Agilent
Trusted Answers

Consumables Workflow Ordering Guide

Host Cell Protein Analysis by RP-LC/MS

Unlike small molecules and oligos, mAbs and other protein-based therapeutics, including adeno-associated virus (AAV) capsids are engineered and produced using a cell line. These cell lines include CHO (Chinese Hamster Ovary), HEK293 (human embryonic kidney) or NS0 (murine) for mAbs and HEK293 and baculovirus for AAVs. These cell lines produce several other proteins, which are called host cell proteins or HCPs. Some HCPs may cause immune responses or cause instability of the therapeutic by interacting with the stabilizers in the formulation buffer. HCPs are considered process-related impurities and numerous purification steps are taken to remove these extra proteins.

Even after purification, small amounts of HCPs remain and the generally accepted limit of HCPs is 1-100 ppm. The US Food and Drug Administration, European Medicines Agency, and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) provide guidance for host cell proteins and other process-related impurities. For example, Q6B from ICH states “impurities should be minimized by the use of appropriate well-controlled manufacturing processes[1]”. Monitoring host cell proteins is a critical quality attribute; detection, identification, and quantitation of host cell proteins is performed by biopharma companies worldwide.

Enzyme-linked immunosorbent assay (ELISA) is the gold standard for quantifying HCPs as it is a sensitive and relatively simple assay to carry out. Liquid chromatography/mass spectrometry (LC/MS) is a rapidly growing orthogonal technique to ELISA, because it can identify and relatively quantify individual host cell proteins.

While LC/MS is an incredibly powerful tool for HCP analysis, it is not without challenges. One of the biggest challenges is the vast difference in abundance between the host cell proteins and the main therapeutic; 1 ppm is equivalent to 1 ng of host cell protein to 1 mg of therapeutic protein. The mass spectrometer must have a wide in-spectrum dynamic range, especially if the peptides are co-eluting (Figure 1). Trap-type mass analyzers can fill quickly and exclude low abundant species.

On the other hand, Q-TOF instruments, such as the AdvanceBio 6545XT Q-TOF have six orders of in-spectrum dynamic range which allows for confident identification of low-level species. The iterative MS/MS function is also useful for characterizing low abundant species (Figure 2). For known HCPs, the triple quadrupole mass spectrometer is ideal for absolute quantitation when paired with stable isotopically labeled peptides.

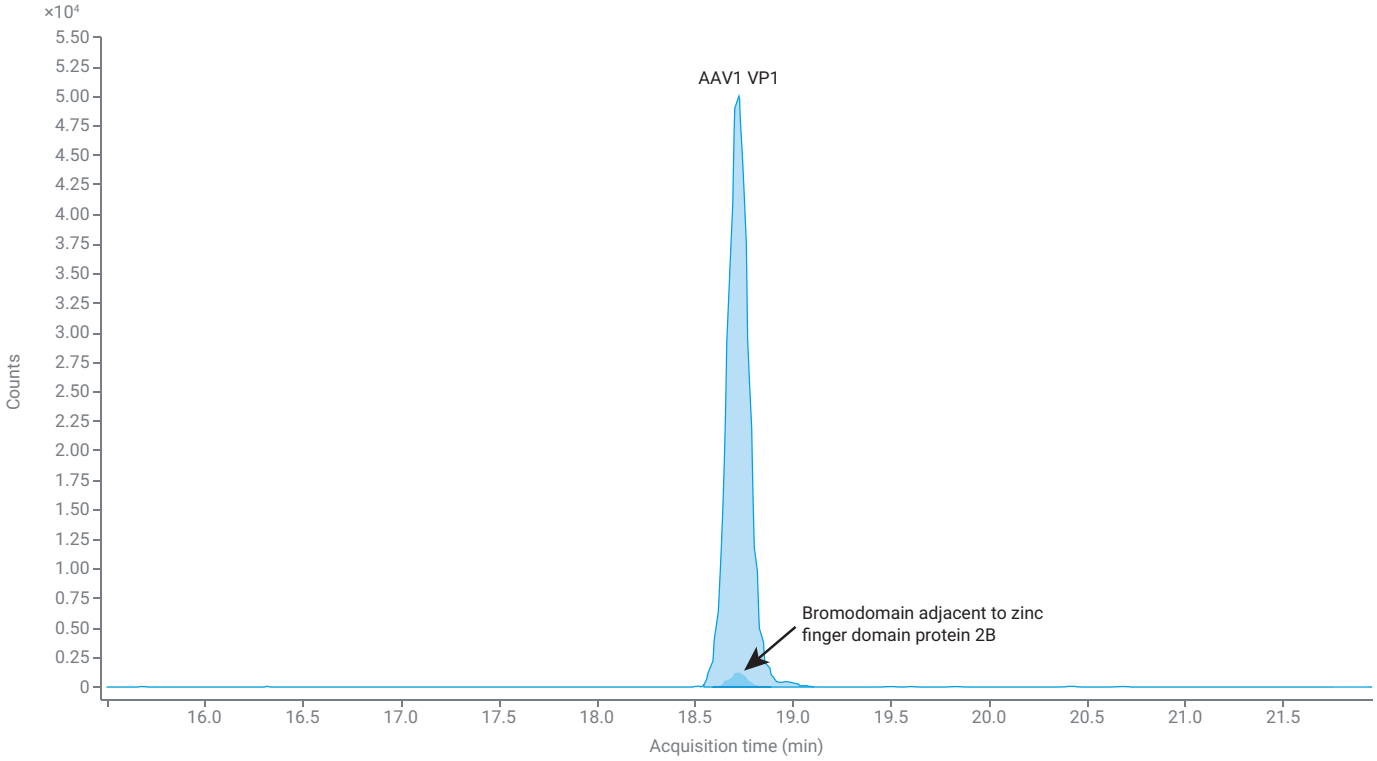

Figure 1. An example of two peptides co-eluting, one from the main therapeutic, the other a low-abundant HCP. Despite the large load, the AAV capsid peptide peak shape is sharp with a low tailing factor.

Another challenge is heavy sample loading on the analytical HPLC column. In order to detect and characterize the HCPs, an excess amount of therapeutic is injected onto the HPLC column. The Agilent AdvanceBio Peptide Plus HPLC column is well suited for host cell protein analysis. The charged surface of the stationary phase is tolerant of high mass loads. In addition, the charged surface allows for use of MS-friendly formic acid and forms sharp peak shapes, avoiding tailing of the high abundant peptides.

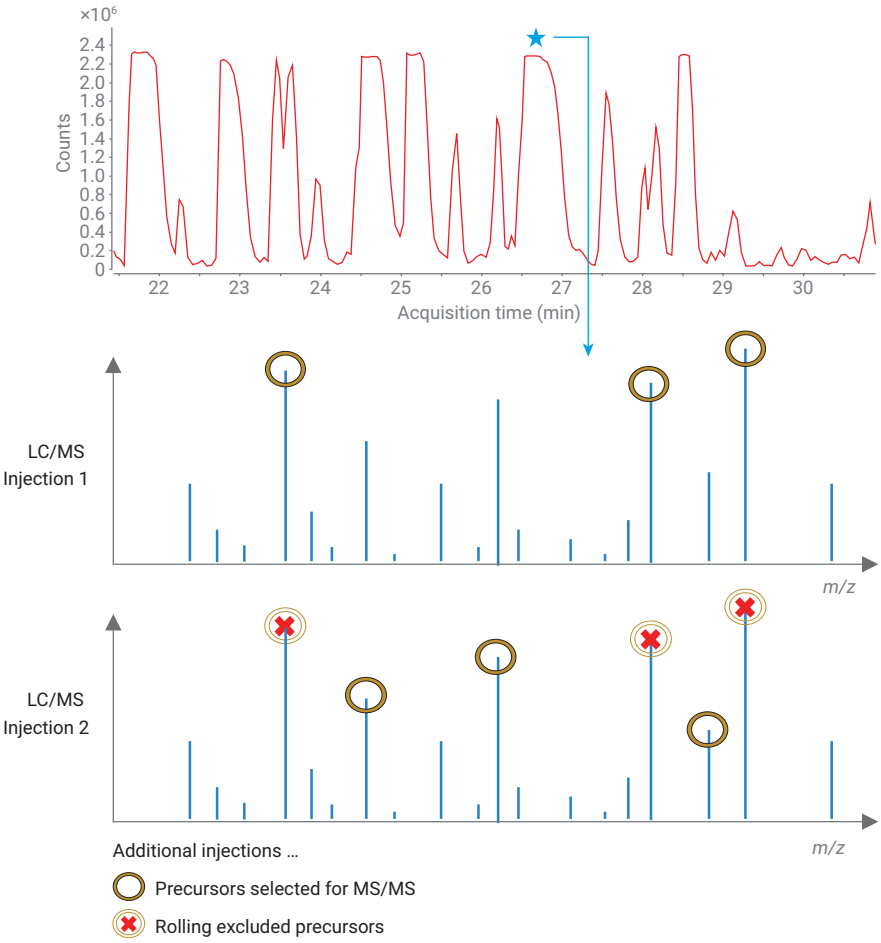

Figure 2. A visualization of the Iterative MS/MS algorithm.

Best Practices:

Sample Preparation:

- Performing a tryptic digest can result in a large sample volume. Consider running the Peptide Cleanup protocol on the Assay Map Bravo to clean up your sample and to reduce the sample volume.
- Samples should be re-constituted in starting mobile phase conditions.

Mobile Phase:

- Formic acid is an ideal modifier because it is mass spec compatible and the charged surface of the peptide plus column was designed to eliminate unwanted silanol interactions with peptides when using formic acid in the mobile phase.
- Use LC/MS grade solvents and pure formic acid (<99% pure) to reduce ion suppression and to keep the mass spectrometer clean.
- Refresh mobile phase frequently to avoid algae growth.

Column Operation/Maintenance:

- Guard columns are available in each inner diameter option for the AdvanceBio Peptide Plus column and can prolong the life of the analytical column.
- Lower the flow ramp rate from the default to 1 mL/min or lower. The gradual increase in flow rate will prolong column lifetime and help prevent sudden over pressuring. In Agilent software this setting can be found in the Advanced section of the LC pump controls.
- Set the maximum pressure limit in the LC method to match that of the column (600 bar for all columns recommended here). This is key for any instance in which the maximum pressure capabilities of the LC exceeds that of the column.
- The AdvanceBio Peptide Plus column is rated up to 60°C. Regularly operating the column below the maximum temperature will prolong the life of the column.
- The AdvanceBio Peptide Plus column should be initially conditioned for at least 4 hours in 95% water, 5% acetonitrile and 0.1% formic acid and then flushed and stored in 100% acetonitrile. The column user guide has instructions on flow rates.
- Long term storage should be in a pure organic solvent.
- Refer to the Agilent BioHPLC and AdvanceBio Reversed-Phase Columns User Guide for cleaning the column.

Mass Spectrometry:

- Clean the mass spectrometer source routinely.
- Keep dry and sheath gas flow rates high (≥11 L/min) when using the Agilent Jet Stream source to keep the instrument cleaner over time.
- Divert the LC stream to waste outside of the retention times of interest, especially at the end of the gradient during the column rinse stage with high organic solvent.

Recommended Starting Conditions

Parameter	Value
Column	Agilent AdvanceBio Peptide Plus 2.1 x 150 mm (p/n 695775-949)
Column Temperature	50°C
Mobile Phase A	Water, 0.1% formic acid
Mobile Phase B	Acetonitrile 0.1% formic acid
Flow Rate	0.4 mL/min
Gradient	0-3 min 3%B, 3-90 min, 3-40%B, 90-93 min 40-90%B, 93-95 90%B, 95-96 min, 90-3% B
Post Time	3 minutes

Table 1. Suggested HPLC Conditions

Parameter	Value
Source	Dual Agilent Jet Stream
Dry Gas Temperature and Flow	325°C and 13 L/min
Nebulizer	35 psig
Sheath Gas Temperature and Flow	275°C and 12 L/min
VCap	4000 V
Nozzle	0 V
Fragmentor	175 V
Acquisition Rate	5/3 spectra/sec for MS and MS/MS
Reference Masses	121.0509, 922.0098

Table 2. Recommended Starting Conditions on an Agilent Q-TOF

Column Selection Criteria: Host Cell Protein Analysis

Column Dimensions:

- A smaller id column is recommended over larger id columns both for sensitivity and compatibility with MS detection. A 2.1 mm id column will be used with a lower flow rate which is conducive to efficient electrospray ionization, which also helps sensitivity.
- For reversed phase columns, longer columns can lead to higher resolution. A 150 mm length column or longer is recommended for this application due to the complexity of the sample

Stationary Phase Chemistry:

- The AdvanceBio Peptide Plus column is the recommended choice for HCP analysis because of its tolerance for high mass load and strong compatibility with formic acid and therefore mass spectrometry.
- The AdvanceBio Peptide Mapping column is an ideal choice for peptide mapping and a secondary choice for HCP analysis. This column excels at balancing retention of smaller hydrophilic peptides and has reasonable elution of more hydrophobic peptides, but is less tolerant of high mass loads, a necessary component of HCP analysis.

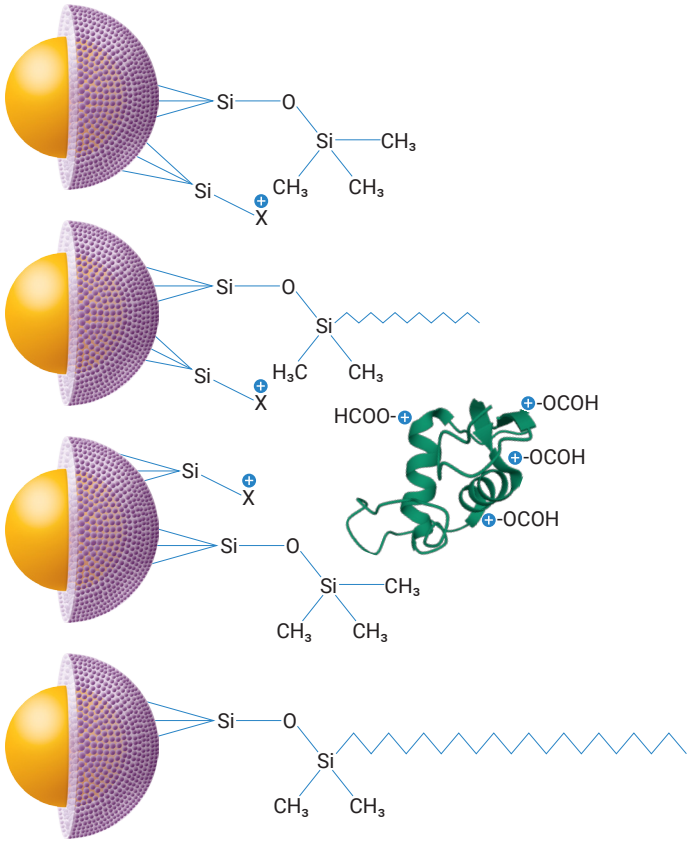

Figure 3. The charged surface of the particle on the AdvanceBio Peptide Plus column prevents deprotonation of the silanol, eliminating silanol interactions with peptides which improves MS sensitivity.

Easy selection and ordering information

To order items from the Agilent online store click on the part number hyperlinks in the table below, add-to-cart and proceed to check-out.

Alternatively, save the items in the table to your Favorite Products list by clicking the corresponding MyList header link. Enter the quantities for the products you need, Add-to-Cart and proceed to check-out. The list will remain under your Favorite Products for future use.

If this is your first time ordering online, you will be asked to enter your email address for account verification. If you have an existing Agilent account, you will be able to log in. If you do not have a registered Agilent account, you will need to register for one.

All items can also be ordered through your regular sales and distributor channels

MyList 1: AdvanceBio Peptide Plus Columns for HCP Analysis

Description	Part No.
AdvanceBio Peptide Plus 2.1 x 150 mm, 2.7 µm	695775-949
AdvanceBio Peptide Plus 2.1 x 250 mm, 2.7 µm	693775-949
UHPLC Guard, AdvanceBio Peptide Plus, 2.1 mm, 2.7 µm, 3 pack	821725-954

MyList 2: Protein/Peptide Standards

Description	Part No.
Agilent NIST mAb, 25 µL	5191-5744
Agilent NIST mAb, 4 x 25 µL	5191-5745
Ten peptide standard, 71 µg, lyophilized	5190-0583
HSA peptide standard	G2455-85001

MyList 3: Supplies & Solvents

Description	Part No.
Connections & Tubing	
Agilent InfinityLab Quick Connect LC fitting	5067-5965
Quick Connect capillary stainless steel 0.12 x 105 mm	5500-1173
Agilent InfinityLab Quick Connect Fitting assembly with pre-fixed 0.12 x 105mm capillary (for connection on column inlet)	5067-5957
Agilent InfinityLab Quick Turn Fitting (for connection on column outlet)	5067-5966
Quick Turn Capillary SST 0.12 x 280 (for Quick Turn fitting)	5500-1191
Mounting tool for quick turn fittings	5043-0915
Ultralow dispersion tubing kit for Agilent 1290 Infinity II	5067-5963
Inline Filters	
InfinityLab Quick Change inline filter assembly, for UHPLC. Including 5 filter discs (2.1 mm id, 0.2 µm pore size), with 90 mm flexible capillary.	5067-1603
InfinityLab Quick Change filter disc, 2.1 mm id, 0.2 µm pore size, 5/pk. for InfinityLab Quick Change inline filter	5067-1610

MyList 3: Supplies & Solvents (continued)

Description	Part No.
Sample Containment	
High recovery vial, screw top, with fixed insert, clear, 300 µL insert volume, 100/pk. Vial size: 12 x 32 mm (12 mm cap)	5188-6591
Cap, screw, blue, PTFE/red silicone septa, 100/pk. Cap size: 12 mm	5182-0717
Vial, crimp/snap top, polypropylene, 250 µL, 1,000/pk. Vial size: 12 x 32 mm (11 mm cap)*	5190-3155
Cap, snap, clear, PTFE/silicone/PTFE septa, 100/pk. Cap size: 11 mm (for 5190-3155)	5182-0566
InfinityLab 96-well plate, 0.5 mL, 30/pk	5043-9310
InfinityLab 96-well plate closing mat, 50/pk	5042-1389
Solvents & Additives	
InfinityLab Ultrapure LC/MS Water, 1 L	5191-4498
InfinityLab Ultrapure LC/MS Acetonitrile, 1 L	5191-4496
Formic acid, 5 mL	G2453-85060
Solvent Handling	
InfinityLab Stay Safe cap starter kit	5043-1222
InfinityLab solvent bottle, clear, 1 L	9301-6524
InfinityLab solvent bottle, amber, 1 L	9301-6526
Solvent bottle, clear, 2 L	9301-6342
Solvent bottle, amber, 2 L	9301-6341
InfinityLab Stay Safe Purging Bottle	5043-1339
InfinityLab waste can, GL45, 6 L with Stay Safe cap	5043-1221
InfinityLab charcoal filter with time strip, 58 g	5043-1193
Stay Safe starter kit and purging bottle, includes InfinityLab Stay Safe purging bottle (PN 5043-1339) and Stay Safe caps starter kit (PN 5043-1222)	5043-1340
Mass Spectrometry	
LC/MS Calibration standard, for ESI-TOF, 100 mL	G1969-85000
API-TOF Reference Mass Solution Kit	G1969-85001
Cloth, lint-free, 23 x 23 cm, 100% cotton, 15/pk	05980-60051
Abrasive mesh, 8000 grit (2 µm), (micro-grit paper)	8660-0852

*Available in select countries

References:

1. *ICH Harmonised Tripartite Guideline Specifications: Test Procedures And Acceptance Criteria For Biotechnological/ Biological Products Q6B.* Step 4 Edition, International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use, 1999.
   https://www.ich.org/page/quality-guidelines
2. Optimizing Adeno-Associated Virus Loading Amounts for Host Cell Protein Analysis using the AdvanceBio Peptide Plus column and the 6545XT AdvanceBio LC/Q-TOF
   5994-6885EN
3. Adeno-Associated Virus Characterization with Agilent 6545XT AdvanceBio LC/Q-TOF and Protein Metrics Byos Software
   5994-5110EN
4. Host Cell Protein Analysis Using Agilent AssayMAP Bravo and 6545XT AdvanceBio LC/Q-TOF
   5991-9300EN
5. Quantification of Host Cell Protein Impurities using the Agilent 6495C Triple Quadrupole LC/MS
   5994-1369EN

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