SUPPLEMENTAL FILES FOR:

MASS SPECTROMETRY-BASED QUANTIFICATION OF PSEUDOURIDINE IN RNA

Balasubrahmanyam Addepalli and Patrick A. Limbach*

Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry, PO Box 210172, University of Cincinnati, Cincinnati, OH 45221-0172

*To whom correspondence should be addressed.

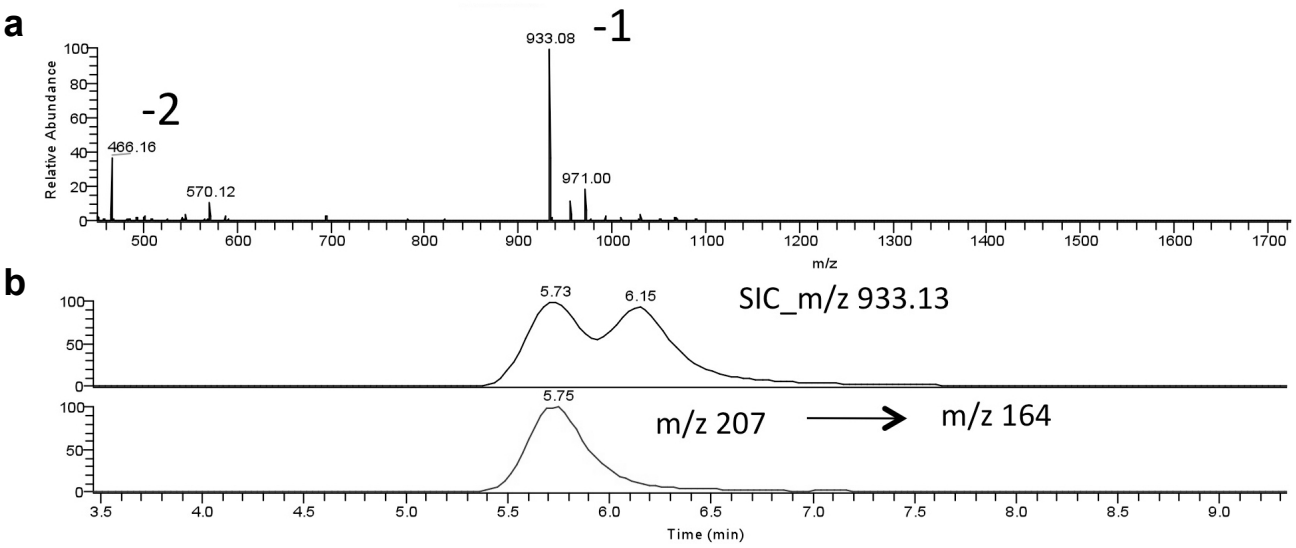
Phone (513) 556-1871
Fax (513) 556-9239
email Pat.Limbach@uc.edu

ABSTRACT

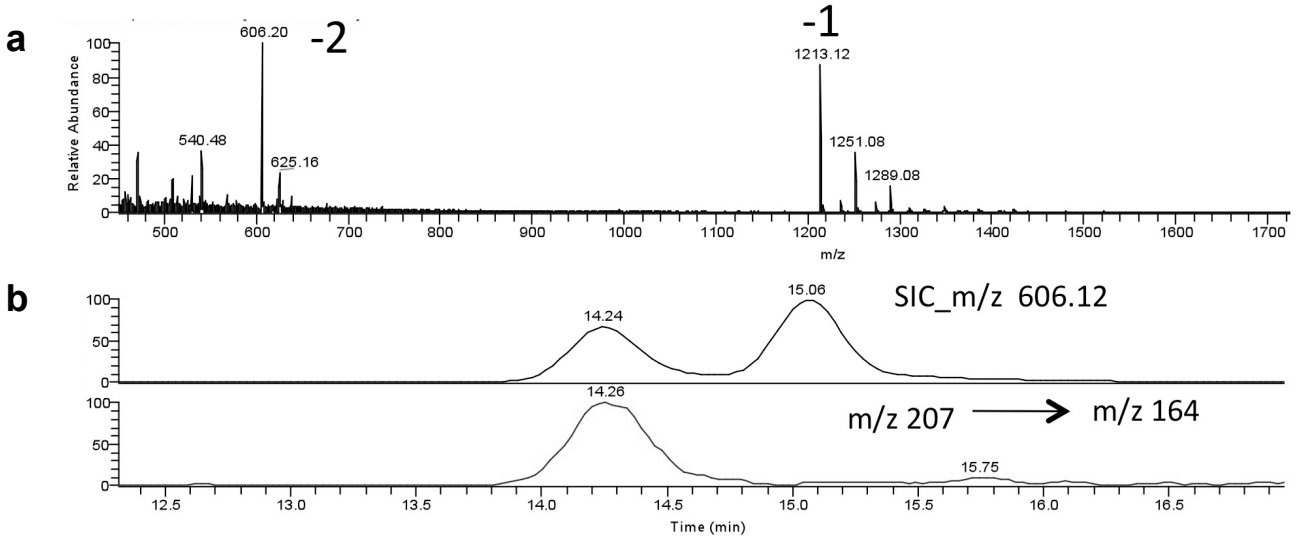
Direct detection of pseudouridine (ψ), an isomer of uridine, in RNA is challenging. The most popular method requires chemical derivatization using N-cyclohexyl-N'-β-(4-methylmorpholinum ethyl) carbodiimide *p*-tosylate (CMCT) followed by radiolabeled primer extension mediated by reverse transcriptase. More recently, mass spectrometry (MS)-based approaches for sequence placement of pseudouridine in RNA have been developed. All of these approaches, however, only yield qualitative information regarding the presence or absence of pseudouridine in a given RNA population. Here, we have extended a previously developed liquid chromatography tandem mass spectrometry (LC-MS/MS) method to enable both the qualitative and quantitative analysis of pseudouridine. Quantitative selected reaction monitoring (SRM) assays were developed using synthetic oligonucleotides, with or without pseudouridine, and the results yielded a linear relationship between the ion abundance of the pseudouridine-specific fragment ion and the amount of pseudouridine-containing oligonucleotide present in the original sample. Using this quantitative SRM assay, the extent of pseudouridine hypomodification in the conserved T-loop of tRNA isolated from two different *Escherichia coli* strains was established.

SUPPLEMENTAL INFORMATION

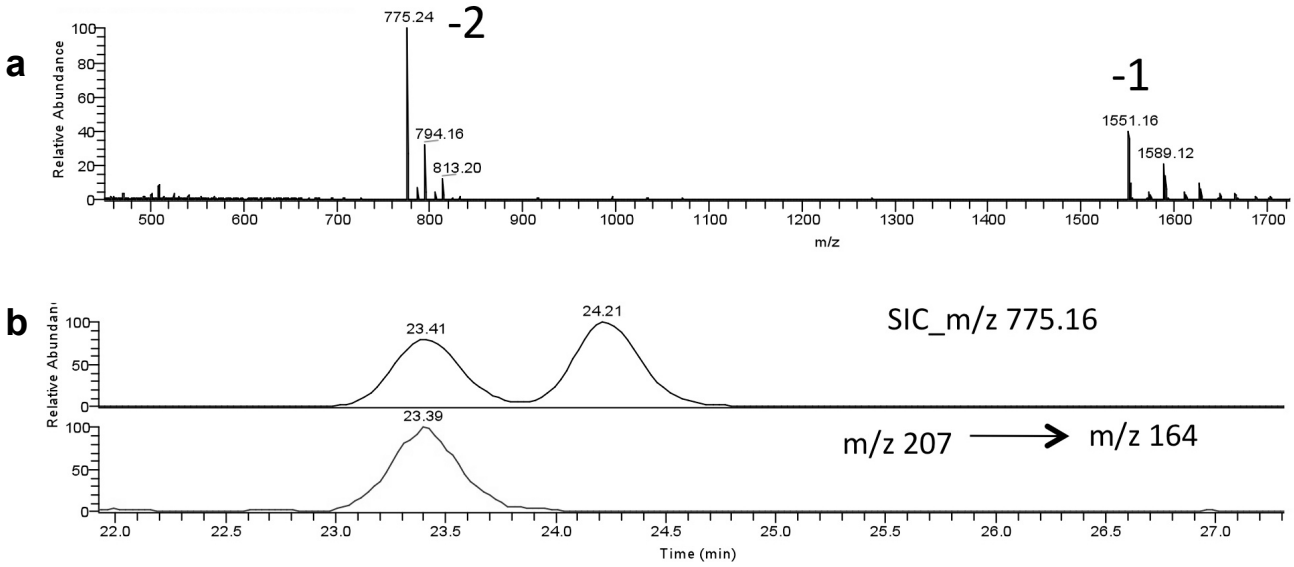
Supplemental Figures S1 – S6



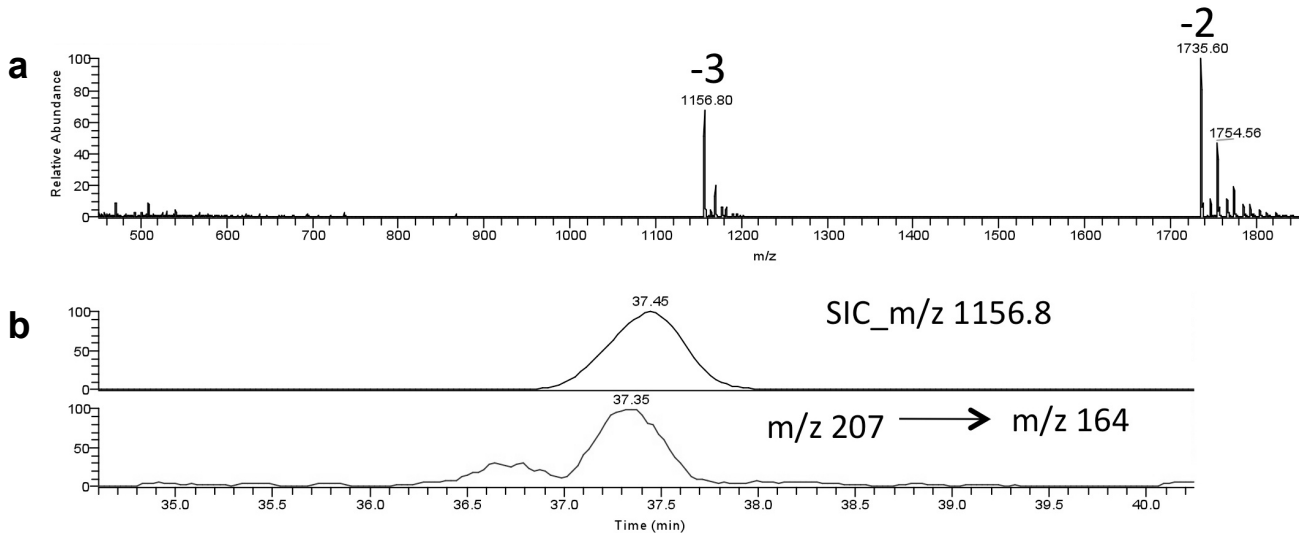
Supplemental Figure S1. Representative mass spectrum and selected ion chromatogram of GψG and GUG. (a) Mass spectrum of the oligonucleotide mixture (charge states are labeled), (b) selected ion chromatogram at 35 pmol concentration of each oligomer (top) and ψ-specific SRM transition (bottom).



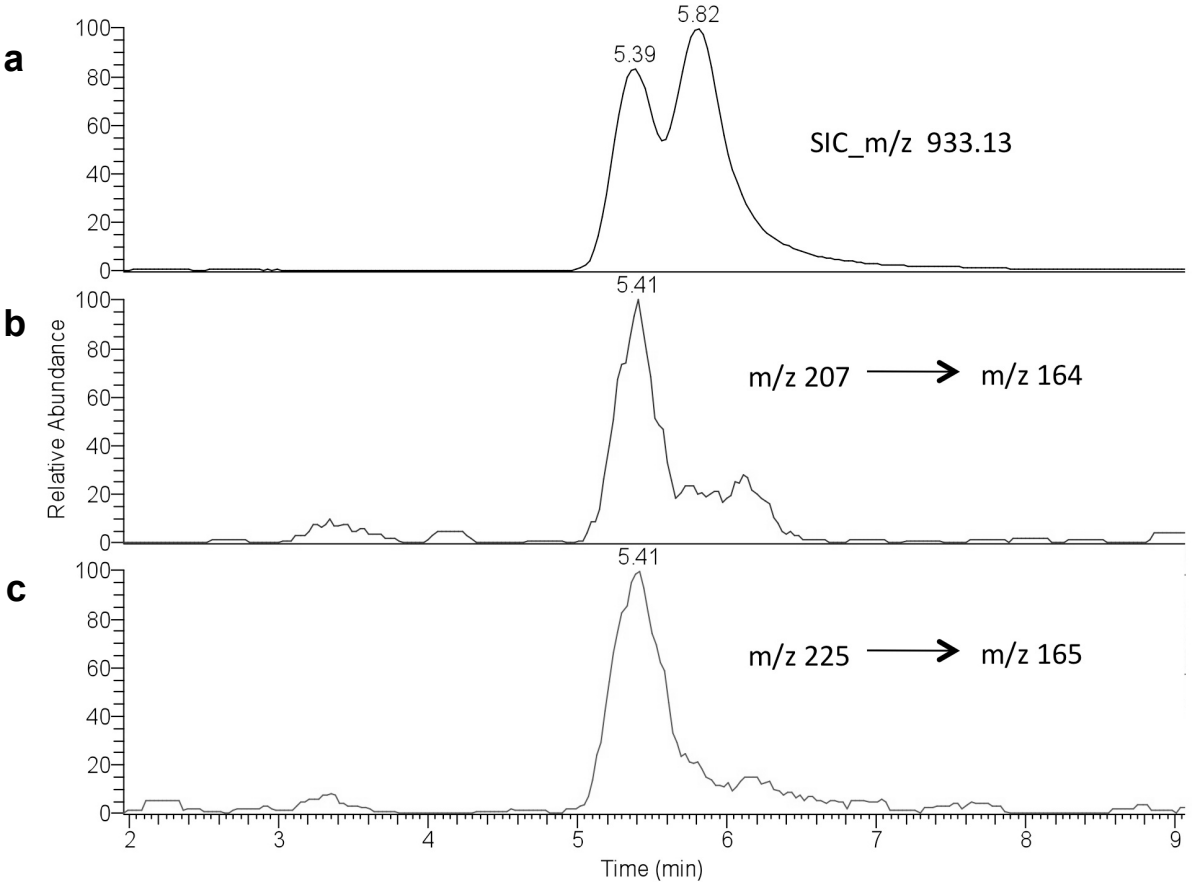
Supplemental Figure S2. Representative mass spectrum and selected ion chromatogram of TψCG and TUCG. (a) Mass spectrum of the oligonucleotide mixture (charge states are labeled), (b) selected ion chromatogram at 35 pmol concentration of each oligomer (top) and ψ-specific SRM transition (bottom).



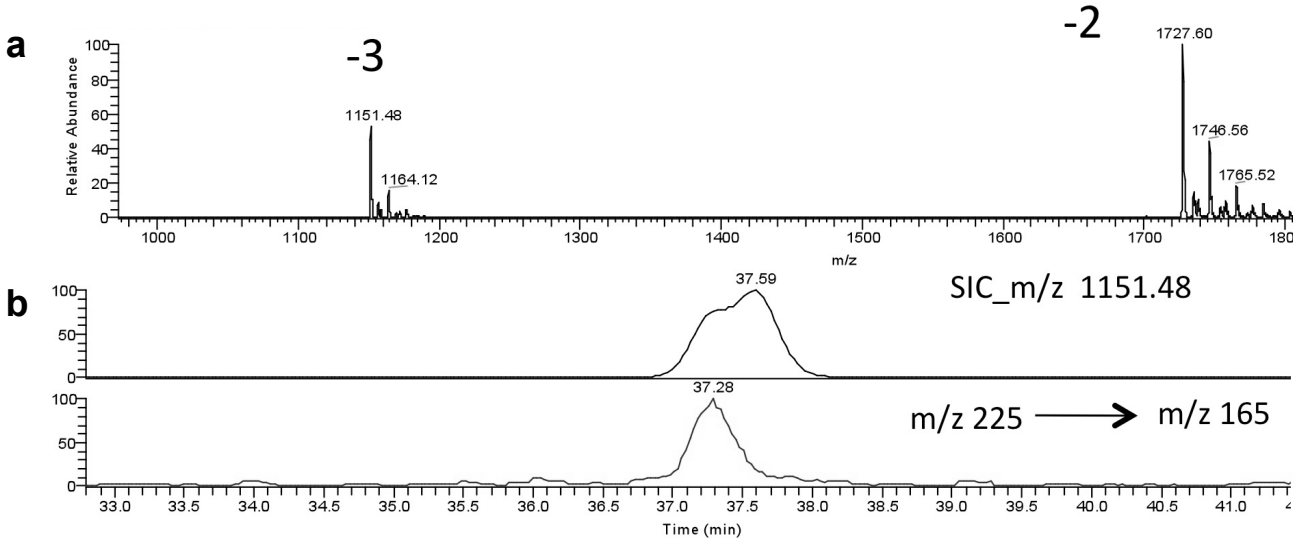
Supplemental Figure S3. Representative mass spectrum and selected ion chromatogram of AψCAG and AUCAG. (a) Mass spectrum of the oligonucleotide mixture (charge states are labeled), (b) selected ion chromatogram at 35 pmol concentration of each oligomer (top) and ψ-specific SRM transition (bottom).



Supplemental Figure S4. Representative mass spectrum and selected ion chromatogram of UAACψAUGACG and UAACUAUGACG. (a) Mass spectrum of the oligonucleotide mixture (charge states are labeled), (b) selected ion chromatogram at 35 pmol concentration of each oligomer (top) and ψ-specific SRM transition (bottom).



Supplemental Figure S5. Representative selected ion chromatogram of ψGG and UGG. (a) selected ion chromatogram at 35 pmol concentration of each oligomer , (b) ψ-specific SRM transition, (c) 5' terminal ψ-specific SRM transition.



Supplemental Figure S6. Representative mass spectrum and selected ion chromatogram of ψAACUAUAACG and UAACUAUAACG. (a) Mass spectrum of the oligonucleotide mixture (charge states are labeled), (b) selected ion chromatogram at 35 pmol concentration of each oligomer (top) and ψ-specific SRM transition (bottom).