High temperature fluoroalcohol-free liquid chromatography mass spectrometry of long RNA (up to 100 kDa) by 7T FT-ICR MS

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Overview of Long RNA Compatible Conditions for LC/MS

- High temperature separations (e.g. 80 °C)
- Triethylammonium bicarbonate
- No fluoroalcohol
- High temperature source for adduct management

Introduction

Liquid chromatography-tandem mass spectrometry (LC-MS) analysis of oligonucleotides often utilizes fluoroalcohol-containing mobile phases. Comprehensive characterization including intact mass and identification of polymerase readthrough failures can inform biosynthesis. Here, we demonstrate a high temperature, fluoroalcohol-free LC method with particular focus on characterizing long RNA. Characterization of longer RNA has been demonstrated in the liquid phase but high-end mass spectrometers are being increasingly applied to longer RNA.

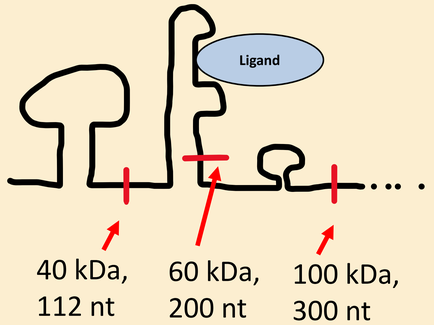

Figure 1. Polymerase transcription of RNA is a process which has druggable response curves for different transcription readthroughs. Quantitative evaluation of production of 112, 200, and 300 nt under varying ligand conditions can indicate efficacy of the ligand to inhibit or promote full or partial readthrough of the polymerase based on the amount of the 112, 200, and 300 nt sequences produced.

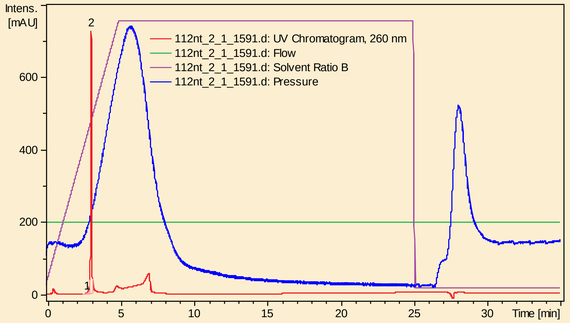

Figure 2. Scouting gradient conditions and results for LC-MS detection of a 36 kDa 112 nt RNA (Fig. 1).

Determination of adequate conditions for chromatographic resolution between targets under preferable and poor MS buffering conditions

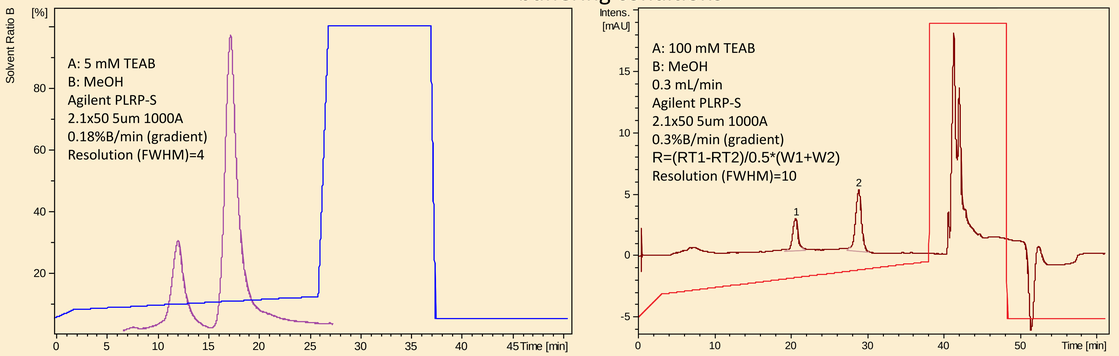

Figure 3. Separation of the 112 nt and 300 nt RNA under ion-pair liquid chromatography conditions at low buffer concentration of 5 mM (left) and high buffer concentration of 100 mM (right).

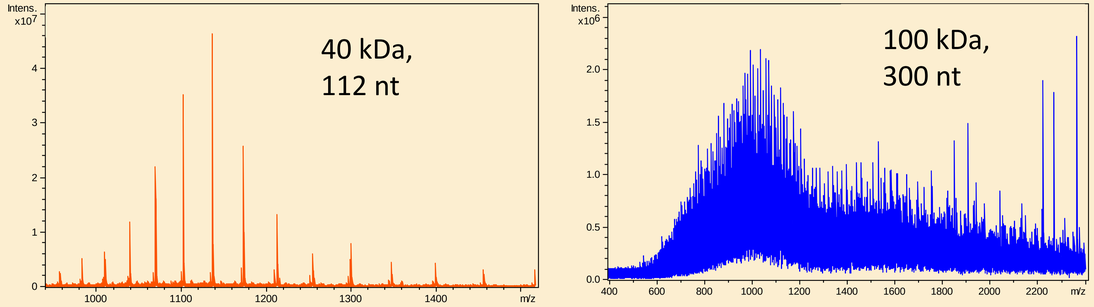

Figure 4. Magnitude mode broadband detection of RNA charge state distributions: 112 nt RNA (left) and 300 nt RNA (right) in LC-MS using 5 mM TEAB.

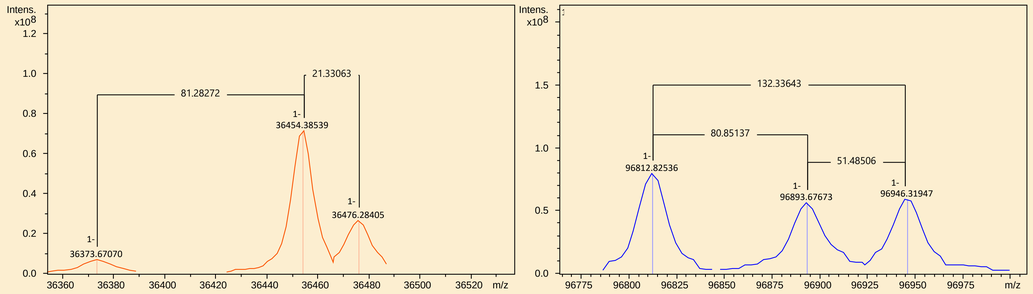

Figure 5. Data were smoothed then deconvoluted to determine the measured analyte molecular weight. The 112 nt RNA (left) shows an intact mass of 36 kDa and the 300 nt RNA shows an intact mass of 97 kDa (right). Mass shifts of 21 and 51 are expected to be sodium and iron respectively.

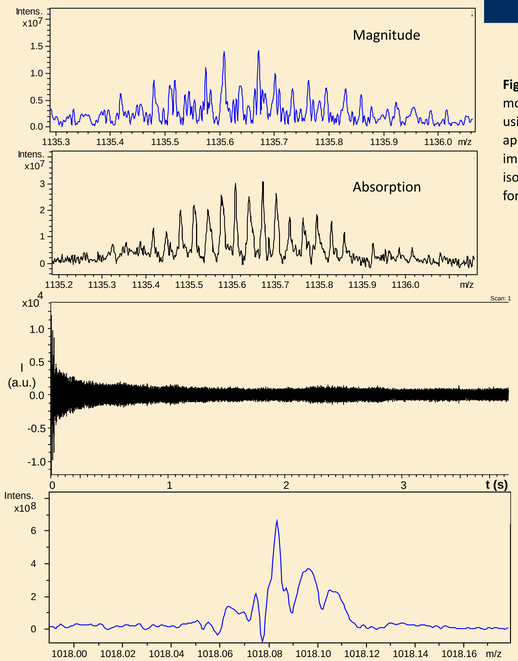

Figure 6. Absorption mode processing using half-sine apodization shows improvement of isotopic resolution for the 112 nt RNA.

Figure 7. Absorption mode processing of the 300 nt sample under current experimental parameters of 4 scan average, 8M, and isolation of three charge states does not show adequate isotopic resolution.

Conclusion

Salt mitigation is critical thus high purity solids ~20 ppm Na/K are likely key for buffer preparation. Solvent Na/K concentrations ≤20 ppb (5 ppb used for desalting here and 20 ppb used for LC-MS here), substitution of glass with polypropylene, and careful solution preparation are all important. Higher scan averaging and narrower isolation for the 300 nt RNA ion populations seems like a probable path to isotopic resolution on the 7T platform.