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Detecting Nucleoside Post Enzymatic Cleavage Modifications in RNA Using Fast Product Ion Scanning Triple Quadrupole Mass Spectrometry

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1. Introduction

Nucleosides (Fig. 1) are building blocks for both DNA and RNA. Modified nucleosides occur in DNA, but they are particularly characteristic of tRNA, rRNA and eukaryotic mRNA. There are more than 79 different nucleosides presently known in tRNA, the most highly modified of the RNAs from all sources. The modified nucleosides have considerable structural variety, from simple methylation of either the base or the *o*-2 hydroxyl of ribose to much more complex types of modification in the base. As selective enzymatic cleavage of RNA produces oligonucleotides and nucleosides, massspectrometry can be an important tool for the detection and sequence localization of post-transcriptionally modified nucleosides in RNA. From a total enzymatic digest of the RNA or RNase T1 hydrolysis fractions, a mixture of nucleosides and modified nucleosides can be obtained.

Scientists rely on LC-MS-MS for detection and quantitation of nucleosides. LC-MS-MS methods use MRM analysis for the highest sensitivity and selectivity; however these methods only detect analytes whose MRM transitions are known in advance. Modified nucleosides are not easily identified by traditional MRM-based methods as many species share common precursor and product ions. Product ion scanning, in conjunction with chromatographic separation, may be used to identify them. We developed LC-MS methods that utilize extremely fast product ion scanning for detection of nucleosides obtained from commercial suppliers. Product ion spectra for each known nucleoside were acquired and inspected for common product ions. A mixture of modified nucleoside standards was analyzed as well.

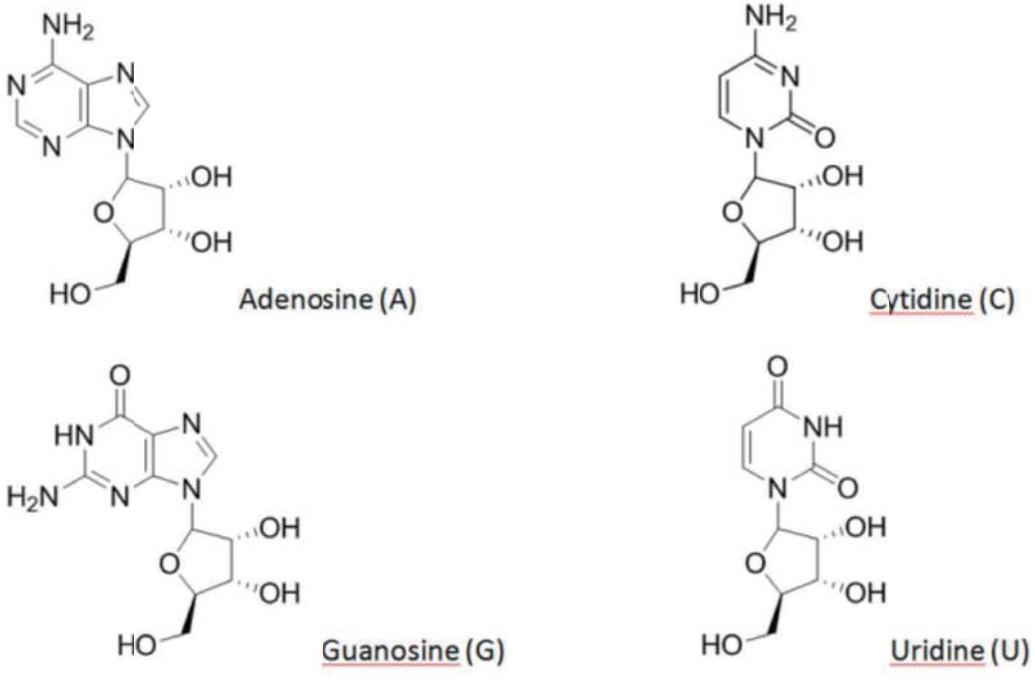

Fig. 1 Common nucleosides: adenosine (A), cytidine (C), guanosine (G) and uridine (U)

2. Method

Electrospray ionization was used on a fast-scanning Shimadzu LCMS-8040 triple quadrupole mass spectrometer. A precursor ion scan for each common product was carried out at a scan speed of 5,000 u/sec. Data dependent tandem mass spectra based on these survey scans were carried out at 15,000 u/sec. A Shimadzu Nexera UHPLC system with a 2 µm C18 column was used for the LC separation. The mobile phase was 10 mM ammonium acetate in water (Pump A) and 10 mM ammonium acetate in acetonitrile (Pump B). The pH was adjusted to 6 with acetic acid. The flow rate was 0.5 mL/min.

3. Results and Discussion

The *m/z* 258 precursor ion corresponds to monomethylated cytidines.
There are four species (m^3C; m^5C; Cm; m^4C) with methylation on the sugar (Cm) and others on the pyrimidine base.
As with all things, there is no substitute for good chromatographic separations for compound identification, but the product ion scans for the precursor were very useful to screen for determination of modified nucleosides in RNA research. Fig. 2 is an example product ion scan used to identify modified cytidine.

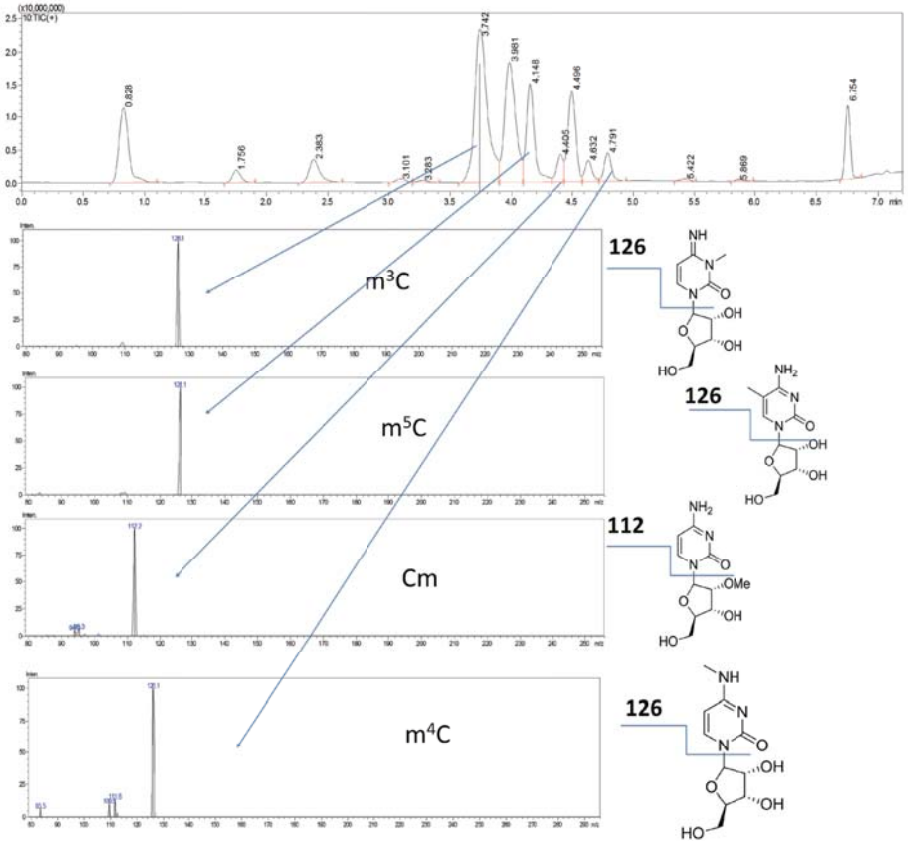

Fig. 2 Product ion scans corresponding to monomethylated cytidines, m^3C; m^5C; Cm; m^4C

3. Results and Discussion (Cont'd)

By using commercially available standards and with product ion scans, MRM transitions can be established. Upon application of CID, most nucleosides have the similar pattern of fragmentation, the breakage of the C-N bond between the base and the sugar moiety.
This is confirmed with standard nucleosides, and some examples are shown in Fig. 3.

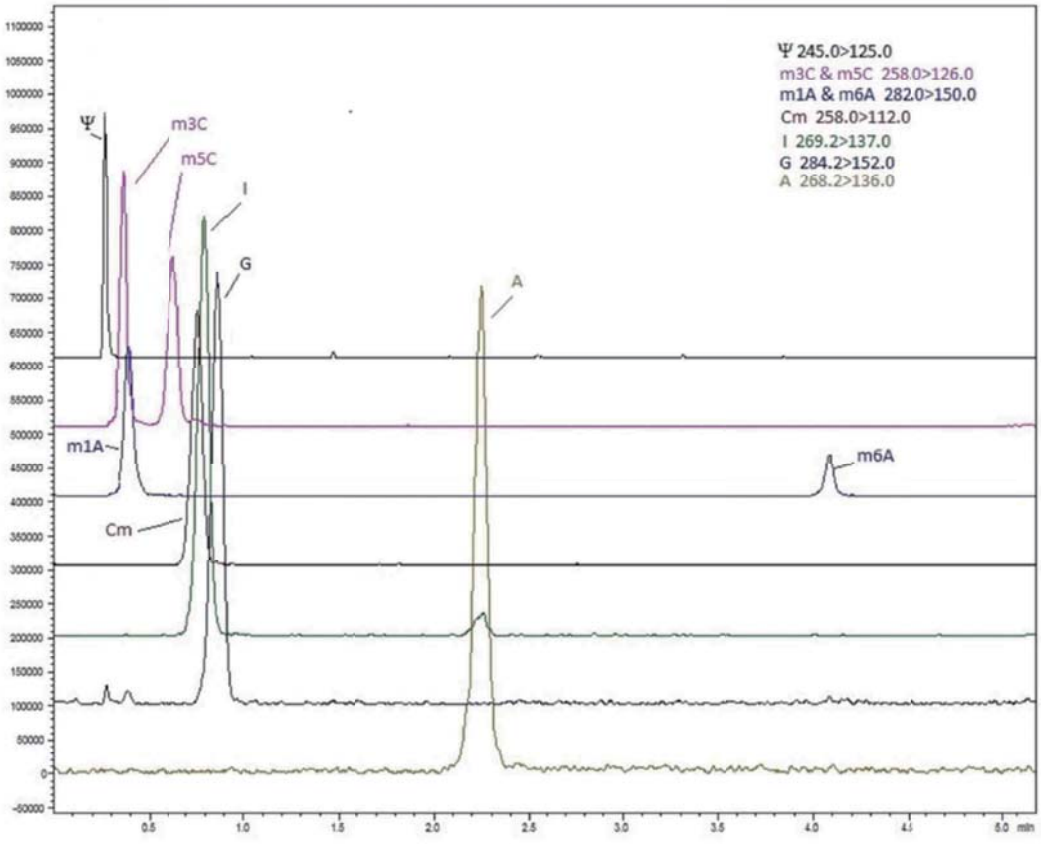

Fig. 3 A standard mixture with MRMs

The most useful utility for this is that one can establish MRM transitions for modified nucleosides for which there are no commercial standards. Although there are many modified nucleosides which have been synthesized and reported in the literature, it is expensive and sometimes impossible to obtain all of the modified nucleosides as authentic standards for identification and quantitation. With this method, one can predict the MRM transition for those modified nucleosides based on the chemical structures. We have demonstrated that it can be used in the analysis of an unknown RNA hydrolysate (Fig. 4).

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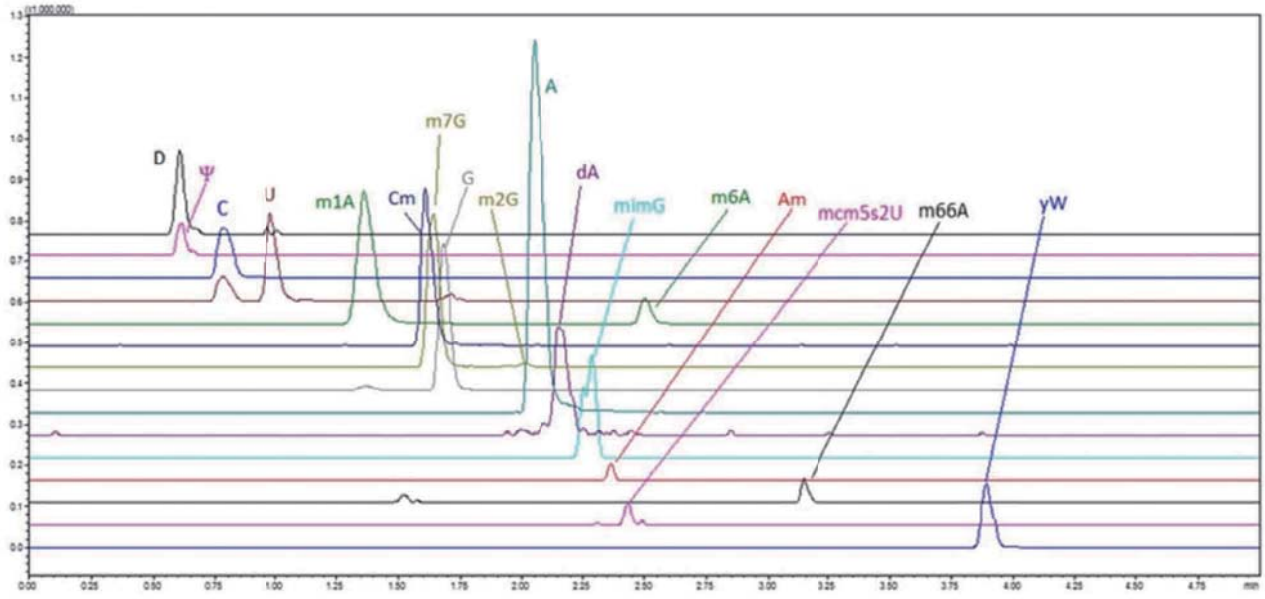

Fig. 4 A sample analysis of modified nucleosides using a Shimadzu UHPLC-LCMS instrument

4. Conclusion

A novel method for detection of both nucleosides and modified nucleosides using fast precursor ion scanning is demonstrated. The fast product ion scanning method will help screening for the identification of the nucleosides, and it can be used as an important tool for structure elucidation of modified nucleosides especially when they are in low abundance.

5. References

• 1. Kowalak, J.A., Pomerantz, S.C., Crain, P.F. and McCloskey, J.A. (1993) Nucleic Acids Res., 19, 4577-4585.
• 2. Pomerantz, S.C. and McCloskey, J.A. (1990) Methods Enzymol., 193, 796-824.

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