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Liquid Chromatograph Mass Spectrometer LCMS-2050

Application News

Analysis of mRNA 5′ Cap Structure Using a Single Quadrupole Mass Spectrometer

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User Benefits

- The LCMS-2050 single quadrupole (SQ) mass spectrometer and the LabSolutions Insight™ Biologics analysis software can confirm the molecular weights of nucleic acids.
- The LCMS-2050 enables a user-friendly operation that is similar to LC systems.
- The software enables users to set analytical parameters for modifications and impurities as desired.

■ Introduction

There has been increasing attention regarding the new drug discovery modality of mRNA because of its efficacy for COVID-19 vaccines. Currently, authorized mRNA vaccines are synthesized using *in vitro* transcriptions to add the Cap-1 structure (m7GpppRm-) on the 5′ end. This modification contributes to mRNA recognition, better efficiency of translation, and mRNA stability in cells, making 5′ cap structure analysis an important element of mRNA quality controls.

The quadrupole time-of-flight mass spectrometer that was introduced in the Application News No. 01-00733 is useful for sequence coverage identification of nucleic acids that SQ cannot perform. However, SQ mass spectrometers are easy to use and similar to operate as an LC, so they are in increasing demand for confirming molecular weights, such as for quality controls. Below is an introduction to 5′ capped mRNA analysis using the LCMS-2050 SQ mass spectrometer and LabSolutions Insight Biologics analysis software.

■ Samples

Given that mRNA is a large molecule, LC/MS analysis is typically done by analyzing fragments generated by cleavage enzyme reactions. In this study, the model samples consisted of Cap-1 structure mRNA with 36 bases (Cap-1 groups) obtained by *in vitro* transcription using plasmid DNA as a template. The 5′ cap modified unreacted RNA (pppR-) was also provided for analysis as an impurity.

■ Analytical Conditions

Analysis was performed using the Nexera™ XS inert and LCMS-2050 systems. The analytical conditions are shown in Table 1. The LCMS-2050 is equipped with a heated DUIS™ ion source for ionization, which combines the advantages of both ESI and APCI.

Table 1 Analytical Conditions

UHPLC (Nexera XS inert)	
Column:	Shim-pack Scepter™ Claris C18-120* (150 mm × 2.1 mm I.D., 1.9 µm)
Mobile Phase A:	95 mM HFIP, 5 mM DIPEA – water
Mobile Phase B:	70 mM HFIP, 5 mM DIPEA, 65 % acetonitrile – water
Gradient Program:	B Conc. 5 % (0 - 2 min) – 25 % (22 min) – 90 % (23 - 24 min) – 5 % (24.1 - 30 min)
Flowrate:	0.3 mL/min
Column Temp.:	60 °C
Injection Volume:	5 µL

MS (LCMS-2050)	
Ionization:	ESI/APCI (DUIS) negative
Interface Voltage:	-2.0 kV
Mode:	MS *m/z* 550-2000
Nebulizing Gas Flow:	2.0 L/min
Drying Gas Flow:	5.0 L/min
Heating Gas Flow:	7.0 L/min
Desolvation Temp.:	250 °C
DL Temp.:	200 °C

* P/N 227-31210-03

■ Setting the Analysis Parameters

LabSolutions Insight Biologics software can analyze nucleic acids and their impurities. First, the user creates a nucleic acid sequence in the parameter configuration window using the software presets for nucleobases, linkers, ribose, and modifications. Nucleobases, linkers, ribose, and base modifications can be added and removed in each tab as required. Once a sequence is entered, the software displays the molecular formula monoisotopic mass, and structural formula (Fig. 1).

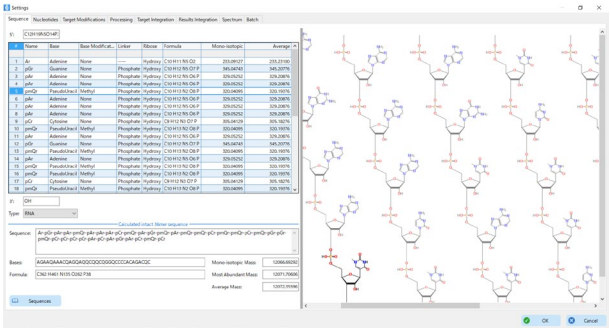


Fig. 1 Parameter Configuration Window

The Target Modifications tab is also used to select the anticipated impurities. In addition to impurities such as different strand lengths, missing nucleobases, depurination/depyrimidination, deamination, protecting groups, additional ions, and unknown modifying groups, the software can also search for molecular changes added by the user as desired. In this study, to enable detection of the 5′ cap modified unreacted group as the impurity, "5′ uncapped" was added as the target modification (Fig. 2).

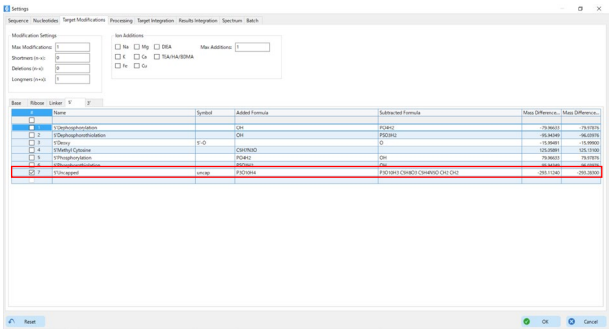


Fig. 2 Setting the Target Modification

■ Identifying Cap-1 and Unreacted Groups

Fig. 3 shows the component chromatogram of analyzed samples obtained by spiking Cap-1 group (0.5 µg) with the unreacted group at 10 % (w/w). The mass chromatogram is displayed as a component chromatogram, based on MS1 spectra and by combining signals from different valences and isotopes. Fig. 4 shows the multivalent ion analysis for the Cap-1 group. Both Cap-1 and unreacted groups were detected with a mass error of less than 1 Da compared to the theoretical molecular weight value.

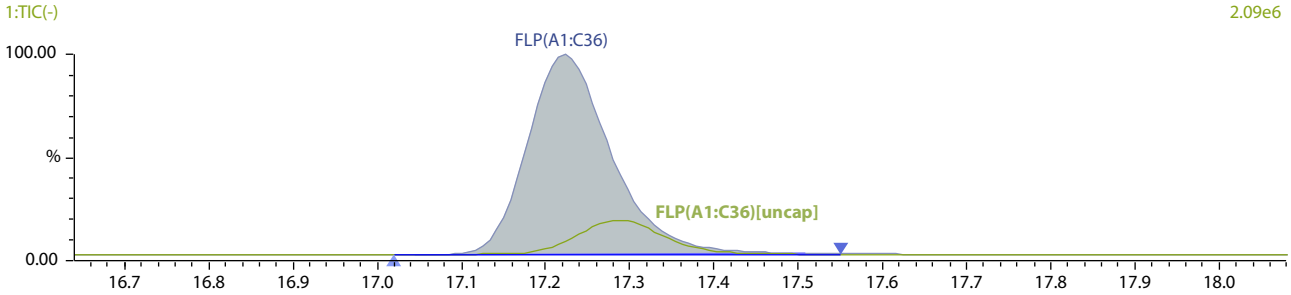
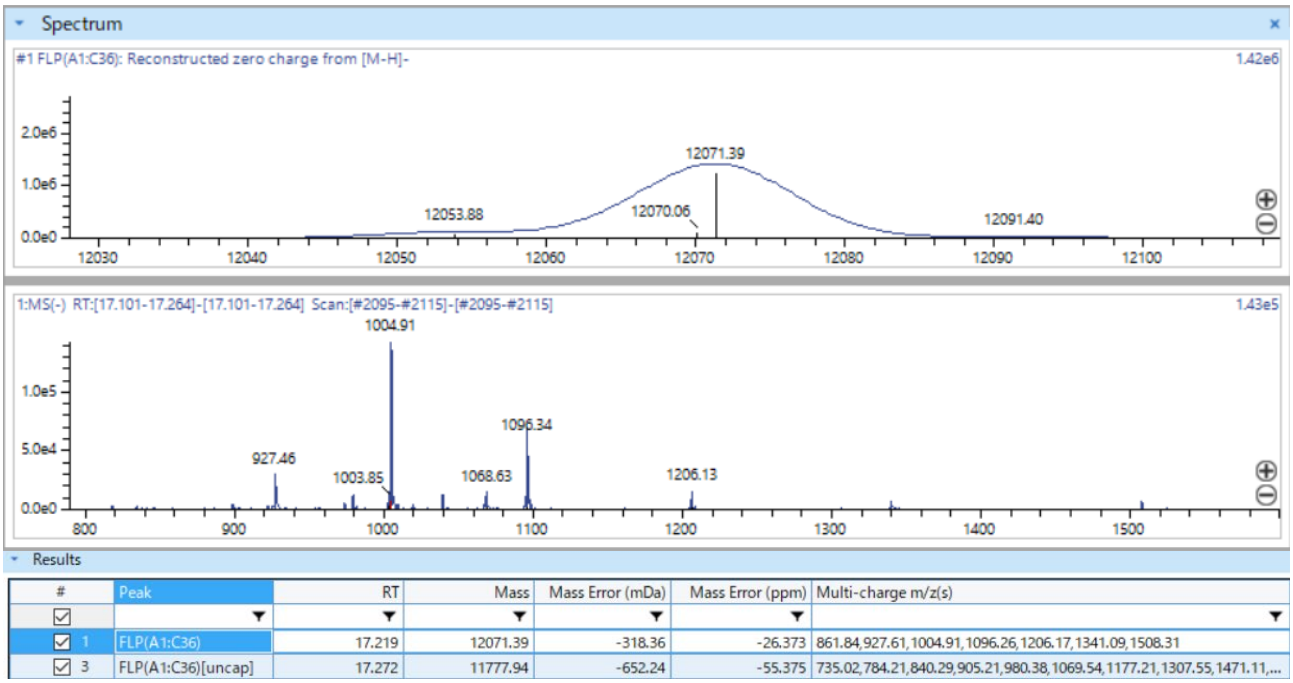


Fig. 3 Component Chromatograms of the Cap-1 Group and the Unreacted Group
FLP (A1:C36) indicates the Cap-1 group, and FLP (A1:36)[uncap] indicates the unreacted group.



#	Peak	RT	Mass	Mass Error (mDa)	Mass Error (ppm)	Multi-charge m/z(s)
1	FLP(A1:C36)	17.219	12071.39	-318.36	-26.373	861.84,927.61,1004.91,1096.26,1206.17,1341.09,1508.31
3	FLP(A1:C36)[uncap]	17.272	11777.94	-652.24	-55.375	735.02,784.21,840.29,905.21,980.38,1069.54,1177.21,1307.55,1471.11,...

Fig. 4 Results of Multivalent Ion Analysis
Top: Deconvoluted mass spectrum; Center: Mass spectrum; Bottom: Identification results

■ Conclusion

In this study, molecular weight identification of 5′ cap modified mRNA was performed using the LCMS-2050 mass spectrometer and LabSolutions Insight Biologics software. Both Cap-1 and unreacted groups were detected with a mass error of less than 1 Da compared to the theoretical molecular weight value. The LCMS-2050 demonstrated it is easy to use and similar to operate as an LC, making it suitable for confirming molecular weights such as for quality controls.

Related Applications

1. Analysis of mRNA 5′ Cap Structure Using a Quadrupole Time-of-Flight Mass Spectrometer
   Application News No. 01-00733-EN
2. Simple Analysis of Impurities in Oligonucleotide Therapeutics Using a Single Quadrupole Mass Spectrometer
   Application News No. 01-00656-EN

01-00753-EN First Edition: Jul. 2024

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