LC-MS/MS quantification of M254, a hyper-sialylated endogenous IgG biotherapeutic: analytical pitfalls and solutions

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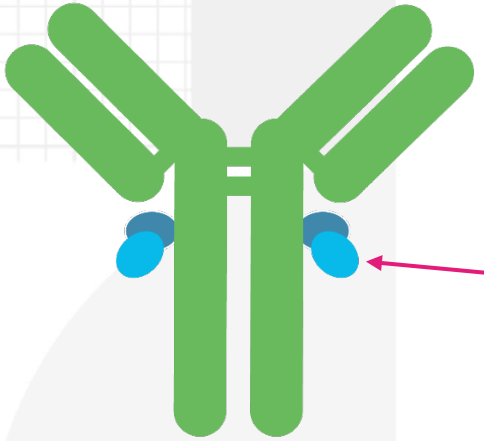
22 November 2019, EBF Open Symposium



MOMENTA

INTRODUCTION

Therapeutic immunoglobulins

- IVIg consists of immunoglobulins from thousands of donors
- Intravenous immunoglobulin (IVIg) is used in a wide variety of immunodeficiency and autoimmune diseases to complement or modulate the immune system of the patient
- In certain autoimmune diseases such as ITP, IVIg is given at doses up to 2000 mg/kg

(70 kg means 140 grams of IVIg!)

- M254 provides a more potent IVIg product
- Enzymatic modification of the glycosylation of immunoglobulins in commercial IVIg

The addition of sialic acid groups has shown increased potency of the IVIg product in pre-clinical studies

✓Possibly longer circulation by prevention of endocytosis and reduced proteolytic degradation

✓Enhancement of immunosuppression

- M254 is in clinical development for the treatment of Immune Thrombocytopenic Purpura (ITP)

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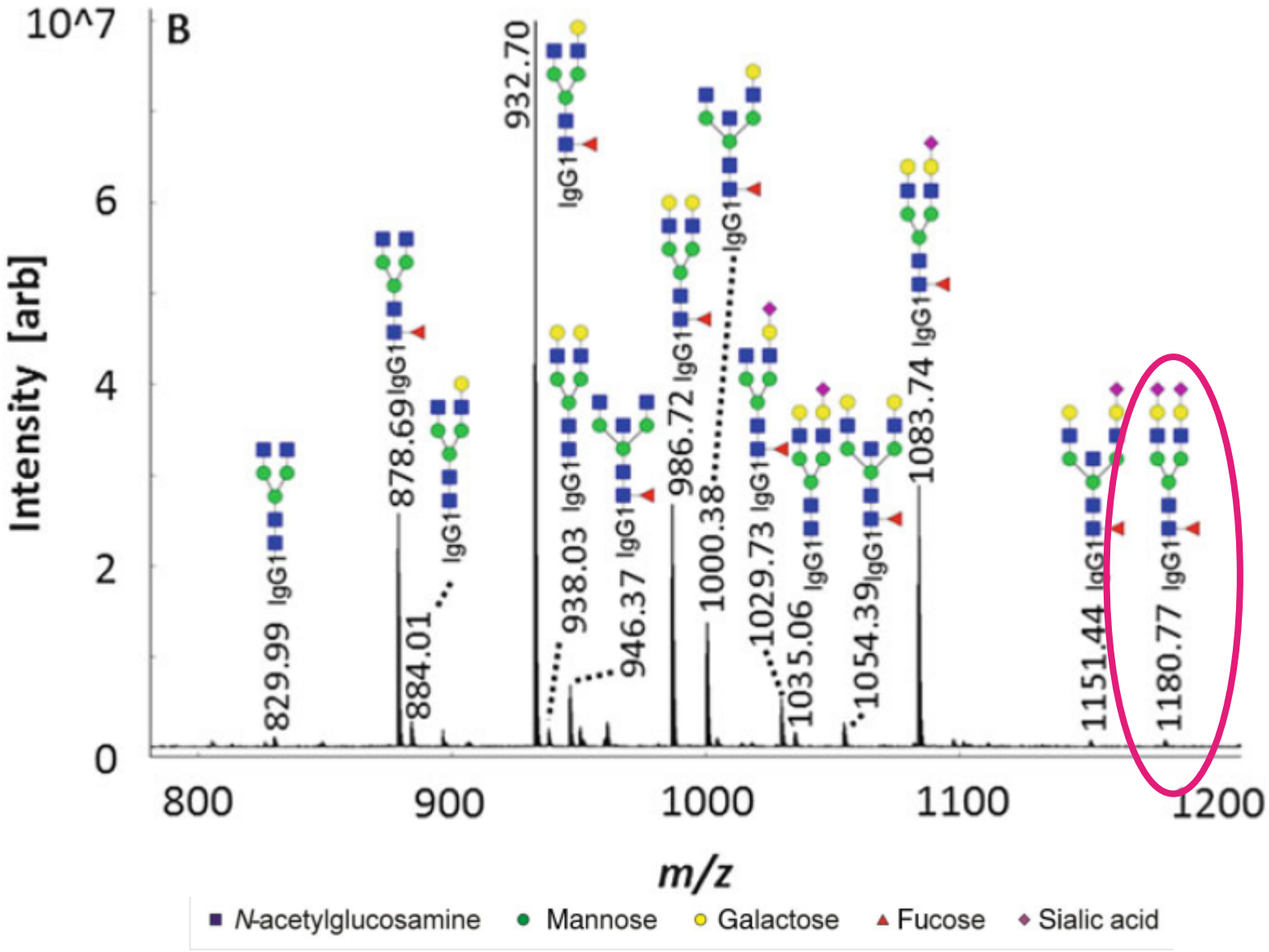
Washburn N, 2015. PNAS 112 (11) E1297-E1306
Buettner MJ, 2018. Front. Immunol. 9:2485.

INTRODUCTION

Distribution of glycoforms of IgG

Example of qualitative analysis of IgG1 glycoforms

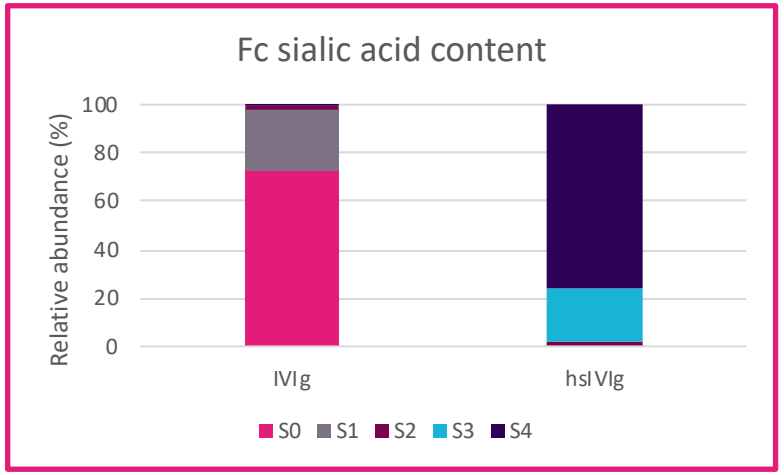


Falck et al. 2016 Methods in Molecular Biology, vol 1503. Humana Press, New York, NY

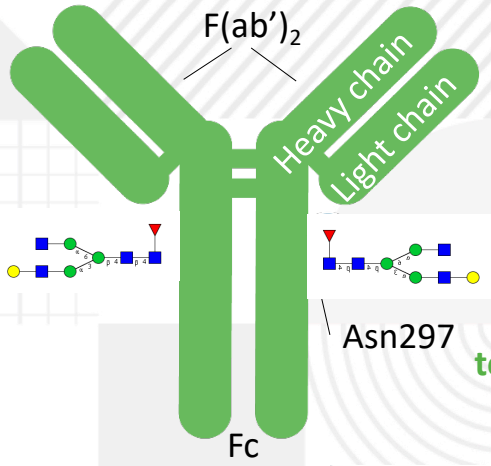
INTRODUCTION

M254 – What are we looking at?

IVIg (Intravenous immunoglobulin) is a **polyclonal mixture** of IgG highly purified from pooled human plasma of at least 1000 donors.



Most abundant "glycan pair" in IVIg

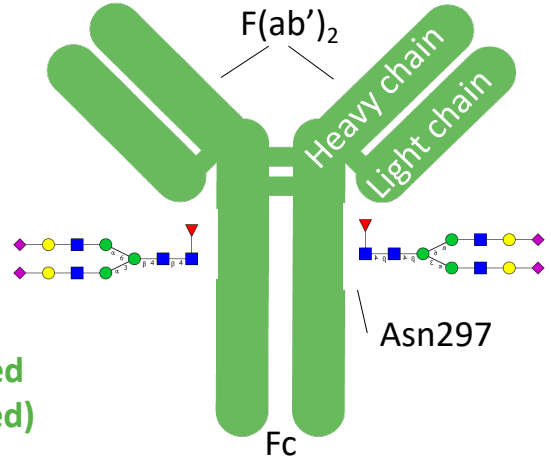


IVIg → In-vitro enzymatic process (B4GalT1, ST6Gal1) → **hsIVIg**

<<1% tetrasialylated

80-90% tetrasialylated (99% sialylated)

Most abundant "glycan pair" in M254

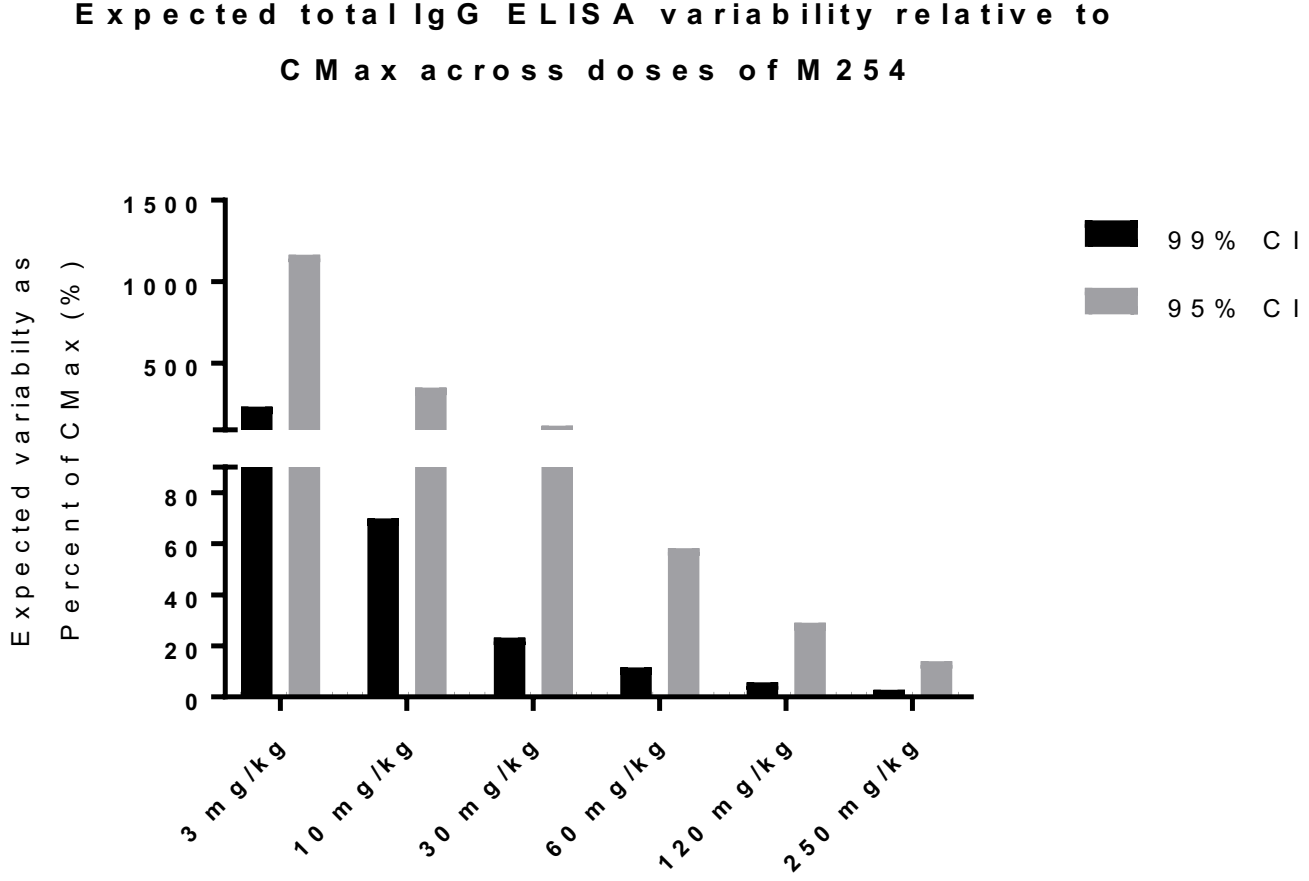


■ *N*-acetylglucosamine ● Mannose ○ Galactose ▲ Fucose ◆ Sialic acid

STANDARD APPROACH TO QUANTIFYING POLYCLONAL IG IS NOT SUITABLE FOR M254

Analytical challenges of M254

- Total IgG ELISA is the standard analytical approach for quantitation of polyclonal IgG mixtures such as IVIg or M254
- Doses of M254 are substantially lower than usual IVIg doses
- The three lowest doses would have expected errors of more than 20% of C_{max} even at the 99% confidence interval (CI).
- The doses selected for the phase I normal healthy volunteer study exclude ELISA as the approach for determination of PK.



Theoretical Cmax calculated based on dose and 70mL/kg blood volume.

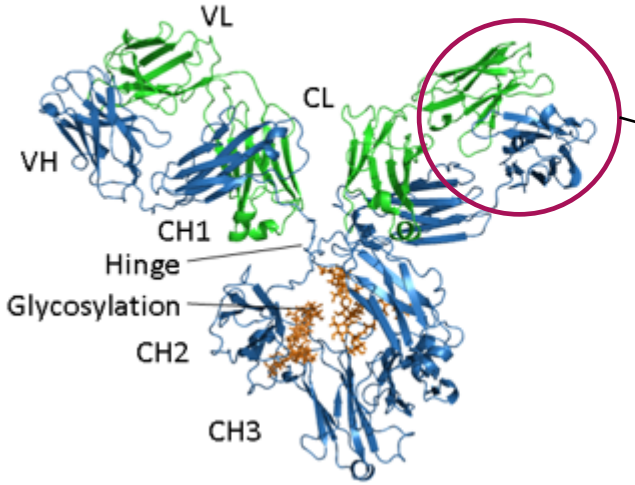
Expected variability for a given confidence interval was calculated based on 10mg/mL total IgG concentration.

ANALYTICAL CHALLENGES

IgG analysis by LC-MS/MS

Our approach for IgG analysis by LC-MS/MS:

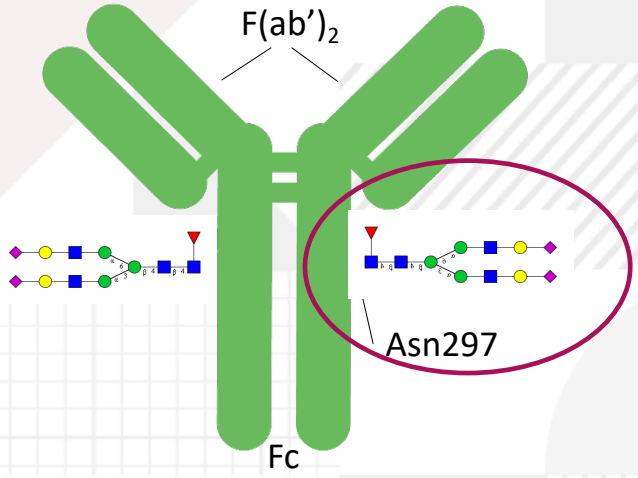
Typical approach for signature peptide selection for IgG analysis by LC-MS/MS:



From hypervariable region with complementarity determining regions (CDR's)

ANALYTICAL CHALLENGES

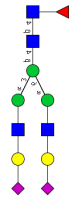
The approach for M254



- High endogenous IgG concentrations precludes a (total) IgG assay

- Selectivity needed for the hyper-sialylated form
  - Drug specific fragment → di-sialylated peptide

EEQYNSTYR

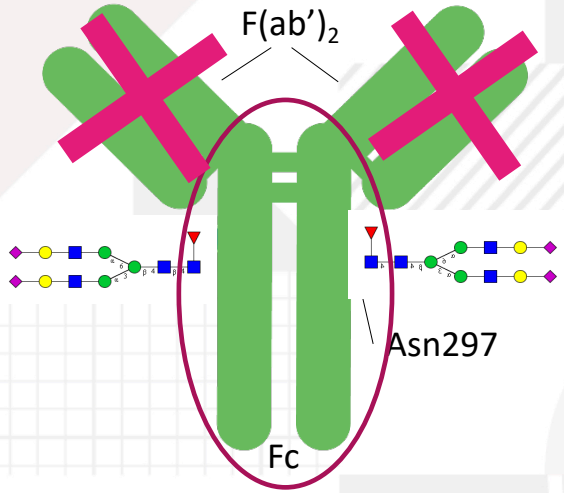


- Low and variable endogenous presence of di-sialylated peptide

- Relatively high expected analyte concentrations

THE M254 ANALYTICAL METHOD

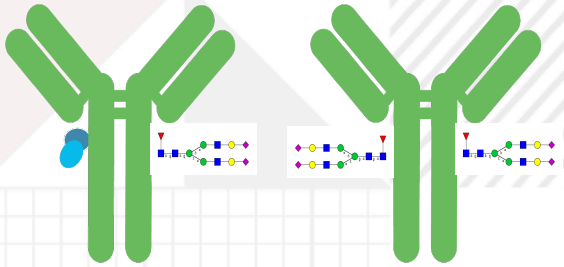
Summary



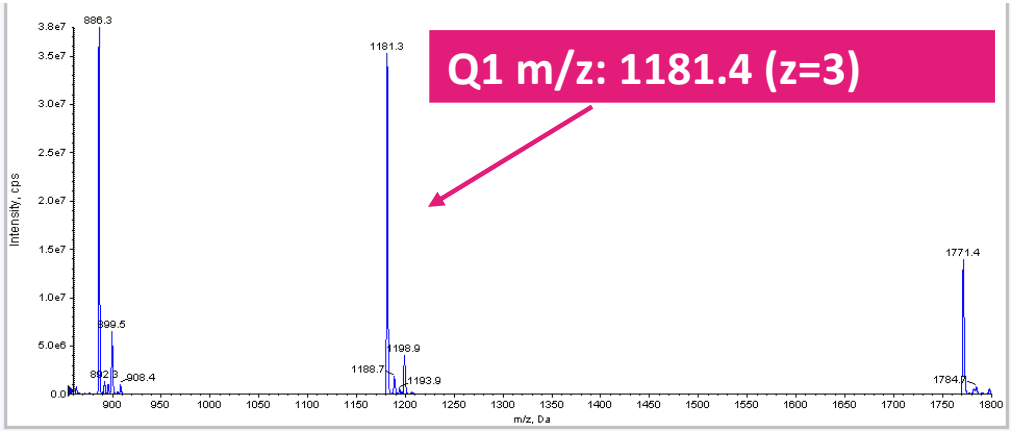
- Range: 5.00 – 5000 µg/mL
  Linear fit with $1/x^2$ weighing
- **Stable isotope labeled hyper-sialylated IgG1 Fc domain** as internal standard
  → digested together with analyte
- Sample preparation by **proteolytic digestion by trypsin**
- 25 µL sample volume
- Column: Acquity CSH C18 2.1 mm x 100 mm, 1.7 µm particles
- MS system: Sciex triple quad 6500
- Standards prepared in **proxy matrix** because of endogenous presence
  - 2% BSA in phosphate buffered saline

THE M254 ANALYTICAL METHOD

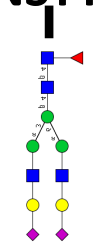
Mass Transitions

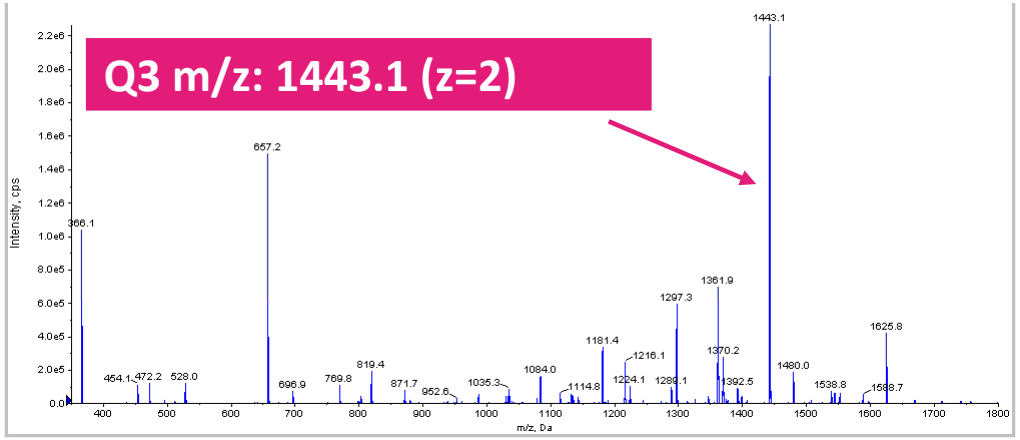


Quantitation of the di-sialylated N-glycopeptide EEQYNSTYR

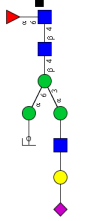


EEQYNSTYR





EEQYNSTYR



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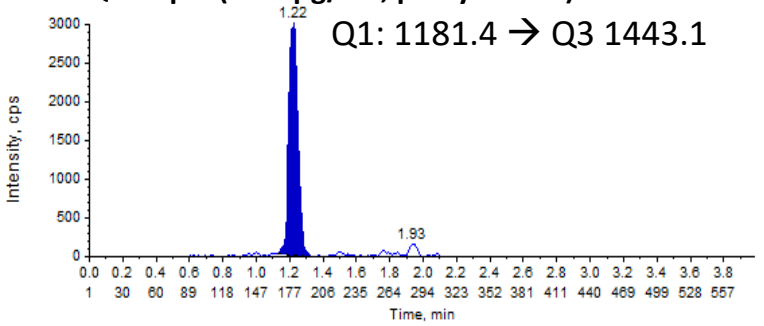
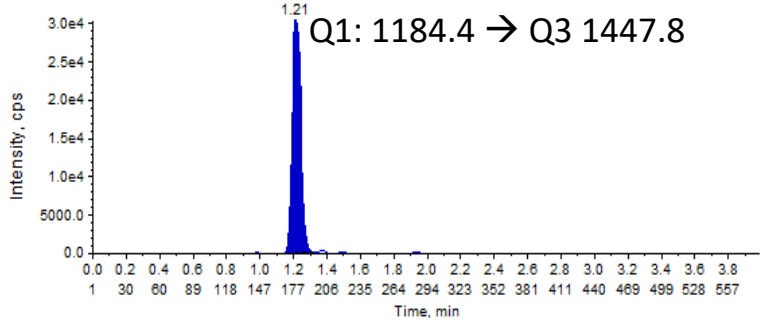
THE M254 ANALYTICAL METHOD

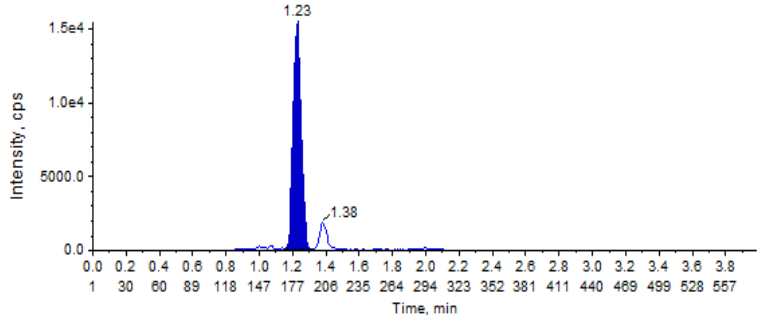
Chromatographic Method

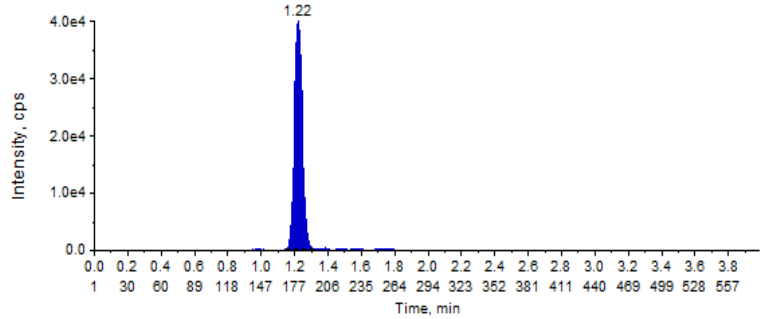
LLOQ sample (5.00 µg/mL, proxy matrix)



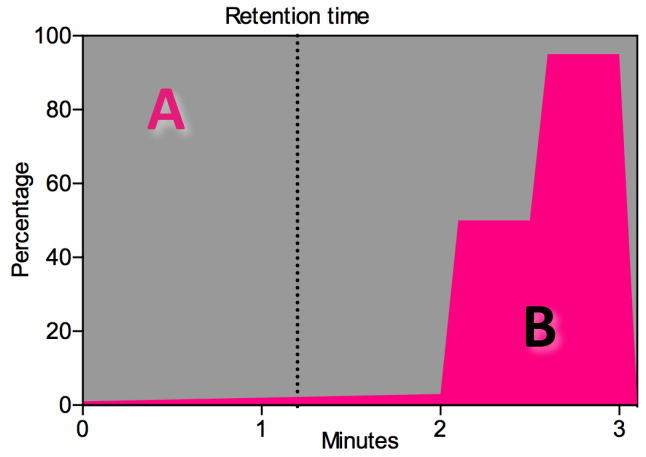


Plasma sample (20.7 µg/mL, endogenous)





Mobile Phase A	10% methanol in 0.1% formic acid in water
Mobile phase B	Acetonitrile



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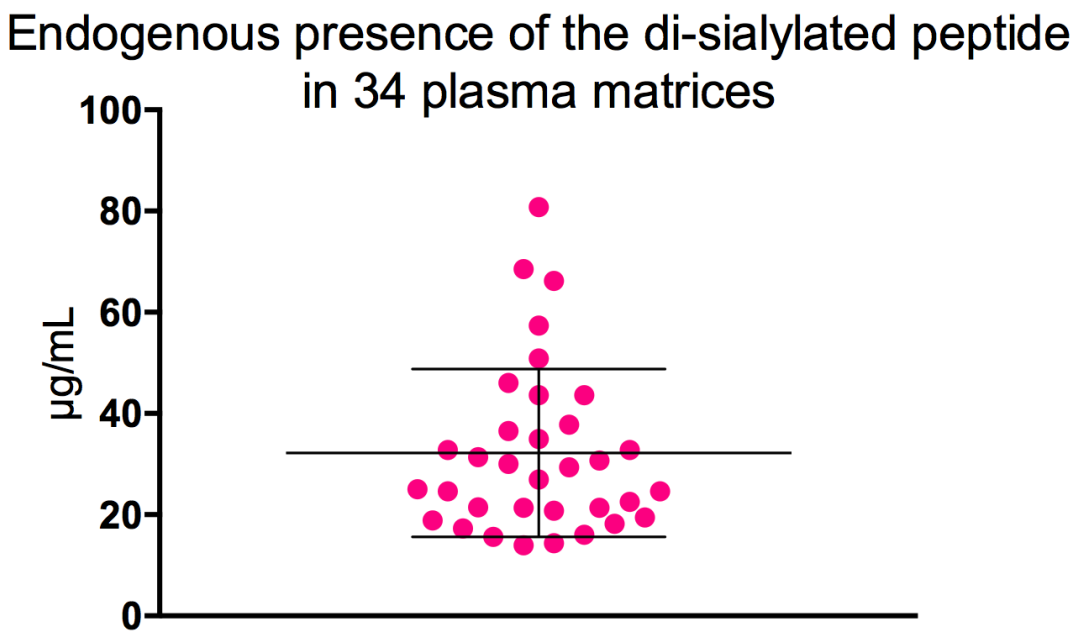
VALIDATION PARAMETERS AND RESULTS

Validation Approach

- Matrix suitability was assessed with 34 lots of commercially available normal human plasma.
- There is a wide spread of endogenous levels of disiaylated IgG1 in normal human plasma.
- Higher levels could result from either higher overall levels of IgG1 or higher overall sialylation.

Screening of several plasma lots to select suitable matrices



VALIDATION PARAMETERS AND RESULTS

Validation Approach

- Experiments that were not performed due to method constraints
  - Selectivity
  - Extraction recovery
  - Matrix Effect

- Screening of several plasma lots to select suitable matrices

- Preparation of validation samples
  - LLOQ samples in proxy matrix
  - Endogenous concentration in a selected matrix (3-10x LLOQ)
  - Higher levels are spiked to the selected matrix

- Endogenous concentration were established in the A&P for use as QC and stability samples
  - 3 runs, 6 repeats
  - QC samples are not fresh!

- Determination of stabilities
  - In endogenous and spiked plasma samples
  - In proxy matrix

- Acceptance criteria: 20.0-25.0%
  - Digestion step

VALIDATION PARAMETERS AND RESULTS

Summary

The following adaptations were necessary to validate with endogenous presence in plasma:

- Matrix variability experiment adapted (50.0 µg/mL added to endogenous plasma)
- A&P results used for establishment of endogenous concentrations to be used for stability assessments

Calibration range	5.00 – 5000 µg/mL
Accuracy (Within-Run Bias)	Ranged from -8.4% to 6.0%
Accuracy (Overall Bias)	Ranged from -6.5% to 2.1%
Precision (Total CV)	Less or equal to 18.7%
Robustness	174 study samples per run
Dilution	2-fold dilution and quantification up to 10000 µg/mL is valid
Matrix Variability/Selectivity	Within criteria
Carry-over	< 20.0% of LLOQ response
Reinjection Reproducibility	126 hours at +10°C in processed sample
Autosampler Stability	118 hours at +10°C in processed sample
Stability in Whole Blood	2 hours at 0°C and at room temperature
<u>Stabilities in plasma and proxy matrix</u>	
Storage Stability	23 hours at room temperature; 183 days at -20°C and at -70°C.
Freeze/thaw Stability	3 cycles at -20°C and at -70°C

VALIDATION PARAMETERS AND RESULTS

Accuracy and Precision, establishment

Validation levels:

- 5 µg/mL (LLOQ, in proxy matrix)
- Endogenous (to be established)
- Endogenous + 250 µg/mL
- Endogenous + 4000 µg/mL

Establishment of endogenous concentration at 18.9 µg/mL

Run	x1	x2	x3	x4	x5	x6	Mean	SD	CV (%)	Within Run Bias (%)
				µg/mL						
1	18.3	18.5	18.1	17.1	17.5	18.6	18.0	0.6	3.2	-4.6
2	18.0	19.1	18.0	19.6	31.8	21.2	21.3	5.3	25.0	12.6
3	17.4	17.1	17.3	17.1	18.3	16.9	17.3	0.5	2.8	-8.4

Overall Statistics	
Mean	18.9
SD	3.4
CV (%)	18.1

Incidental lower IS response

VALIDATION PARAMETERS AND RESULTS

Accuracy and Precision, establishment

Validation levels:

- 5 µg/mL (LLOQ, in proxy matrix)
- Endogenous (to be established)
- Endogenous + 250 µg/mL
- Endogenous + 4000 µg/mL

- Establishment of endogenous concentration at 18.9 µg/mL in 3 runs
- 4th A&P run was performed

Run	x1	x2	x3	x4	x5	x6	Mean	SD	CV (%)	Within Run Bias (%)
				µg/mL						
1	18.3	18.5	18.1	17.1	17.5	18.6	18.0	0.6	3.2	-4.6
2	18.0	19.1	18.0	19.6	31.8	21.2	21.3	5.3	25.0	12.6
3	17.4	17.1	17.3	17.1	18.3	16.9	17.3	0.5	2.8	-8.4
4	17.4	17.3	18.6	18.8	17.1	16.8	17.7	0.8	4.6	-7.0

Overall Statistics	
Mean	18.6
SD	3.0
CV (%)	16.1
Bias (%)	1.7
Accuracy (%)	98.3

After successful A&P, establishment of endogenous concentration is performed in a single run in 6-fold

VALIDATION PARAMETERS AND RESULTS

Matrix Variability / Selectivity

- 6 independent matrix lots were spiked with 10x LLOQ (50.0 µg/mL)
- Unspiked and spiked sample were analyzed to determine the selectivity and matrix variability
- Absolute mean bias should be ≤20.0%

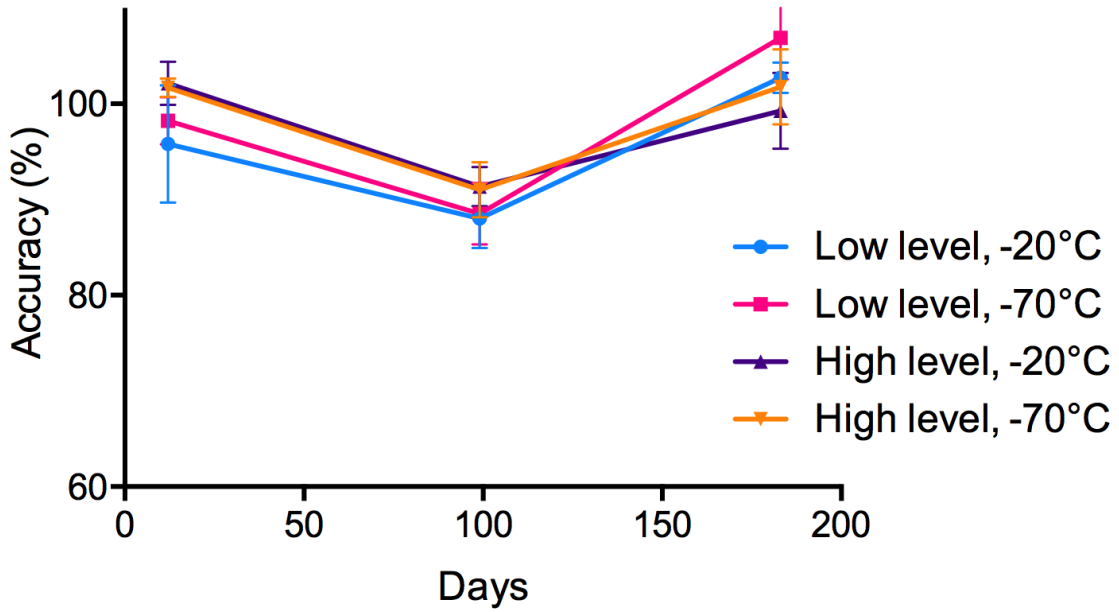
Matrix	Endogenous concentration (µg/mL)	Theoretical concentration (µg/mL)	Observed concentration (µg/mL)	Bias (%)
1	42.8	92.8	85.0	-8.3
2	37.9	87.9	87.7	-0.2
3	18.5	68.5	64.0	-6.7
4	18.4	68.4	62.0	-9.4
5	14.6	64.6	60.8	-5.8
6	24.4	74.4	71.6	-3.8
			Mean bias (%)	**-5.7**

VALIDATION PARAMETERS AND RESULTS

Stability assessment

Long-term frozen stability in plasma

Nominal Concentration Low Level (endogenous)	18.9 µg/mL
Nominal Concentration High Level	4020 µg/mL



CONCLUSIONS

- LC-MS/MS analysis of the polyclonal IVIg product M254 is possible because of the specific glycoform

- The endogenous presence of the peptide required a biomarker-like approach for method development and validation
  - Consideration of different proxy matrices
  - Establishment of endogenous concentrations
  - Incorporation of the endogenous presence in the experiments (A&P, stability assessments and matrix variability)

- Successful application for the PK in the M254 clinical study

MOMENTA

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