

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.

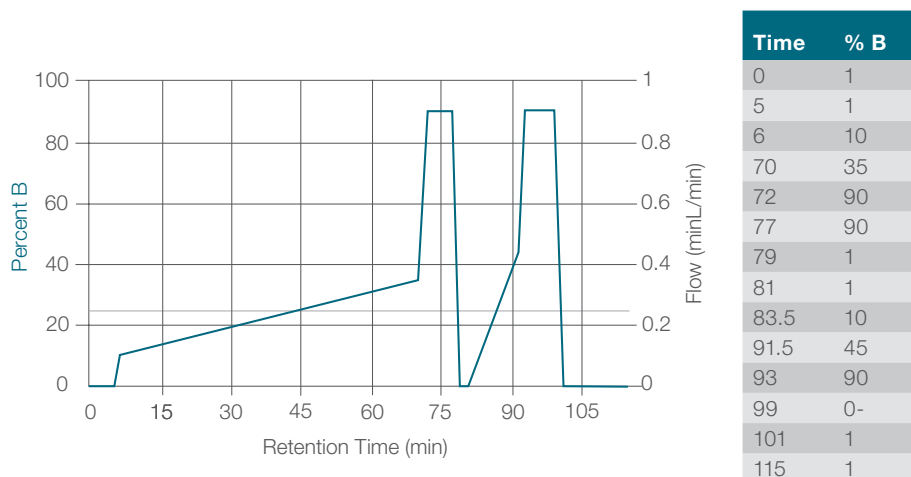


Figure 1. Typical LC gradient in a MAM workflow for the separation of NIST mAb peptides with a binary mobile phase: A: 0.1% formic acid in water (Cat. No. LS118); B: 0.1% formic acid in acetonitrile (Cat. No. LS120), from Reference [1].

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 reagents 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 reagents 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 Chemical 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 20% Water and 0.1% FA	Amber Glass Bottle	500 mL	LS122-500
Acetonitrile with 0.1% TFA	Amber Glass Bottle	500 mL, 1 L, 2.5 L, 4 L	LS121
Acetonitrile with 0.05% TFA	Amber Glass Bottle	4 L	LS1174
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
Water with 0.05% TFA	Amber Glass Bottle	4 L	LS1154

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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