LC-MS-based host cell protein (HCP) identification and monitoring during biopharmaceutical downstream process development

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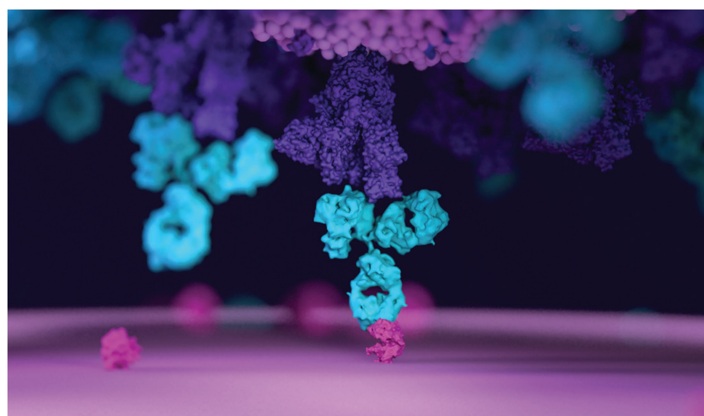
Application benefits

- A streamlined workflow for host cell protein (HCP) identification and relative quantitation using non-denaturing digestion conditions, followed by LC-MS/MS analysis.
- Detection and identification of HCPs across a large dynamic range with high sensitivity, down to ~1 ppm, using a UHPLC system coupled with a high-resolution accurate mass (HRAM) mass spectrometer.



- Qualitative and quantitative information for individual HCPs that can be used to guide downstream process development and optimization decisions.
- High selectivity of POROS resin for effective removal of HCP content and species.
- A single software solution to provide complete peptide mapping and HCP analysis in the same analysis.

Goals

To illustrate the benefit of applying LC-MS based HCP analysis to aid downstream process development, including monitoring of high risk HCPs in downstream pools.

To introduce a streamlined HCP analysis solution from sample preparation, LC-MS data acquisition through data processing and review.

Introduction

Host cell proteins, which are biologic drug product impurities released during cell growth and subsequent processing, can detrimentally affect final drug product safety and efficacy. Therefore, these HCPs must be removed post-harvest through a series of purification steps.[1]

A generalized platform for monoclonal antibody purification processing contains a series of affinity chromatography and polishing steps that target process- and product-related impurity removal and reduction. Many HCPs are removed after multiple steps of purification, but it can be challenging to completely remove all of them, and trace amounts of HCPs can be present in the drug substance. Although the acceptable limits for HCP contamination in final product is below 100 ppm and the overall safety and purity track record of the industry is excellent, there have been some harmful incidents and publicly disclosed reports on patients taking drug substances that contain minute levels of these impurities.[2] Most of these impurities were discovered during clinical development, and changes were made to the manufacturing process to remove the HCP impurity (Table 1). Identification of the high-risk HCPs and evaluation of the process removal ability in process characterization is needed for safety and potency consideration. A complete understanding of HCP clearance during characterization would assist in understanding the effects of process and raw material changes.

Table 1. Adverse event cases caused by HCP for biotherapeutic proteins

Product	Impurity	Effect
Lebrikuzumab (Genentech)	PLBL2	Anti-PLBL2 immune response. No increase in ADA response. Impurity reduction required to proceed to Phase III trial.
CTLA4-Ig (BMS)	MCP-1	Acute toxicity associated with MCP-1 activity. Clinical hold.
Hgh (Sandoz)	RPI and perhaps other ECPs	Anti-RPI antibodies and increased ADA. Suggesting an adjuvant like effect. Purer drug substituted in clinical study.
Multiple (Ipsen)	Proteinase or Tweenase	Degradation of product or excipients upon storage.
FIX (Ixinity and Rixubis) (Baxter)	Furin and/or other co-expressed proteins	Non-transient anti- CHOP antibodies with rising titers are of greatest concern.

All major regulatory authorities, like the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH)[3], U.S. Food and Drug Administration (U.S. FDA)[4], European Medicines Agency (EMA)[5], and others, require a strict and state-of-the-art monitoring of HCP residues. The conventional enzyme-linked immunosorbent assay (ELISA) has been considered as the gold standard for HCP monitoring due to its inherent advantages, such as high sensitivity, throughput, ease of operation, and automation[7]. However, this approach lacks individual HCP identification and quantitation information and might miss or underestimate non-immunogenic HCPs. Thus, LC-MS/MS based HCP analysis has emerged as an orthogonal approach that can both identify and quantitate individual HCPs independent from their immunogenicity.

A scalable platform process for mAb purification was established in the Shanghai Bioprocess Design Center, and three intermediate product samples from this platform process were used in this study for LC-MS/MS based HCP analysis, using the new host cell protein analysis workflow in Thermo Scientific™ BioPharma Finder™ 4.1 software. The goal of this application note is to gain additional knowledge of influencing factors affecting upstream processing (USP) and downstream processing (DSP) into individual HCP clearance in recombinant mAb products, thus supporting decisions on most suitable HCP control strategies.

Experimental

Instrumentation

Thermo Scientific™ Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (P/N 0726030)

Thermo Scientific™ Vanquish™ Flex Binary UHPLC system consisting of:

- Thermo Scientific™ System Base Vanquish™ Horizon/Flex (P/N VF-S01-A-02)
- Thermo Scientific™ Vanquish™ Binary Pump F (P/N VF-P10-A-01)
- Thermo Scientific™ Vanquish™ Split Sampler FT (P/N VF-A10-A-02)
- Thermo Scientific™ Vanquish™ Column Compartment H (P/N VH-C10-A-02)

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.2.10 with the following components:

- Chromeleon Enterprise Client (P/N 7200.0300)
- Biopharma QC Package (P/N 7200.0044)
- Thermo Scientific Instrument Control (P/N 7200.1000)
- License Key New (P/N 7050.0104A)

BioPharma Finder 4.1 software (OPTON-30986)

Reagents and consumables

- Thermo Scientific™ Water, UHPLC-MS grade (P/N W8-1)
- Thermo Scientific™ Acetonitrile, UHPLC-MS grade (P/N A956-1)
- Thermo Scientific™ Pierce™ Trypsin protease MS grade (P/N 90058)
- Thermo Scientific™ Pierce™ Formic acid, LC-MS grade (P/N 28905)
- Invitrogen™ UltraPure™ 1 M Tris-HCl buffer, pH 7.5 (P/N 15567027)
- Sigma, DL-Dithiothreitol (DTT) BioXtra ≥99% purity (P/N D-5545)
- Thermo Scientific™, POROS™ MabCapture™ A Select affinity chromatography resin (P/N A26457)
- Thermo Scientific™, POROS™ XS strong cation exchange resin (P/N 4404335)
- Thermo Scientific™, POROS™ XQ strong anion exchange resin (P/N 4467818)
- Sinopharm, Tris (Base)
- Sinopharm, Tris-HCl
- Qinfen pharm, Sodium chloride
- Nanjing Chemical Reagent, Acetic acid
- Nanjing Chemical Reagent, Anhydrous sodium acetate
- Thermo Scientific™ Acclaim™ VANQUISH™ C18 column, 2.2 µm, 2.1 × 250 mm (P/N 074812-V)

Sample purification

Three mAb samples from different purification steps were used in this study. The total purification steps included three chromatographic units used to capture mAb from cell culture fluid and gradually remove the process- and product-related impurities. The Protein A step is the first chromatographic unit operation in the purification process. This step used an immobilized Protein A resin that binds the mAb from the harvested cell culture fluid (clarified harvest). Process impurities such as HCP, DNA, and small molecules are removed in the flow through or wash. A low pH buffer elutes the mAb, and the elution pools are moved to a low pH viral inactivation step at pH 3.6 ± 0.1 for 1.5 h at ambient temperature. Samples of the elution pools are collected and kept alone for LC-MS analysis. The Protein A column is packed with POROS MabCapture A Select affinity resin to a bed height of 20 ± 2 cm and the loading density is 30–40 g/L. The elution buffer is 100 mmol/L acetate buffer with pH 3.5 ± 0.1, and the peak collection criteria are based on UV absorbance at 280 nm wavelength detection of the elution peak.

The anion exchange (AIEX) chromatography step is the first polishing step. The chromatography unit is operated in flow-through mode, binding impurities such as HCP, DNA, and endotoxins to the resin while the antibody passes through. Following the low pH viral inactivation step, the Protein A elution pool is adjusted to pH 7.0 ± 0.1 and loaded onto a pre-equilibrated AIEX chromatography column at ambient temperature. Next, the column is washed with equilibration buffer to collect the AEX pool based on monitoring by UV absorbance at 280 nm wavelength detection. The column is packed with POROS XQ resin to a height of approximately 20 cm and the loading density is 60–70 g/L. The equilibration and wash buffer are 20 mmol/L Tris-HCl buffer with pH 7.0 ± 0.1, and the peak collection criteria are based on UV absorbance at 280 nm wavelength detection of the flow-through and washing peak. Also, part of the elution pool samples is collected for LC-MS.

The cation exchange (CIEX) chromatography step is the final chromatographic purification step in the process. It is operated in the Bind-Elute mode to capture the mAb. The step is operated with a step elution designed to provide separation of HCP and aggregate, while also providing clearance of DNA and leached Protein A. Following the AIEX chromatography step, the product pool is adjusted to pH 5.0 ± 0.1 in acetate buffer and loaded onto a pre-equilibrated 20 ± 2 cm bed height column at ambient temperature. The column is washed and subsequently eluted by equilibration buffer with 175 mmol/L NaCl concentration. The column is packed with POROS XS resin to a height of approximately 20 cm and the loading density is 40–50 g/L. The equilibration and wash buffer are 20 mmol/L acetate buffer with pH 5.0 ± 0.1 and the peak collection criteria are based on UV absorbance at 280 nm wavelength detection of the elution peak. Details are shown in Figure 1.

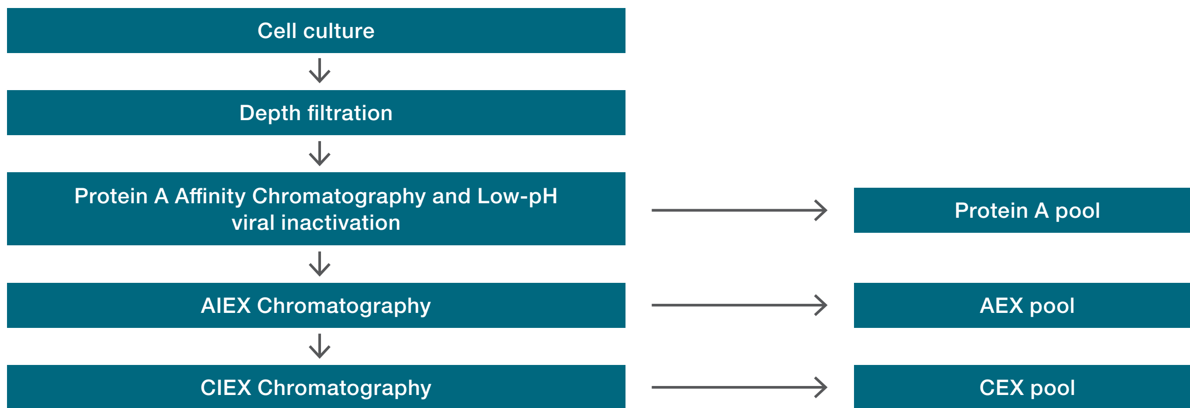


Figure 1. Total purification steps

Sample preparation

Four intact standard proteins were spiked into samples for quantitative purposes, as shown in Table 2.

Table 2. Spike-in standards. A 1 µg/µL stock of each protein was prepared in 50 mM Tris-HCl (pH 7.9). The stock solutions of the individual proteins were used to prepare a STD mix solution of the four standard proteins in 50 mM Tris-HCl (pH 7.9).

Uniprot accession	Description	Organism	MW (Da)	pmol/ injection
P00915	Carbonic anhydrase 1	*Homo sapiens*	28870	1.5
P04040	Catalase from human erythrocyte	*Homo sapiens*	59756	0.15
P68082	Myoglobin	*Equus caballus*	17083	0.15
P00711	Alpha-lactalbumin	*Bos taurus*	16247	0.015

In this study, we optimized the non-denaturing tryptic peptide sample preparation protocol published by Huang et al. in 2017[8]. Digestion conditions are described in Table 3. For three technical replicates, 500 µg of sample were used in each digestion. Undigested protein was removed via precipitation at 95 ˚C for 10 min, followed by centrifugation at 14,000 × g for 10 min. Each sample had multiple digestions (biological replicates).

Table 3. Optimized non-denatured digestion protocol

Millipore™ 3k cut-off filter
500 µg sample + STD mix + 200 µL digestion buffer (50 mM Tris-HCl, pH = 7.9) 21,000 g * 10 min centrifugation 4 times Final volume ~80 µL
Trypsin:mAb = 1:200, 37 ˚C, 2 h
5 mM DTT, 95 ˚C, 10 min
14,000 g * 10 min centrifugation
Supernatant ~70 µL

Liquid chromatography

For each analysis, peptides in a 22 µL injection (equivalent to 167 µg mAb, if digestion was complete) were loaded onto a 2.1 x 250 mm Accucore Vanquish UHPLC column with 2.2 µm particle size (P/N 074812-V) and separated with a linear gradient using a Vanquish Flex Binary UHPLC system. The autosampler temperature was set to 5 ˚C while the column temperature was held at 60 ˚C (Still Air Thermostatting Mode).

The LC gradient used in this study is shown in Table 4.

- Solvent A: 0.1% formic acid in water
- Solvent B: 0.1% formic acid in acetonitrile
- Flow rate: 0.3 mL/min

Table 4. LC gradient for tryptic peptides separation

Time	%B
0.0	3.0
1.0	3.0
90.0	35.0
95.0	85.0
100.0	85.0
105.0	3.0
110.0	85.0
115.0	85.0
115.1	3.0
135.0	Stop run

Mass spectrometry

All experiments presented in this application note were performed on the Q Exactive Plus mass spectrometer controlled by Chromeleon CDS. Ion source settings and MS method parameters are summarized in Table 5.

Table 5. Mass spectrometry tune and method settings

MS source setting	Value
Sheath gas	35
Aux gas	10
Sweep gas	0
Spray voltage (kV)	3.9
S-lens RF level (%)	50
Aux gas temperature (˚C)	350
Capillary temperature (˚C)	320
Properties of Full MS	Value
General	
Runtime (min)	0–100
Polarity	Positive
Full MS	
Resolution	70,000
AGC target value	3.00E+06
Maximum injection time (ms)	100
Scan range (*m/z*)	390–1,200
Properties of Full MS/dd-MS² (top5)	**Value**
dd-MS²	
Resolution	35,000
AGC target value	1.00E+05
Maximum IT (ms)	150
TopN	10
Isolation window (*m/z*)	1.2
NCE (%)	27
dd settings	
Minimum AGC target	2.00E+03
Intensity threshold	1.00E+04
Charge exclusion	Unassigned
Peptide match	Preferred
Exclude isotopes	On
Dynamic exclusion (s)	10.0

The data for peptide mapping were acquired with a data-dependent Top10 tandem mass spectrometry (ddMS²) method.

Data processing

The ddMS² data was processed by BioPharma Finder software using the host cell protein workflow, a new feature within peptide mapping analysis. The default CHO database in BioPharma Finder software was used, and the sequence of protein standards was added at the beginning of the FASTA file. The processing parameters are listed in Tables 6 and 7.

Table 6. BioPharma Finder software parameter settings for peptide mapping analysis

Component detection	Setting	Identification	Setting
S/N threshold	1	Maximum peptide mass	11,000
Typical chromatographic peak width	0.29	Mass accuracy (ppm)	6
Relative MS signal threshold (% base peak)	1.00	Minimum confidence	0.80
Relative analog threshold (% of highest peak)	1.00	Maximum number of modifications for a peptide	1
Width of Gaussian filter (represented as 1/n of chromatographic peak width)	3	Unspecified modification	-
Minimum valley to be considered as two chromatographic peaks	80.00	N-Glycosylation	CHO
Minimum MS peak width (Da)	1.20	Protease specificity	High
Maximum MS peak width (Da)	4.20	**Static modifications**	**Setting**
Mass tolerance (ppm for high-res or Da for low-res)	6.00	Side chain N-term	Gln → Pyro-Glu
Maximum retention time shift (min)	4.29	**Variable modifications**	**Setting**
Maximum mass (Da)	30,000.00	C-term	None
Mass Centroiding cut off (% from base)	15.00	Side chain	Deamidation (N), Oxidation (MW)

Table 7. BioPharma Finder software parameter settings for HCP analysis

Basic parameters	Setting	Modifications	Setting
Protein database	Default CHO database in BioPharma Finder software with the following protein standards: P00915 Carbonic anhydrase 1 *(Homo sapiens)* P04040 Catalase from human Erythrocyte *(Homo sapiens)* P68082 Myoglobin *(Equus caballus)* P00711 Alpha-lactalbumin *(Bos taurus)*	Static side chain	None
Acquisition type	High-High (MS^1 and MS^2)	Max # of variable modification per peptide	1
Precursor mass tolerance	10 ppm	Variable side chain	Deamidation (N)
Ions to search	b ion, y ion, NL ion	-	Oxidation (MW)
Mass range (MH^+ peptide mass)	350 to 5,000	Protein terminal modification	Acetylation (N-term)
E-value cutoff	0.100	Enable methionine protein N-term clip	Yes
Protease termini	Fully digested	Enable methionine protein N-term clip	Yes
Max num internal miscleavages	2	-	-
Enable decoy search	Yes	-	-

Host cell protein analysis workflow: new feature in BioPharma Finder software

Data were processed using BioPharma Finder software. Host cell protein analysis capability was recently added to the peptide mapping workflow in the software. The sequence manager contains a new subpage, “Host Cell Protein Database Manager” (Figure 2). Some popular host cell line databases, such as Chinese hamster, human, and mouse, are built in and can be used directly. Users may also add their customized database here. For protein standard spiked-in experiments, it is recommended to add the sequences of standards at the beginning of the database.

The component detection and mAb identification are the same as before. However, a new tab for host cell protein database searching parameters in the parameters page of the peptide mapping analysis part can now be enabled (Figure 3).

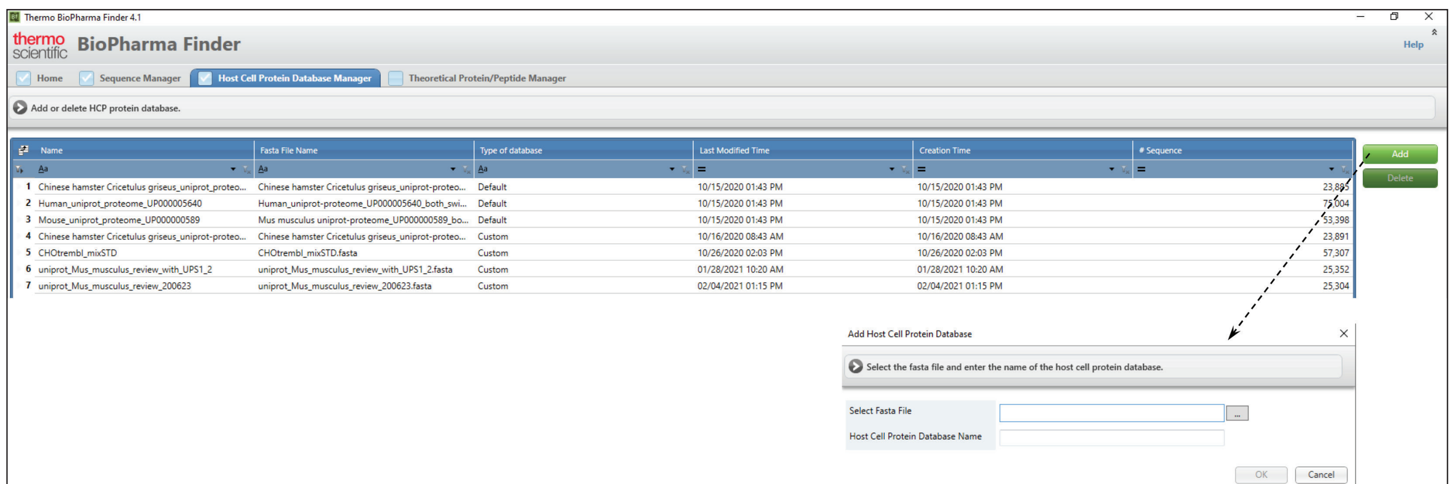


Figure 2. Newly added Host Cell Protein Database Manager page. Customized database can be added by left-clicking the “Add” button.

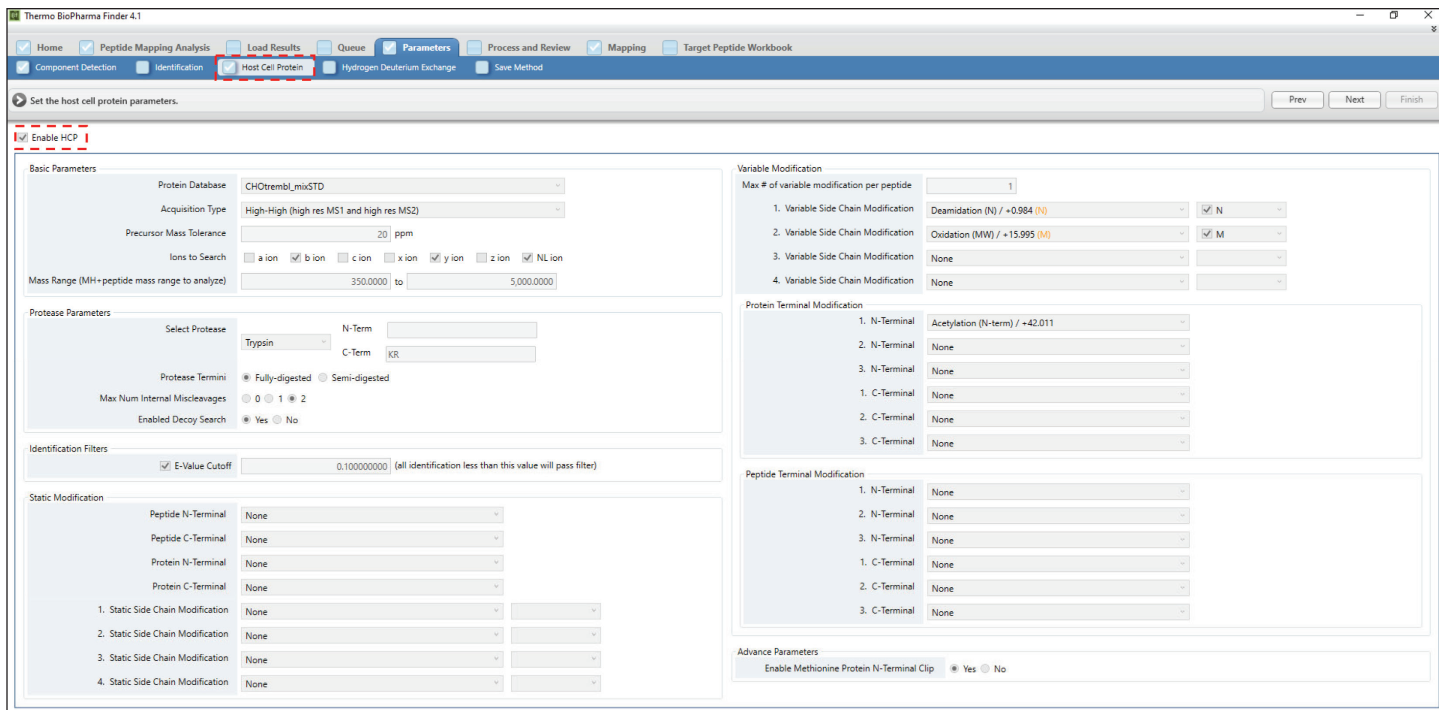


Figure 3. Host Cell Protein database searching parameters. All host cell protein related database searching parameters should be set here.

To set HCP searching parameters, "Enable HCP" must be checked. HCP parameters are divided into several categories. Protein database, precursor mass tolerance, ions to search, and mass range (MH^+ peptide mass range to analyze) can be set in the basic parameters. The acquisition type is automatically read from the raw file by the software. In most instances, b/y and neutral loss (NL) ions are used for the search. Protease parameters include four parameters, select protease/protease termini/max num internal miscleavages/enabled decoy search, which should be set properly in alignment with the digesting conditions. The parameter E-Value cutoff is used to evaluate the credibility of the identification results, and it can be modified after the searching is finished. Both static and variable modifications can be set in corresponding parts of the protein or sections.

A Top3 peptide-based quantitation method[9] was used in the software. Spiked-in proteins were used as the quantitation standards.

Results and discussion

In this study, we report a case study utilizing an LC-MS workflow to identify and quantify host cell proteins from different purification stages to support mAb downstream CEX process (DSP) development. ELISA quantitation results show that the HCPs were efficiently removed from the mAb product step by step, and the levels in the CEX elution pool had already reached the final product standards (<100 ppm).

To increase the dynamic range of HCP detection, non-denatured tryptic digestion was performed by following an optimized non-denaturing tryptic peptide sample preparation protocol based on publication of Huang *et.al* in 2017[8]. Using this procedure, the antibody is not or only minimally digested, while residual HCPs in the sample are digested, which means the interference of mAb peptides are reduced compared to traditional denatured digestion. Through optimization, we were able to shorten the digestion time from overnight to two hours, which significantly reduces the overall sample preparation time. This also means that more HCPs can be loaded on-column without negative effect on the separation.

Tryptic peptides were then analyzed by a Vanquish Flex Binary UHPLC system connected to Q Exactive Plus mass spectrometer. Peptide mapping workflow in BioPharma Finder software was used for HCP identification, relative quantitation, and visualization. Figure 4 shows the entire workflow from sample digestion to data processing.

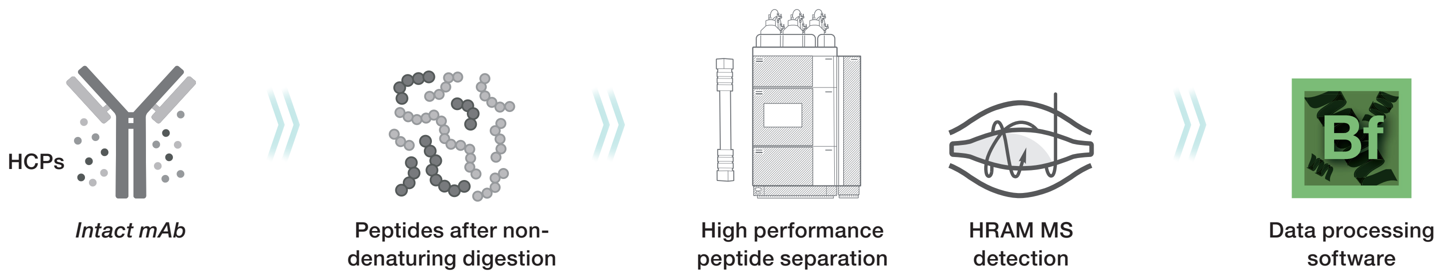


Figure 4. Overview of experimental set-up

Figure 5 shows the base peak chromatogram (BPC) comparison of Protein A/AEX/CEX pool samples. Although non-denaturing digestion was employed to minimize the digestion of mAb, due to the dominant in absolute amount, the major peaks are from mAb digested peptides (Figure 5A). Since HCPs are at low levels compared to mAb product (~10^3 ppm after Protein A affinity purification and ~10 ppm or lower in final product), the HCP peptide peaks intensity are quite low. Benefiting from the UHPLC separation and sensitivity of high-resolution MS platform, these low abundance components can be identified and further quantitated. Figure 5B shows clearly that the intensity and peak numbers of HCPs decrease following step-by-step HCP clearance.

Figure 5. Base peak chromatogram (BPC) comparison of Protein A/AEX/CEX pool samples. (A) Overview. The major peaks are from the mAb. (B) XIC and MS spectra of an HCP peptide. The intensity decreases from Protein A pool to CEX pool sample, which is due to step-by-step HCP clearance.

Robustness and system stability play important roles in HCP identification and quantitation. Figure 6 shows two replicate non-denaturing digests of Protein A pool sample, which demonstrated excellent reproducibility of digestion and chromatographic separation in this study.

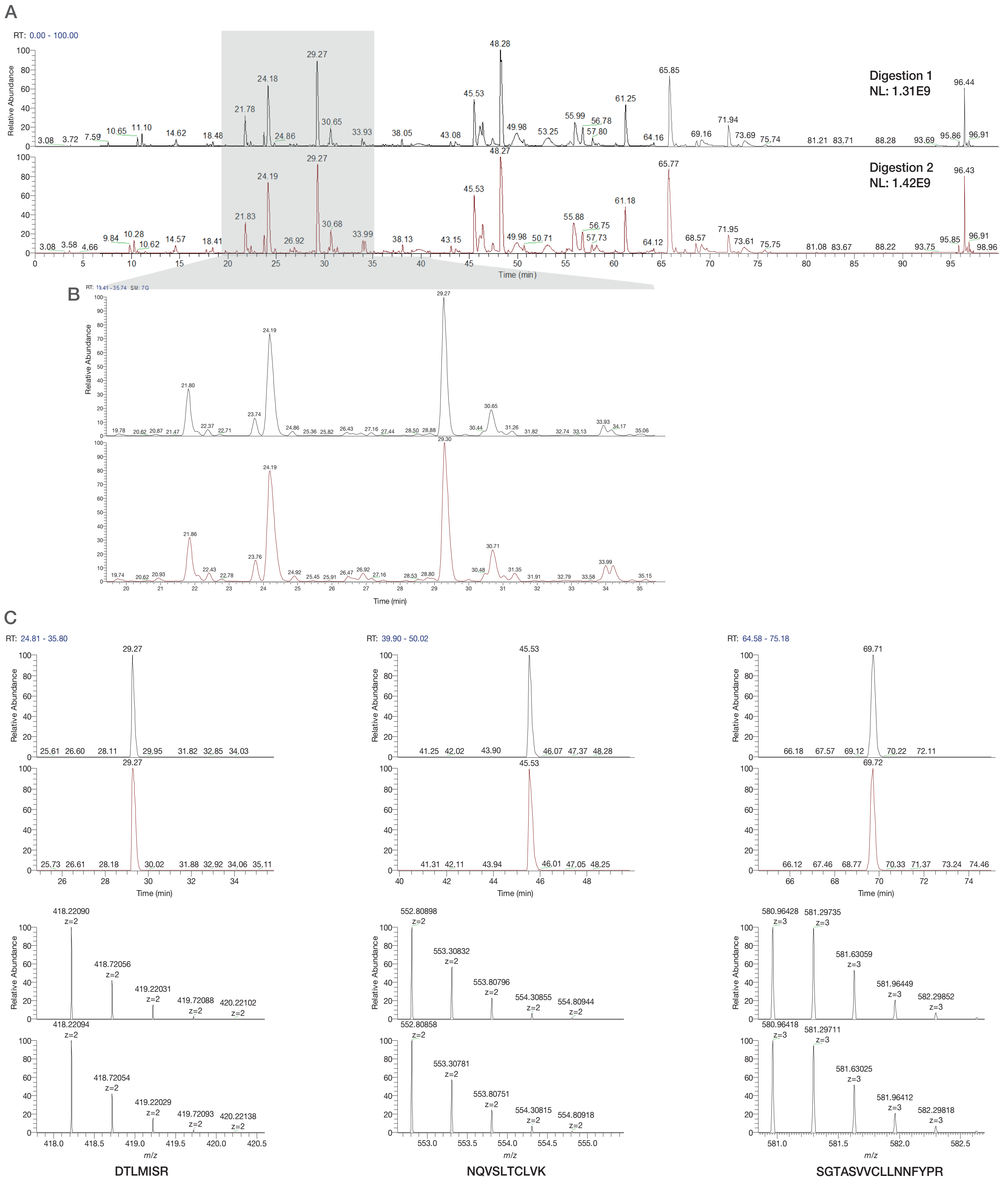


Figure 6. Two replicate non-denatured digests of Protein A pool sample. (A) Whole time range. (B) Expanded view, time range 20–35 min. (C) Three mAb peptides across different RT session from early to late elution.

HCP identification and quantitation results of samples from different purification steps

Multiple previously reported HCPs were identified in our results, such as clustering, phospholipase B-like, and peroxiredoxin families[10]. Table 8 shows the quantitated HCP number and total HCP (ppm) measured by MS. For LC-MS/MS based HCP analysis, the total HCP level was averaged across biological replicates. Top number of peptides ≥3 was set as a quantitation threshold, which means protein must be quantitated with at least three peptides present. 676 HCPs (total HCP 49473.27 ppm) in Protein A pool sample, 111 HCPs (total HCP 1253.38 ppm) in AEX pool sample, and 7 HCPs (total HCP 89.66 ppm) in CEX pool sample passed this criterion. The decreasing trend of HCP number after each purification step provides clear evidence of HCP clearance. There might be a difference in total HCP level between MS and ELISA assays, which was reported before[10,11]. A possible explanation of this phenomenon may be the difference in algorithms used for HCP level calculation and the ELISA kit may fail to detect some HCPs[22].

Table 8. Quantitated HCP number and total HCP (ppm) measured by MS. Total HCP level is averaged across biological replicates.

Sample	Quantitated HCP number (Top # of Peptides ≥ 3)	Total HCP (average) by MS, ppm
Protein A pool	676	49,473.27
AEX pool	111	1,253.38
CEX pool	7	89.66

Database search results of HCP identification and relative quantitation can be reviewed under the Mapping tag within the BioPharma Finder software peptide mapping workflow. Search results of one CEX pool sample digestion contain three technical replicates (Figure 7) as an example. An overview of both mAb and HCP identification and quantitation is displayed in the coverage subpage of the mapping page, including the chromatogram, results table, and sequence map. In comparison to the mAb peaks, it is clear that the HCP peaks are at a low level, which indicates the importance of dynamic range and sensitivity of the analysis method.

A

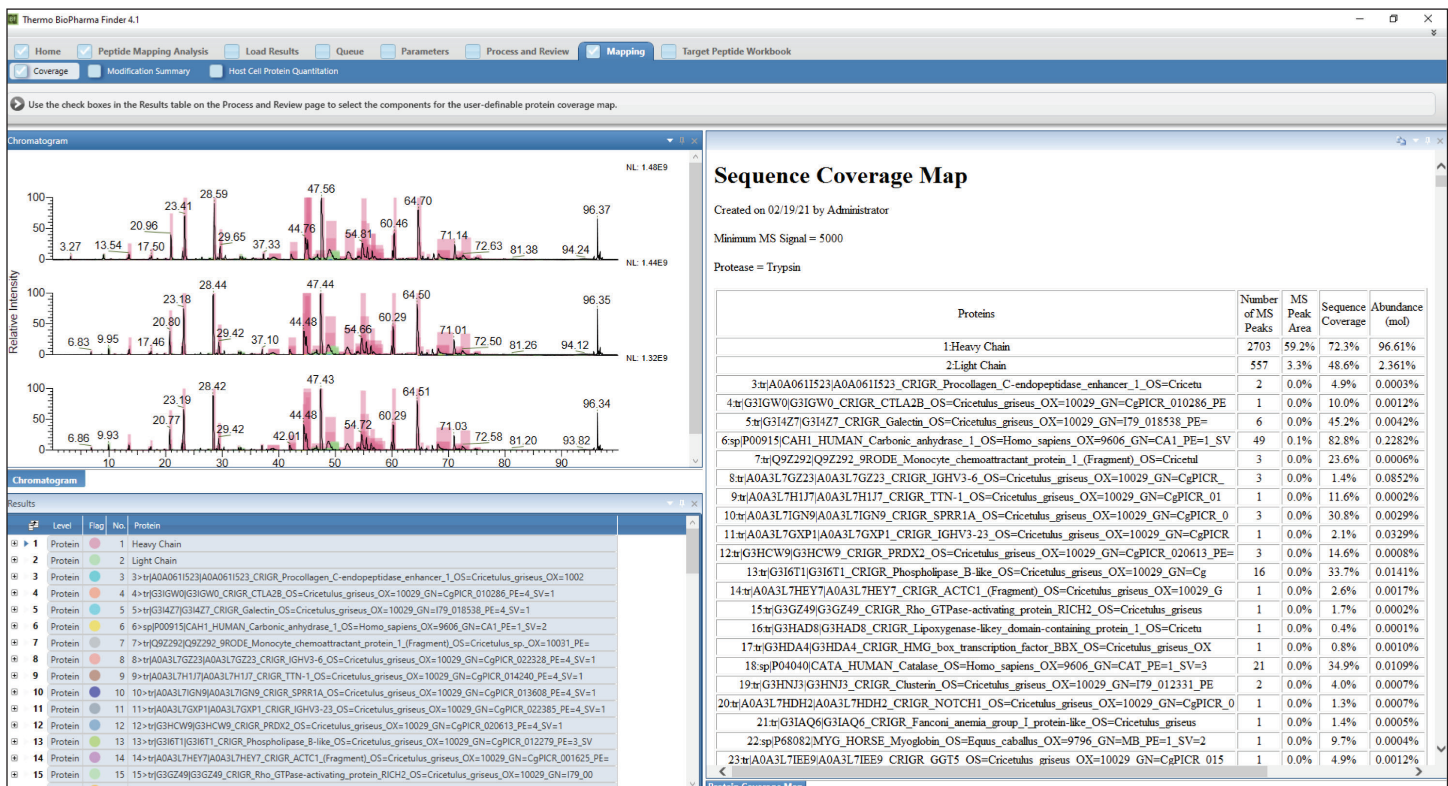


Figure 7. A new feature of the HCP identification and sequence coverage on the mapping page. A digestion of the CEX pool sample is shown as an example. (A) Whole page overview. Upper left, chromatogram of technical replicates. Both mAb and HCP identified peaks are shaded in color. Lower left, protein identification result. Right, sequence coverage map. (Part B on next page)

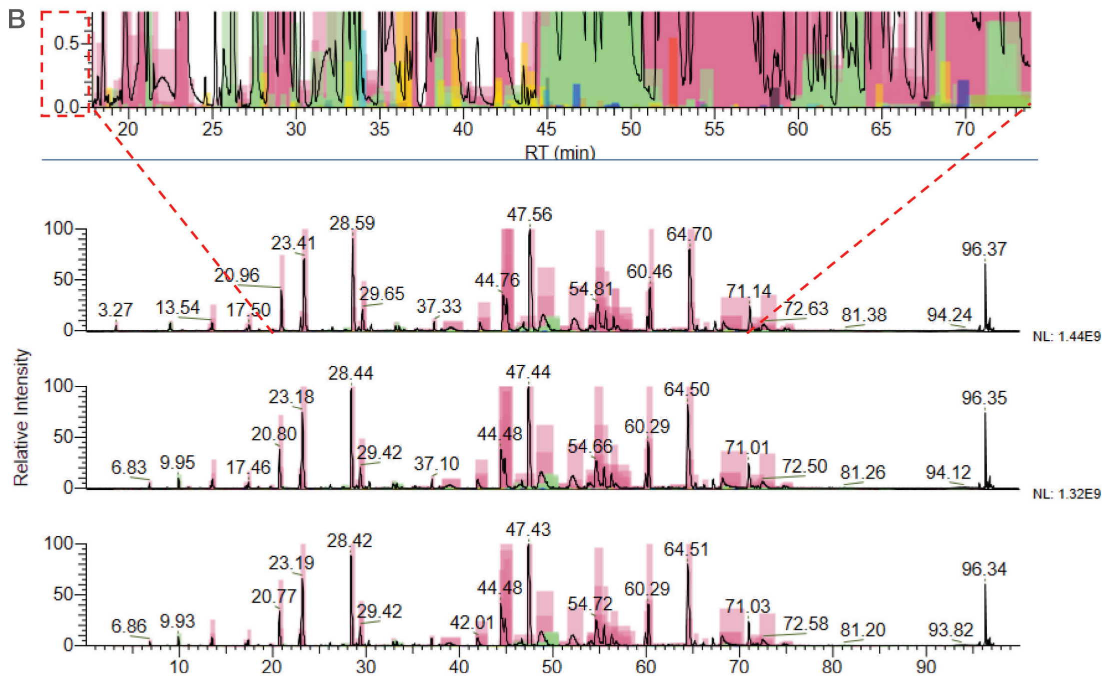


Figure 7. A new feature of the HCP identification and sequence coverage on the mapping page. A digestion of the CEX pool sample is shown as an example. (B) Expanded view of low abundance HCP peaks.

Figure 8 displays the host cell protein quantitation page, which is also a new feature in BioPharma Finder software. Here, we chose phospholipase B-like, which had been implicated as a potentially immunogenic HCP[12] to demonstrate the details. All HCP quantitation results and corresponding peptides can be found on this page, and peptides used for quantitation can be customized.

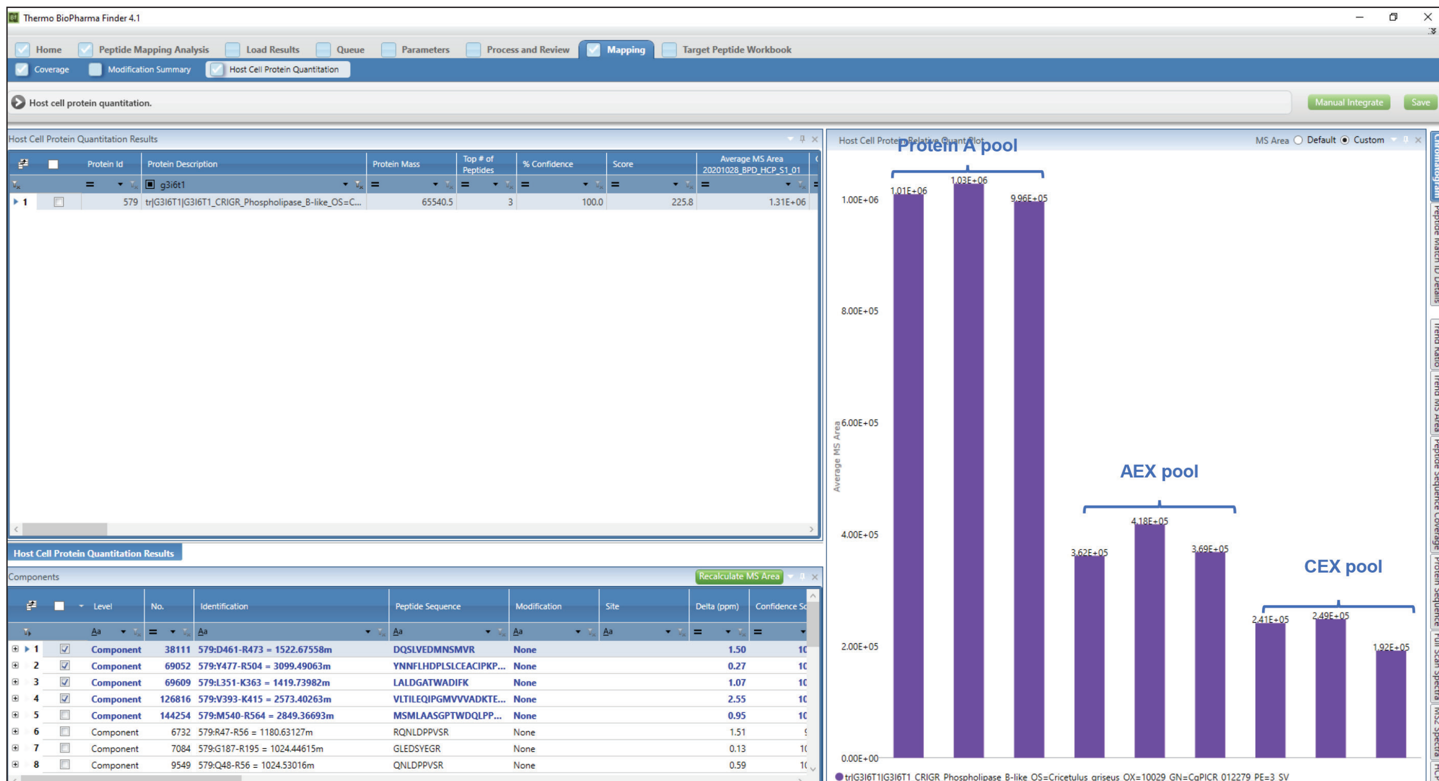


Figure 8. A new feature of the host cell protein quantitation on the mapping page. Upper left, host cell protein quantitation results. Once an HCP is selected, the peptides of this protein will be displayed in component table and peptides used for quantitation will be highlighted and checked automatically. Here we filtered phospholipase B-like as an example. Lower left, components table. Users can customize quantitation peptides by check/uncheck peptides here. Right, host cell protein relative quant plot. This example shows the trend of the selected HCP among different purification steps in one biological replicate.

High-risk HCPs selection and quantitation

HCP level is regarded as a critical quality attribute (CQA) in biopharmaceuticals as it might bare immunogenicity risks for patients[13], constrain drug efficiency in vivo[14], negatively impact product quality through proteolytic activity[15,16,17] or have potential to degrade polysorbate[18,19] typically used as a solubilizing agent in mAb products[20]. Therefore, the level of these high-risk HCPs needs to be monitored during the entire upstream and downstream manufacturing processes.

In this study, according to protein functions and previously published papers,[21,22] several high-risk HCPs were selected for monitoring in all samples during process development. 30 high-risk HCPs are quantitated in Protein A pool sample, indicating that these HCPs may be co-purified with mAb at the Protein A affinity step. However, only 15 high-risk HCPs existed in the AEX pool and 3 are detected in the CEX pool sample (Figure 9), which means the following polishing steps such as AEX and CEX can remove the majority of the high-risk HCPs.

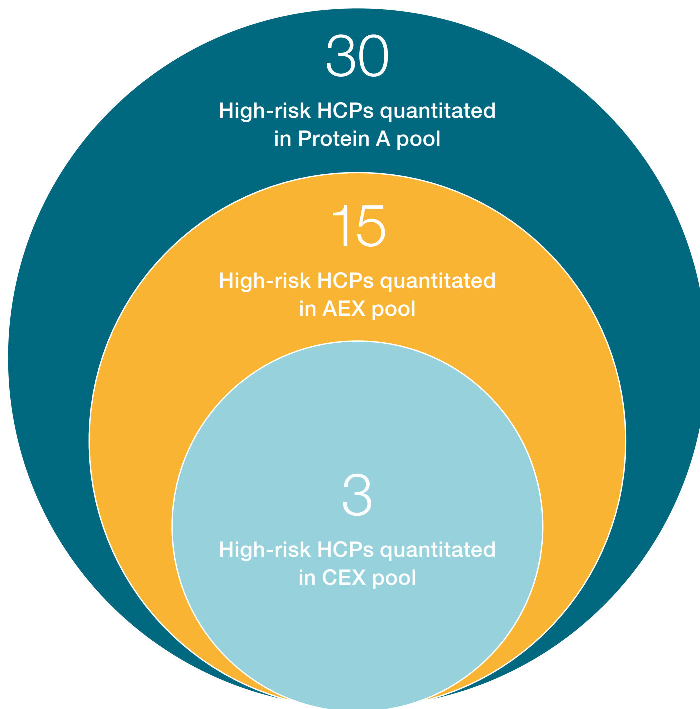
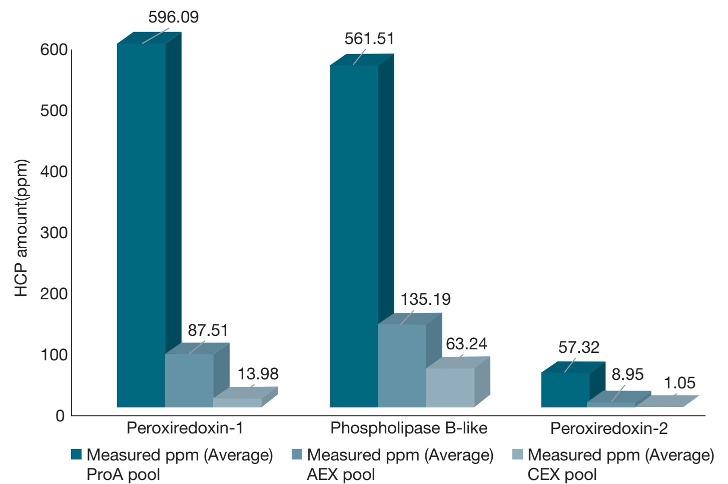


Figure 9. Overview of quantitated high-risk HCP numbers in Protein A/AEX/CEX pool samples. All HCPs are identified and quantitated with at least two peptides.

Figure 10 shows the concentration trends of three HCPs (phospholipase B-like, peroxiredoxin-1, and peroxiredoxin-2) across different purification steps. All HCP levels were averaged among biological replicates. CVs represent the deviation across technical replicates, proving the stability of the system.



Uniprot accession	Description	CV Measured ppm Protein A pool	CV Measured ppm AEX pool	CV Measured ppm CEX pool
Q9JKY1	Peroxiredoxin-1	1.48%	4.71%	1.44%
G3I6T1	Phospholipase B-like	2.59%	9.35%	5.66%
Q8K3U7	Peroxiredoxin-2	0.11%	2.87%	0.26%

Figure 10. Three high-risk HCP trends across different purification steps. The HCP level (ppm) was averaged on biological replicates.

As we mentioned before, 676 HCPs can be found in Protein A elute and the total HCP is 49473.27 ppm. The concentration of phospholipase B-like and peroxiredoxin-1 are 500–600 ppm and peroxiredoxin-2 is 57.32 ppm in Protein A elute. After anion ion exchange chromatography, the total HCP reduces to 1253.38 ppm and the concentrations of phospholipase B-like and peroxiredoxin-1 decrease to 135.19 ppm and 87.51 ppm, respectively, and the level of peroxiredoxin-2 is lower than 10 ppm. Then, cation exchange chromatography is used as the last polishing step and the total HCP in the CEX elute is 89.66 ppm while peroxiredoxin-1 is reduced to 13.98 ppm, and peroxiredoxin-2 to 1.05 ppm. LC-MS based HCP analysis can provide identification and quantitation information of individual HCP while ELISA only shows the total HCP amount.

Phospholipase B-like has attracted attention as a highly immunogenic HCP[12,21]. This HCP binds to humanized mAbs, in particular the IgG4 isotype, and is not detected in some widely used anti-CHOP immunoassays. This indicates the necessity of an LC-MS/MS based method for HCP identification and quantitation as a complementary of ELISA. Figure 11 shows the top abundance peptide of phospholipase B-like used for quantitation in a CEX pool sample digestion. Many fragment ions were identified with high S/N, which ensures highly confident identification result.

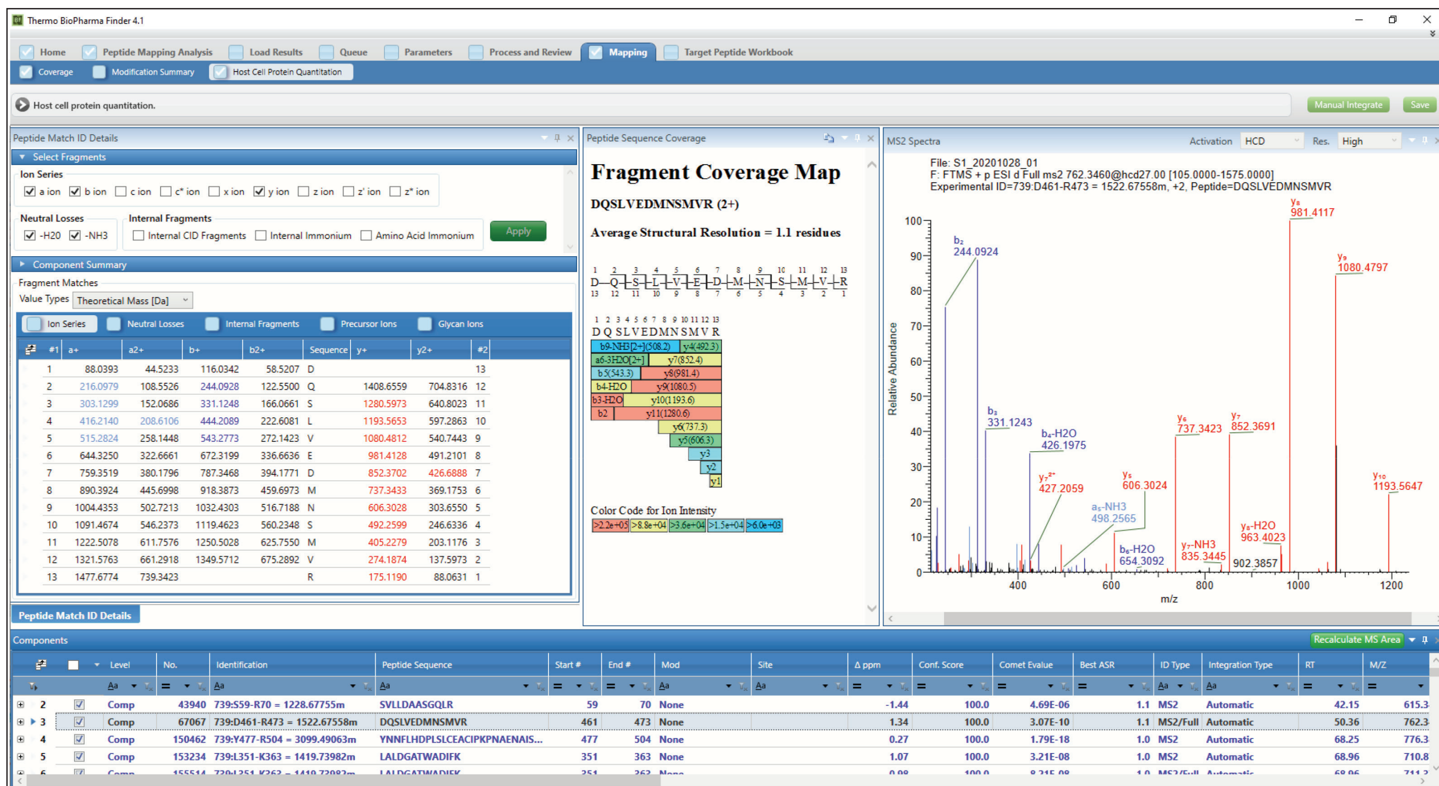


Figure 11. Peptide (DQSLVEDMNSMVR) of phospholipase B-like used for quantitation in a CEX pool sample digestion. Upper left, fragment ions list. Middle, fragment coverage map. Right, MS^2 spectrum. Lower, component table.

Conclusion

Specific HCP identification, quantitation, and monitoring, especially for high-risk HCPs, provides a meaningful method to support biologics downstream process development by focusing on individual HCP clearance at each step. This method allows for better process understanding and more rapid process improvements throughout the drug product lifecycle.

In this study, we demonstrated that the optimized non-denaturing protein digestion facilitates detection of HCPs. Removal of undigested protein with heat-treatment prior to MS analysis reduces the dynamic range of the sample, increasing the probability of HCP identification.

- 1D LC-MS based HCP analysis workflow provides both efficiency and robustness.
- New workflow for Host Cell Protein analysis in BioPharma Finder software enables data analysis of peptide mapping, both identification and relative quantitation of HCP, and monitoring of biotherapeutic products in a single software.
- Based on the result, three high-risk HCPs were selected for monitoring in further optimization of downstream process.

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