Incorporating Stable Isotope-Labeled IgG Internal Standard and Affinity Purification for Analyzing Human IgG4 and Fc-glycan Profiles by UHPLC-MS/MS

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Introduction

It is reported that IgG Fc-glycosylation is distinct between patients with autoimmune pancreatitis (AIP) and pancreatic ductal adenocarcinoma (PDAC), which has the potential to be used to benefit differential diagnosis in clinical application. Since type I AIP is categorized as IgG4 related disease (IgG4-RD), we aimed to purify IgG4 from human serum and focused on analyzing the Fc-glycosylation profiles. The purification of IgG4 can also solve the issue of isobaric Fc-glycopeptides from human IgG3 and IgG4 of Asia population.

Methods

- Purification : CaptureSelect Human IgG4
- Target serum volume: 10 µL
- Trypsin digestion
  (1)DTT (Dithiothreitol)
  (2)IAA (Iodoacetamide)
  (3)Trypsin digestion
- UHPLC: Waters ACQUITY UPLC system
- Mass spectrometry : Xevo TQ-XS triple quadruple mass spectrometry
- Column: Kinetex C18, 2.1 x 50 mm

Compound name	Precursor (m/z)	Product [a] (m/z)	Cone (V)	Collision (V)
IgG4	951.5	850.4	35	34
		1293.6		
H3N3F1-IgG4	805.7	204.1	35	25
		366.1		
H3N4F1-IgG4	873.4	204.1	35	25
		366.1		
H4N4-IgG4	878.7	204.1	35	25
		366.1		
H3N5-IgG4	892.4	204.1	35	25
		366.1		
H4N4F1-IgG4	927.4	204.1	35	25
		366.1		
H3N5F1-IgG4	941.0	204.1	35	25
		366.1		
H4N5-IgG4	946.4	204.1	35	25
		366.1		
H5N4F1-IgG4	981.4	204.1	35	25
		366.1		

Compound name	Precursor (m/z)	Product [a] (m/z)	Cone (V)	Collision (V)
H4N5F1-IgG4	995.1	204.1	35	25
		366.1		
H4N4F1S1-IgG4	1024.4	204.1	35	25
		366.1		
H5N4S1-IgG4	1029.7	204.1	35	25
		366.1		
H4N5S1-IgG4	1043.4	204.1	35	25
		366.1		
H5N5F1-IgG4	1049.1	204.1	35	25
		366.1		
H5N4F1S1-IgG4	1078.4	204.1	35	25
		366.1		
H4N5F1S1-IgG4	1092.1	204.1	35	25
		366.1		
H5N5S1-IgG4	1097.4	204.1	35	25
		366.1		
H5N4S2-IgG4	1126.8	204.1	35	25
		366.1		
H5N5F1S1-IgG4	1146.1	204.1	35	25
		366.1		

Results

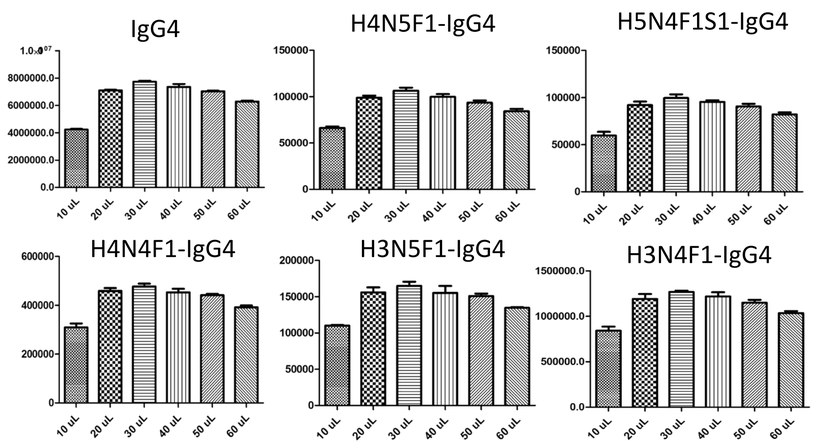

Figure 1. Optimization of affinity bead volume

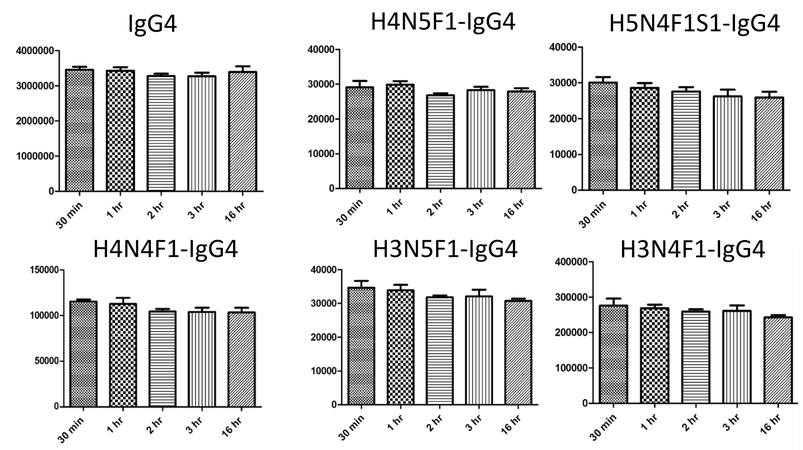

Figure 2. Optimization of incubation time

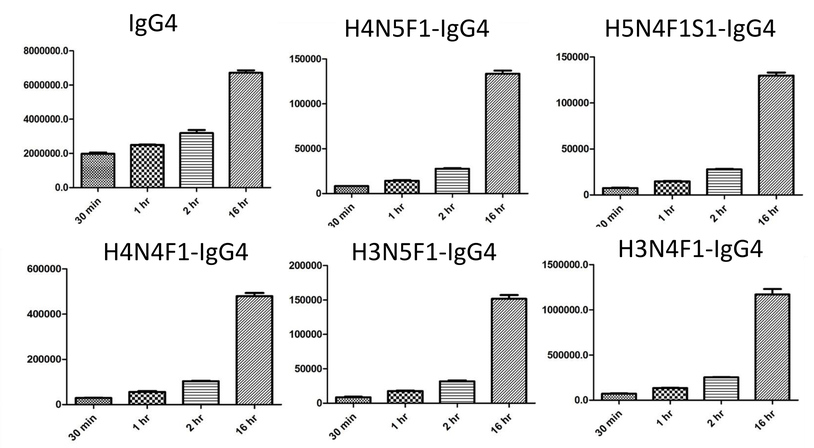

Figure 3. Optimization of on-bead digestion time

Table. Part of validation results

Compound name	Conce. (µg µL^{-1})	Intra-day Precision (RSD, %)	Intra-day Accuracy (%Rec)	Inter-day Precision (RSD, %)	Inter-day Accuracy (%Rec)
IgG4	0.14	4.8	101.8±5.0	3.9	100.7±4.1
	0.41	3.0	101.5±3.0	2.7	100.5±3.0
	3.30	2.8	100.6±2.8	3.0	99.8±2.9
	6.60	1.6	95.7±1.5	1.3	96.8±1.9

Calibration curves ($y = ax^2 + bx + c$)					
Target	Range (µg µL^{-1})	a	b	Intercept	r
IgG4	0.14~8.80	-0.0228	7.2679	0.155	0.999

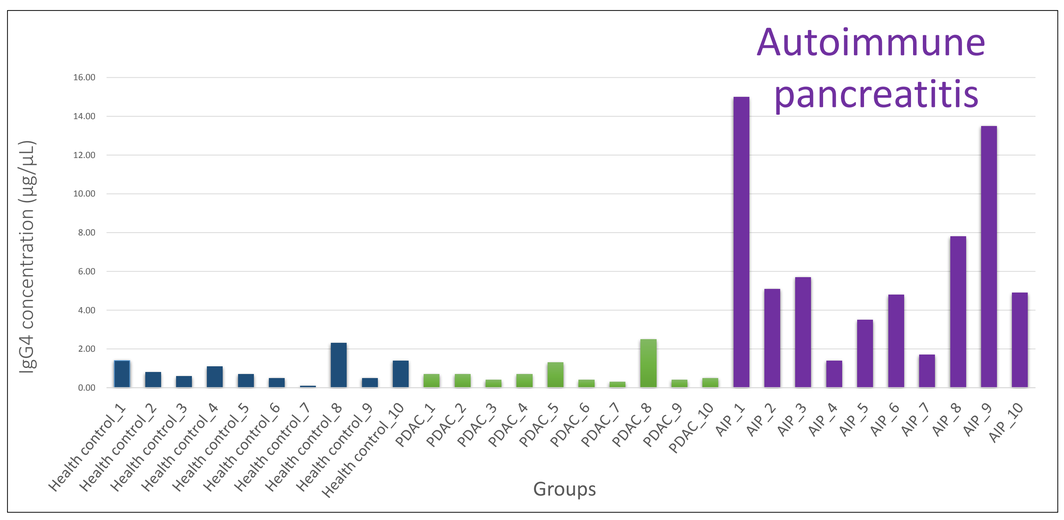

Figure 4. Clinical applications

Conclusions

IgG4 quantification and the monitoring of Fc-glycan profiles can be achieved efficiently by using UHPLC-MS/MS with MRM mode. And the developed workflow is suitable for clinical applications.

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