

MRNA CRITICAL QUALITY ATTRIBUTE ANALYSIS USING A UPLC-TOF-MS SYSTEM AND CUSTOMIZED SOFTWARE

Ying-Qing Yu; Rebecca J. D'Esposito; Catalin E Doneanu; Leslie Napoletano; Michael Zagleboyo
Waters Corporation, Milford USA 01757

INTRODUCTION

Recent development and approval of the two COVID mRNA-based vaccines has brought RNA therapeutics to the forefront of the biopharma industry. As such, development of analytical methods for monitoring of the CQA of RNA-based therapeutics has become a high priority for ensuring proper control of manufacturing process.

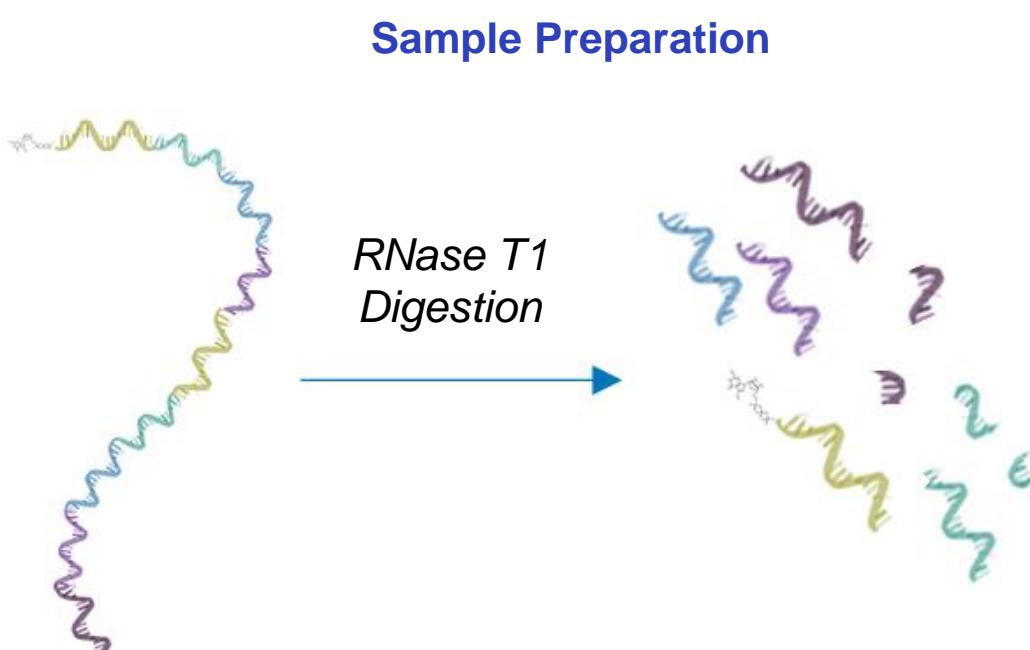
This study illustrated three CQAs (Critical Quality Attributes) analytical workflow for mRNA using a bench top UPLC-TOF MS instruments and customized data processing software.¹⁻³

These CQAs are:

- 5' capping
- Poly A tail heterogeneity
- Sequence mapping



METHODS



INTACT Mass App (software) was the main software used for 5' capping analysis, sequence mapping and poly A tail heterogeneity profiling.



Figure 1: BioAccord™ UPLC-TOF MS System with Max Peak™ High Performance Surfaces.
Column used: ACQUITY™ Premier OST Column 1.7 μ m, 130 \AA , 2.1 x 150 mm
More method information can be found in the three application notes listed under the reference.

Sample used for RNA Mapping:

•sgRNA (100 mer, IDT) - contains six PTMs, such as 2'-O-methylguanosine, 2'-O-methyluridine and 2'-O-methyladenosine and each modification has a phosphorothioate group.

•Cypidine mRNA

Sample used for Poly A Tail Heterogeneity

•Firefly Luciferase (Fluc) mRNA

Sample used for 5' Capping demonstration

5'-GUAGAACUUCGUCGAGUACGUCAA (New England Biolab)

5'capping

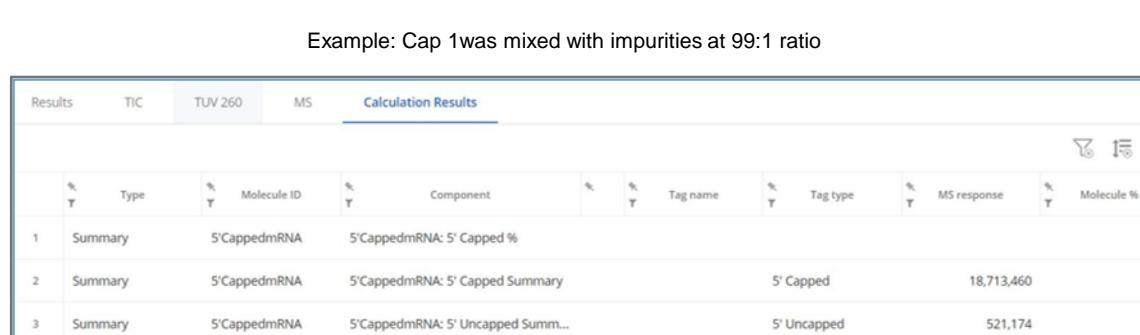
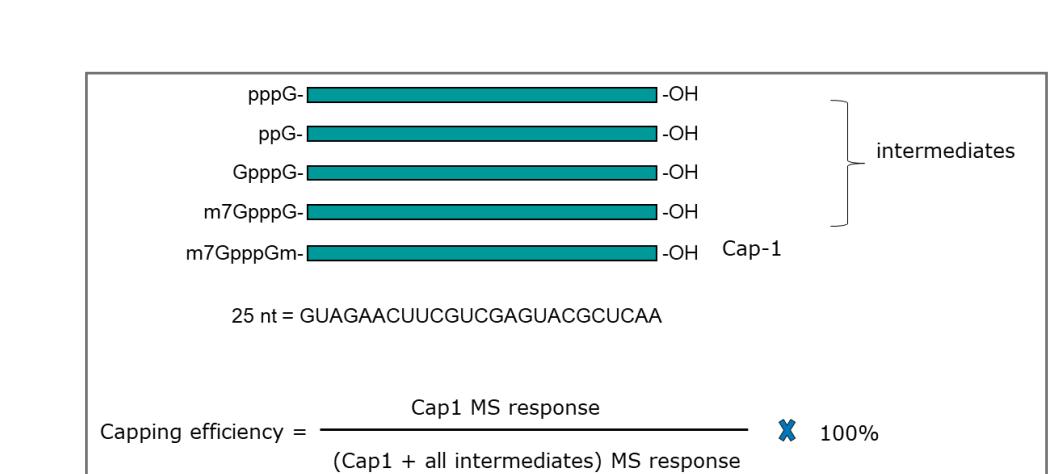
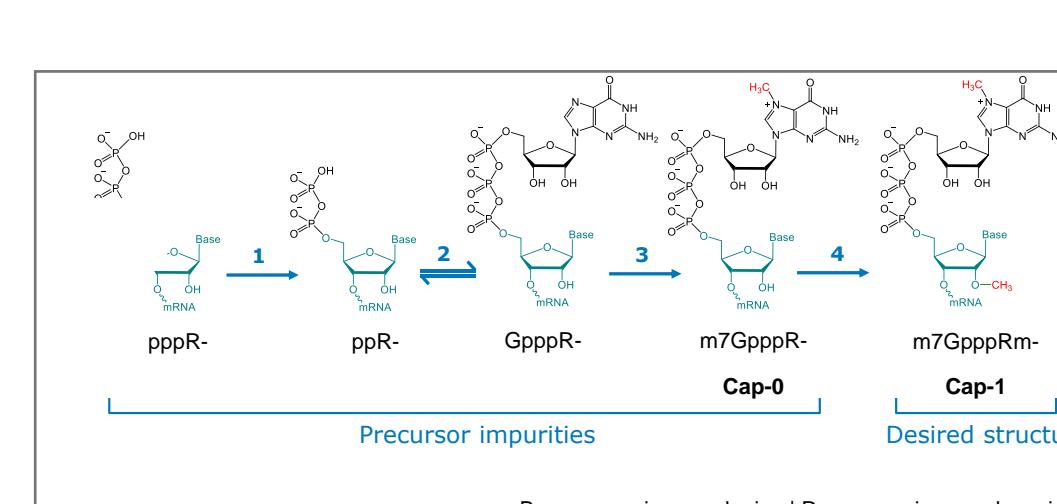


Figure 2. (A) Illustration of the Cap-1 vs. precursor impurities; (B) Synthetically made Cap-1 (25 mer long) was mixed with synthetically made intermediate byproducts in 99:1, 99:1 and 9:1 ratio before LC-MS analysis to quantify the Cap-1 product rel% using MS signals. C) Result table from Cap-1 mixed in at 99:1 ratio with other impurities. LC-MS analysis and data processing using the INTACT Mass App generated the rel% calculation of the Cap-1 species. The data processing gave 97.3% Cap 1 vs 2.7% of byproducts (for the 99:1 mixed sample). Differences in ionization efficiency could play a role in the MS-based quantitation.

RESULTS

Poly A Tail

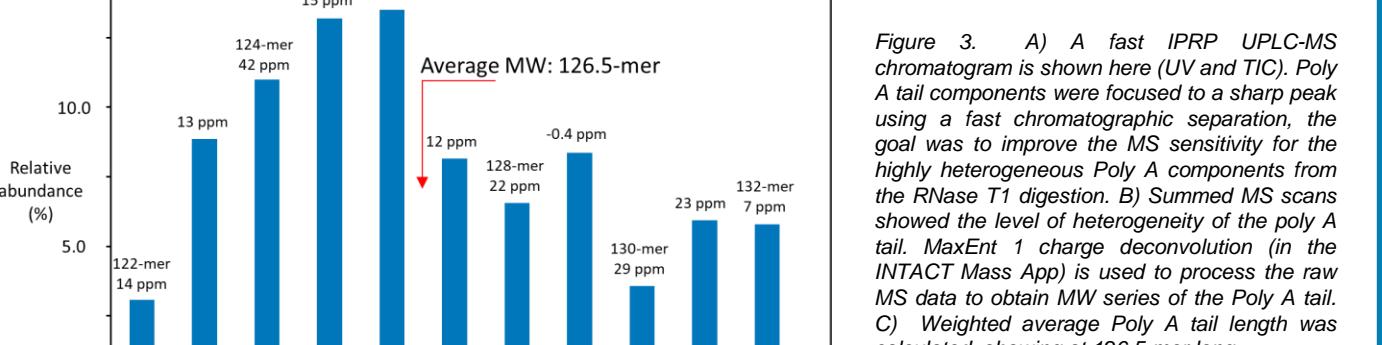
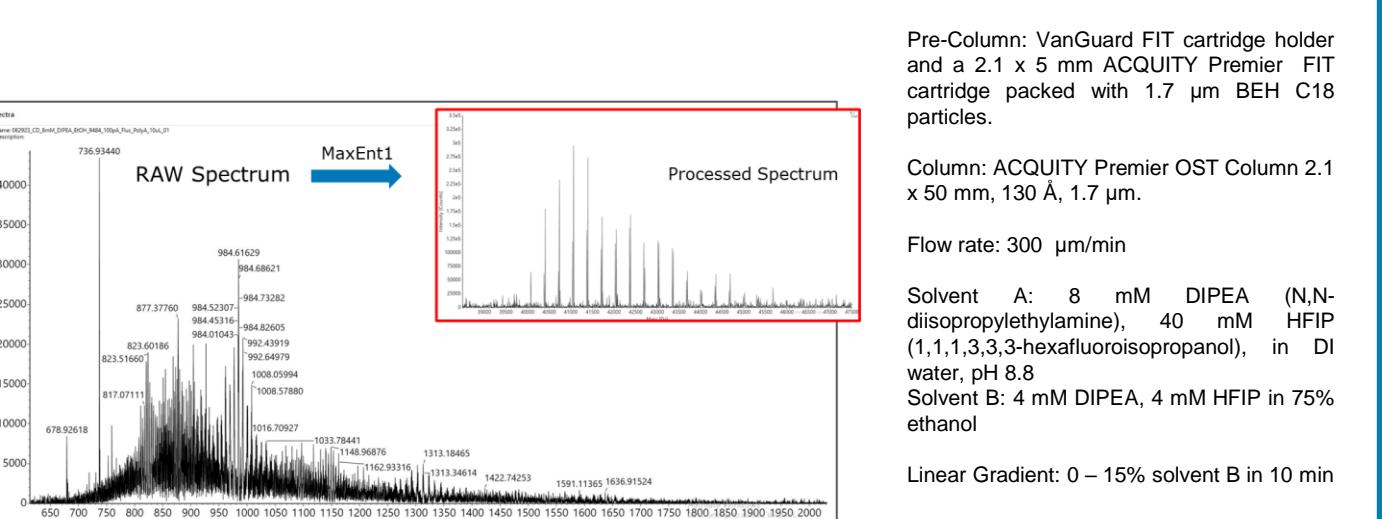
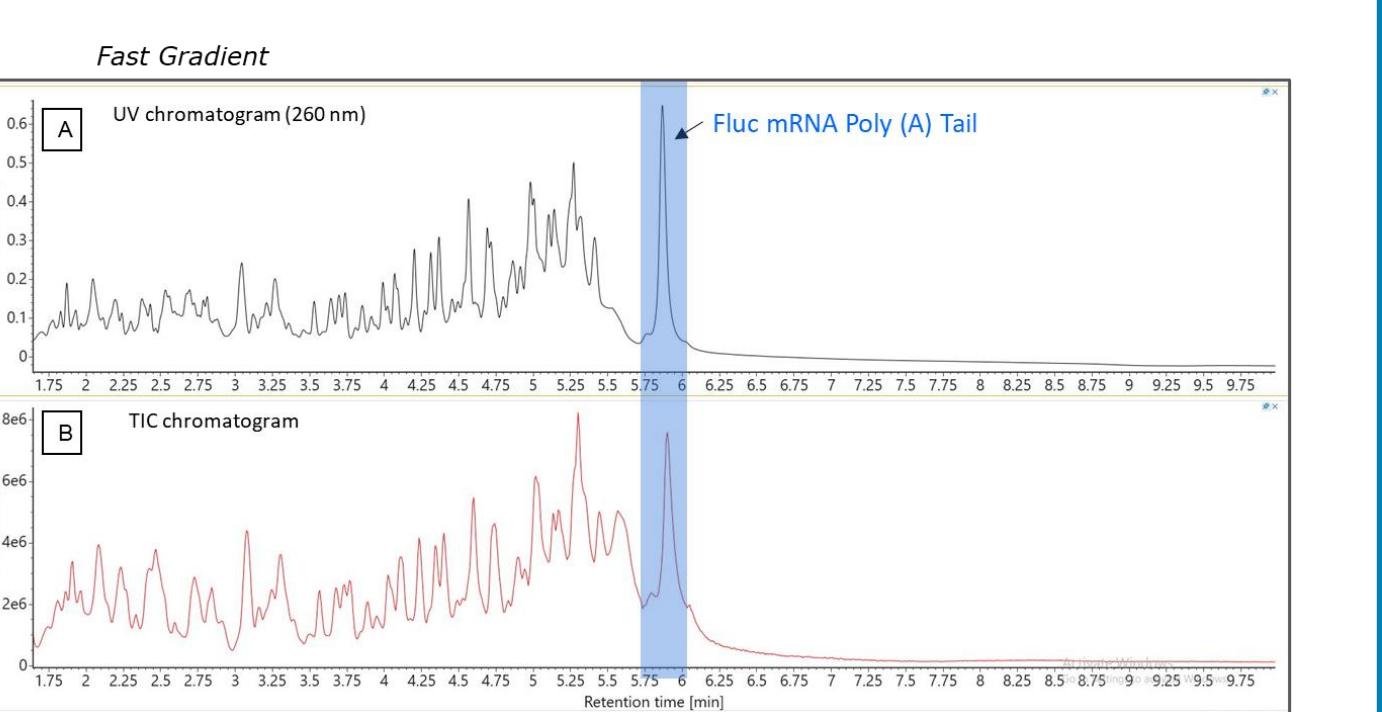


Figure 3. A) A fast IPRC UPLC-MS chromatogram is shown here (UV and TIC). Poly A tail components were focused as a sharp peak using a fast chromatographic separation, the good separation improved the MS analysis of the highly heterogeneous Poly A components from the RNase T1 digestion. B) Summed MS scans showed the level of heterogeneity of the poly A tail. MaxEnt 1 charge deconvolution (in the INTACT Mass App) is used to process the raw MS data to obtain MW series of the Poly A tail. C) Weighted average Poly A tail length was calculated, showing at 126.5 mer long.

Sequence Confirmation

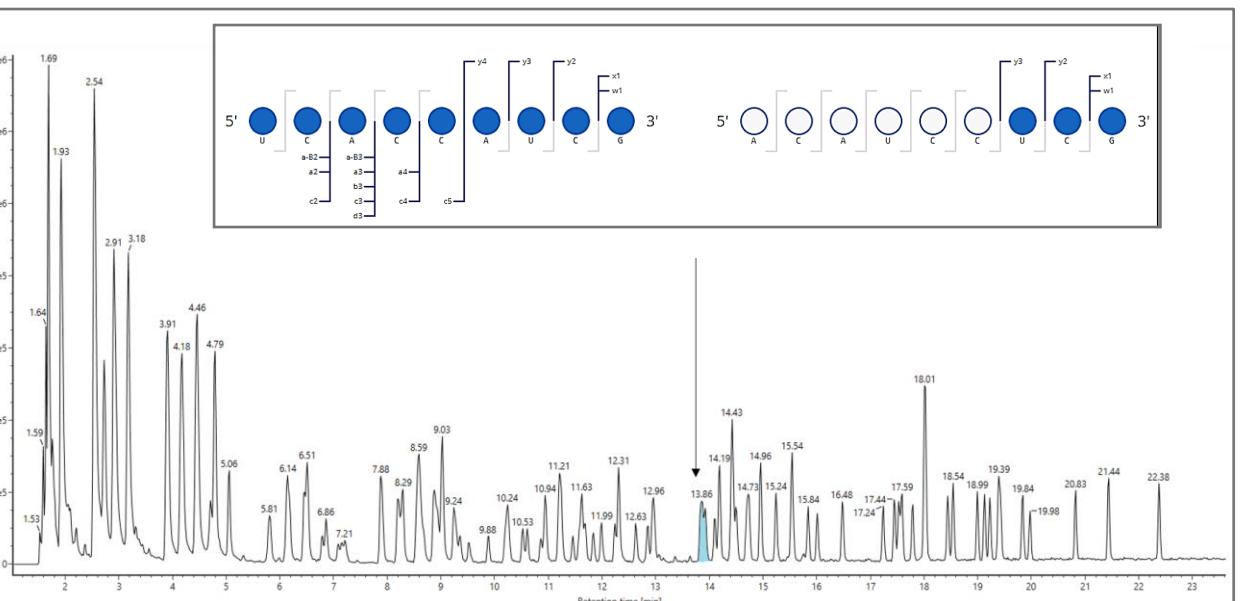
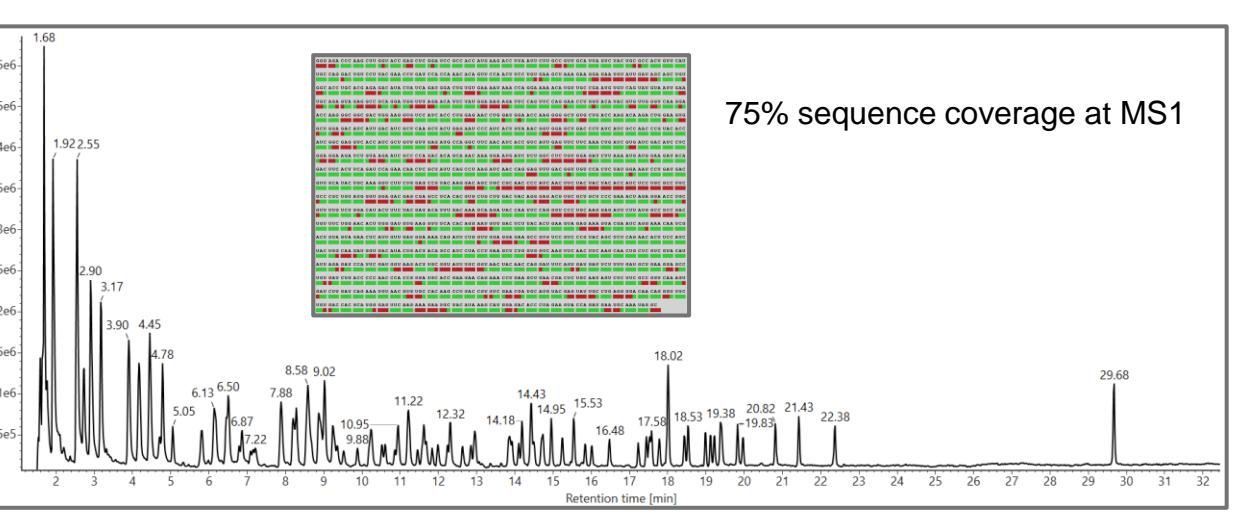
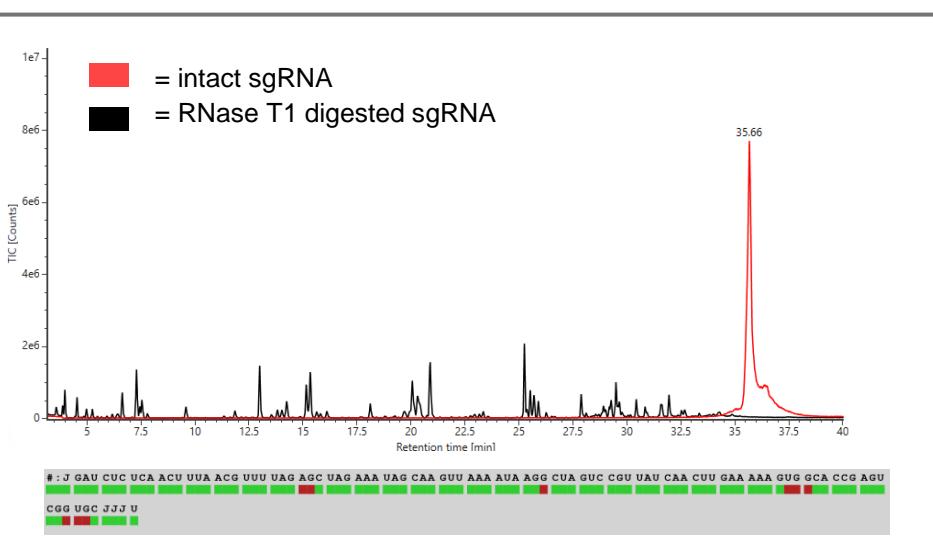


Figure 4. sgRNA and mRNA were digested by RNase T1 and analyzed using the BioAccord LC-MS system. Data analysis is performed using INTACT Mass App with the assistance of mRNA Cleaver App (to generate theoretical mass of the digested fragments) and Sequence Viewer (for coverage% calculation and data review). A) Example data were shown from a ~100 mer sgRNA. B) LC-MS chromatogram of RNase T1 digested Luciferase mRNA (~4000 mer) and the sequence coverage in a Map viewer. We included the ambiguous sequence in the calculation. Data generated from elevated energy fragmentation can be processed in another software in the waters_connect software platform, CONFIRM Sequence to differentiate ambiguous sequence. For example, C) A better match from two candidate sequences was highlighted based on the fragment ion coverage. These two candidate are the so-called ambiguous sequence since they have same MW, but scrambled sequence.

CONCLUSION

- THREE ANALYTICAL WORKFLOW ARE DEVELOPED ON THE BIOACCORD LC-MS SYSTEM AND WATERS_CONNECT INFORMATICS PLATFORM FOR THREE MRNA CQA ANALYSIS. THESE CQAS ARE 5' CAPPING EFFICIENCY, POLY A TAIL HETEROGENEITY AND SEQUENCE MAPPING ANALYSIS.
- SOFTWARE TOOL PLAYS CRITICAL ROLE IN REDUCING THE TIME USED FOR DATA INTERPRETATION. THE SOFTWARE USED IN THIS STUDY IS INTACT MASS APP WITH NEWLY ADDED CAPABILITIES SUCH AS ENZYME CLEAVAGE TOOL AND SEQUENCE MAPPING VIEWER.
- FUTURE WORK WILL BE FOCUSED ON IMPROVING THE SEQUENCE COVERAGE BY 1) COMBINING MS1 AND MS2 RESULTS; 2) AUTOMATED THE ENTIRE DATA PROCESSING WITH LESS MANUAL INTERVENTION TO REDUCE THE LEVEL OF AMBIGUOUS ASSIGNMENTS.

References

1. WATERS APPLICATION NOTE: 720008130 "RNA CQA ANALYSIS USING THE BIOACCORD LC-MS SYSTEM AND INTACT MASS WATERS_CONNECT APPLICATION".
2. WATERS APPLICATION NOTE: 720007329 "RAPID ANALYSIS OF SYNTHETIC MRNA CAP STRUCTURE USING ION-PAIRING RPLC WITH THE BIOACCORD LC-MS SYSTEM".
3. WATERS APPLICATION NOTE: 720007925 "ION PAIRING REVERSED PHASE LC-MS ANALYSIS OF POLY (A) TAIL HETEROGENEITY USING THE BIOACCORD LC-MS SYSTEM".