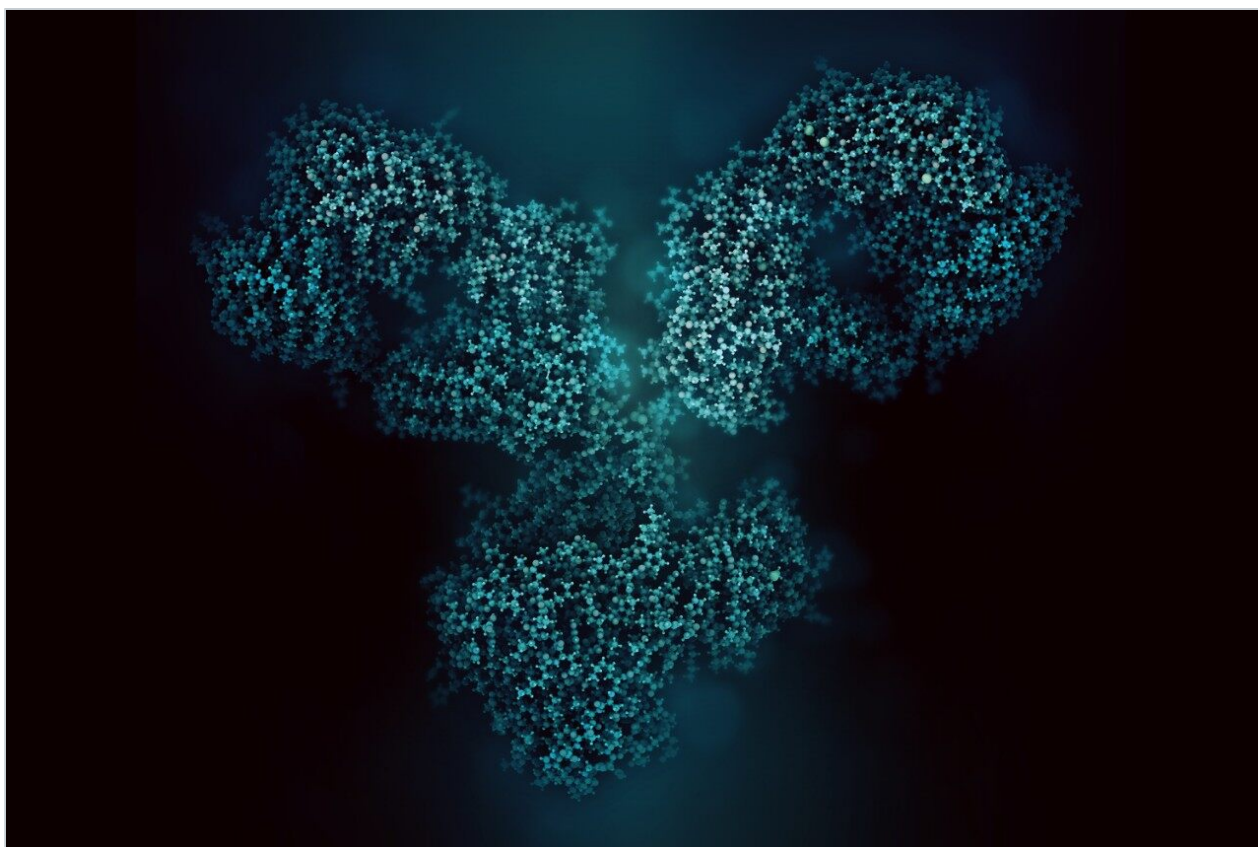


Nota de aplicación

Intact Trastuzumab Monoclonal Antibody Quantification Using the Xevo G2-XS QTof High Resolution Mass Spectrometer

Yun Alelyunas, Henry Shion, Ian Edwards, Mark Wrona

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrate the performance of the Xevo G2-XS QTof platform for intact level quantification of the trastuzumab monoclonal antibody and the use of the UNIFI Scientific Information System for rapid data processing and analysis.

Benefits

In this study, a monoclonal antibody is quantified at the intact level through integration of extracted ion chromatograms (XICs).

Introduction

The discovery and development of biotherapeutic modalities of increasing complexity and high molecular weight creates new quantitative and qualitative analytical challenges. Intact protein quantification using a high resolution mass spectrometer (HRMS) is increasingly performed in discovery bioanalytical labs. Intact mass analysis offers direct quantitative measurement of the whole molecule potentially including sub-species and variant information, as well as providing information about molecular stability and sites of modification. In this technology brief, intact quantification of trastuzumab is demonstrated using the Xevo G2-XS QTof HRMS and the UNIFI Scientific Information System. Quantitative performance attributes including sensitivity, accuracy, and linear dynamic range are presented.

Results and Discussion

A dilution series of trastuzumab prepared in 0.1% formic acid in the presence of 0.1 mg/mL BSA carrier protein was analyzed using the ACQUITY UPLC H-Class Bio System and Xevo G2-XS QTof. LC-MS acquisition conditions are summarized in Figure 1. The data was processed post acquisition in UNIFI, a next generation informatics platform offering a friendly user interface for data processing, review, and reporting.¹ Here, we show a complete quantitative workflow for processing of intact protein data. By scrolling through the workflow, one can quickly review the analysis of a batch of samples including various quantitative

performance attributes (Figure 2).

LC conditions:

Column: Polystyrene divinylbenzene based column, 4 μ M, 1500 \AA , 3 x 50 mm

Column temp.: 80 $^{\circ}$ C

Sample temp.: 8 $^{\circ}$ C

Injection vol.: 10 μ L

Flow rate: 0.2 mL/min

Mobile phase A: Water with 0.1 % formic acid

Mobile phase B: Acetonitrile with 0.1 % formic acid

Gradient table:	Time (min)	Flow Rate (mL/min)	A (%)	B (%)	Curve
	0.0	0.2	98	2	Initial
	1.0	0.2	98	2	6
	2.0	0.2	65	35	6
	7.0	0.2	55	45	6
	7.5	0.2	10	90	6
	8.0	0.2	98	2	6
	8.5	0.2	10	90	6
	9.0	0.2	98	2	6
	11.0	0.2	98	2	6

MS conditions:

Ionization mode: ESI+

Acquisition mass range: 500–4000 m/z

Experiment: MS full scan, sensitivity mode

Capillary voltage: 2.25 kV

Cone voltage: 150 V

Cone gas flow: 0 L/h

Source temp.: 150 $^{\circ}$ C

Source offset: 150 V

Desolvation gas temp.: 500 $^{\circ}$ C

Desolvation gas flow: 700 L/h

Scan time: 0.5 s

Figure 1. Summary of LC-MS acquisition parameters (ACQUITY UPLC H-Class Bio System or ACQUITY UPLC I-Class System and Xevo G2-XS QToF HRMS).

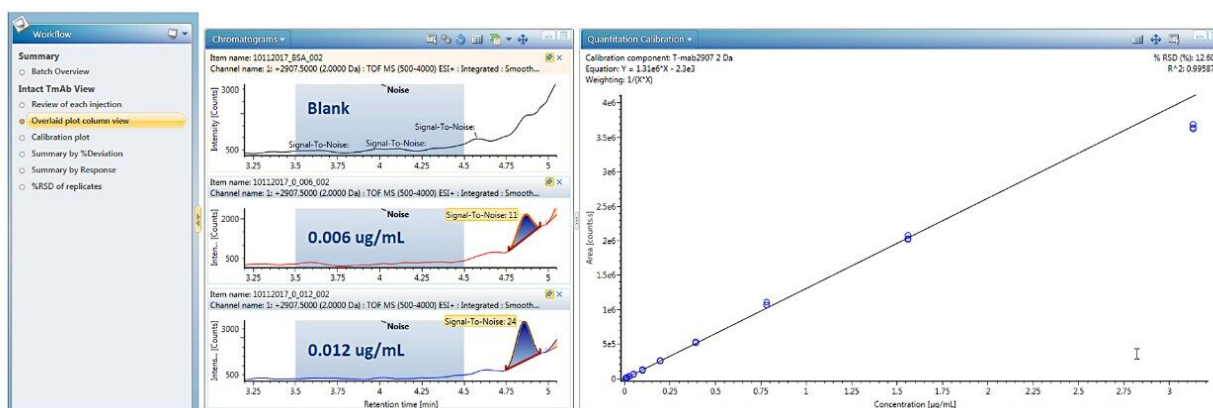


Figure 2. (Left) Data review workflow; (Middle) XICs of trastuzumab at and near the LLOQ concentration; (Right) Linear calibration curve: individual concentrations from three replicate injections are plotted.

Data processing for quantification of intact proteins was performed by automatically generating and summing extracted ion chromatograms (XICs) from the dataset. Single or multiple peaks can be selected from one or multiple charge states using a mass tolerance window. A detailed investigation of these options on quantitative performance including LLOQ is summarized in a recent Waters Application Note (720006222EN).² For the present data set, a mass tolerance window of 2 Da for both a single peak at m/z 2907 and the sum of 9 GF0/GF1 peaks across multiple charge states were found to produce the same quantitative results; single peak data is reported here.

Trastuzumab eluted at 4.9 min, as a symmetrical peak, with a linear dose response curve for concentrations ranging from 0.006 to 3.13 $\mu\text{g/mL}$ or 2.7 orders of magnitude. Using $1/X^2$ weighting, the linear regression produced an $R^2 = 0.996$ (Figure 2). The LLOQ was 0.006 $\mu\text{g/mL}$ from triplicate injections all with a signal-to-noise ratio >10 . The calibration table in Figure 3 shows that calculated %accuracy for triplicate injections across all concentrations is within 15%, with no data outliers (Figure 3).

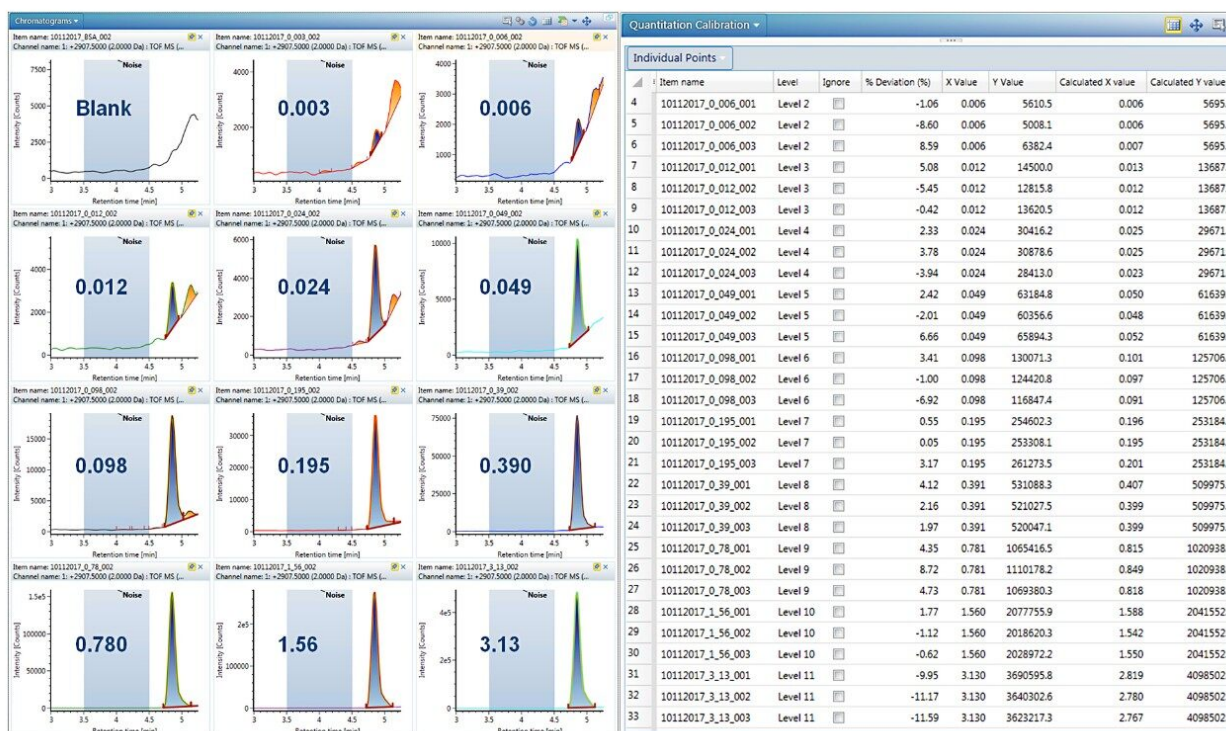


Figure 3. (Left) Review of XICs for trastuzumab across the concentration range ($\mu\text{g/mL}$). The noise region used for calculating S/N values is highlighted in blue. (Right) Calibration table showing % deviation values.

Conclusion

HRMS with its extended mass range makes it an ideal platform for intact quantification of larger proteins such as monoclonal antibodies. In the present study, a monoclonal antibody, trastuzumab, was quantified at the intact level through integration of XICs, which follows common practice in small molecule and peptide bioanalysis. The Xevo G2-XS QToF (HRMS) platform was shown to provide sensitive detection, achieving a LLOQ of 0.006 µg/mL or 60 pg load on column, and a linear dynamic range of 2.7 orders of magnitude that is suitable for routine intact monoclonal antibody quantifications.

References

1. Kellie JF, Kehler JR, Karlinsey MZ, Summerfield SG. "Toward Best Practices in Data Processing and Analysis for Intact Biotherapeutics by MS in Quantitative Bioanalysis", *Bioanalysis* 2017, 23, 1883.
2. High Sensitivity Intact Monoclonal Antibody (mAb) HRMS Quantification. Waters Application Note. 720006222EN. March 2018.

Appendix I. Instructions for data import from MassLynx into UNIFI (1.9 service release 3)

UNIFI offers direct data import of MassLynx .raw data files. Instructions for importing data are provided as follows:

STEP 1.

Click the drop-down arrow of the main menu, a panel shown in Figure A will appear. Choose "Tools", "Import MassLynx data". A new dialog "Create Import Task" shown in Figure B will appear. From this dialogue, one can choose either "MassLynx raw folders" for importing data only (*.RAW), or "MassLynx SPL files" for importing through a MassLynx sample list file (*.SPL). The advantage of using the sample list file type is that the injection sequence of the imported samples is the same as the original.

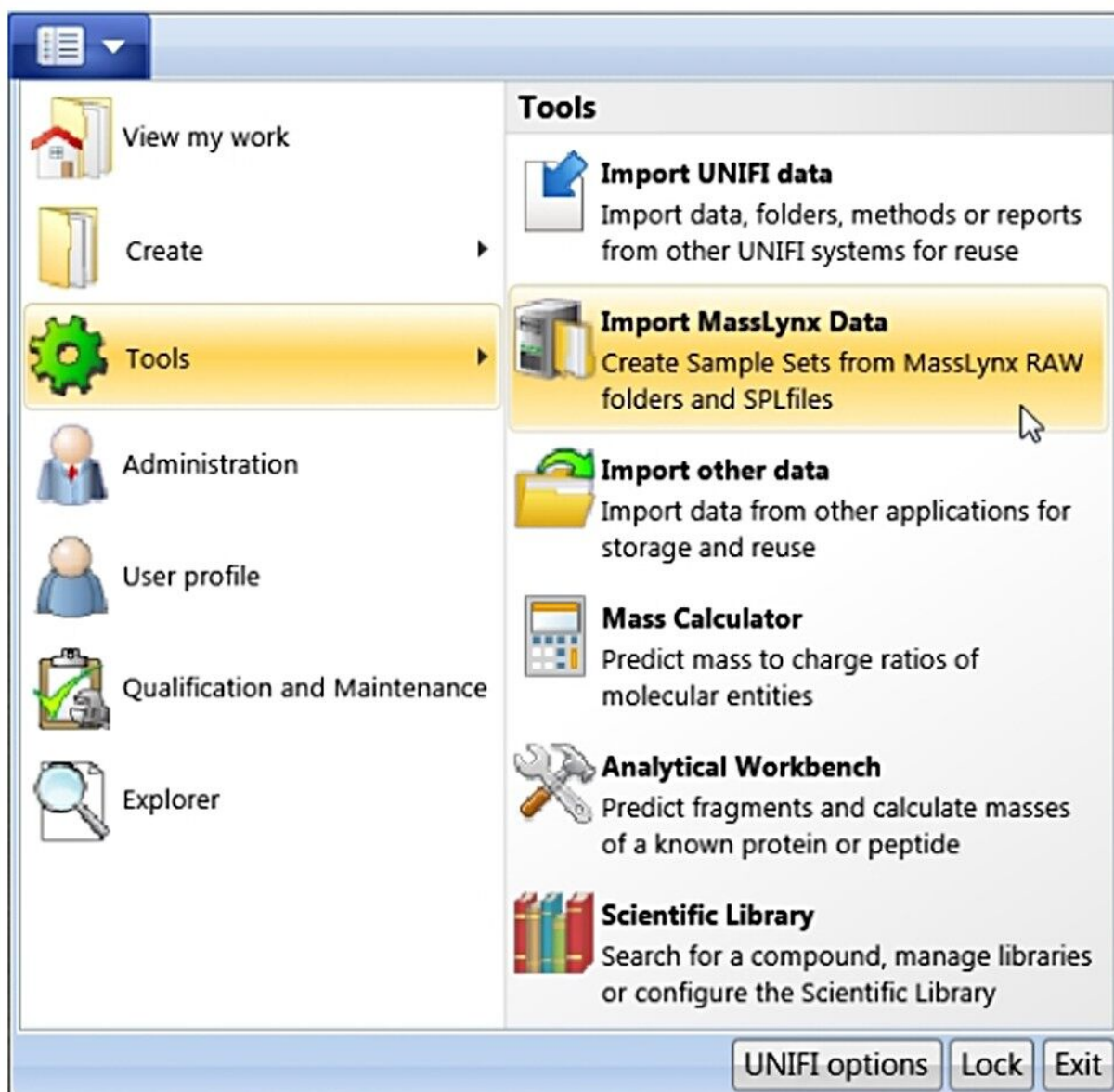


Figure A. Panel from Main Menu.

With the "MassLynx SPL file" import type chosen, one can import directly from a MassLynx.PRO project folder or from copied data. Note: In the case of copied data, the sample list file .SPL needs to reside in a folder called "SampleDB", and .raw files need to reside in a folder called "Data" as shown in Figure B (right). In this way, the sample list will correctly associate each entry with a data file.

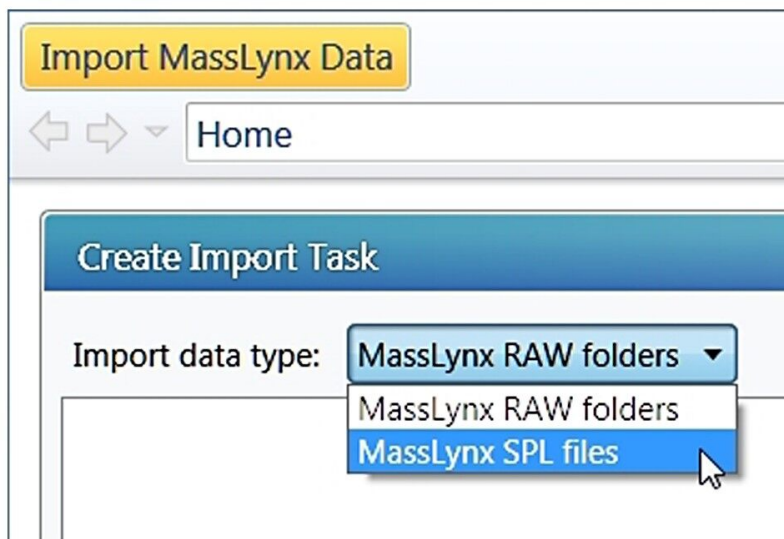


Figure B. (left) Import data type choices from "Import MassLynx Data." (right) Minimum folder structure for using "MassLynx SPL files" import data type. The Data folder contains .raw files, and the SampleDB folder contains .SPL files.

STEP 2.

With choosing the "MassLynx SPL data files", click the "+" button, a file browsing window will appear (not shown). Browse to the .SPL file location and click OK, the sample list file and corresponding .raw data files will populate the left panel of Figure C. Choose a destination folder and enter the sample set name, pressing the "Create UNIFI Sample Set" button will start the import. A green bar shown in the right panel shows the progression of the import.

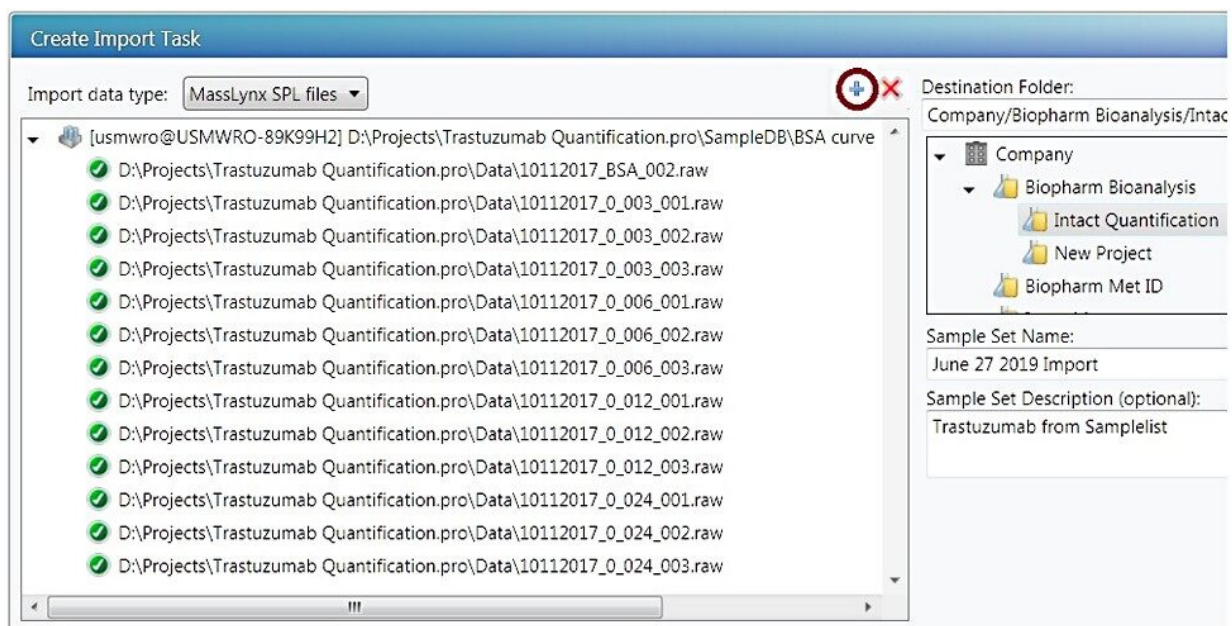


Figure C. Data import for "MassLynx SPL Files" data type.

STEP 3.

After the import is completed, the files will appear in UNIFI Explorer with the sample set format. The user can then create an analysis and perform data processing.

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[UNIFI Scientific Information System <https://www.waters.com/134801359>](https://www.waters.com/134801359)

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