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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.

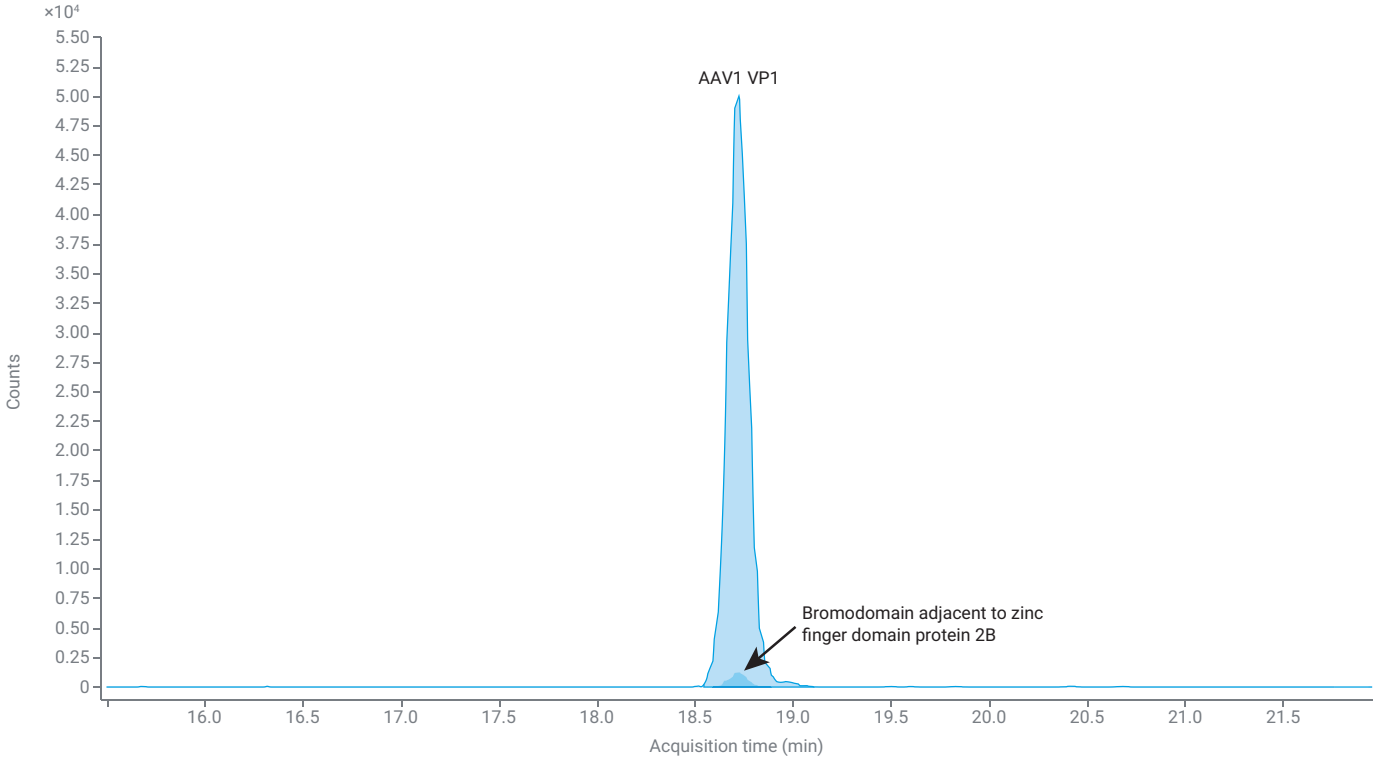


**Figure 1.** An example of two peptides co-eluting, one from the main therapeutic, the other a low-abundant HCP. Despite the large load, the AAV capsid peptide peak shape is sharp with a low tailing factor.

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.

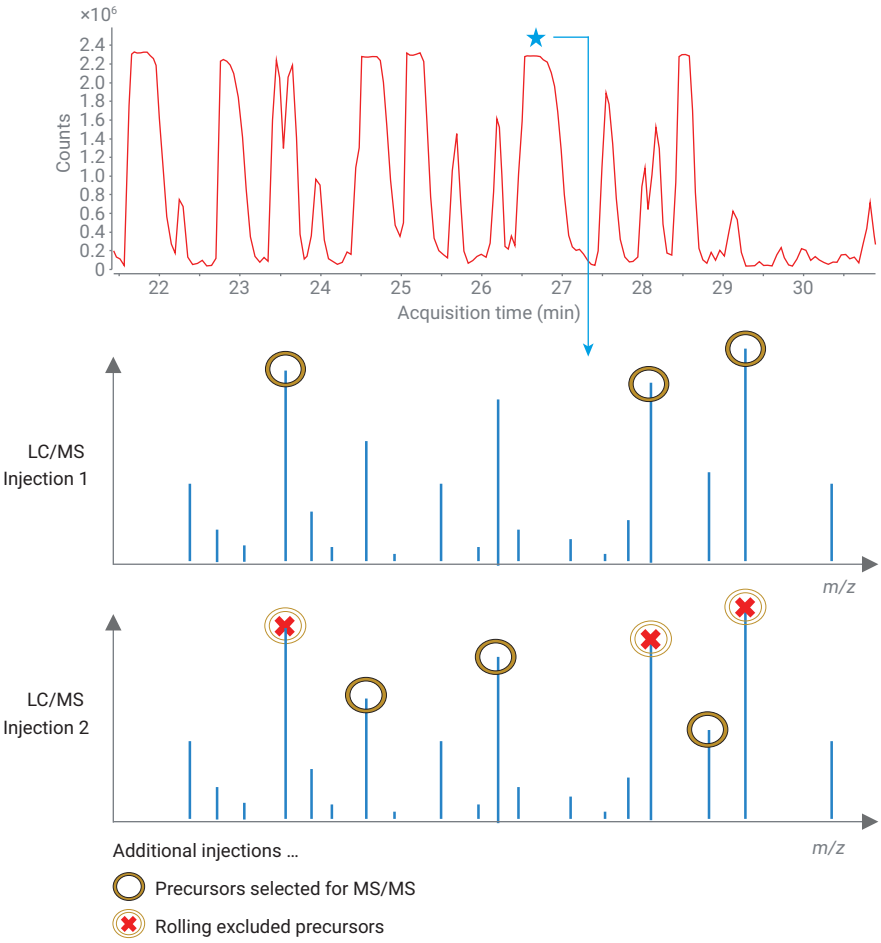


**Figure 2.** A visualization of the Iterative MS/MS algorithm.

## 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.

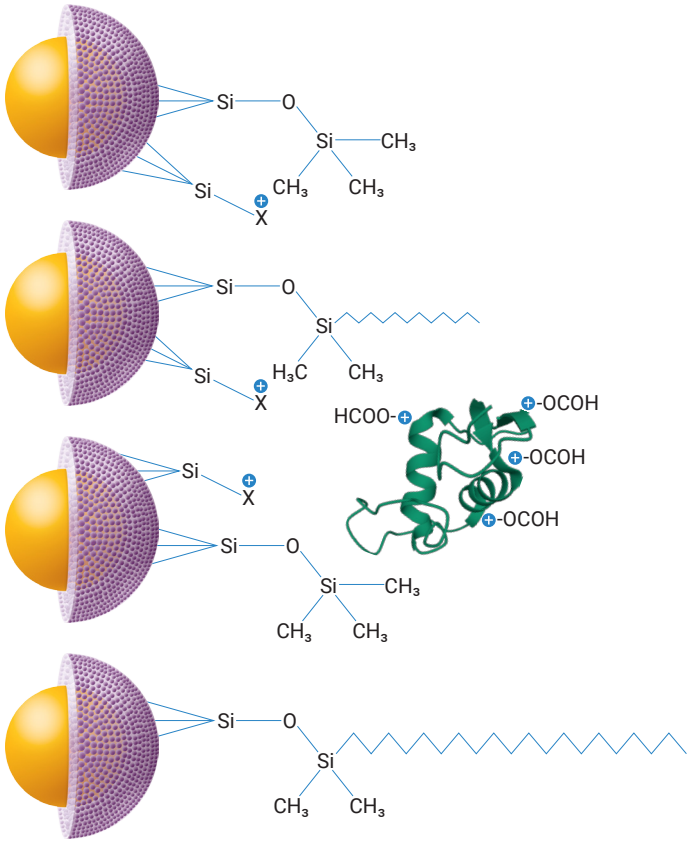


**Figure 3.** The charged surface of the particle on the AdvanceBio Peptide Plus column prevents deprotonation of the silanol, eliminating silanol interactions with peptides which improves MS sensitivity.

## 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: Sample Preparation

| Description | Part No. |
|---|---|
| AdvanceBio Spin columns for desalting or buffer exchange, <100 µL samples, 25/pk, collection tubes included | 1980-1103 |
| AdvanceBio Spin 96-sample plate for desalting or buffer exchange, 10 to 50 µL samples, 1/pk | 1980-1104 |
| 96-well plate, polypropylene, 1.2 mL, 27 mm, round wells, U shape, 25/pk<br>Recommended for wash steps with p/n 1980-1104 | 5043-9308 |
| 96-well plate, polypropylene, 0.33 mL, 14 mm, round wells, V shape, 25/pk<br>Recommended for final collection step with p/n 1980-1104 | 5043-9312 |
| Sealing mat, 96 wells, round, preslitted, silicone, 50/pk | 5042-1389 |

MyList 4: 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 |
| **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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