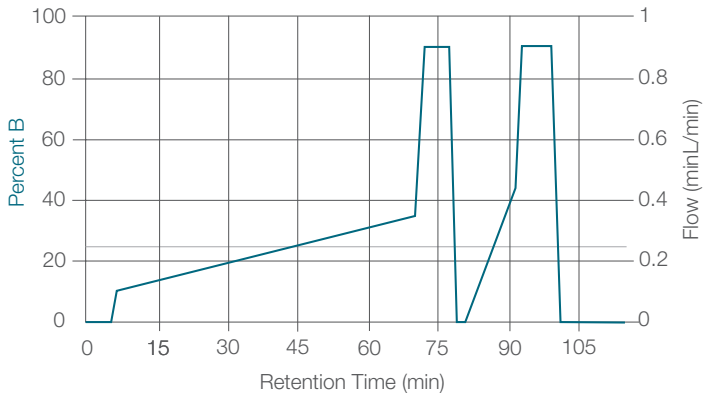
APPLICATION NOTE

# Mobile phase solvents and reagents for LC-MS biopharmaceutical applications

Biopharmaceutical drugs are a unique class of therapeutic proteins that have far more complex structures than small molecule drugs. Liquid chromatography (LC) coupled with mass spectrometry (MS), LC-MS, has become increasingly popular for characterizing biopharmaceutical drugs. LC-MS is widely used for characterizing the primary structure of protein drugs such as amino acid sequencing for peptides/proteins, profiling of process-related impurities such as host cell proteins (HCPs), disulfide bond mapping, N-glycan profiling of monoclonal antibodies (mAbs), and nucleotide sequencing for oligonucleotides.

At the primary structure level, the protein post-translational modifications (PTMs) that arise at different stages of manufacturing and storage can impact drug efficacy and safety; thus, PTMs are classified as critical quality attributes (CQA) that should be monitored and controlled. Combining the power of LC separation with the sensitivity and specificity of MS detection, LC-MS is the tool of choice for peptide mapping to detect multiple PTMs in a single analytical run, i.e., the multi-attribute method (MAM) workflow [1].

A critical component of the MAM workflow is to separate peptides of various lengths from the digested therapeutic protein. This is typically achieved by employing a long LC gradient of water and acetonitrile with formic acid added as a modifier, as shown in Figure 1. Mobile phase solvents and reagents must be of high purity with low organic impurities to ensure no interference with detection by electrospray (ESI) MS.




| Time | % B |
|---|---|
| 0 | 1 |
| 5 | 1 |
| 6 | 10 |
| 70 | 35 |
| 72 | 90 |
| 77 | 90 |
| 79 | 1 |
| 81 | 1 |
| 83.5 | 10 |
| 91.5 | 45 |
| 93 | 90 |
| 99 | 0- |
| 101 | 1 |
| 115 | 1 |

**Figure 1. Typical LC gradient in a MAM workflow for the separation of NIST mAb peptides with a binary mobile phase: A: 0.1% formic acid in water (Cat. No. LS118); B: 0.1% formic acid in acetonitrile (Cat. No. LS120), from Reference [1].**

Thermo Fisher Scientific offers two grades of LC-MS solvents. Fisher Chemical™ Optima™ grade LC-MS solvents are high purity and meet all the needs for LC-MS analysis. Pre-made LC-MS blends are also available in Fisher Chemical Optima grade and are precisely mixed with lot-to-lot consistency and tested by LC-MS, making these blends convenient for use in biopharma QC labs. The Thermo Scientific™ UHPLC-MS grade solvents are a higher grade with more stringent specifications to meet the most sensitivity-demanding analyses. Both LC-MS and UHPLC-MS grades of solvents and blends have been fully tested to ensure low organic impurities to minimize interference and ion suppression.

Another important quality attribute of our high-purity LC-MS regents is the extremely low levels of metal impurities. Trace metal impurities can cause poor chromatography peak shape, peak tailing, and diminished recovery via the metal-ion mediated adsorption on the LC column media [2]. The alkali metal ions such as sodium ($Na^+$) and potassium ($K^+$) are electrostatically attracted to the negatively charged carboxyl group of the C-terminal amino acid in peptides/proteins and the polyanionic backbone of oligonucleotides [3]. The $Na^+$ and $K^+$ adducts decrease the overall detection sensitivity and complicate the peptide identification in LC-MS analysis. Minimizing the metal contamination in mobile phase solvents and regents has become ever more critical with the growing demand for higher LC-MS sensitivity.

All Thermo Fisher Scientific LC-MS grade solvents and reagents are subject to strict control of trace metal contamination during the production process and thoroughly tested to stringent specifications with ICP-MS. A comparison of the four most common alkali and alkaline contaminant metal impurities in pre-blended LC-MS grade 0.1% formic acid in water from three competitors are listed along with the Fisher Chemical Optima offering in Table 1. As shown, the Fisher Chemical Optima pre-blended mobile phase has the lowest allowable overall metal ions present.

**Table 1. Specification of alkali and alkaline metals in four LC-MS grade 0.1% formic acid in water (in ppb) from four vendors.**

| Metal impurity | Fisher Chemcial Optima (Cat. No. LS118) | Competitor M | Competitor H | Competitor J |
|---|---|---|---|---|
| Na | ≤50 | ≤700 | ≤5000 | ≤50 |
| K | ≤ 50 | ≤40 | ≤2000 | ≤50 |
| Mg | ≤10 | ≤40 | ≤500 | ≤50 |
| Ca | ≤50 | ≤50 | ≤500 | ≤50 |

The Fisher Chemical Optima LC-MS grade mobile phase additives are equally thoroughly tested and subject to the same strict quality control paradigm as the solvents. As shown in Table 2, the Optima LC-MS grade trifluoroacetic acid (Cat. No. A116) also has the lowest overall allowable metal ion content among the six different LC-MS grade trifluoroacetic acids surveyed. Note that two competitor offerings do not even have specifications on metal ions.

**Table 2. Metal impurity specifications of six different LC-MS grade trifluoroacetic acid offerings (in ppb) from five vendors.**

| Metal impurity | Fisher Chemical Optima (Cat. No. LS118) | Competitor M | Competitor H | Competitor H (Ultra) | Competitor R | Competitor P |
|---|---|---|---|---|---|---|
| Na | ≤50 | ≤500 | ≤500 | ≤500 | No spec | No spec |
| K | ≤20 | ≤100 | ≤100 | ≤100 | No spec | No spec |
| Mg | ≤10 | ≤500 | ≤500 | ≤500 | No spec | No spec |
| Ca | ≤50 | ≤200 | ≤200 | ≤200 | No spec | No spec |

## Purity grades for every LC-MS application

Fisher Chemical Optima LC-MS Grade Solvents: general purpose for all LC-MS applications, low organic and metal impurities, tested with stringent LC-MS gradient suitability tests.

| Description | Packaging | Quantity | Cat. No. |
|---|---|---|---|
| Acetonitrile | Amber Glass Bottles | 500 mL, 1 L, 2.5 L, 4 L | A955 |
| Methanol | Amber Glass Bottles | 500 mL, 1 L, 2.5 L, 4 L | A456 |
| Water | Amber Glass Bottles | 500 mL, 1 L, 2.5 L, 4 L | W6 |
| 2-Propanol | Amber Glass Bottles | 500 mL, 1 L, 2.5 L, 4 L | A461 |

Thermo Scientific™ UHPLC-MS Grade Solvents: ultrapure quality solvents to meet your most demanding UHPLC and LC-MS applications for low background and high sensitivity.

| Description | Packaging | Quantity | Cat. No. |
|---|---|---|---|
| Acetonitrile | Clear Borosilicate Glass Bottles | 1 L | A956-1 |
| Methanol | Clear Borosilicate Glass Bottles | 1 L | A458-1 |
| Water | Clear Borosilicate Glass Bottles | 1 L | W8-1 |

Thermo Scientific UHPLC-MS Reagent Installation Kit (Cat. No. UHPLCMSKIT): recommended for setting up and passing the specification tests during the installation of a new LC-MS system. The kit includes:

- 1 L Acetonitrile (Cat. No. A956-1)
- 2 × 1 L Methanol (Cat. No. A458-1)
- 2 × 1 L Water (Cat. No. W8-1)
- 1 L Thermo Scientific™ ChromaCare™ Instrument Flush Solution (Cat. No. T111101000): a blend of 25% acetonitrile, 25% methanol, 25%, water, and 25% 2-propanol (IPA) to clean the flow path of an LC-MS system.


Fisher Chemical Optima LC-MS Grade Mobile Phase Blends: precisely pre-blended for convenience and tested for lot-to-lot consistency.

| Description | Packaging | Quantity | Cat. No. |
|---|---|---|---|
| Acetonitrile with 0.1% FA | Amber Glass Bottle | 500 mL, 1 L, 2.5 L, 4 L | LS120 |
| Acetonitrile with 0.1% TFA | Amber Glass Bottle | 500 mL, 1 L, 2.5 L, 4 L | LS121 |
| Water with 0.1% FA | Amber Glass Bottle | 500 mL, 1 L, 2.5 L, 4 L | LS118 |
| Water with 0.1% TFA | Amber Glass Bottle | 500 mL, 1 L, 2.5 L, 4 L | LS119 |

Fisher Chemical Optima LC-MS Grade Mobile Phase Additives: tested for high impurity and low trace metals for LC-MS use.

| Description | Packaging | Quantity | Cat. No. |
|---|---|---|---|
| Formic Acid | Poly Bottles | 50 mL | A117-50 |
| | Ampules | 0.5, 1, 2, 10 × 1 mL | A117 |
| Acetic Acid | Poly Bottles | 50 mL | A113-50 |
| | Ampules | 0.5, 1, 2, 10 × 1 mL | A113 |
| Trifluoroacetic Acid | Amber Glass Bottle | 50 mL | A116-50 |
| | Ampules | 0.5, 1, 2, 10 × 1 mL | A116 |
| Ammonium Formate | Glass Bottle | 50 g | A11550 |
| Ammonium Acetate | Glass Bottle | 50 g | A11450 |

## References


1. Haichuan Liu, et al, *"A high-resolution accurate mass multi-attribute method for critical quality attribute monitoring and new peak detection,"* Thermo Scientific Application Note 72916 (2019)
2. Robert E. Birdsall, et al, *"Application of mobile phase additives to reduce metal-ion mediated adsorption of non-phosphorylated peptides in RPLC/MS based assays,"* Journal of Chromatography B, Vol. 1126-1127 (2019)
3. Joachim Emmert, et al, *"Influence of $Na^+$ and $K^+$ Concentration in Solvents on Mass Spectra of Peptides in LC–ESI-MS,"* Spectroscopy, Vol 21, Issue 2, Feb 1 (2006)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