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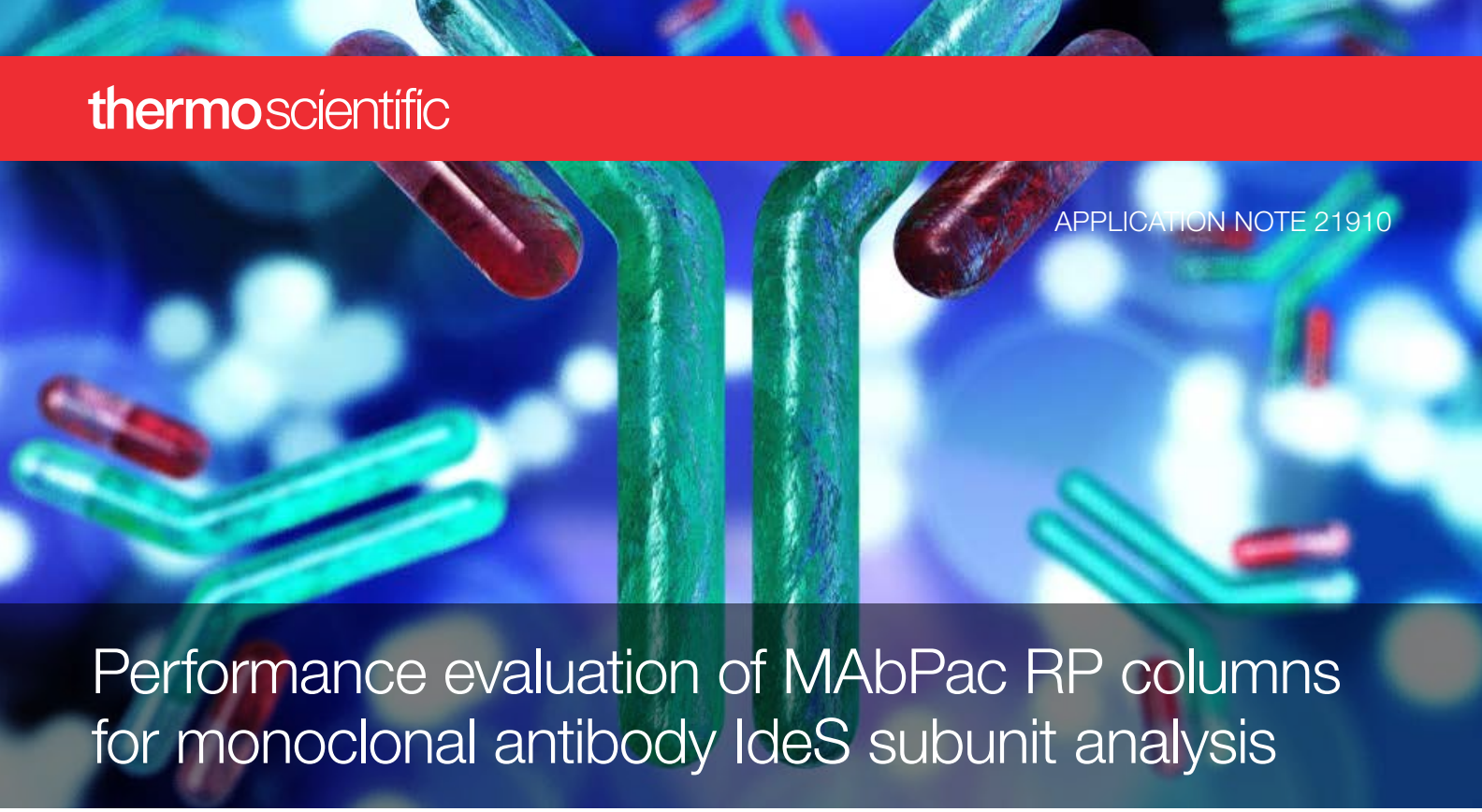

APPLICATION NOTE 21910

Performance evaluation of MAbPac RP columns for monoclonal antibody IdeS subunit analysis

Authors

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Keywords

NIBRT, biopharmaceutical, biotherapeutic, monoclonal antibody (mAb), IgG, middle-up, MAbPac RP columns, Vanquish Flex Binary UHPLC system, Vanquish Horizon UHPLC, IdeS, trastuzumab

Application benefits

- Exceptionally high efficiency, high resolution for separation of mAb fragments
- Fast analysis
- Ideal for stability and QA/QC testing

Goal

To demonstrate the applicability of middle-up techniques for biotherapeutics characterization. To show fast, efficient separation using reversed-phase chromatography with Thermo Scientific™ MAbPac™ RP columns for the characterization of mAb variants. To demonstrate the effect of column i.d. on the resolution, loading capacity, mobile phase composition, and ease of use.

Introduction

Monoclonal antibody (mAb) therapeutics are a promising class of therapeutic agents for the treatment of autoimmune disease or cancers. Therapeutic mAbs are mostly produced by mammalian cells and are heterogeneous due to post-translational modifications.

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A suitable way of evaluating mAb heterogeneity and simplifying the analysis is to analyze the mAb variants and locate the modifications through characterization of mAb fragments. The use of a middle-up approach has the potential to minimize sample handling and artefacts, while providing quicker and confirmatory information with respect to more widely used techniques, such as peptide mapping.

Considering their inherent structural complexity there is a need to develop analytical methods to guarantee mAb purity, and to evaluate aggregates, variants, and batch-to-batch reproducibility. Recent advances in mass spectrometry technologies have facilitated the characterization of biotherapeutics by employing reversed-phase liquid chromatography (RPLC) coupled with high-resolution mass spectrometry as one of the most informative analytical tools for the analysis of intact mAb and their associated subunits.[1,2]

In the last years some significant advances were made in terms of RPLC column technology for mAbs characterization, such as the MAbPac RP columns, which are based on supermacroporous 4 µm polymer particles that are stable at extreme pH (0–14) and high temperature (up to 110 °C). The large pore size polymeric resin enables efficient separation of protein molecules with very low carryover.

In the current study, fast separation methods are presented for variants of trastuzumab subunits using two versions of the MAbPac RP column, 1.0 mm and 2.1 mm i.d. formats with 100 mm length. This was performed using liquid chromatography coupled to UV detection (LC-UV) and also coupled to high-resolution mass spectrometry (LC-HRMS). Samples were digested with IdeS protease cleaving the monoclonal antibody in the hinge region and generating, after reduction of disulfide bonds, two pairs of polypeptides from the heavy chain (Fd' and scFc) along with the light chain portion.

All analyses were performed on ultra-high-resolution analytical platforms consisting of a Thermo Scientific™ Vanquish™ Flex quaternary or a Thermo Scientific™ Vanquish™ Horizon binary UHPLC hyphenated with a variable wavelength UV detector. This demonstrates the differences in low-pressure gradient (LPG) mixing and high-pressure gradient (HPG) mixing at low flow rates. The fast LC-UV approach described here can be generally applied to any mAb characterization.

Experimental

Recommended consumables

- Deionized water, 18.2 MΩ/cm resistivity
- Water, Optima™ LC/MS grade (Fisher Chemical) (P/N 10505904)
- Water with 0.1% formic acid (v/v), Optima™ LC/MS grade (Fisher Chemical) (P/N 10188164)
- Acetonitrile with 0.1% formic acid (v/v), Optima™ LC/MS grade (Fisher Chemical) (P/N 10118464)
- FabRICATOR® (Genovis) (P/N A0-FR1-020)
- MAbPac RP, 4 µm, 1.0 × 100 mm column (P/N 303183)
- MAbPac RP, 4 µm, 2.1 × 100 mm column (P/N 088647)
- Thermo Scientific™ Virtuoso™ vial, clear 2 mL kit with septa and cap (P/N 60180-VT405)
- Thermo Scientific™ Virtuoso™ Vial Identification System (P/N 60180-VT100)
- Thermo Scientific™ Bond-Breaker™ TCEP Solution, Neutral pH (P/N 77720)

Sample handling equipment

Vanquish Flex Binary Flex UHPLC system including:

- Quaternary Pump (P/N VF-P20-A)
- Column Compartment H (P/N VH-C10-A)
- Split Sampler FT (P/N VF-A10-A)
- Variable Wavelength Detector F (P/N VF-D40-A)
- Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (P/N IQLAAEGAAPFALGMBDK)
- System Base Vanquish Horizon (P/N VH-S01-A)

Vanquish Horizon UHPLC system including:

- Binary Pump H (P/N VH-P10-A)
- Column Compartment H (P/N VH-C10-A)
- Split Sampler HT (P/N VH-A10-A)
- Variable Wavelength Detector F (P/N VF-D40-A)

Middle-up analysis of IdeS-digested mAb

First, 40 µg of mAb were diluted with 48 µL of PBS, then combined with 0.5 µL of the FabRICATOR enzymatic digestion solution (67 units IdeS/µL in Optima grade water), and incubated at 37 °C for 2 hours at 500 rpm. Disulfide bonds were reduced by the addition of 60 µL of 8 M guanidine HCl and 10 µL of 500 mM TCEP. The solution was incubated for 45 minutes at 56 °C. Following incubation, samples were reduced to dryness *via* vacuum centrifugation and reconstituted in water with 0.1% formic acid prior to LC-UV analysis.

LC conditions

Middle-up

Mobile phase A: H_2O/FA (99.9:0.1 v/v)
Mobile phase B: Acetonitrile/H_2O/FA (90:9.9:0.1 v/v/v)
Column temperature: 80 °C (active pre-heater) (Still air)
Autosampler temp.: 5 °C
Injection volume: 1 µL
Injection wash solvent: Methanol:water, 10:90
Needle wash: Enabled pre-injection
Gradient: See Table 1 for details

Table 1. Mobile phase gradient for UHPLC separation of peptides

Time (min)	Flow rate 2.1 mm i.d. (µL/min)	Flow rate 1.0 mm i.d. (µL/min)	%A	%B	Curve
0	0.300	0.150	80	25	5
1	0.300	0.150	80	25	5
16	0.300	0.150	80	45	5
17	0.300	0.150	55	45	5
18	0.300	0.150	80	25	5
25	0.300	0.150	80	25	5

MS conditions

Table 2. Summary of tune parameters

MS source parameters	Setting for middle-up analysis
Source	HESI
Sheath gas flow rate	25
Auxiliary gas flow	10
Probe heater temperature	150 °C
Source voltage	3.8 kV
Capillary temperature	320 °C
S-lens RF voltage	60.0

Table 3. Summary of MS parameters

General	Setting middle-up analysis
Run time	0 to 25 min
Polarity	Positive
Full MS parameters	
Full MS mass range	600–2400 *m/z*
Protein mode	On
Resolution Settings	140,000
AGC target value	3e6
Max injection time	200 ms
SID	0.0 eV
Microscans	5

MS data analysis

Detailed parameter settings are shown in Table 4.

Table 4. Thermo Scientific™ Biopharma Finder™ 3.0 software parameter settings for analysis of IdeS subunits. Default Thermo Scientific™ Xtract – Average over selected region method used.

Component detection	
Output mass range	20,000 to 30,000
Output mass	M
S/N threshold	3.00
Rel. abundance threshold	0.00
Charge range	5 to 50
Min. num detected charge	3
Isotope table	Protein
Fit factor (%)	80
Remainder threshold (%)	25
Consider overlap	Yes
Resolution at 400 *m/z*	Raw File Specific
Charge carrier	H^+ (1.00727663)
Minimum intensity	1
Expected intensity error	3
m/z range	600.00 to 2,400.00
Chromatogram trace type	TIC
Sensitivity	High
Rel. intensity threshold (%)	1
Identification	
Sequence matching mass tolerance	20.00 ppm
Mass tolerance	10.00 ppm
RT tolerance	1.000 minutes
Min. number of required occurrences	1

Results and discussion

Separation of mAb fragments variants

The column dimension can affect the resolution, sensitivity, loading capacity, efficiency, and time of analysis. The column with a 2.1 mm i.d. requires higher flow rates and higher amounts of sample. Scaling down the column diameter to 1.0 mm i.d. reduces the flow rate, volume of mobile phase, and sample amount without increasing the retention time, if the linear velocity is the same for both columns. However, the normal low running flow rates of 1 mm columns can be challenging to achieve with standard UHPLC systems. Flow rates as low as 50 µL/min increases the problems seen with dispersion, often requiring a reduction in tubing internal diameter. Low flow rates can also add considerable delay times in the gradient reaching the column, significantly increasing run times.

In this study, the effect of column diameter on resolution, peak shape and loading capacity were evaluated employing MAbPac RP, 4 µm columns (2.1 × 100 mm and 1 × 100 mm) for analysis of monoclonal antibodies (mAb) subunits. The flow rates on the 1 mm column were optimized for use on the Vanquish systems without the need to modify the systems.

To optimize the UHPLC–UV method for separation of mAb subunits, different starting percentages of the organic eluent were evaluated. The shallower gradient of 1.33% B/minute from 25 to 45% B was chosen [a] because it provided better resolution and shorter run times when compared to the 1.66% B/minute from 20 to 45% B gradient.

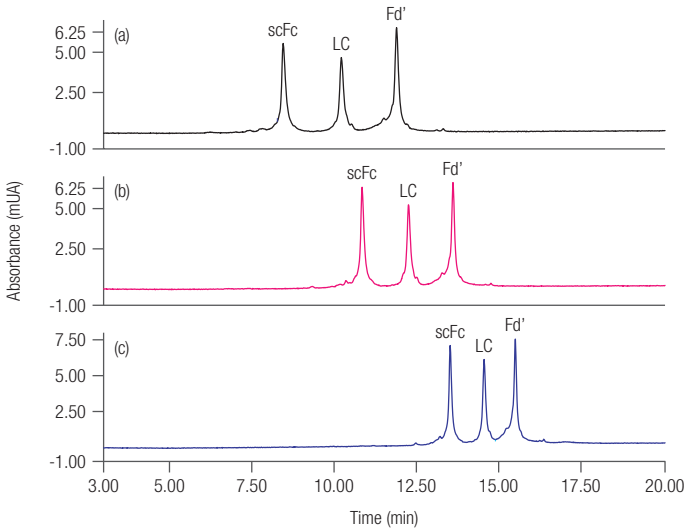


Figure 1. LC-UV separation of the three subunits obtained after IdeS digestion and separated on a MAbPac RP 4 µm, analytical column, 2.1 × 100 mm, under different gradient conditions. The top trace (a) shows the LC-UV separation employing a gradient range 25%–45% mobile phase B, (b) for the middle trace the range utilized is 20%–45% mobile phase B and (c) the bottom trace uses a gradient of 10%–45% mobile phase B.

When columns with different diameters are compared it is important to take into account several parameters such as the dimensions, the dead volume of the system, and linear velocity in order to optimize column performances. These are significant factors that can affect the retention time and peak shape when narrow columns are used. The system Gradient Delay Volume (GDV) shifts the gradient onset, and its effect is more pronounced when narrow columns at low flow rate are used. The mixer, placed between the pump and the injector device, plays an important role in determining the GDV. The system dispersion depends on the geometry of the flow path and, if not optimized, will affect peak width and peak shape especially with the narrow columns. Two millimeter columns have a flow rate that is well within the flow rate range of standard UHPLC systems. One millimeter columns have positive advantages in sensitivity and reduced solvent consumption but normally require flow rates as low as 50 µL/min. This is on the edge of the optimized abilities of a standard UHPLC system. To remedy this, we used a higher flow of 150 µL/min for the 1 mm column to reduce dispersion and delay problems. Doing this, the UHPLC systems did not have to be optimized further for 1 mm column use.

Different types of UHPLC systems were evaluated for acquiring the data to show these effects in Figure 2. The Vanquish Horizon system contains an advanced high-pressure mixing gradient pump, which has low gradient delay and high precision gradient flow control of the pumps. Using this system, the 1 mm column shows reduced retention times compared to the 2.1 mm column due to the higher linear flow rate of 150 µL/min chosen for the 1 mm column combined with the low gradient delay of the Vanquish Horizon system. The LPG mixing pump in the Vanquish Flex system has a much larger gradient delay volume due to mixing before the pump head. This is apparent in Figure 2 with the 1 mm column displaying higher retention times than the 2.1 mm column despite the higher linear flow rate used. The increased time delay in the gradient reaching the column at the lower flow of 150 µL/min compared to 300 µL/min for the 2.1 mm column, overcomes the effect of the faster comparable flow rate used. Both systems however give very good results with both columns in terms of resolution and peak shape.

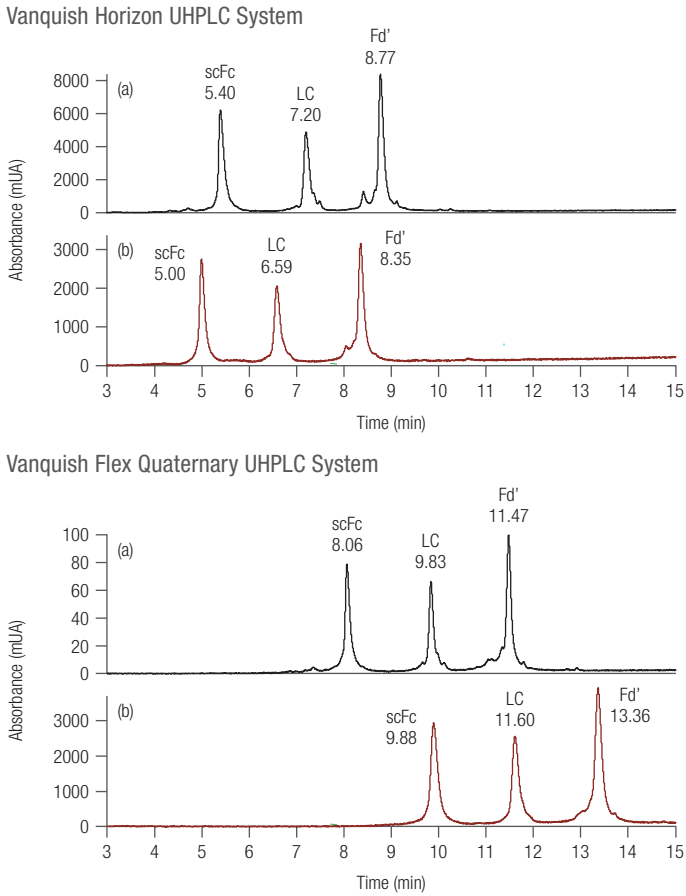
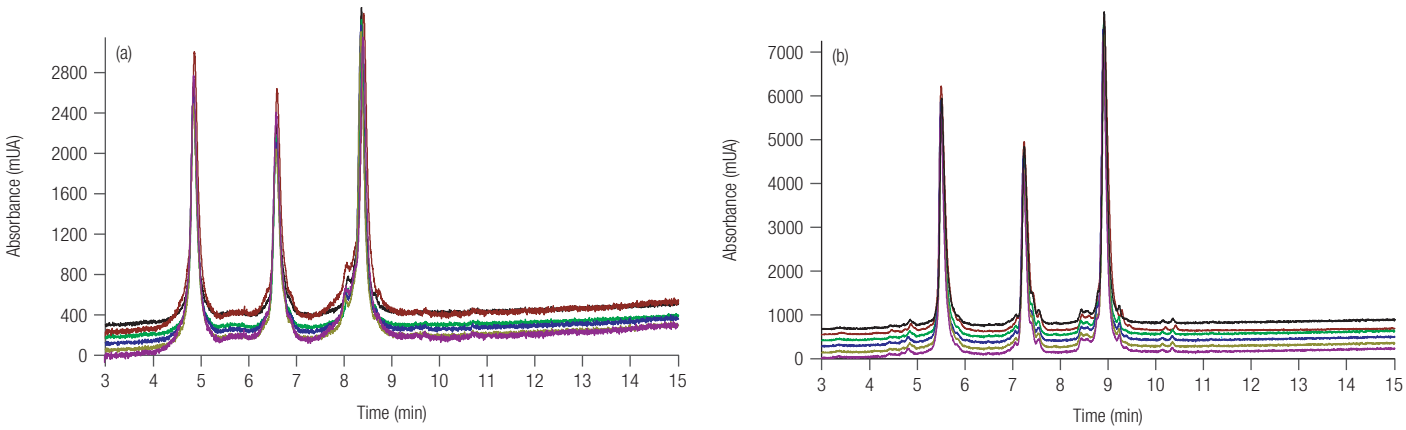


Figure 2. Chromatograms obtained with MAbPac RP, 4 µm, 2 × 100 mm column (a) and MAbPac RP, 4 µm, 1 × 100 mm column (b) for separation of trastuzumab fragments obtained after enzymatic digestion

Figure 3 shows the chromatograms for six replicates of trastuzumab fragment analysis with both Vanquish systems. The reproducibility of retention time and other peak parameters are clear; when checking RSD values (%) for retention time, they are in a range lower than 0.20 for the 1 mm column and 0.15 for the 2.1 mm column (Table 5). The high-pressure mixing pump of the Horizon system returns better RSD values on retention time for the lower flow rate 1 mm column. However, both Vanquish Flex and Vanquish Horizon systems have excellent performances for this analysis.

As for the values of peak width (50%), asymmetry, and resolution (Table 5), both columns were similar when the Vanquish Horizon system was utilized. On the Vanquish Flex system the peaks were narrower on the MAbPac RP column, 2.1 × 100 mm, than on the MAbPac RP column, 1.0 × 100 mm, which is due to the increased dwell volumes of this system that can influence mobile phase composition and dispersion of the proteins to be separated.

Vanquish Horizon UHPLC System



Vanquish Flex Quaternary UHPLC System

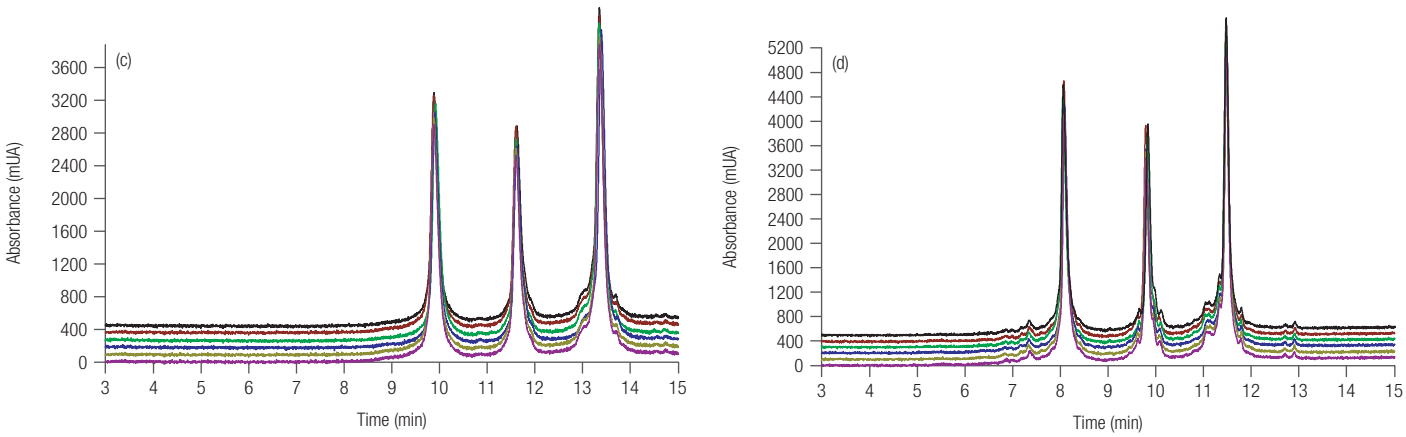


Figure 3. Chromatograms obtained with MAbPac RP, 4 µm, 2.1 × 100 mm (a and c) and MAbPac RP, 4 µm, 1 × 100 mm (b and d) columns for separation of trastuzumab fragments obtained after enzymatic digestion

In terms of retention time, peak shape, and resolution, both columns show similar performance on both Vanquish systems. The two different column formats however meet different analytical needs for this application. The MAbPac RP, 4 µm, 1 × 100 mm column provides lower consumption of mobile phase and sample with an increase in absolute sensitivity. On the other hand, the MAbPac RP, 4 µm, 2.1 × 100 mm column requires higher flow rates resulting in higher consumption of mobile phase but is easier to set up and use on standard UHPLC systems with either high-pressure or low-pressure mixing pumps. The 1 mm column run at 150 µL/min is a higher flow rate than would be standard for a 1 mm column, however reducing the flow to 50 µL/min increases the gradient delay significantly. Particularly with low-pressure mixing pumps, which have inherently larger GDV, this effect would lead to substantially longer methods. The 150 µL/min flow rate showed a good compromise for this column with both LPG and HPG systems and prevented system modifications when changing between the two column dimensions. Lower flow rates are still more achievable with the HPG system due to the lower GDV.

Table 5. Evaluation of MAbPac RP, 2.1 × 100 mm and MAbPac RP, 1.0 × 100 mm columns on low-pressure and high-pressure mixing Vanquish systems

Vanquish Horizon MAbPac RP, 1.0 × 100 mm						Vanquish Horizon MAbPac RP, 2.1 × 100 mm					
Injection	Fragment	RT	Width (50%)	Resol. (EP)	Asym. (EP)	Injection	Fragment	RT	Width (50%)	Resol. (EP)	Asym. (EP)
1	LC	7.259	0.105	9.10	1.71	1	LC	6.585	0.119	8.84	1.61
2	LC	7.252	0.097	9.67	1.71	2	LC	6.581	0.125	8.64	1.35
3	LC	7.248	0.100	9.48	1.66	3	LC	6.571	0.134	8.01	1.36
4	LC	7.240	0.102	9.56	1.21	4	LC	6.586	0.126	8.76	1.30
5	LC	7.229	0.099	9.40	1.22	5	LC	6.584	0.131	8.41	1.38
6	LC	7.243	0.130	7.38	1.87	6	LC	6.574	0.142	7.85	1.31
RSD (%)	**LC**	**0.11**				**RSD (%)**	**LC**	**0.09**			
1	scFc	5.519	0.106	9.72	1.51	1	scFc	5.003	0.114	8.01	1.24
2	scFc	5.514	0.104	10.20	1.45	2	scFc	4.859	0.127	8.57	1.23
3	scFc	5.508	0.111	9.74	1.41	3	scFc	4.835	0.132	7.71	1.13
4	scFc	5.510	0.111	9.60	1.45	4	scFc	4.847	0.125	8.18	1.13
5	scFc	5.501	0.107	9.88	1.41	5	scFc	4.873	0.128	7.78	1.20
6	scFc	5.509	0.132	7.82	1.53	6	scFc	4.845	0.136	7.34	1.13
RSD (%)	**scFc**	**0.08**				**RSD (%)**	**scFc**	**0.13**			
1	Fd'	11.489	0.092	n.a.	1.32	1	Fd'	8.353	0.117	n.a.	1.05
2	Fd'	11.471	0.093	n.a.	1.39	2	Fd'	8.363	0.118	n.a.	1.23
3	Fd'	11.469	0.092	n.a.	1.30	3	Fd'	8.346	0.127	n.a.	1.06
4	Fd'	11.485	0.089	n.a.	1.34	4	Fd'	8.367	0.114	n.a.	1.08
5	Fd'	11.485	0.091	n.a.	1.38	5	Fd'	8.377	0.120	n.a.	1.31
6	Fd'	11.473	0.090	n.a.	1.39	6	Fd'	8.358	0.126	n.a.	1.02
RSD (%)	**Fd'**	**0.06**				**RSD (%)**	**Fd'**	**0.12**			

Table 5 (Continued). Evaluation of MAbPac RP, 2.1 × 100 mm and MAbPac RP, 1.0 × 100 mm columns on low-pressure and high-pressure mixing Vanquish systems.

Vanquish Flex MAbPac RP, 1.0 × 100 mm						Vanquish Flex MAbPac RP, 2.1 × 100 mm					
Injection	Fragment	RT	Width (50%)	Resol. (EP)	Asym. (EP)	Injection	Fragment	RT	Width (50%)	Resol. (EP)	Asym. (EP)
1	LC	9.796	0.09	10.99	1.11	1	LC	11.647	0.144	7.29	1.28
2	LC	9.787	0.096	10.50	1.56	2	LC	11.666	0.145	7.01	1.31
3	LC	9.828	0.089	10.71	1.06	3	LC	11.618	0.146	7.10	1.43
4	LC	9.800	0.089	11.13	1.07	4	LC	11.600	0.144	7.21	1.55
5	LC	9.793	0.097	10.61	1.50	5	LC	11.603	0.147	7.13	1.48
6	LC	9.801	0.096	10.65	1.52	6	LC	11.622	0.146	7.11	1.34
RSD (%)	**LC**	**0.15**				**RSD (%)**	**LC**	**0.22**			
1	scFc	8.064	0.102	10.63	1.22	1	scFc	9.956	0.147	6.85	1.18
2	scFc	8.077	0.101	10.27	1.21	2	scFc	9.928	0.153	6.86	1.15
3	scFc	8.057	0.103	10.91	1.28	3	scFc	9.878	0.156	6.80	1.17
4	scFc	8.087	0.101	10.61	1.25	4	scFc	9.920	0.153	6.66	1.14
5	scFc	8.079	0.104	10.05	1.23	5	scFc	9.883	0.152	6.80	1.18
6	scFc	8.088	0.100	10.31	1.21	6	scFc	9.884	0.154	6.83	1.22
RSD (%)	**scFc**	**0.08**				**RSD (%)**	**scFc**	**0.19**			
1	Fd'	11.489	0.092	n.a.	1.32	1	Fd'	13.410	0.142	n.a.	1.05
2	Fd'	11.471	0.093	n.a.	1.39	2	Fd'	13.359	0.140	n.a.	1.06
3	Fd'	11.469	0.092	n.a.	1.30	3	Fd'	13.347	0.141	n.a.	1.05
4	Fd'	11.485	0.089	n.a.	1.34	4	Fd'	13.342	0.141	n.a.	1.16
5	Fd'	11.485	0.091	n.a.	1.38	5	Fd'	13.356	0.143	n.a.	1.03
6	Fd'	11.473	0.090	n.a.	1.39	6	Fd'	13.347	0.140	n.a.	1.08
RSD (%)	**Fd'**	**0.08**				**RSD (%)**	**Fd'**	**0.19**			

Loadability

The increase of injection volume and amount can potentially compromise the resolution and peak shape in the separation. The mass capacity of both columns MAbPac RP, 2.1 × 100 mm and MAbPac RP, 1 × 100 mm columns were evaluated using LC-UV analysis for separation of trastuzumab subunits scFc, LC, and Fd', as demonstrated in Figure 4.

Increasing the injection volume results in the width of the peaks increasing on both MAbPac RP columns, however this does not affect the resolution and performance for this application. As expected, the column with small diameter offers a lower loadability.

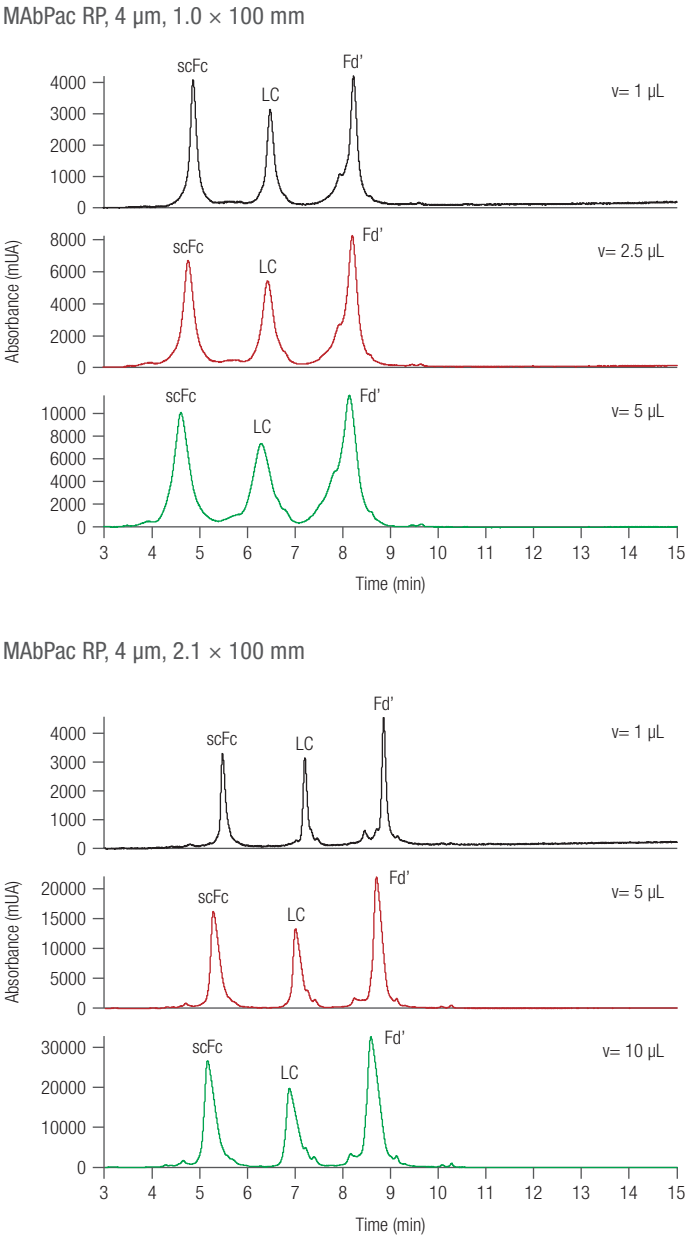


Figure 4. LC-UV separation of the three subunits of trastuzumab obtained after enzymatic digestion and separated on a MAbPac RP, 4 µm, 2.1 × 100 mm column of trastuzumab fragments (1 mg/mL) using different injection volumes

Sensitivity

It is expected with a decrease of the column i.d. that the peak height response should be higher. Figure 5 shows the behavior of the two columns and a ~1.7-fold increase of the UV absorbance on the column with smaller i.d., resulting in increased sensitivity when injecting the same amount of sample even with the increased linear flow used for the 1 mm column.

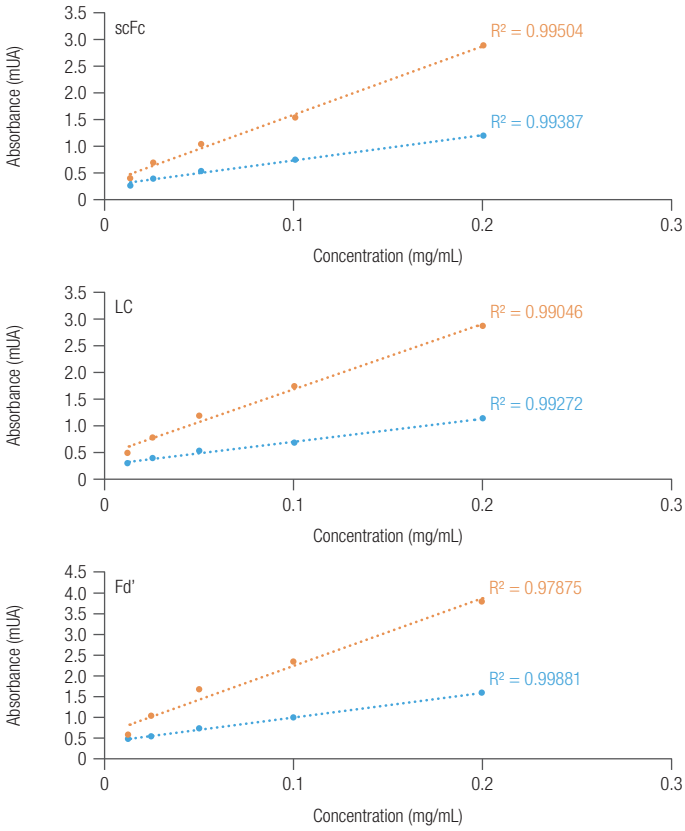


Figure 5. Curve of the three subunits of trastuzumab from 0.025 to 0.20 mg/mL obtained after enzymatic digestion and separated on a MAbPac RP, 4 µm, 2.1 × 100 mm (blue) and 1.0 × 100 mm column (orange)

LC/MS analysis of mAb fragments

The reversed-phase chromatographic analysis can be coupled to high-resolution mass spectrometry systems to confirm the accurate molecular weight of the target product and identify variants.

Figure 6 shows the LC-MS analysis of trastuzumab subunits such as light chain (LC) and heavy chain (HC), scFc and Fd' using a MAbPac RP columns coupled to a Q Exactive Plus hybrid quadrupole-Orbitrap mass spectrometer.

Figure 6 shows the total ion chromatogram for trastuzumab subunits on the two columns (2.1 mm, 1.0 mm) with flow rates of 0.3 mL/min for the 2.1 mm and 0.15 mL/min for the 1.0 mm. The same linear gradient was used for each column (25–45% B). As the column i.d. is decreased, the peak height response is 1.3 times higher, which is a benefit to the analyst looking for increased sensitivity. This sensitivity could be increased further with a reduction in flow rate, however the run times would increase.

MAbPac RP, 4 µm, 1.0 × 100 mm

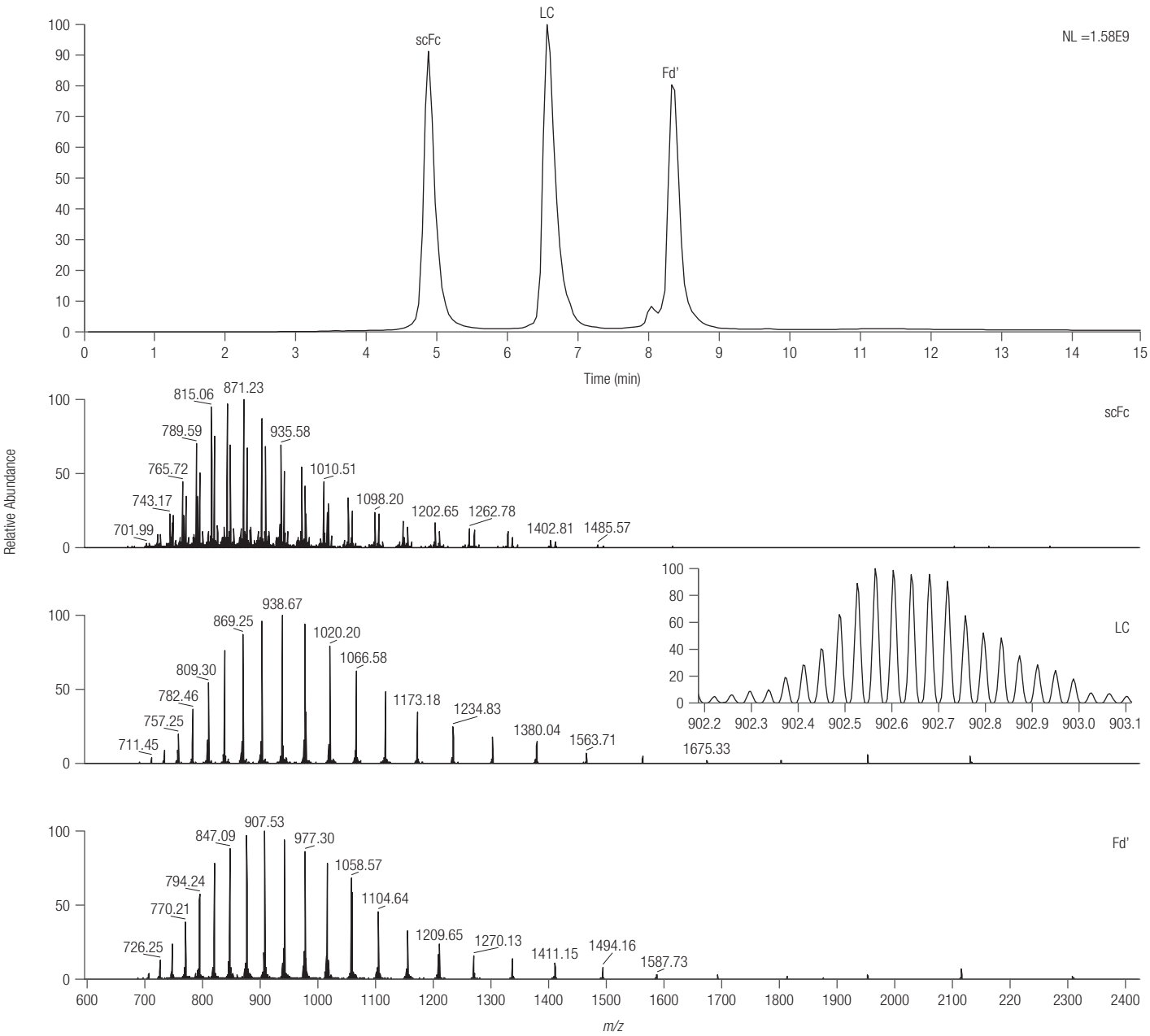


Figure 6. LC-MS analysis of IdeS digested trastuzumab using MAbPac RP, 4 µm, 1 x 100 mm (left) and MAbPac RP, 4 µm, 2.1 x 100 mm (right) columns. The top panels show the TIC traces while the bottom panels show the charge envelopes for scFc, LC, and Fd' subunits, respectively.

MAbPac RP, 4 µm, 2.1 × 100 mm

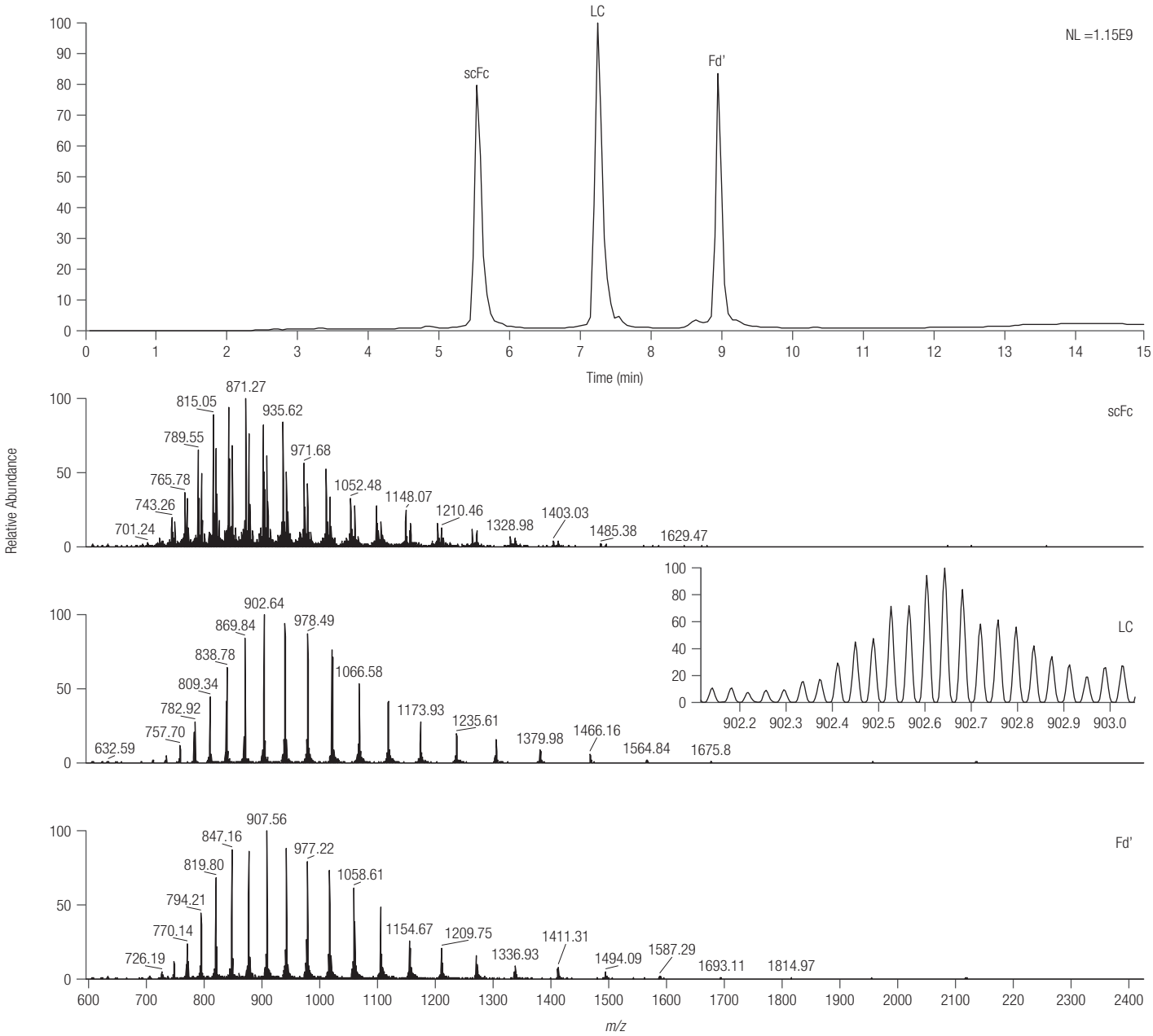


Figure 6 (Continued). LC-MS analysis of IdeS digested trastuzumab using MAbPac RP, 4 µm, 1 x 100 mm (left) and MAbPac RP, 4 µm, 2.1 x 100 mm (right) columns. The top panels show the TIC traces while the bottom panels show the charge envelopes for scFc, LC, and Fd' subunits, respectively.

Table 6 shows the evaluation of the columns' performance in terms of mass accuracy. Table 6 and Figure 7 show the MAbPac RP, 1 × 100 mm column has comparable mass accuracy and results to the MAbPac RP, 2.1 × 100 mm column, which is the most commonly used column dimension.

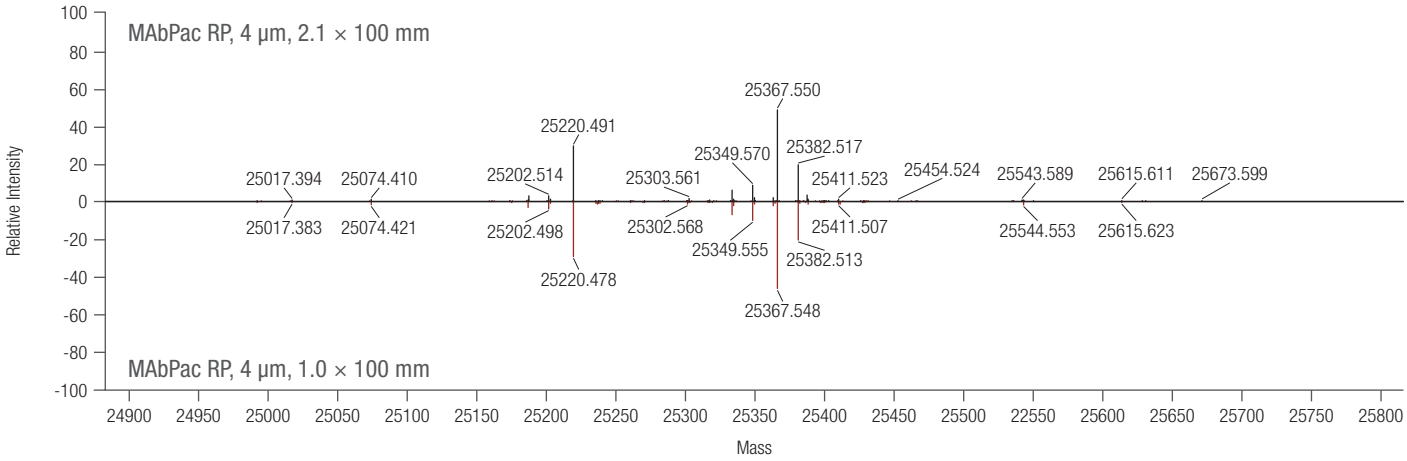


Figure 7. Deconvoluted mass spectra of IdeS digested trastuzumab using MAbPac RP, 4 µm, 2.1 x 100 mm column (top) and MAbPac RP, 4 µm, 1.0 x 100 mm column (bottom)

Table 6. Experimental and theoretical masses (Da) obtained for IdeS digested trastuzumab using MAbPac RP, 4 µm, 2.1 × 100 mm column and MAbPac RP, 4 µm, 1.0 × 100 mm column

Column MAbPac RP, 4 µm	Chain	Trastuzumab		
		Experimental mass	Monoisotopic mass	Mass difference (Δ ppm)
1.0 X 100 mm	LC	23428.5015	23428.5238	0.95
	Fd'	25367.5208	25367.5174	0.13
	scFc-G0F	25074.3805	25074.4055	1.00
	scFc-G1F	25382.5086	25382.5162	0.30
2.1 X 100 mm	LC	23428.5323	23428.5238	0.36
	Fd'	25367.5381	25367.5174	0.82
	scFc-G0F	25220.4667	25220.4634	0.13
	scFc-G1F	25382.5138	25382.5162	0.09

Conclusions

- mAb scFc, Fc, and Fd' fragments are successfully separated using MAbPac RP column with a 15 min gradient.
- High performances were obtained for IdeS subunit analysis using Thermo Scientific Vanquish systems and MAbPac RP, 4 µm columns in different formats.
- The workflow is fast and efficient with a relatively simple sample preparation and a rapidly optimized LC-UV method suitable for routine analysis.
- The MAbPac RP, 2.1 × 100 mm column has higher loadability than the MAbPac RP, 1 × 100 mm column, while the lower i.d. columns could be useful for low sample availability or lower solvent consumption.
- The combination of the IdeS digestion with the MAbPac columns allows fast and efficient separation, evaluation, and characterization of mAb variants, especially when hyphenated with a Thermo Scientific Q Exactive Hybrid Orbitrap mass spectrometer.
- Both LPG and HPG Vanquish pumping systems can be utilized with the smaller 1 mm column. The HPG system is better suited to lower flow rates.

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