Two-Dimensional Chromatography for Quantitation and MS Analysis of Monoclonal Antibodies in a Complex Mixture

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INTRODUCTION

Identification, characterization, and quantitation of monoclonal antibodies (mAbs) is required at many stages of biopharmaceutical research and development. Commonly used analytical tools are liquid chromatography coupled with UV or mass spectrometry (MS). Both techniques can be compromised by interferences in the sample matrix. Affinity chromatography on immobilized Protein A can be used to isolate the antibody from a complex matrix while reversed phase LC is useful for introducing a salt free, concentrated sample into an MS ion-source.

In this presentation we describe the use of an ACQUITY UPLC® system with 2D technology. The 2D system allows for automated purification and quantitation of monoclonal antibodies by Protein A affinity chromatography and determination of mass profile by MS analysis after desalting on a short reversed phase column. The peak of interest is eluted from the first dimension affinity separation to a second dimension reverse phase separation. Our data demonstrates the linearity and reproducibility of response for mAbs from complex matrices. We also discuss carryover and chromatographic reproducibility observed when performing protein A affinity chromatography with the 2D system. 2D UPLC system optimization for peak collection, desalting, and MS detection of antibody samples is also discussed.

INSTRUMENTS AND METHODS

Instrument:

ACQUITY UPLC® with 2D Technology composed of:

BioQuaternary Solvent Manager
BioSample Manager FTN
CM-A
Tunable UV (TUV) Detector
Binary Solvent Manager

First Dimension:
Mobile Phase A: 50 mM phosphate, pH 7.0, 150 mM NaCl
Mobile Phase B: 12 mM HCl, pH 1.9, 150 mM NaCl
Flow Rate: 1.0 ml/min
Column: Poros A 20 µm, 2.1x30 mm
Column Temp: 20 °C
UV: 280 nm

Second Dimension:
Mobile Phase A: 0.1 % Formic Acid
Mobile Phase B: 0.1 % Formic Acid in Acetonitrile
Flow Rate: 0.5 ml/min (desalting), 0.2 ml/min (elution)
Column: Waters Mass Prep Micro Desalting Column
Column Temp: 80 °C

Mass Spectrometer (Xevo G2 QTof MS)

Polarity	ES+
Capillary	2.5 kV
Sampling Cone	55 V
Source Temp.	130 °C
Desolv. Temp.	350 °C
Desolv. Flow	800.0 L/hr
Mass Range	1000 to 4000
Scan Time	1 sec.

Sample: monoclonal antibody in cell culture media spike with 1mg/ml BSA

INSTRUMENT CONFIGURATION

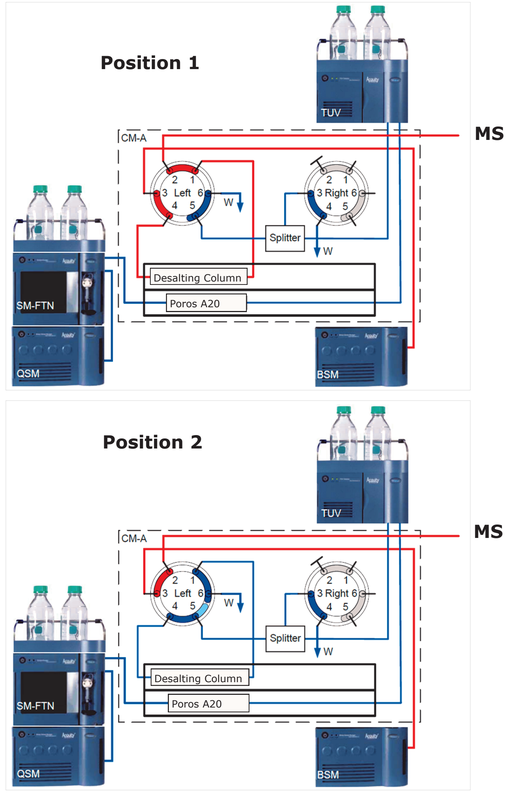

Figure 1. Instrument plumbing configuration. The sample is introduced to the 1D column in position 1. At the specified time, the left valve is switched to position 2 to collect divert flow from the 1D to the 2D column. After collection, the valve is returned to position 1 and the second dimension gradient is initiated. A splitter is used between the 1D and 2D to compensate for the differences in mass load needed for each analysis. The splitter can be bypassed when the 1D mass load is compatible with the 2D.

INLET METHOD

	First Dimension (QSM)						Second Dimension (BSM)				Valve Position
Time	Flow Rate	%A	%B	%C	%D	Curve	Flow Rate	%A	%B	Curve	
Initial	1.0	100	0	0	0		0.5	100	0		Left 1
1.00	1.0	0	100	0	0	11					
1.50											Left 2
1.80											Left 1
3.50							0.2	100	0	11	
4.00	1.0	100	0	0	0	11					
5.00							0.2	5	95		
5.10							0.5	100	0		
5.70							0.5	5	95		
5.80							0.5	100	0		
6.40							0.5	5	95		
6.50							0.5	100	0		
7.00	1.0	100	0	0	0	1	0.5	100	0	6	Left 1

Table 1. Chromatographic method for 2D affinity purification/mass profiling. Both first and second dimension separations occur on the same time scale. Entries are listed chronologically and values are entered according to entries made in inlet method editor.

RESULTS

First Dimension: Protein A purification of mAb from cell culture media spiked with BSA

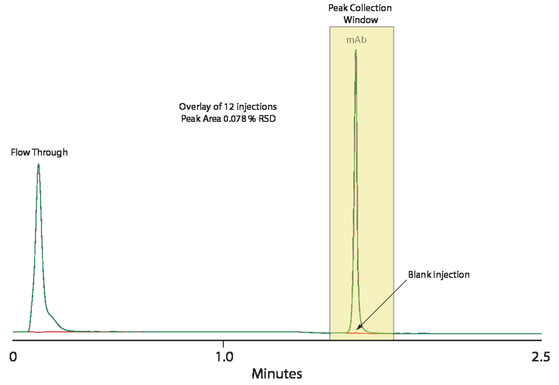

Figure 2. Overlay of twelve 1D Protein A affinity chromatograms The sample is trastuzumab, 1 mg/ml in cell culture media (DMEM) with 1mg/ml BSA. Excellent reproducibility of retention time, peak shape, and peak area were found. Blank injections did not indicate any carryover. Analyte of interest is efficiently purified from sample matrix.

RESULTS CONT.

First Dimension: Linearity of Response

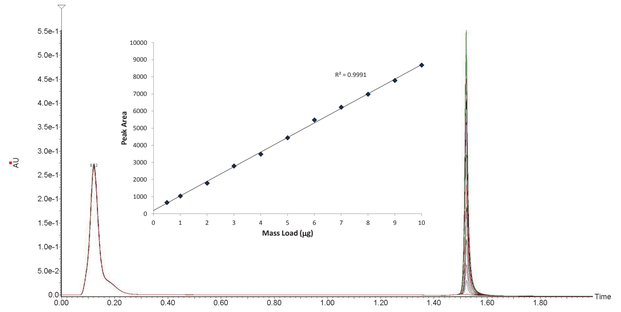

Figure 3. Overlay of serially diluted trastuzumab in cell culture media with 1mg/ml BSA. Response for mAb is linear over entire mass load range. Samples run in triplicate with RSD for each mass load <0.1%.

Spectral Deconvolution

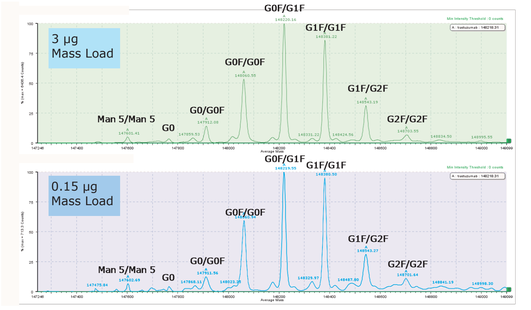

Figure 4. Intact mass data can be processed using Waters bioinformatics tool, Biopharmalynx 1.2, for automated intact protein mass deconvolution. Data can be batch processed and quickly screened for mass and glycoprofile. Additionally, data can be compared to a control sample to identify quickly assess similarities and differences between samples.

CONCLUSIONS

- **Using the ACQUITY UPLC with 2D Technonogy a method was developed to determine mAb concentration and mass profile from a single injection.**
- **Chromatographic methods are easily defined using inlet method editor.**
- **Very good reproducibility in retention time and recovery for samples from complex matricies was found.**
- **UV response is linear over a typical working range allowing for quantitation of analytes prior to MS analysis.**
- **MS TIC, raw, and processed data show reproducible collection and desalting of analyte from first dimension.**

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