# 13 LC-UV and LC-MS workflow fixes for oligonucleotide and mRNA analysis



In oligonucleotide and mRNA LC workflows, small details matter. Digestion control, HFIP handling, column materials, system configuration, and data filtering can all influence retention, carryover, and mapping confidence. This tip sheet includes 13 practical steps labs can take to help improve performance from sample preparation through sequence mapping.

## HPLC and UHPLC

### Sample preparation

1. **Standardize mRNA digestion protocols**
   mRNA mapping requires enzymatic digestion to generate smaller fragments before LC–MS analysis. Applying controlled, standardized digestion reactions helps generate reproducible cleavage patterns suitable for routine or QC use.
2. **Control partial RNase digestion with immobilized enzymes**
   Partial RNase T1 digestions are difficult to control in solution. However, immobilizing RNase T1—available on magnetic beads or in a column format—enables precise control over digestion time by allowing you to either remove the magnetic beads or adjust the column temperature and flow rate.
3. **Automate workflows and protect the LC system**
   When using enzymatic digestion, magnetic-bead-based protocols support automation on liquid-handling platforms and enable effective removal of RNase T1, preventing nuclease buildup on the LC column that could cause further digestion during analysis.

### Sample handling

4. **Choose vial materials that protect mRNA integrity**
   Use low-adsorption, polypropylene autosampler vials and borosilicate solvent bottles to minimize degradation and unwanted surface interactions during storage and analysis. This helps preserve sample integrity throughout the workflow.

### Column selection

5. **Extend column lifetime with polymer-based stationary phases**
   Oligonucleotide separations often benefit from high pH and elevated temperature to improve peak shape and reduce secondary interactions. However, these same conditions rapidly degrade silica-based columns.

   Selecting stationary phases designed for harsh environments can improve method stability under high pH and elevated temperature. Polymer-based columns tolerate high pH and elevated temperature more effectively than silica, improving method robustness and reducing the need for frequent column replacement.

## Sample analysis

6. **Standardize ion-pair mobile phase preparations**
   Ion-pair reversed-phase methods rely on alkylamines and hexafluoroisopropanol (HFIP), where small differences in preparation can lead to significant chromatographic changes. Using a consistent preparation approach across runs and systems helps stabilize retention and reduce variability during method transfer.
7. **Prevent HFIP evaporation during routine use**
   HFIP is highly volatile, and concentration changes directly affect mobile-phase strength. Keep solvent bottles fully sealed and plug unused ports into the bottle caps to prevent evaporation.
8. **Document eluent preparation and manage lifetimes**
   Include detailed eluent preparation instructions in protocols, assign expiration dates to HFIP-containing mobile phases, and refresh eluents regularly to avoid unintended variability.
9. **Account for system gradient delay volume**
   Some oligonucleotides may begin eluting during the isocratic hold, making retention sensitive to system gradient delay volume. This effect is mostly pronounced for short oligos and cannot always be corrected post-run.
10. **Maintain leak-free inert flow path**
    Use a chromatography system with a biocompatible (metal-free) flow path to minimize unwanted interactions, carryover, and signal instability. Ensure fittings are properly tightened and leak-free.
11. **Reduce carryover to protect quantitation**
    Residual analyte can persist between injections, inflating signal—especially for large, intact mRNA. Focus on reducing carryover to a reproducible level by comparing separation modes and optimizing run time.

## Data processing and sequence mapping

12. **Use Thermo Scientific™ BioPharma Finder™ Software for confident sequence mapping**
    Apply software designed for oligonucleotide and mRNA workflows to automate fragment annotation and map detected species to known sequences.
13. **Apply data filters to improve identification confidence**
    mRNA mapping workflows can generate large numbers of annotated fragments, including low-confidence or spurious matches. Applying data filters helps remove false identifications and improves confidence in sequence coverage and mapping results.

**Read more**


1. Accurate and consistent analysis of poly(A) tails of mRNA therapeutics on a UHPLC-HRAM-MS platform
2. Identification and quantitation of oligonucleotides, impurities, and degradation products using high-resolution accurate-mass spectrometry


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