Poster Reprint

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A Comprehensive Software Ecosystem for End-to-End Monoclonal Antibody and Antibody–Drug Conjugate Data Acquisition, Analysis, and Reporting

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Introduction

Large and complex biomolecules, such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs), constitute a critical class of biopharmaceuticals with diverse therapeutic applications. Comprehensive analytical characterization of these molecules necessitates advanced instrumentation, including specialized hardware, consumables—particularly separation columns—and robust software solutions. This work focuses on developing an integrated data system that enables end-to-end analysis of mAbs and ADCs at multiple levels, including intact molecules and peptide fragments. The results demonstrate the software's ease of use and its capability to provide a complete solution for analyzing complex biopharmaceutical molecules and their critical quality attributes (CQAs).

Experimental

Trastuzumab (Herceptin®), trastuzumab emtansine (T-DM1) and NISTmAb were used to evaluate the performance of the solution. The software, designed to enable end-to-end data acquisition, analysis, and reporting, was implemented in a client–server architecture, allowing instruments, acquisition methods, data analysis workflows, results, reporting templates, and reports to be shared and updated easily while maintaining regulatory compliance across multiple users (Figure 1).

Experimental

For intact-level and peptide-level analyses, non-deglycosylated Herceptin, T-DM1, NISTmAb were prepared according to the specific analytical objectives and diluted to 1 mg/mL. The LC and (Q-)TOF conditions are presented in Table 1. A sequence configured to run automatically over three days is summarized in Table 2.

Table 1. LC and (Q-)TOF conditions.

	Native MS	Intact protein	Peptide mapping
Column	Altura SEC 200Å, 4.6 x 30 mm, 1.9 µm	Altura PLRP-S 1000Å, 2.1 x 50 mm, 5 µm	Altura ZORBAX Eclipse Plus C18, 2.1 x 150 mm, 1,8 µm
Flow rate	0.2 mL/min	0.4 mL/min	0.4 mL/min
Column temperature	25 °C	80 °C	50 °C
Injection volume	5/10 µL	0.5/1 µL	5 µL
Mobile phase	A: water + 100mM AA pH7 B: -	A: water + 0.1% FA B: ACN + 0.1% FA	A: B:
Gradient	Time (min) %B 0 0	Time (min) %B 0 5 0.1 20 4 50 5 70	Time (min) %B 0 2 5 2 38 38 47 95 50 95 51 2 60 2
Stop time	3 min	5 min	60 min
Post time	1 min	1.5 min	-
Drying gas temperature	300 °C	350 °C	350 °C
Drying gas flow	12 L/min	13 L/min	13 L/min
Nebulizer	60 psi	60 psi	35 psi
Sheath gas temperature	300 °C	400 °C	375 °C
Sheath gas flow	12 L/min	12 L/min	12 L/min
Capillary voltage	5500 V	5500 V	3750 V
Nozzle voltage	2000 V	2000 V	0 V
Fragmentor	380 V	380 V	45 V
Skimmer	220 V	140 V	35 V
Instrument mode	High mass stable	High mass stable	System tune
Acquisition mode	MS	MS	Auto MS/MS
Mass range	*m/z* 1000-10000	*m/z* 800-6000	*m/z* 100-3000
Acquisition rate	0.5 spectra/s	1 spectra/s	3 spectra/s
Reference mass	-	-	*m/z* 322.048, 922.0097

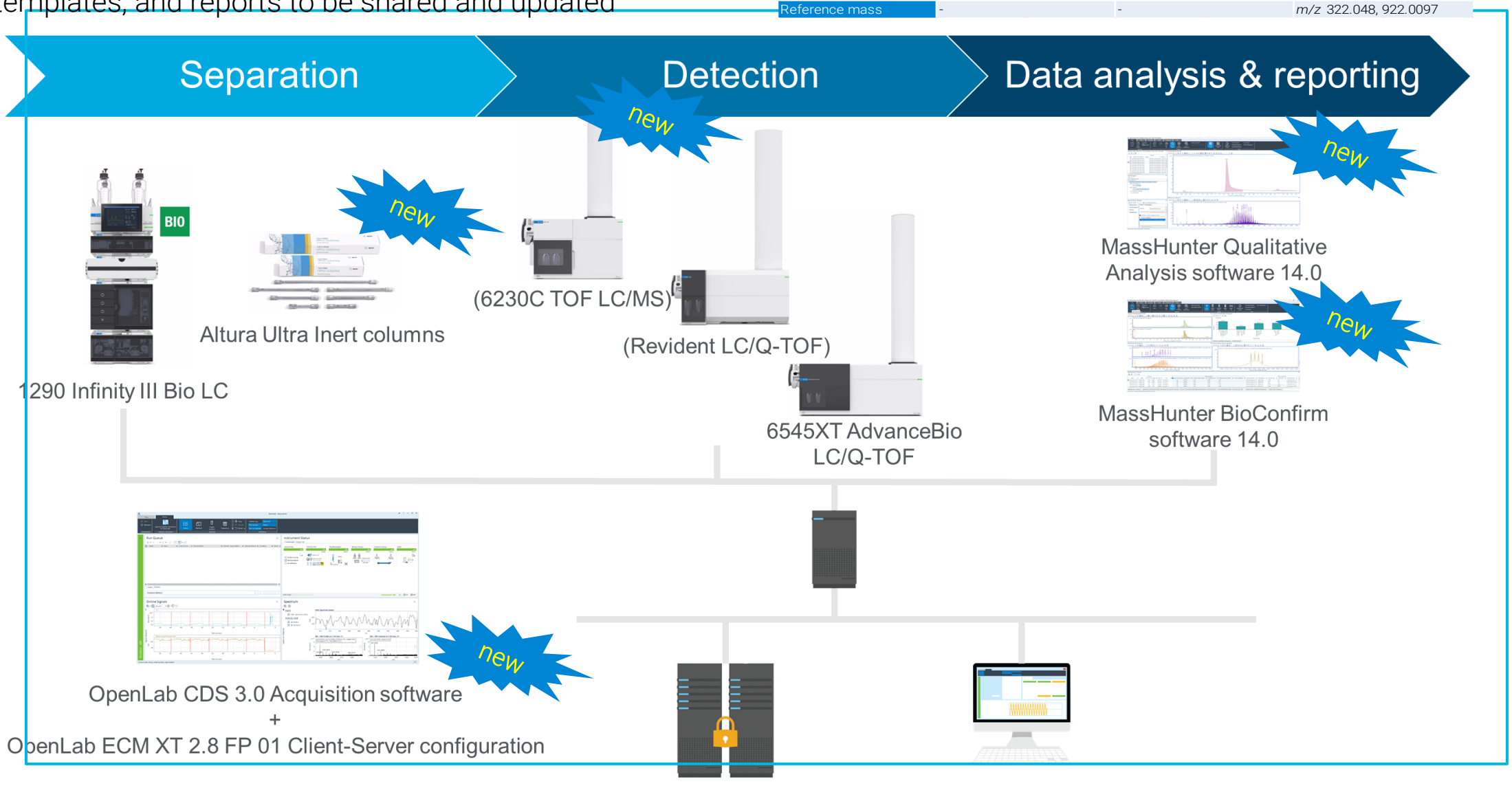


Figure 1. Solution overview.

Seamless sample processing: from data acquisition through data analysis to reporting

A combination of task actions, wait actions, and custom sample parameters enables the sample sequence to run without human intervention for several days (see Table 2). Unattended processing allows samples to be processed automatically immediately after data acquisition is complete, using predefined data analysis and reporting methods.

Table 2. A sequence for automated data acquisition, processing, reporting, and instrument control over several days.

	Action	Sample name	Inj/ Vial	Acquisition method	Processing + Reporting method	Sample Custom Parameters			
						BioConfirm Sequence	BioConfirm DAR 0 Mass	BioConfirm Drug/Linker Mass	BioConfirm Modification Profiles, and more...
Day 1	Inject	T-DM1	25	IntactProtein.amx	IntactProtein-DAR.bcpmx		148059	957.5	
	Inject	Water	3	IntactProtein.amx					
	Inject	Herceptin	25	IntactProtein.amx	IntactProtein.bcpmx	Herceptin sequence			
	Inject	Water	3	IntactProtein.amx					
	Inject	NISTmAb	25	IntactProtein.amx	IntactProtein.bcpmx	NISTmAb sequence			
	Inject	Water	3	IntactProtein.amx					
	Task	Turn off the LC modules, switch MS to Eco Standby							
	Wait	Wait until next morning, e.g. 8:00							
	Task	Turn on all instruments, run mass calibration for MS							
Day 2	...								
Day 3	Inject	T-DM1	25	IntactProtein.amx	IntactProtein-DAR.bcpmx		148059	957.5	
	Inject	Water	3	IntactProtein.amx					
	Inject	Herceptin	25	IntactProtein.amx	IntactProtein.bcpmx	Herceptin sequence			
	Inject	Water	3	IntactProtein.amx					
	Inject	NISTmAb	25	IntactProtein.amx	IntactProtein.bcpmx	NISTmAb sequence			
	Inject	Water	3	IntactProtein.amx					

Native MS vs. denatured intact protein and DAR analysis

Excellent intact protein analysis of Herceptin and accurate DAR analysis of T-DM1 were achieved under both native and denaturing conditions using newly developed Altura Ultra Inert columns coupled with a new 6230C TOF LC/MS system and the OpenLab CDS and MassHunter software suites.

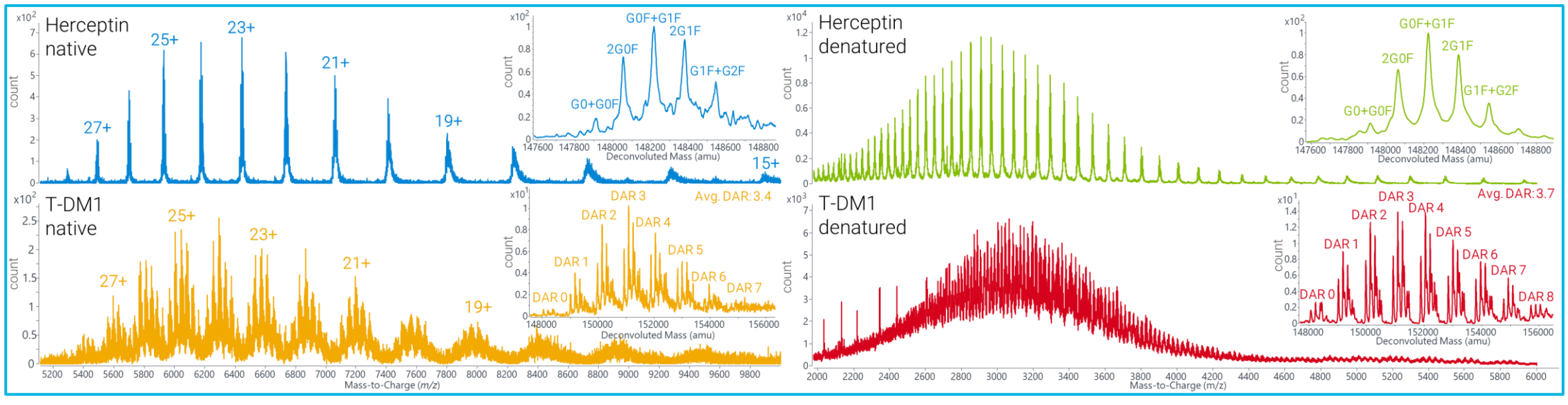


Figure 2. Native MS vs. denatured intact protein/DAR analysis for Herceptin and T-DM1, measured by 6230C TOF LC/MS.

Robustness of the solution

Figure 3 demonstrates the minimal retention time shifts over a three-day period. Figure 4 summarizes the relative quantity of each glycoform, averaged over 75 samples, for Herceptin and NISTmAb, showing excellent reproducibility.

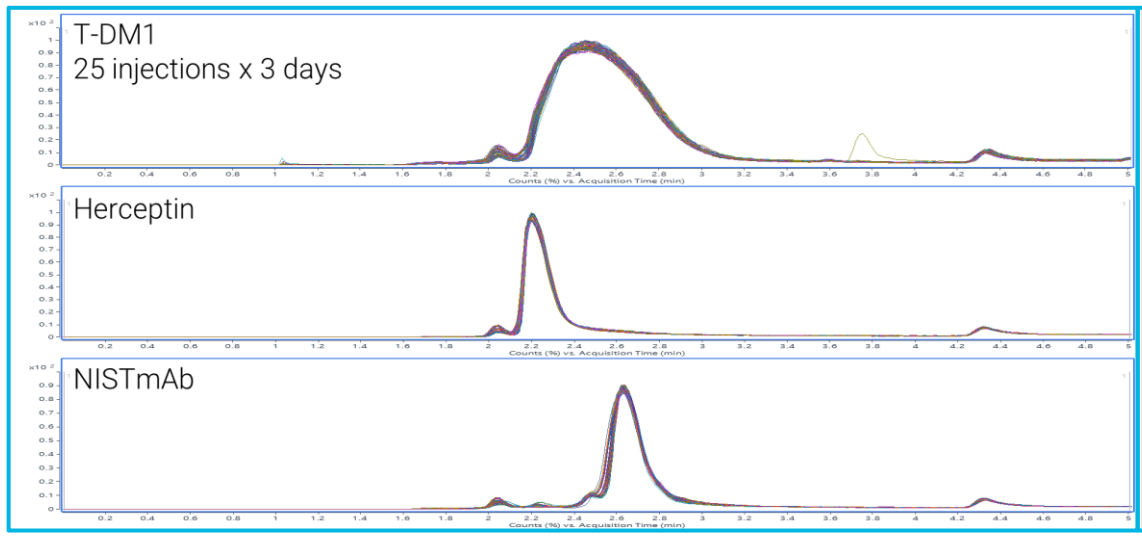


Figure 3. Overlaid 75 TICs over 3 days for reversed-phase separation of T-DM1, Herceptin, and NISTmAb.



Figure 4. Quantitation results from intact Herceptin and NISTmAb glycoforms analysis, 75 replicates over 3 days.

Results and Discussion

Peptide mapping

The total ion chromatogram and sequence coverage of trypsin-digested Herceptin are presented in Figure 5 and 6, respectively. Fragment coverage for one representative peptide is shown in Figure 7.

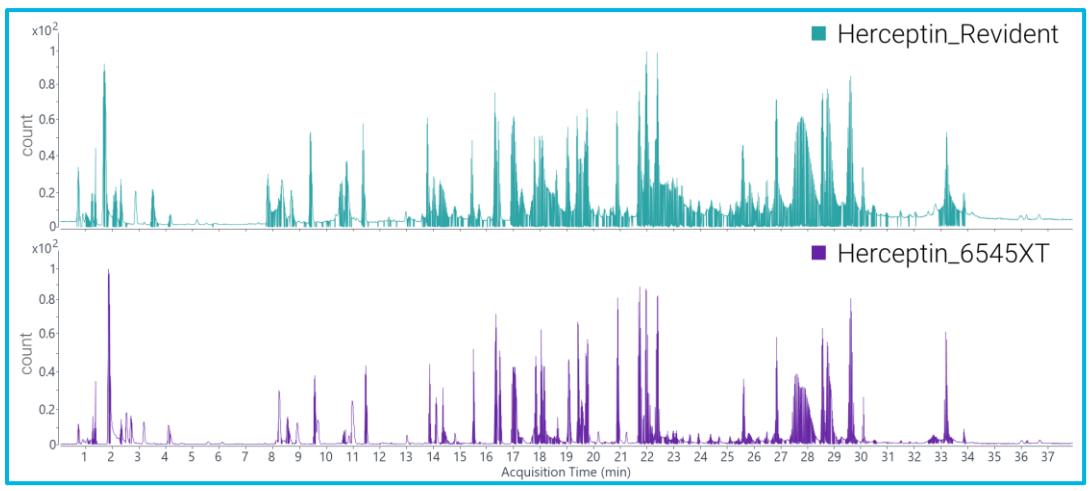


Figure 5. Total ion chromatogram for trypsin-digested Herceptin, measured by Revident and 6545XT.

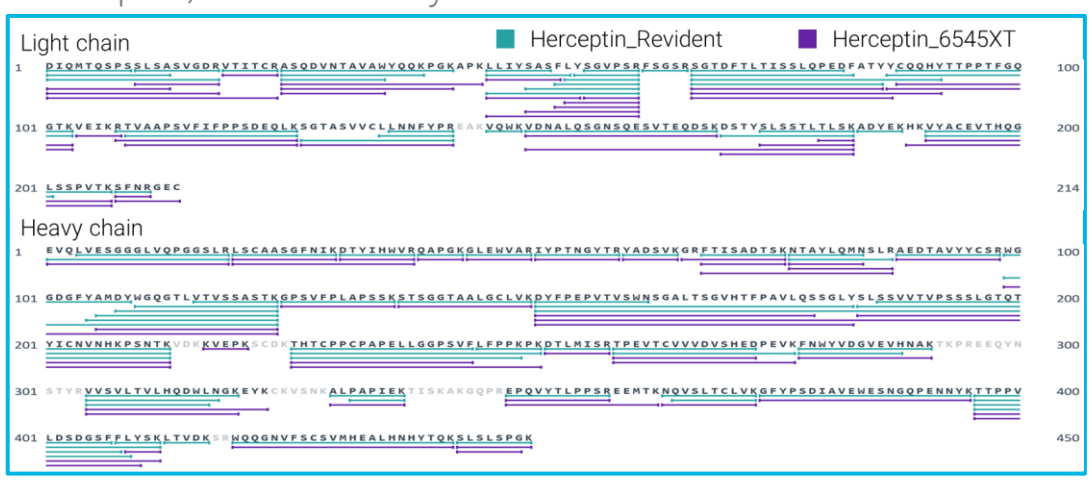


Figure 6. Sequence coverage map for Herceptin, measured by Revident (88.9%) and 6545XT (91.0%).

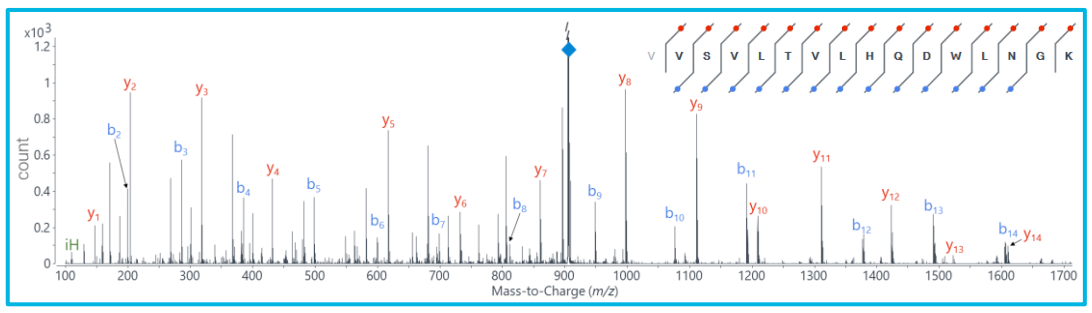


Figure 7. MS/MS spectrum and fragment coverage map for peptide VVSVLTVLHQDWLNGK, measured by 6545XT.

Table 3. Consumables for Inert LC/MS solution.

Description	Part Number
Agilent Altura SEC 200Å, 4.6 x 30 mm, 1.9 µm	PL1580-1201A
Agilent Altura PLRP-S 1000Å, 2.1 x 50 mm, 5 µm	PL1912-1502A
Agilent Altura ZORBAX Eclipse Plus C18, 2.1 x 150 mm, 1,8 µm	204215-308
Agilent InfinityLab bio solvent bottle, PP, 1 L, with cap	9301-6028
Agilent InfinityLab bio solvent inlet filter, 10 µm, PEEK	5320-0070
Agilent InfinityLab Quick Connect LC fitting	5067-5965
Agilent InfinityLab Acetonitrile for LC/MS	5191-5101
Agilent InfinityLab Water for LC/MS	5191-5121
Agilent LC/MS Grade Formic Acid	5191-4549
Agilent Polypropylene Vials	5188-2788
Agilent Snap Top Caps	5182-3458

Reporting

Ready-to-use report templates are available for the analysis of intact protein, DAR, protein digest, released glycans, with built-in report builder supporting customized designs. This enables reports to be generated automatically and efficiently.

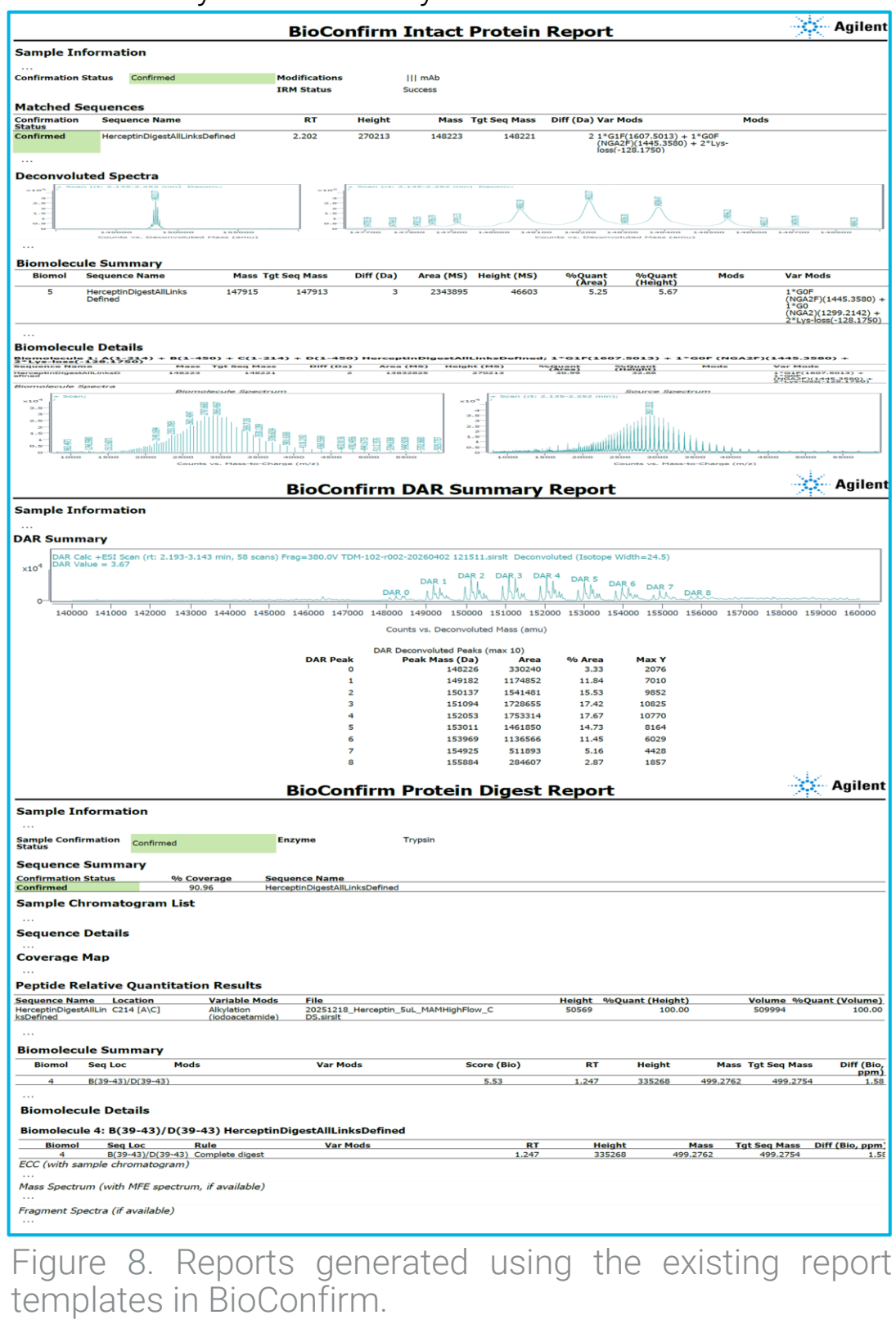


Figure 8. Reports generated using the existing report templates in BioConfirm.

Conclusions

- An integrated software platform, together with new hardware and consumables, enables end-to-end data acquisition, analysis, and reporting for mAbs and ADCs.
- Intelligent sequence management is supported through task actions, wait actions, sample custom parameters, and additional advanced features.
- Outstanding instrumental robustness, reproducibility and accuracy are achieved.

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