Oligonucleotides

Comparison of biocompatible and inert UHPLC systems for LC-UV quantitation of large RNA

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Abstract

Purpose: Evaluate the performance of the Thermo Scientific™ Vanquish™ Amplify UHPLC system for analysis of metal-sensitive analytes such as AMPcP and large RNA.

Methods: Ion-pairing reversed-phase liquid chromatography (IP-RPLC) separation of large RNA.

Results: The Vanquish Amplify UHPLC system reduces metal–analyte interactions, improving chromatographic performance and reproducibility, especially for low-concentration analytes.

Introduction

Accurate LC-UV analysis of large RNA and oligonucleotide therapeutics requires highly inert chromatographic flow paths because interactions between the negatively charged phosphodiester backbone and metallic surfaces can cause analyte loss, peak broadening, and reduced quantitative sensitivity, particularly at low concentration.

Here, an inert UHPLC system – Vanquish Amplify system, incorporating inert-coated flow path components was evaluated against a biocompatible UHPLC configuration using MP35N alloy capillaries and additionally against a commercially available inert UHPLC platform. Large RNA fragments of 500 and 5000 nucleotides were analyzed by IP-RPLC to assess the difference between these systems. To further investigate flow path interactions, adenosine 5′-(α,β-methylene) diphosphate (AMPcP), a metal-sensitive analyte, was analyzed alongside adenosine, a non-interacting reference compound.

Materials and methods

Sample preparation

Invitrogen™ RNA 500 & 5,000 nt; Adenosine 5-(α,β-methylene) diphosphate (Sigma-Aldrich); Thermo Scientific™ Adenosine.

Sample preparation

AMPcP and adenosine were weighed and co-dissolved together in water to generate an equimolar solution with a concentration of 11.7 µM.

RNA sample was diluted separately in RNase-free water and then combined in a 1:1 ratio at a final concentration of 12 ng/µL (12 ng on column) per component.

Test methods

10 mM ammonium acetate mobile phase with pH adjusted to 5.2 / 6.7 / 8.7 was used for AMPcP/Adenosine testing.

Mobile phase for large RNA testing:

Eluent A: 100 mM triethylammonium acetate (TEAA)

Eluent B: 100 mM TEAA/acetonitrile (ACN) 75/25 (v/v)

Column

Thermo Scientific™ SurePac™ Oligo RP MDi™ HPLC column, 2.5 µm 2.1 X 50 mm (P/N: 43712-052132)

Data Analysis

The Thermo Scientific™ Chromeleon™ Software 7.3.2 MUc Chromatography Data System (CDS) was used for all data acquisition and analysis.

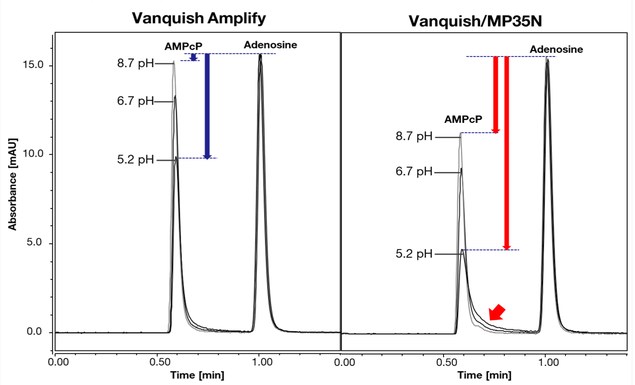
Results

Probe analyte testing

Secondary interactions between the phosphodiester backbone and metallic surfaces have been widely observed and are well documented. These interactions vary depending on the nature of the sample, its concentration, and the pH of the mobile phase used.

AMPcP, used as a probe analyte, together with adenosine (which is not sensitive to metallic surfaces), was tested to assess the impact of mobile phase pH on these secondary interactions (Figure 1). The results show that acidic conditions create the most favorable environment for strong interactions. Under these sensitive conditions, the Vanquish Amplify system provides a twofold increase in sensitivity compared to conventional biocompatible MP35N Vanquish system.

Figure 1. Chromatographic profiles obtained from the 11.76 µM AMPcP/ adenosine sample under three different mobile phase pH conditions.

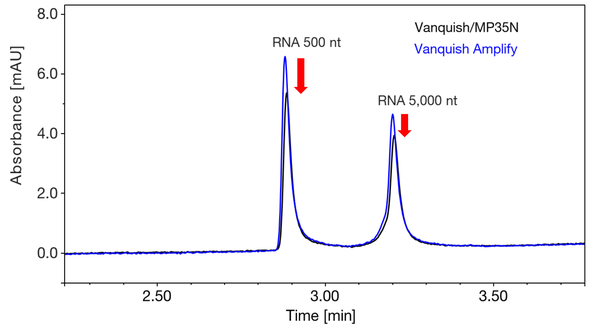


Large RNA testing

While AMPcP is widely used as a sensitive probe for metallic surface testing, it doesn't represent the expected interaction of long phosphodiester backbone.

To assess a practically more relevant sample, a mix of 500- & 5000-nucleotide large RNAs were tested to compare Vanquish Amplify flow path to a standard Vanquish system (Figure 2).

Figure 2. Chromatogram of the first injection of 500 & 5,000 nt RNA sample on the Vanquish Amplify (blue) and Vanquish system with MP35N fluidics (black).



These results indicate the presence of secondary interactions even with the use of a biocompatible system; however, these interactions are minimized whenever the inert Amplify system is used.

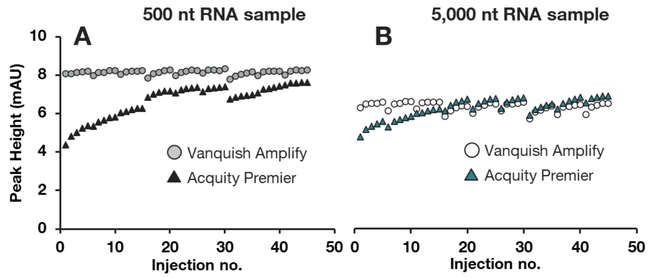
Large RNA, cross-vendor comparison of systems

The performance of the Vanquish Amplify UHPLC system and the Acquity Premier UPLC system for large RNA analysis was evaluated by comparing peak heights of the 500 nt and 5,000 nt RNA. Each system was equipped with a matched SurePac Oligo RP MDi column to ensure comparable chromatographic conditions. Results were obtained using identical DAD UV detectors installed downstream of the system UV detector to ensure consistent detection conditions.

Testing was performed in cycles of five injections and repeats within three days, totaling almost up to 50 injections per system.

While the results for the 5,000 nt RNA sample are similar for both systems, the 500 nt sample proved to be different (Figure 3). Initial injections on Vanquish Amplify had almost a two fold increase in peak height, resulting in better sensitivity and proving lower secondary interactions. Consequently, the Acquity Premier showed a consistent conditioning effect which wasn't observed on Vanquish Amplify flow path.

Figure 3. Peak heights of 500 & 5,000 nt RNA sample on Vanquish Amplify and Acquity Premier systems. Both systems had an identical DAD UV detector installed downstream, to ensure consistent conditions.



Conclusions

- Using AMPcP with the Vanquish Amplify system demonstrated up to a twofold increase in sensitivity compared to systems with MP35N fluidics under conditions where metal interactions are most pronounced
- For large RNA, the Vanquish Amplify UHPLC system delivered 1.25–1.35× higher signal intensity compared to systems with MP35N fluidics, enabling reduced sample consumption and improved quantitative reliability.
- The Vanquish Amplify UHPLC system demonstrated comparable system inertness under the tested conditions for larger RNA fragments (5,000 nt) and slightly better system inertness for smaller fragments (500 nt) in this study than the MaxPeak HPS containing system, based on peak height and area comparisons using an external UV detector.

References

1. Lovejoy, K., Žvirblis, A., Grübner, M., Fehrenbach, T., & Steiner, F. (2026). *LC-UV analysis of large RNA using an inert UHPLC system* (Technical Note No. 004606). Thermo Fisher Scientific. Available at:

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