Quantitative Measurement of a Candidate Antibody Therapeutic in Human Plasma by LC/MS/MS using Formic Acid Digestion

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Overview

Purpose:

- To develop a liquid chromatography with tandem mass spectrometry (LC/MS/MS) assay for quantification of a candidate antibody therapeutic, DX-2930, in human plasma.

Method:

- DX-2930 was isolated from sodium citrate-treated human plasma using a bead-based immunoprecipitation extraction. The extracted samples were reduced with *tris*(2-carboxyethyl)phosphine (TCEP) and digested with formic acid and heat.
- Nanoscale chromatography and ionization combined with the selected reaction monitoring (SRM) mode of a Thermo TSQ Vantage mass spectrometer were utilized.

Results:

- A highly selective signature peptide was produced from DX-2930 by means of formic acid digestion, which could not be achieved with tryptic digestion.
- A 100- to 1,500-ng/mL calibration curve and 100-fold dilution cover a sample concentration range from 100 ng/mL to 150 µg/mL.
- Good assay statistics, including inter-individual selectivity and accuracy, were achieved with this developed method.

Introduction

DX-2930 is a human monoclonal antibody inhibitor of plasma kallikrein under investigation for long-term prophylaxis of hereditary angioedema. Although a ligand binding assay (LBA) was successfully validated and used to assay pharmacokinetic samples, an LC/MS/MS assay following a "bottom-up" quantitative approach was developed due to early LBA method development issues. In-silico sequence homology searches utilizing various enzymatic digestion reagents (including trypsin) did not provide unique peptides for this candidate therapeutic antibody.

It has long been known that aspartyl peptide bonds can be cleaved with dilute acid and heat.[1] Applications of this protein digestion approach have mostly been limited to protein characterization.[2,3] To the authors' knowledge, formic acid digestion has not been employed for quantitative measurement of proteins in complex biological matrices. Here, we present data showcasing the utility of formic acid digestion in producing a signature, quantitative peptide for the measurement of a candidate monoclonal antibody (mAb) therapeutic in human plasma.

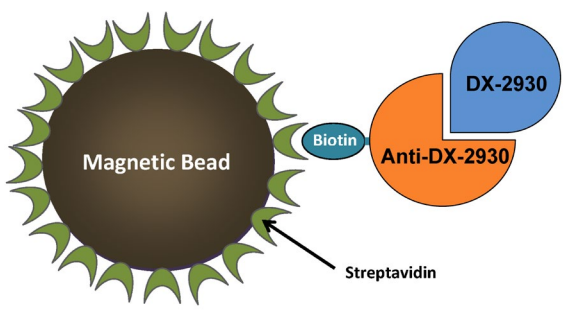
Method

Sample Preparation

- 100-µL samples were incubated with 400 ng of anti-DX-2930 antibody.
- 300 µL of 0.1% bovine serum albumin in phosphate buffered saline was added to dilute the sample.
- Sample mixture was incubated at 4 °C overnight.

Extraction Procedure

- Streptavidin-coated magnetic beads were prepared using custom-made magnetic plates and automated Hamilton Microlab STAR liquid handling.
- Biotinylated anti-DX-2930 complex was captured with the magnetic beads.
- Beads were collected on the magnetic stand and washed.
- DX-2930 was eluted from the magnetic beads and collected.
- ^{13}C, ^{15}N stable isotope-labeled internal standard (SIL IS) and reducing agent TCEP were added, and digestion with 2% formic acid was carried out overnight at 95 °C.



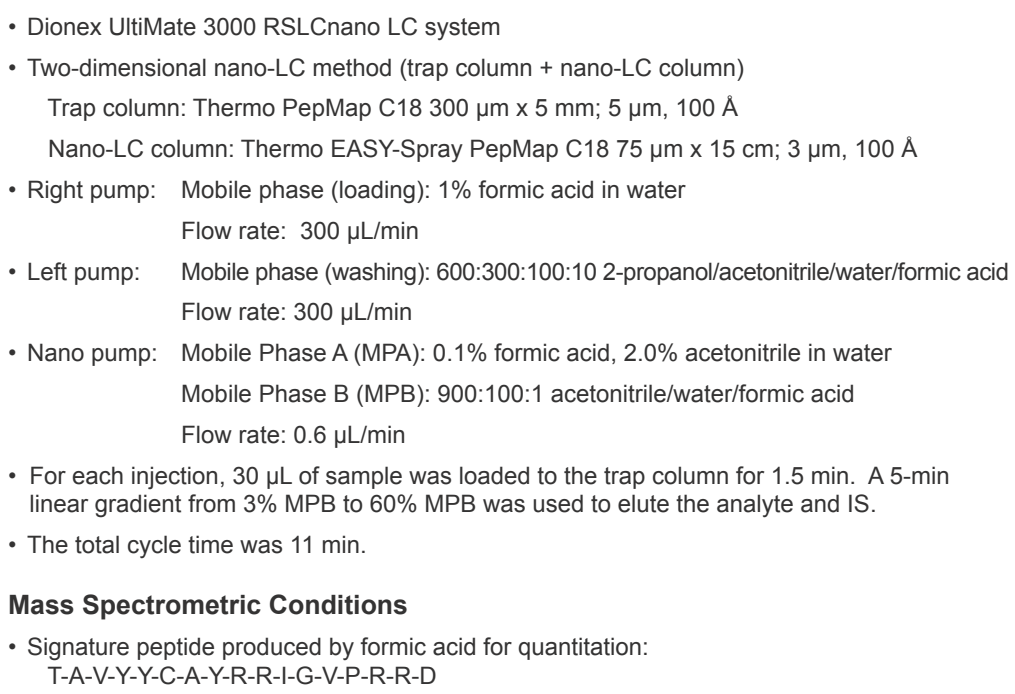
Chromatographic Conditions

- Dionex UltiMate 3000 RSLCnano LC system
- Two-dimensional nano-LC method (trap column + nano-LC column)
  - Trap column: Thermo PepMap C18 300 µm x 5 mm; 5 µm, 100 Å
  - Nano-LC column: Thermo EASY-Spray PepMap C18 75 µm x 15 cm; 3 µm, 100 Å
- Right pump: Mobile phase (loading): 1% formic acid in water
  Flow rate: 300 µL/min
- Left pump: Mobile phase (washing): 600:300:100:10 2-propanol/acetonitrile/water/formic acid
  Flow rate: 300 µL/min
- Nano pump: Mobile Phase A (MPA): 0.1% formic acid, 2.0% acetonitrile in water
  Mobile Phase B (MPB): 900:100:1 acetonitrile/water/formic acid
  Flow rate: 0.6 µL/min
- For each injection, 30 µL of sample was loaded to the trap column for 1.5 min. A 5-min linear gradient from 3% MPB to 60% MPB was used to elute the analyte and IS.
- The total cycle time was 11 min.

Mass Spectrometric Conditions

- Signature peptide produced by formic acid for quantitation:
  T-A-V-Y-Y-C-A-Y-R-R-I-G-V-P-R-R-D
- ^{13}C, ^{15}N-labeled IS:
  R-A-E-D-T-A-V-Y-Y-C-A-Y-[^{15}N, $^{13}C_6$]R-[^{15}N, $^{13}C_6$]R-I-G-V-P-R-R-D-E-F-D-I
- Spray source: Thermo EASY-Spray ionization source
- Spray voltage: 2.3 kV
- SRM transitions (TSQ Vantage triple quadrupole):
  DX-2930 fingerprint peptide: *m/z* 687.3 → *m/z* 649.0 [collision energy (CE): 23 eV; scan time: 100 ms]
  SIL IS: *m/z* 694.3 → *m/z* 656.0 (CE: 23 eV; scan time: 100 ms)

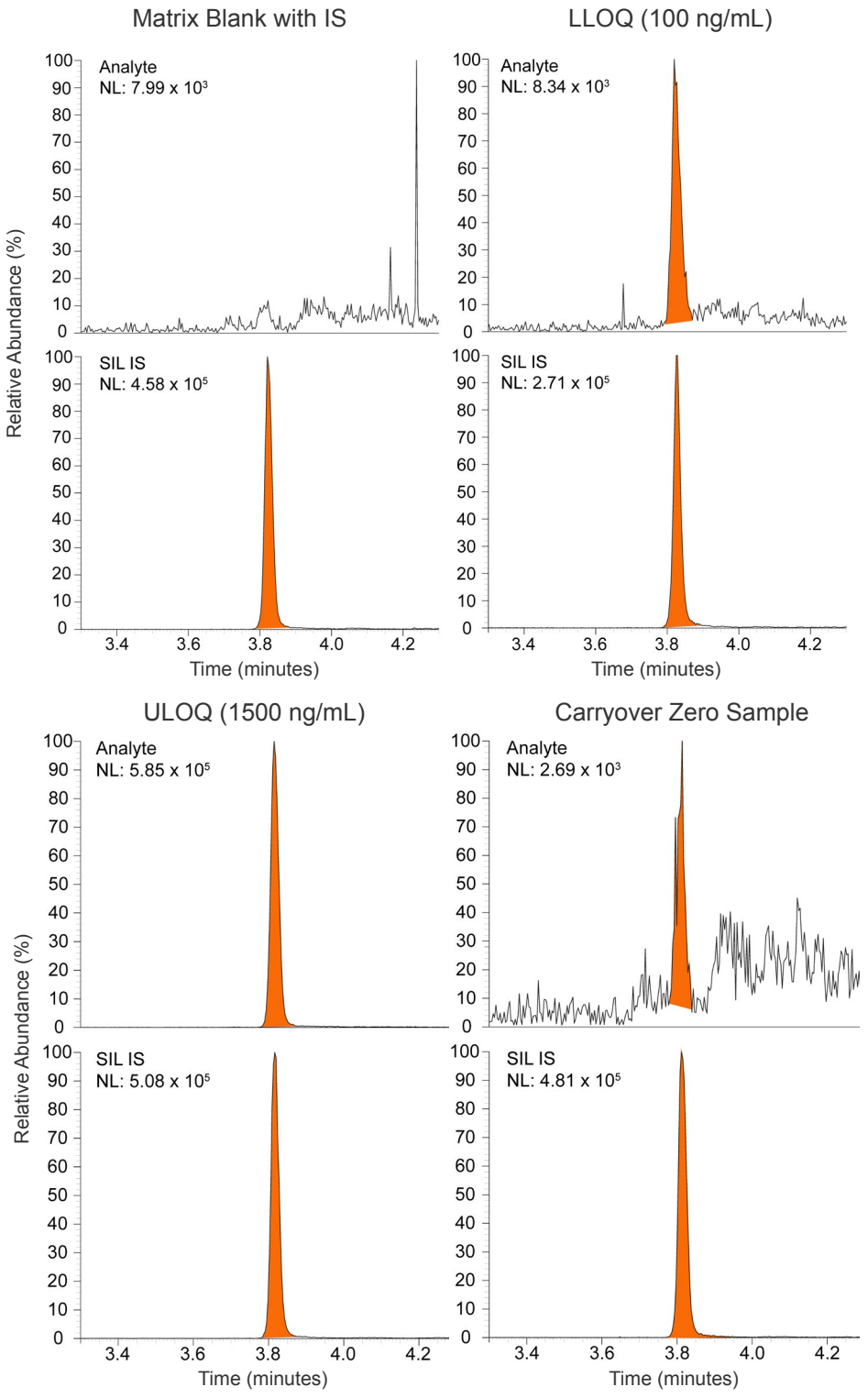
Formic Acid Digestion Optimization: Varying Concentration and Time

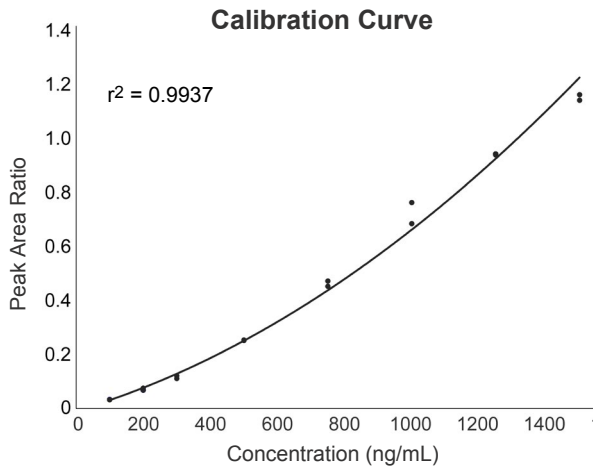


- The formic acid digestion was optimized to determine the concentration of formic acid and duration required to produce the signature peptide in the greatest abundance.
- Optimal formic acid concentration: 2%.
- Optimal digestion time: Overnight (>12 hours).

Results

Chromatograms of a matrix blank with IS (zero sample), sample at the lower limit of quantitation (LLOQ), sample at the upper limit of quantitation (ULOQ), and carryover zero sample are shown below. The analyte appears in the upper panel and the IS in the lower panel.





Inter-lot Accuracy and Precision at the LLOQ

	DX-2930 Concentration (ng/mL)					
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6
Replicate 1	108	117	116	114	113	115
Replicate 2	98.4	117	121	107	105	106
Replicate 3	109	117	113	111	93	104
Mean	105	117	117	111	104	108
%CV	5.6	0.0	3.5	3.2	9.7	5.4
%Theoretical	105.1	117.0	116.7	110.7	103.7	108.3

Inter-lot accuracy and precision was evaluated by analyzing six individual lots of sodium citrate-treated plasma.
LLOQ: Lower limit of quantitation (100 ng/mL).
Precision: %CV (coefficient of variation) = (standard deviation/mean) x 100
Accuracy: %Theoretical = (mean/nominal) x 100

Inter-lot Selectivity

	DX-2930			
	Control Blank Peak Area	% of mean LLOQ area	Zero Sample Peak Area Ratio	% of mean LLOQ response
Lot 1	631	1.0%	0.001426	1.9%
Lot 2	166	0.3%	0.000401	0.5%
Lot 3	1235	2.0%	0.001335	1.8%
Lot 4	229	0.4%	0.000744	1.0%
Lot 5	405	0.7%	0.000593	0.8%
Lot 6	159	0.3%	0.000168	0.2%

Inter-lot selectivity was evaluated by analyzing six individual lots of sodium citrate-treated plasma.

Accuracy and Precision in Quality Control (QC) Samples

	DX-2930 Concentration (ng/mL)					
	LLOQ QC 100	LQC 300	GMQC 500	MQC 900	HQC 1200	DilQC (100-fold dilution) 100000
Replicate 1	102	277	467	898	1140	108000
Replicate 2	110	285	432	875	1280	114000
Replicate 3	99.2	297	444	888	1330	114000
Replicate 4	110	311	453	971	1250	109000
Replicate 5	106	295	477	879	1210	110000
Replicate 6	109	303	465	899	1260	107000
Mean	106	295	456	902	1250	110000
%CV	4.3	4.1	3.6	3.9	5.2	2.7
%Theoretical	106.0	98.3	91.2	100.2	104.2	110.0

Precision: %CV (coefficient of variation) = (standard deviation/mean) x 100
Accuracy: %Theoretical = (mean/nominal) x 100

Conclusions

- We demonstrate that a 2% formic acid digestion can produce a selective signature peptide for antibody quantification using nano-LC/MS/MS.
- Clinical samples will be run to compare the performance of the presented nano-LC/MS/MS method to the existing LBA method.

References:

1. Inglis AS (1983) Cleavage at Aspartic Acid. *Methods in Enzymology* **91**:324-332.
2. Li A, Sowder RC, Henderson LE, Moore SP, Garfinkel DJ, and Fisher RJ (2001) Chemical Cleavage at Aspartyl Residues for Protein Identification. *Analytical Chemistry* **73**:5395-5402.
3. Hua L, Low TY, and Sze SK (2006) Microwave-assisted specific chemical digestion for rapid protein identification. *Proteomics* **6**:586-591.

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