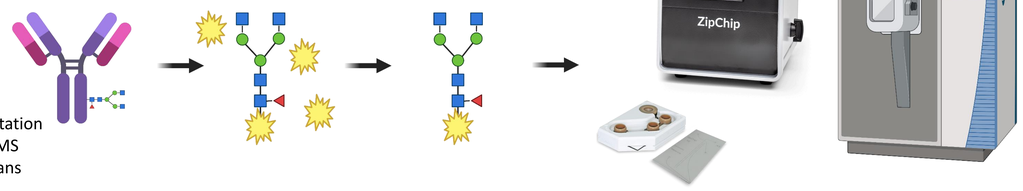


Figure 1: Schematic representation of the workflow used for CE-MS analysis of RF labelled N-glycans

Results

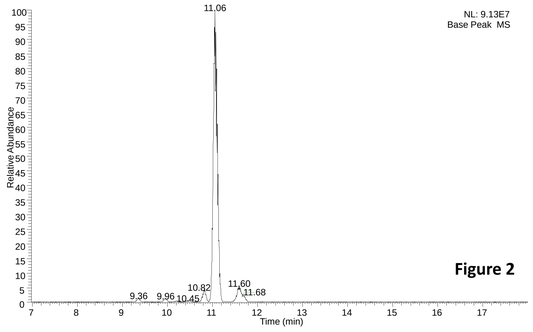
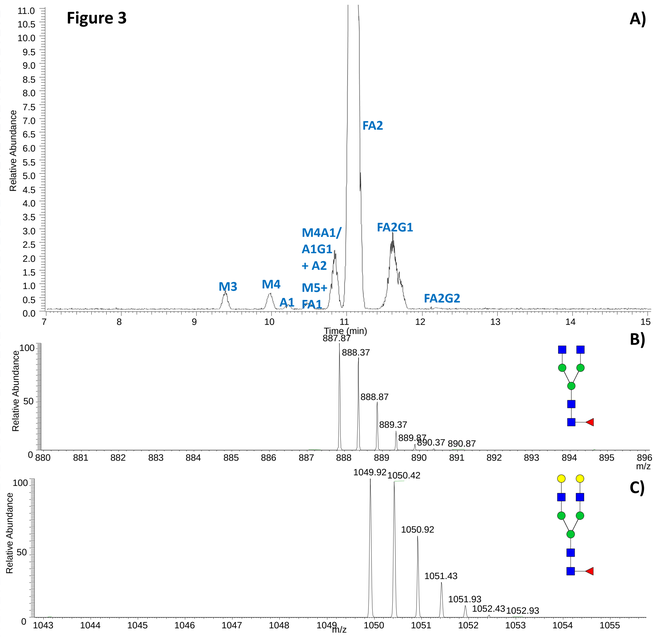


Figure 2

CE-MS analysis of released N-glycans from an in-house produced IgG1 was performed as described above. The resulting base peak chromatogram is displayed in Figure 2, indicating the prevalence of a single major glycoform and other minor N-glycans present in lower abundance. Although some N-glycans were observed to co-migrate (Figure 3A), high resolution accurate mass enables the correct identification of these low abundant species. In total, 17 N-glycans were detected and identified using a 20 minute method (Table 1). As an example, spectra obtained from the FA2 and FA2G2 species are displayed in Figure 3B and C, respectively.



In-house IgG1 -					
t_M (min)	Theoretical Mass (m/z)	Observed Mass (m/z)	Error (ppm)	Ion	Assignment
9.36	611.7512	611.7595	13.57	[M+2H]	M3
9.96	692.7906	692.7858	6.93	[M+2H]	M4
10.19	713.3039	713.3030	1.26	[M+2H]	A1
10.45	692.7906	692.7906	0.00	[M+2H]	M4
10.45	786.3328	786.3285	5.47	[M+2H]	FA1
10.45	773.8170	773.8128	5.43	[M+2H]	M5
10.63	794.3303	794.3268	4.41	[M+2H]	M4A1/A1G1
10.82	814.8435	814.8388	5.77	[M+2H]	A2
10.97	854.8434	854.8490	6.55	[M+2H]	M6
11.05	887.8725	887.8678	5.29	[M+2H]	FA2
11.32	895.8700	895.8657	4.80	[M+2H]	A2G1
11.48	935.8698	935.8658	4.27	[M+2H]	M7
11.60	786.3328	786.3277	6.49	[M+2H]	FA1
11.60	968.8989	968.8949	4.13	[M+2H]	FA2G1
	646.2684	646.2650	5.26	[M+3H]	
12.06	1016.8962	1016.8938	2.36	[M+2H]	M8
12.04	1070.4386	1070.4371	1.40	[M+2H]	FA3G1
12.17	1049.9253	1049.9219	3.24	[M+2H]	FA2G2
	700.2860	700.2843	2.43	[M+3H]	

Table 1: N-glycans obtained from the CE-MS analysis performed on an IgG1

To enhance the detection and separation of sialylated species, we performed DMT-MM derivatization on the released and labelled N-glycans to obtain information on the sialic acid linkage type as well as to neutralize the negative charge on the sialic acid, that may potentially interfere with the migration of those species.

Results presented in Table 2 showed the additional sialylated species detected after DMT-MM derivatization, while Figure 4 shows an example of the extracted ion chromatogram and associated spectrum for species α(2,3) FA2G1S1, showing the potential of this approach for the CE-MS analysis of sialylated N-glycans.

Figure 4

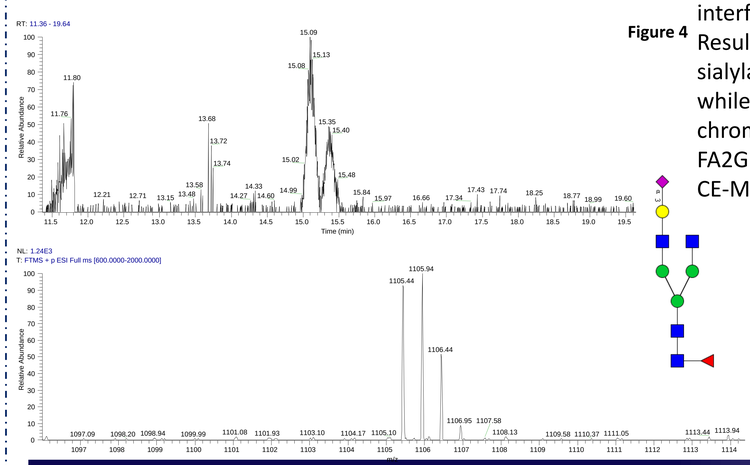


Table 2: DMT-MM derived N-glycans obtained from the CE-MS analysis performed on an IgG1

t_R (min)	Theoretical Mass (m/z)	Observed Mass (m/z)	Error	Ion	Assignment
15.09	1105.4413	1105.4401	1.09	[M+2H]	α(2,3) FA2G1S1
	737.2933	737.2954	2.85	[M+3H]	
15.40	1105.4413	1105.4409	0.36	[M+2H]	α(2,3) FA2G1'S1
	737.2933	737.2956	3.12	[M+3H]	
15.91	1186.4677	-	-	[M+2H]	α(2,3) FA2G2S1
	791.3143	791.3133	1.26	[M+3H]	

Conclusions and Future Work

CE-MS analysis of released N-glycans was achieved using a rapid sample preparation workflow and coupling this with rapid microfluidic CE-MS separation. Preliminary data obtained on an in-house produced IgG1 showed release N-glycans analysis was completed in less than one hour (excluding samples drying down time) and allowed the identification of 17 species. Moreover, to obtain critical information on sialic acid containing glycans, an additional derivatization with DMT-MM was performed to identify 2 additional species.

Future work will involve the validation of the method and evaluation of the quantitative aspect, as well as test the workflow on different classes of glycoproteins containing a wider range of N-glycoforms.

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[3] Tousi et al. Differential chemical derivatization integrated with chromatographic separation for analysis of isomeric sialylated N-glycans: a nano-hydrophilic interaction liquid chromatography-MS platform Anal Chem. 2013. 85(17):8421-8.