Identification and Quantification of Host Cell Proteins in Biopharmaceuticals using a Novel Data-Independent Acquisition Mode and MS/MS Spectral Library Search

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INTRODUCTION

- Here we investigated the capabilities of a novel data independent acquisition mode recently implemented on a Waters quadrupole/time-of-flight (QTOF) mass spectrometer, namely SONAR™ data acquisition.
- Instead of transmitting all peptide ions produced by the electrospray source, in SONAR mode the quadrupole slides over the mass range of interest during the time required for recording a single mass spectrum by the TOF analyzer. The precursor ions from co-eluting peptides with different m/z are separated by the quadrupole and their corresponding fragmentation spectra are therefore acquired individually. In this way, SONAR offers additional selectivity, by producing cleaner MS/MS spectra with less interferences.
- Spectral libraries are composed of peptide precursors, their RT and the corresponding MS/MS fragment ions recorded in a single LC/MS SONAR acquisition.
- Our results show that SONAR improves the identification of low-abundance HCPs when 1D LC-MS assays are used for identification and quantification of HCP impurities in biopharmaceuticals when coupled with spectral library searches.

METHODS

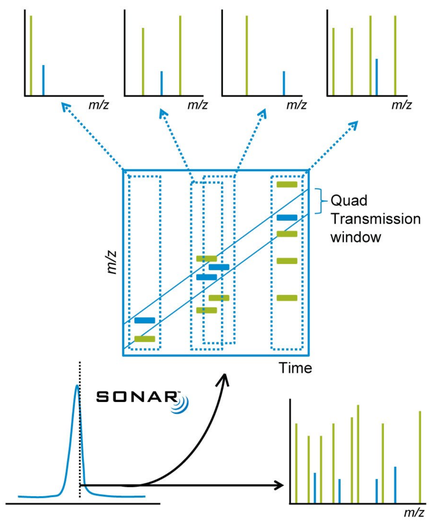
Sample preparation:
A highly purified mAb (NIST mAb LRM 8671, 100 mg/mL) was denatured with RapiGest surfactant (60°C, 15 min), DTT reduced (60°C, 1h), alkylated with IAM (RT, 30 min) and digested with a mixture of Lys-C and porcine trypsin (Promega) overnight. Three protein digest standards (ADH—yeast alcohol dehydrogenase, PHO - rabbit phosphorylase b, and BSA - bovine serum albumin) were spiked post-digestion.

LC Conditions:
The LC/MS assay was performed on an ACQUITY™ UPLC® I-class system equipped with a CSH (charged-surface hybrid) C18 column (2.1 x 150 mm, 1.7 µm particles, P/N 186005298). Peptide separations were performed at a flow rate of 0.2 mL/min with a gradient of from 0% to 40% Solvent B in 90 min, at a column temperature of 60 deg C. The mobile phases were: 0.1% FA (formic acid) in DI water (Solvent A) and 0.1% FA in acetonitrile (Solvent B).

Mass Spectrometry:
Data-independent acquisitions were performed on a Xevo® G2-XS QTof mass spectrometer using MS^E and SONAR™ acquisition modes. MS^E data was acquired with 0.5 s scans over a mass range of 100-2000 Da. SONAR acquisitions (also 0.5 s scans) had a quadrupole mass range of 400-900 m/z, a 50 Da mass window and a TOF mass range of 100-2000 m/z. For both acquisition modes low-energy scans were acquired with a CE of 6 eV, while the high-energy fragmentation scans used CE ramping from 15 to 40 V. Three replicate LC/MS injections were performed in each mode.

Data processing:

Progenesis QI for proteomics 4.0 was used for data processing. The SONAR and MS^E datasets was searched against a mouse protein database containing 16,644 entries.
A spectral library of 42 HCP peptides (precursors m/z, RT and major fragment ions) was compiled and used for identification of NIST mAb HCPs.



SONAR™ features:

- *the resolving quadrupole slides over a selected mass range during each MS scan;*
- *from scan to scan the collision cell is alternating between low and high collision energies;*
- *MS and MS/MS spectra are acquired in two data channels which are used by the Progenesis QI for proteomics software to align precursors and fragment ions;*
- *data interpretation is greatly simplified because SONAR produces very clean MS and MS/MS spectra*

1D LC-MS platform used for HCP identification and quantification in conjunction with Progenesis QI for proteomics 4.0

RESULTS

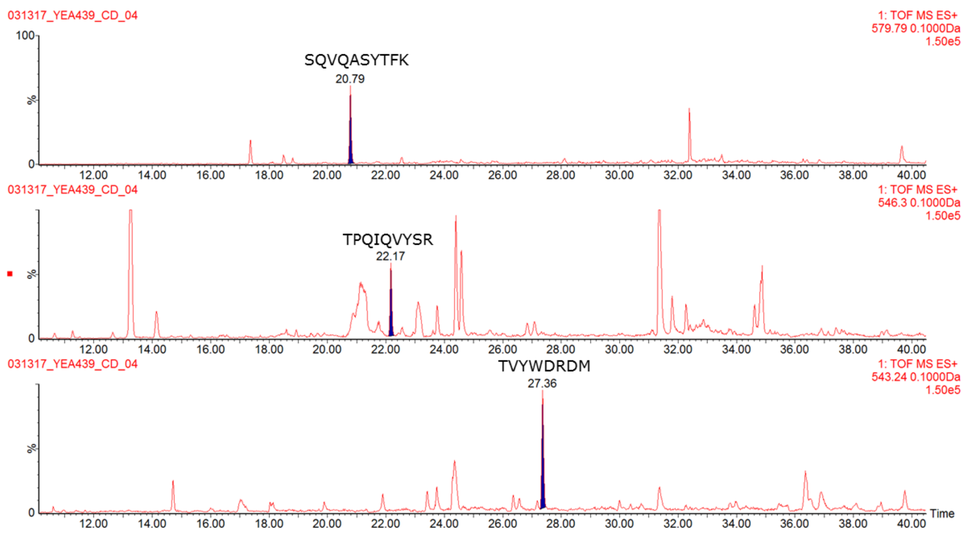


Figure 1. Extracted mass chromatograms of three low-abundance HCP peptides identified in the NIST mAb using SONAR acquisition: (A) SQVQASYTFK peptide (precursor 579.79, +2) from low affinity IgG gamma Fc region receptor protein; (B) TPQIQVYSR (546.30, +2) from beta-2-microglobulin; (C) TVYWDRDM (543.24, +2), also from beta-2-microglobulin.

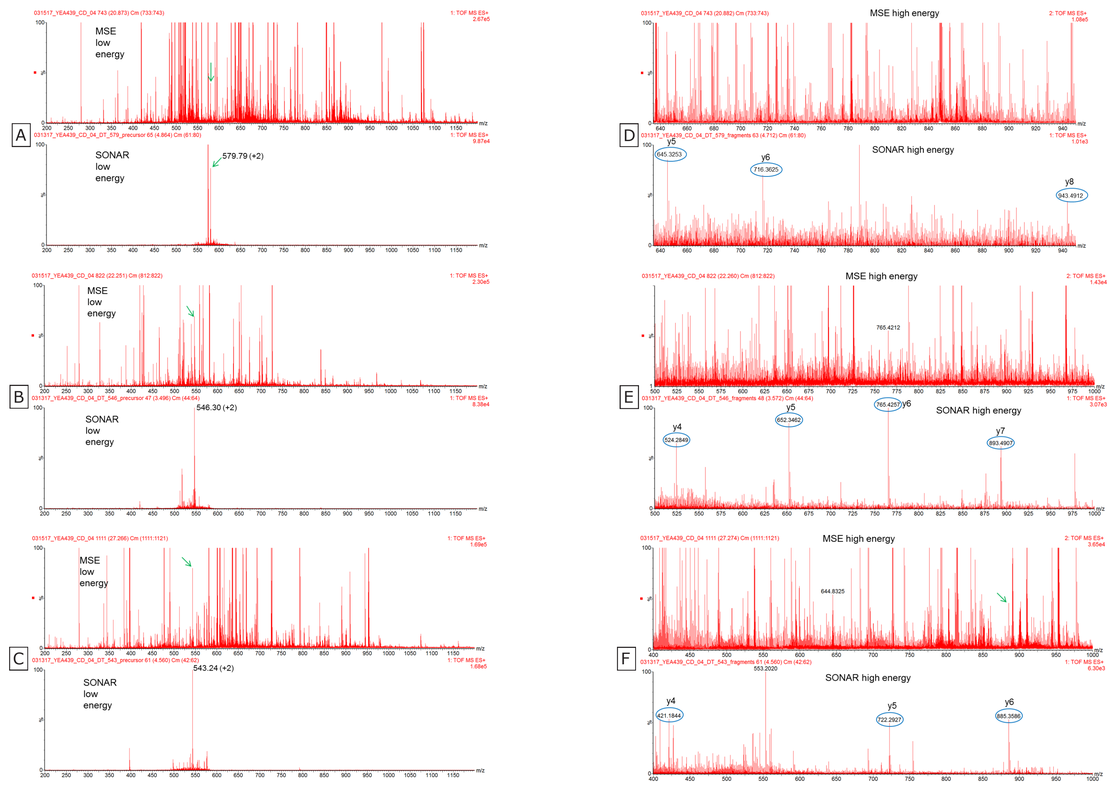


Figure 2. Comparison of low and high energy MSE and SONAR spectra recorded for three low-abundance HCP peptides: (A, D) Precursor and fragmentation mass spectra of SQVQASYTFK peptide; (B,E) MS and MS/MS spectra of TPQIQVYSR (C,F) MS and MS/MS spectra of TVYWDRDM. In all three cases, the precursor MS spectra as well as the MS/MS fragmentation spectra are significantly less complex than the corresponding MSE spectra, enabling Progenesis QI for proteomics 4.0 to easily identify two low level (10-20 ppm) HCPs from NIST mAb: low affinity IgG gamma Fc receptor protein and beta-2-microglobulin.

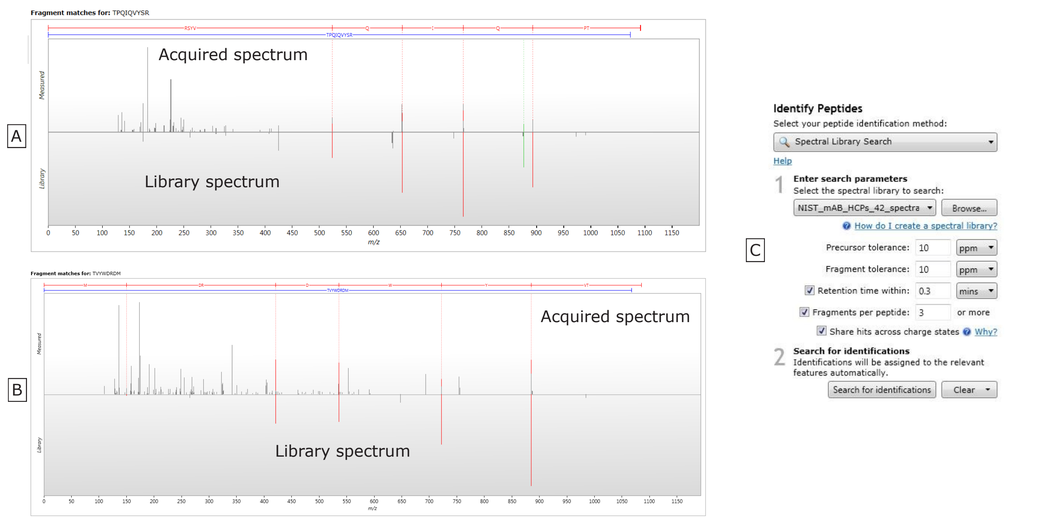


Figure 3. Comparison between the acquired MS/MS spectra against the SONAR MS/MS library spectra for two HCP peptides: (A) TPQIQVYSR peptide and (B) TVYWDRDM peptide. The library search parameters from Progenesis QI for proteomics 4.0 are highlighted in panel C.

No crt	Accesssion Number	Protein Description	Sequence Coverage (%)	Average MW (kDa)	Amount on column fmoles	Amount on column ng	Concentration ng/mL	Concentration ppm	RSD (%)
1	P00330	Alcohol dehydrogenase yeast (ADH) - 5000 fmoles	61.3	36.7	4533	166	26618	266	23.6
2	P00489	Glycogen phosphorylase rabbit (PHO) - 1000 fmoles	31.2	97.1	1000	97	15536	155	0.0
3	P05064	Fructose biphosphate aldolase A isoform	58.7	39.3	2817	111	17713	177	16.8
4	P05063	Fructose biphosphate aldolase C isoform	32.5	39.4	1742	69	10982	110	6.0
5	P02769	Bovine serum albumin (BSA) - 250 fmoles	30.7	66.3	292	19	3098	31	7.4
6	P08101	Low affinity immunoglobulin gamma Fc region receptor	11.6	36.7	367	13	2155	22	24.1
7	P01887	Beta-2-microglobulin	16.0	13.8	482	7	1064	11	15.7

Table I. Host cell proteins (HCPs) identified and quantified in the NIST mAb using SONAR acquisition. Three spiked proteins (ADH, BSA and BSA) along with 4 HCPs (highlighted in red) were identified in a single LC-MS injection. The data presented in Figures 1 and 2 is centered on the two low-level (10-20 ppm) HCPs: low affinity IgG gamma Fc region receptor and beta-2-microglobulin.

CONCLUSIONS

- SONAR acquisition significantly reduced spectral complexity at both precursor and fragment ion level.
- Three spiked proteins as well as four HCPs were identified and quantified in a single LC-MS injection with an LLOQ of the assay of 10 ppm.
- Spectral libraries containing all the HCP peptides (m/z, RT and MS/MS fragment ions) identified in a single SONAR acquisition can be used for fast screening/HCP monitoring for bioprocess monitoring of therapeutic mAbs
- Results show that HCPs can be quickly and robustly identified, quantified and monitored in biopharmaceutical samples using the 1D LC-MS HCP workflow with Progenesis QI for proteomics 4.0.

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