In-Depth Impurity Assessment of Synthetic Oligonucleotides Enabled by HRMS

SBIA 2022: Advancing Generic Drug Development: Translating Science to Approval

Day 1, Session 1B: Oligonucleotide Active Pharmaceutical Ingredient (API) Sameness and Impurity Assessment Considerations

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9/20/2022

Learning Objectives

- Identify unique scientific and regulatory challenges in synthetic oligonucleotides
- Understand in-depth assessment of product-related oligonucleotide impurities
- Resolve complex impurity mixtures by high resolution mass spectrometry (HRMS)

Disclaimer: This presentation reflects the views of the authors and should not be construed to represent FDA's views or policies.

Challenges in Synthetic Oligonucleotides

- *Target a broad range of mRNAs* that encode critical cellular proteins (“undruggable”; great variety)

- *Pose unique scientific and regulatory challenges* (“Big small molecules”; currently no ICH regulatory guidelines or FDA general CMC guidances)

  - ✓ 1st PSG – Nusinersen, draft guidance in Feb 2022

Challenges in Impurity Assessment

Product-related Impurities

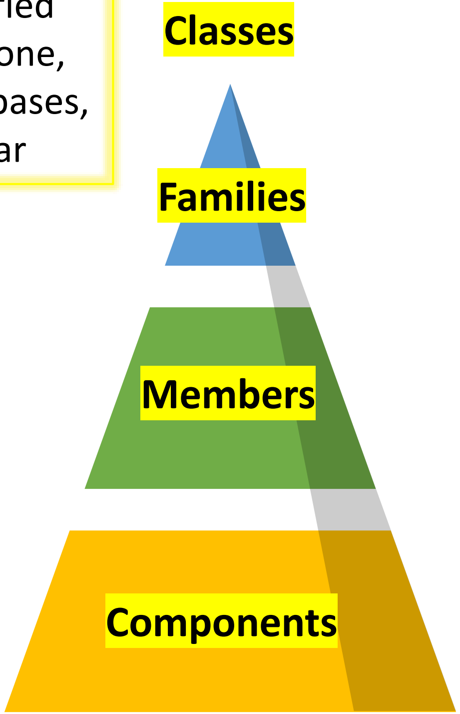
Deletion/Addition Sequences

n-1, n-2
n+1, n+2
…

n-U, n-C,
n-A, n-G
…

n-U_1, n-U_2, …
n-C_1, n-C_2, …
… …

Modified Backbone, Nucleobases, Sugar


Classes

Families

Members

Components

Impurity Profiling

In-depth assessment

Comprehension


NDA, ANDA Review

Quality Assessment

Confidence

Challenges in Impurity Assessment

Product-related Impurities

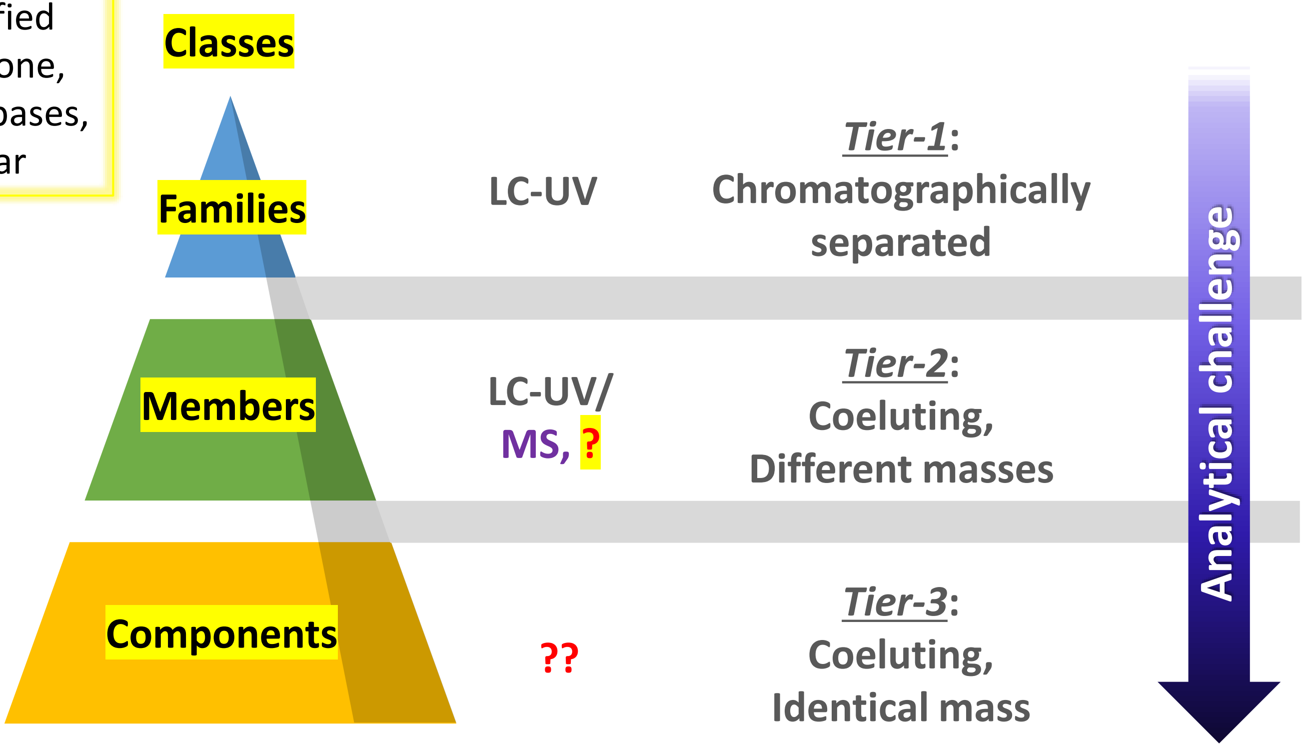
Deletion/Addition Sequences

n-1, n-2
n+1, n+2
…

n-U, n-C,
n-A, n-G
…

$n\text{-}U_1$, $n\text{-}U_2$, …
$n\text{-}C_1$, $n\text{-}C_2$, …
… …

Modified Backbone, Nucleobases, Sugar



Classes

Families

Members

Components

LC-UV	***Tier-1***: Chromatographically separated	
LC-UV/ MS, ?	***Tier-2***: Coeluting, Different masses	
??	***Tier-3***: Coeluting, Identical mass	

Outline

- In-depth impurity assessment case study
  - HRMS-enabled identity and quantitation
  - Isobaric impurity case: deletion sequence variants
- High *vs* low resolution MS

HRMS-enabled identity

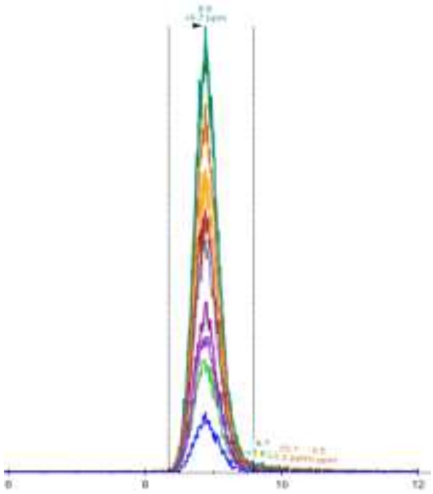
Full-length product (FLP): *2’-O-MOE, PS modified RNA 18-mer* (nusinersen)

U-C-A-C-U-U-U-C-A-U-A-A-U-G-C-U-G-G (C and U are methylated)

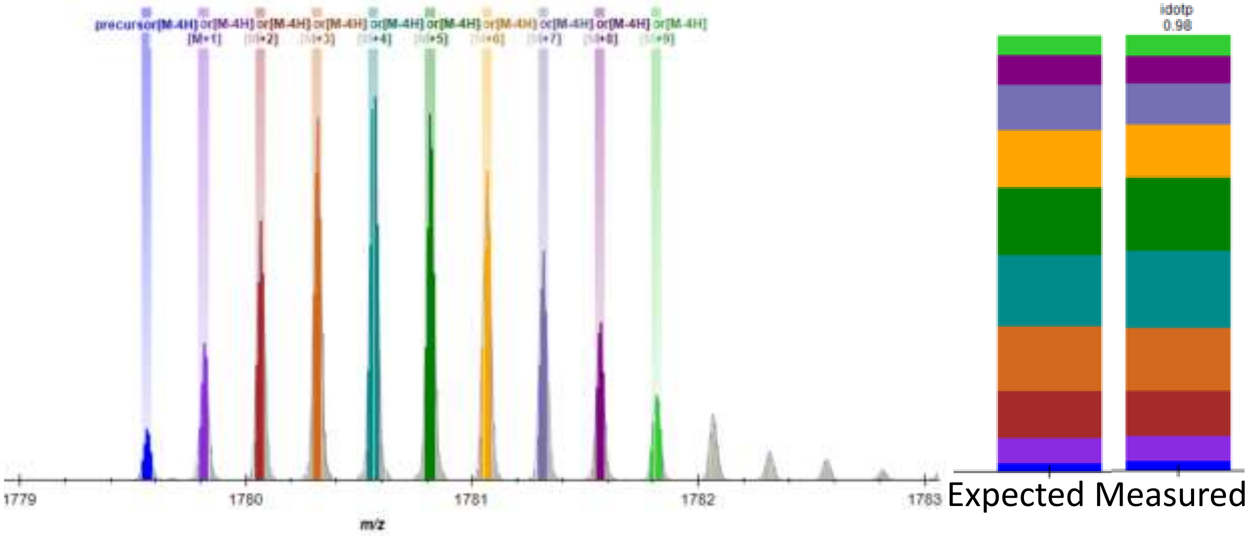
Exact mass: 7122.2763; m/z (**-4** charge state): 1779.5618

🟦 precursor - 1779.5618[M-4H]
🟪 precursor [M+1] - 1779.8125[M-4H]
🟥 precursor [M+2] - 1780.0628[M-4H]
🟧 precursor [M+3] - 1780.3131[M-4H]
🟦 precursor [M+4] - 1780.5633[M-4H]
🟩 precursor [M+5] - 1780.8135[M-4H]
🟨 precursor [M+6] - 1781.0636[M-4H]
🟪 precursor [M+7] - 1781.3138[M-4H]
🟪 precursor [M+8] - 1781.5639[M-4H]
🟩 precursor [M+9] - 1781.8140[M-4H]

Accurate mass

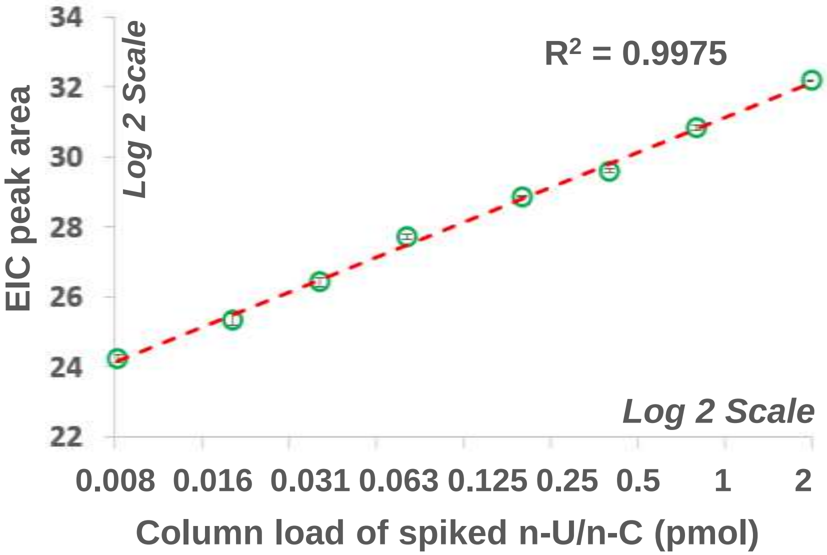


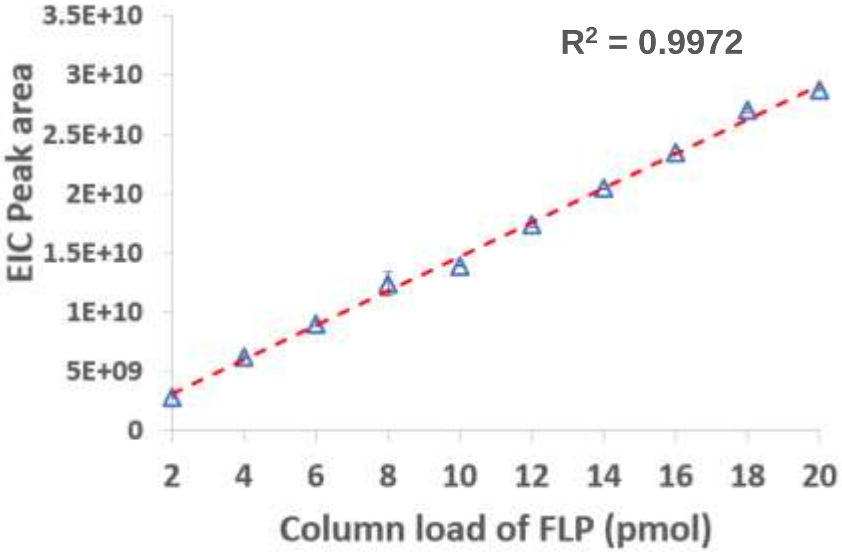
Isotopic envelope / distribution



HRMS-enabled quantitation

HILIC-HRMS: Extracted ion chromatogram (EIC) of isotopic peaks





RSD (%) of EIC peak area < 15%

RSD (%) of EIC peak area < 10%

A challenging scenario …

Case study: resolve isobaric sequence deletion impurities

Isobaric: equal nominal mass

Full-length product (FLP)*:*

2’-O-MOE, PS modified RNA 18-mer (nusinersen)

U-C-A-C-U-U-U-C-A-U-A-A-U-G-C-U-G-G (C and U are methylated)

Exact mass: 7122.2763; m/z (**-4** charge state): 1779.5618

Isobaric sequence deletion impurities

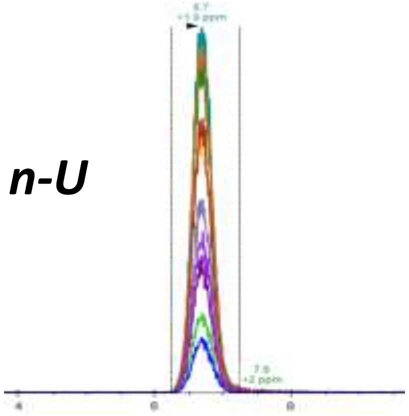
Impurities	Exact mass (Da)	m/z (-4 charge state)
n-U	6728.2163	**1681**.0468
n-C	6729.2003	**1681**.2928

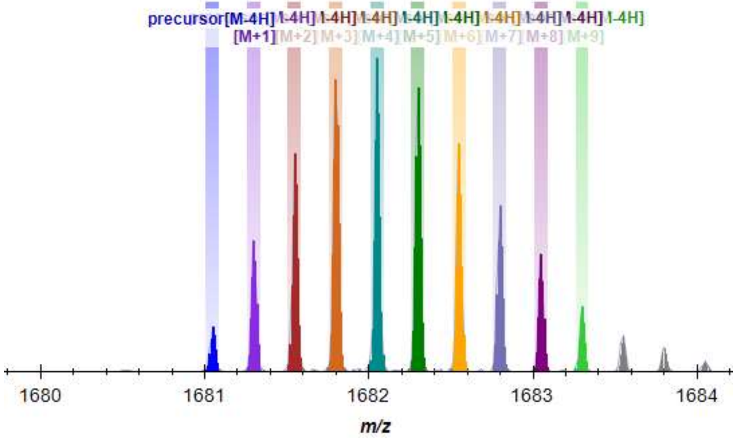
Individual n-U and n-C

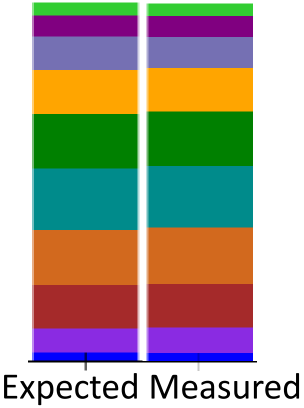
Accurate mass

Isotopic envelope / distribution

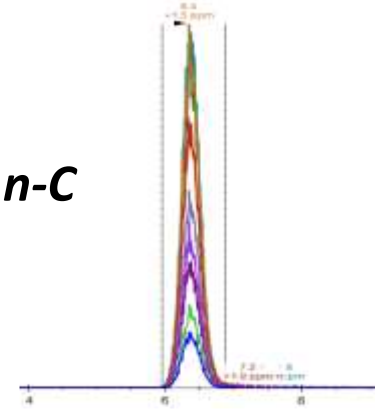
n-U

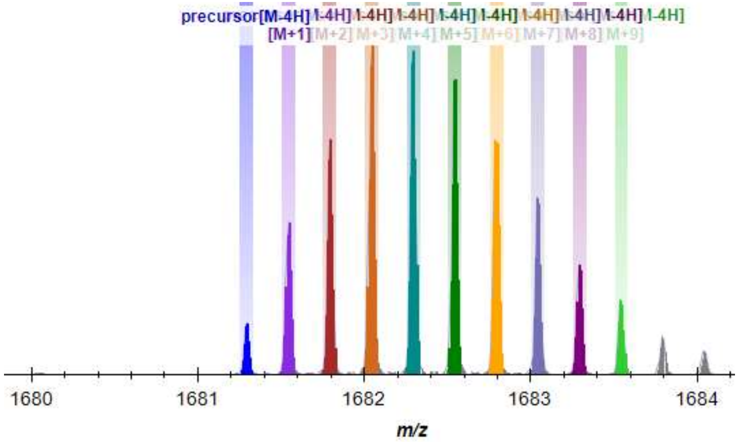


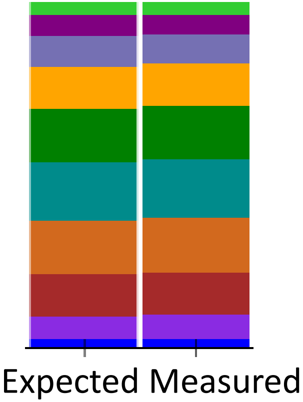




n-C



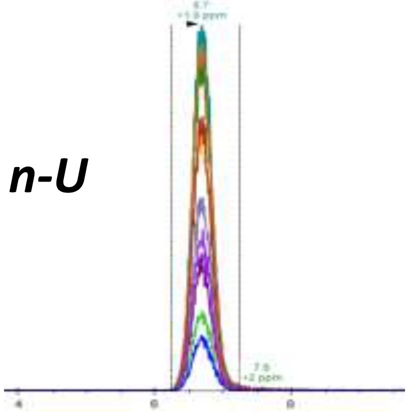




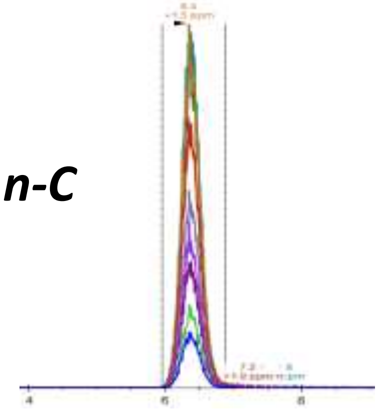
Individual n-U and n-C

Accurate mass

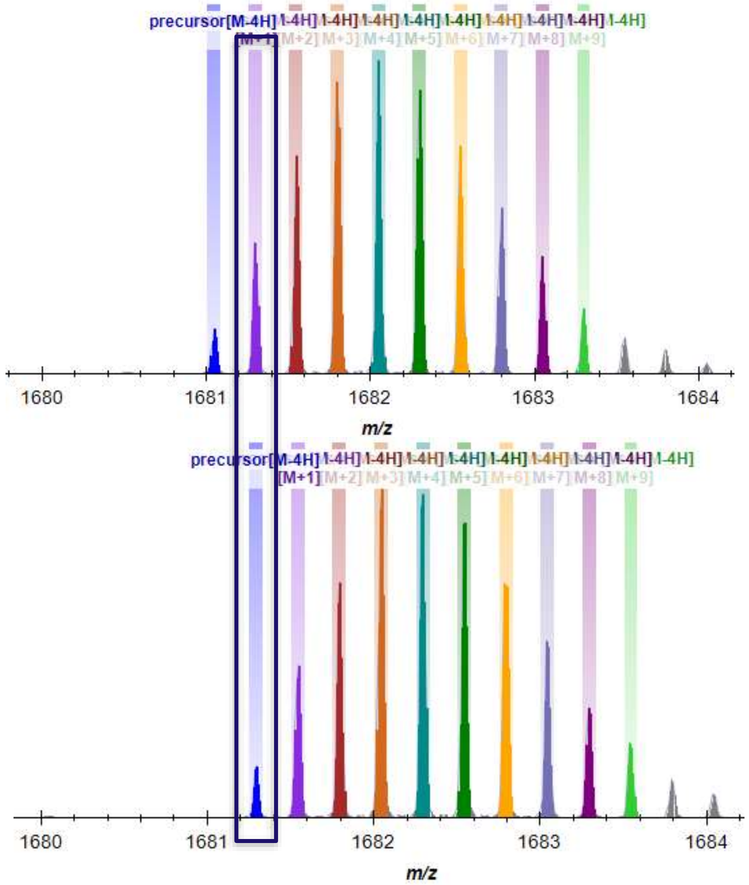
n-U



n-C

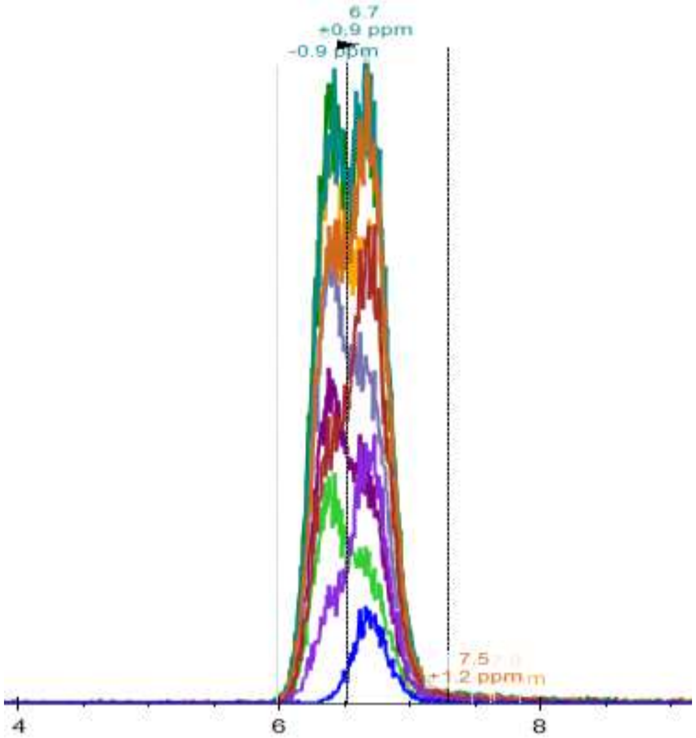


Isotopic envelope / distribution



n-U		*n-C*		*Δ*
M	**1681.0468**			
M+1	1681.2975	M	1681.2928	0.0047
M+2	1681.5478	M+1	1681.5435	0.0043
M+3	1681.7981	M+2	1681.7938	0.0043
M+4	1682.0483	M+3	1682.0441	0.0042
M+5	1682.2984	M+4	1682.2943	0.0041
M+6	1682.5486	M+5	1682.5445	0.0041
M+7	1682.7987	M+6	1682.7946	0.0041
M+8	1683.0488	M+7	1683.0447	0.0041
M+9	1683.2989	M+8	1683.2948	0.0041

n-U / n-C mixtures



Coexisting *n-U* / *n-C* in a mixture:

- Not chromatographically separated
- Not mass differentiated
- Not individually reported in QC testing or specification

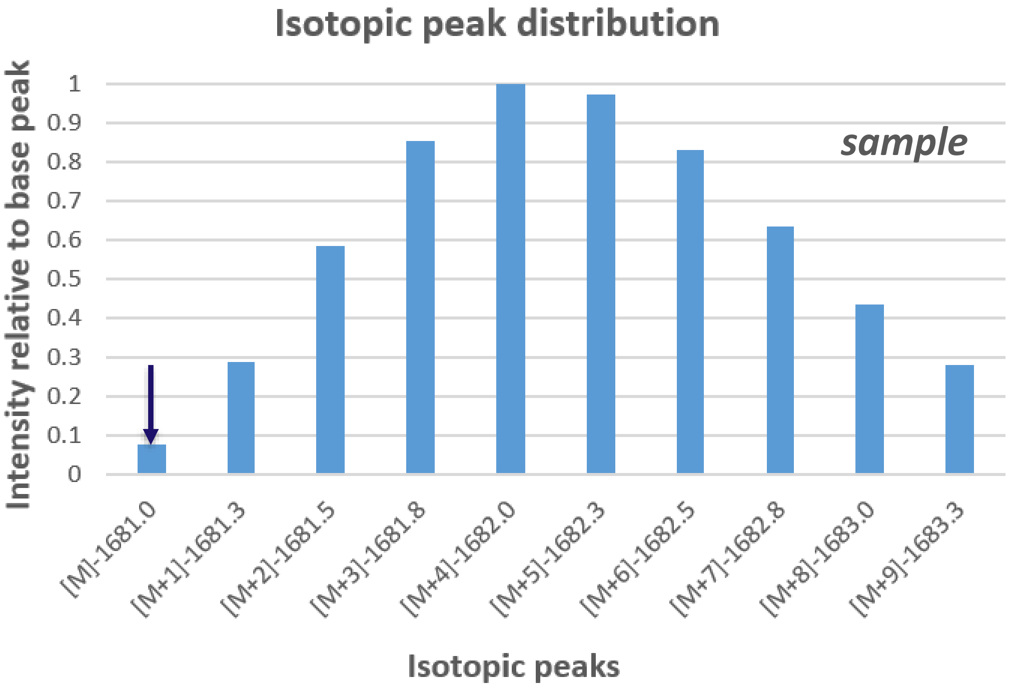
n-p(MOE^{Me}C) / n-p(MOE^{Me}U)

@ m/z 1682.2

n-U / n-C mixtures

n-U		***n-C***	
M	**1681.0468**		
M+1	1681.2975	M	1681.2928
M+2	1681.5478	M+1	1681.5435
M+3	1681.7981	M+2	1681.7938

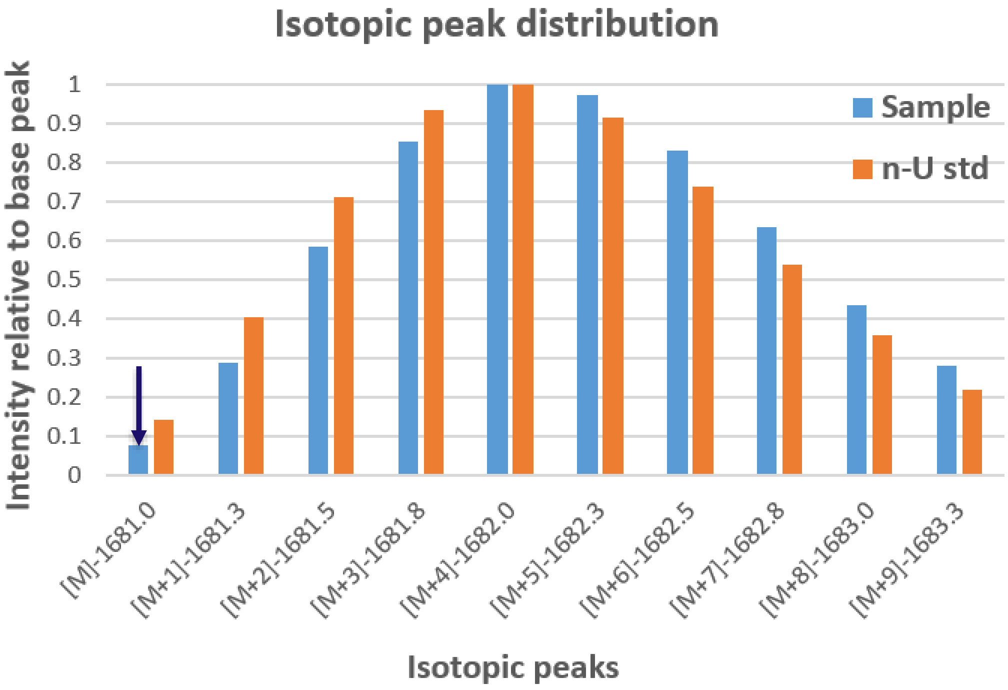
- Monoisotopic peak at 1681.0 indicates **the presence of n-U**



n-U / n-C mixtures

n-U		***n-C***	
M	**1681.0468**		
M+1	1681.2975	M	1681.2928
M+2	1681.5478	M+1	1681.5435
M+3	1681.7981	M+2	1681.7938

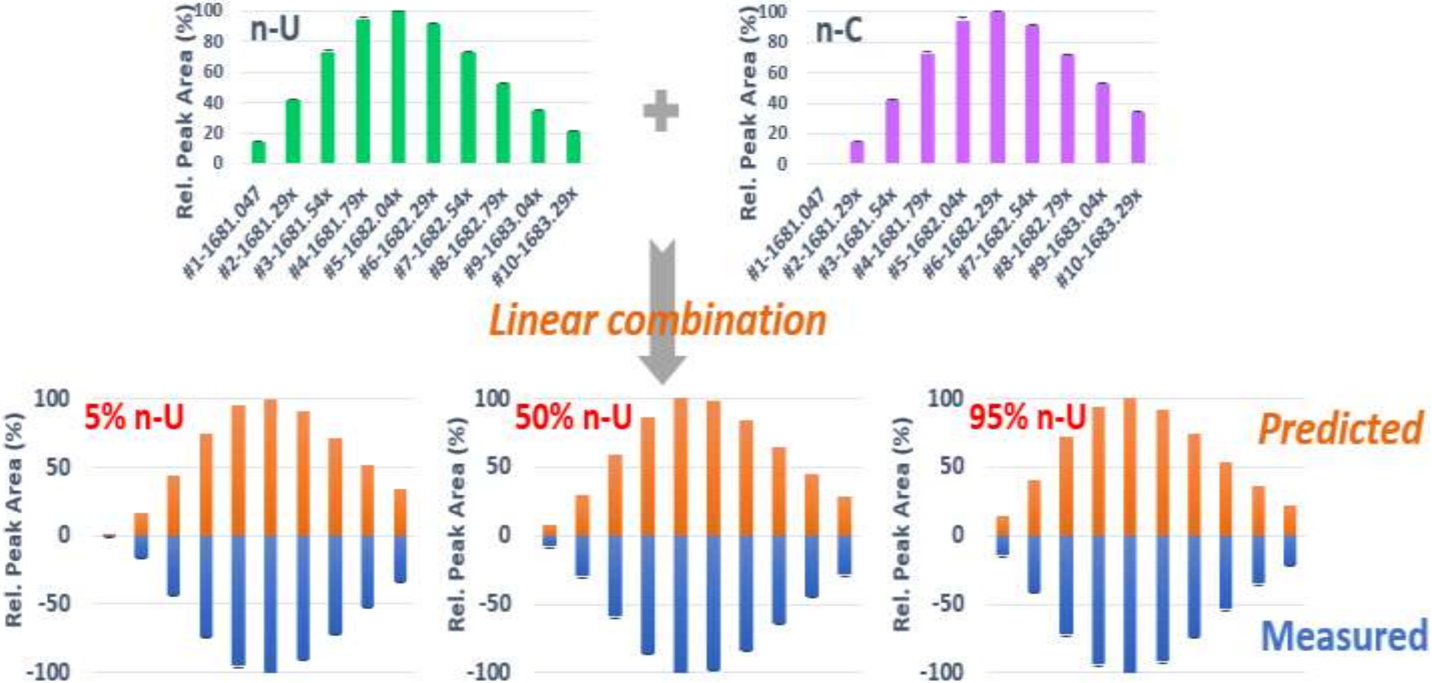
- Monoisotopic peak at 1681.0 indicates **the presence of n-U**
- Mismatching isotopic pattern indicates **the coexistence** of n-U with n-C in the mixture


Isotopic peak distribution

Calculated (predicted) *vs* **Measured** isotopic distributions

Individual
n-U and n-C

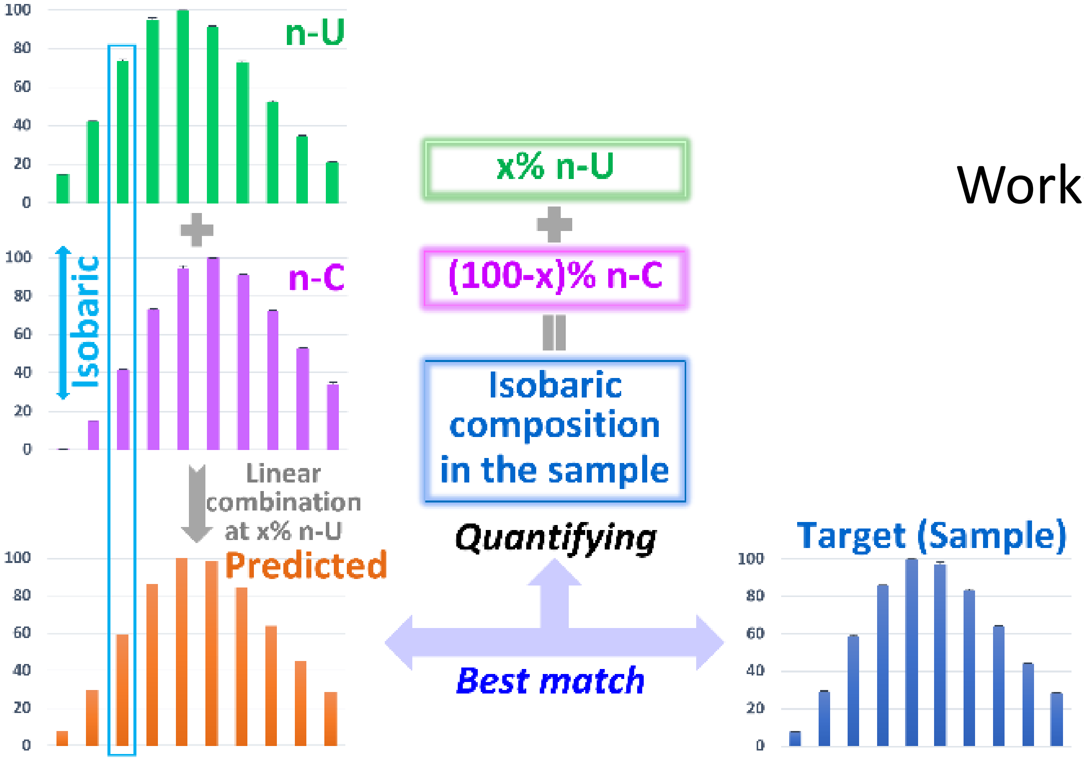
n-U / n-C
mixtures



Unpublished data: A.M. Abdullah, C. Sommers, J. Rodriguez, D. Zhang, D. Kozak, J. Hawes, M. Sapru, and K. Yang. Manuscript under revision.

Quantification of isobaric composition of n-U/n-C mixtures

Workflow



x% n-U

(100-x)% n-C

Isobaric composition in the sample

Quantifying

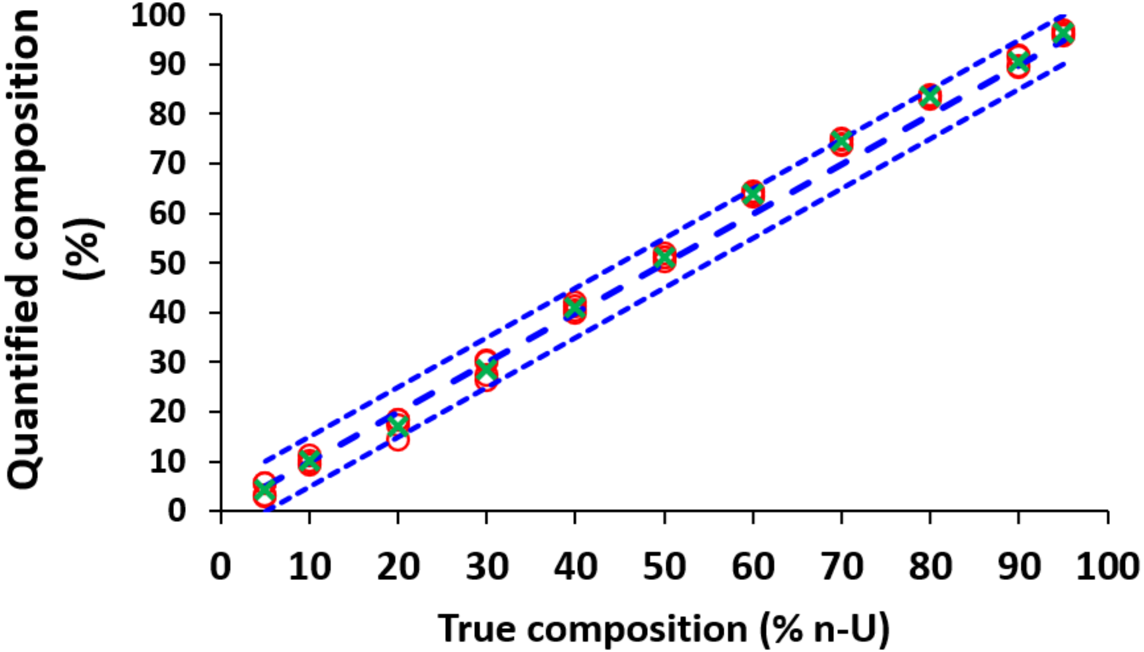
Best match

Best match:

Defined by: the difference between the predicted and the targeted is minimum.

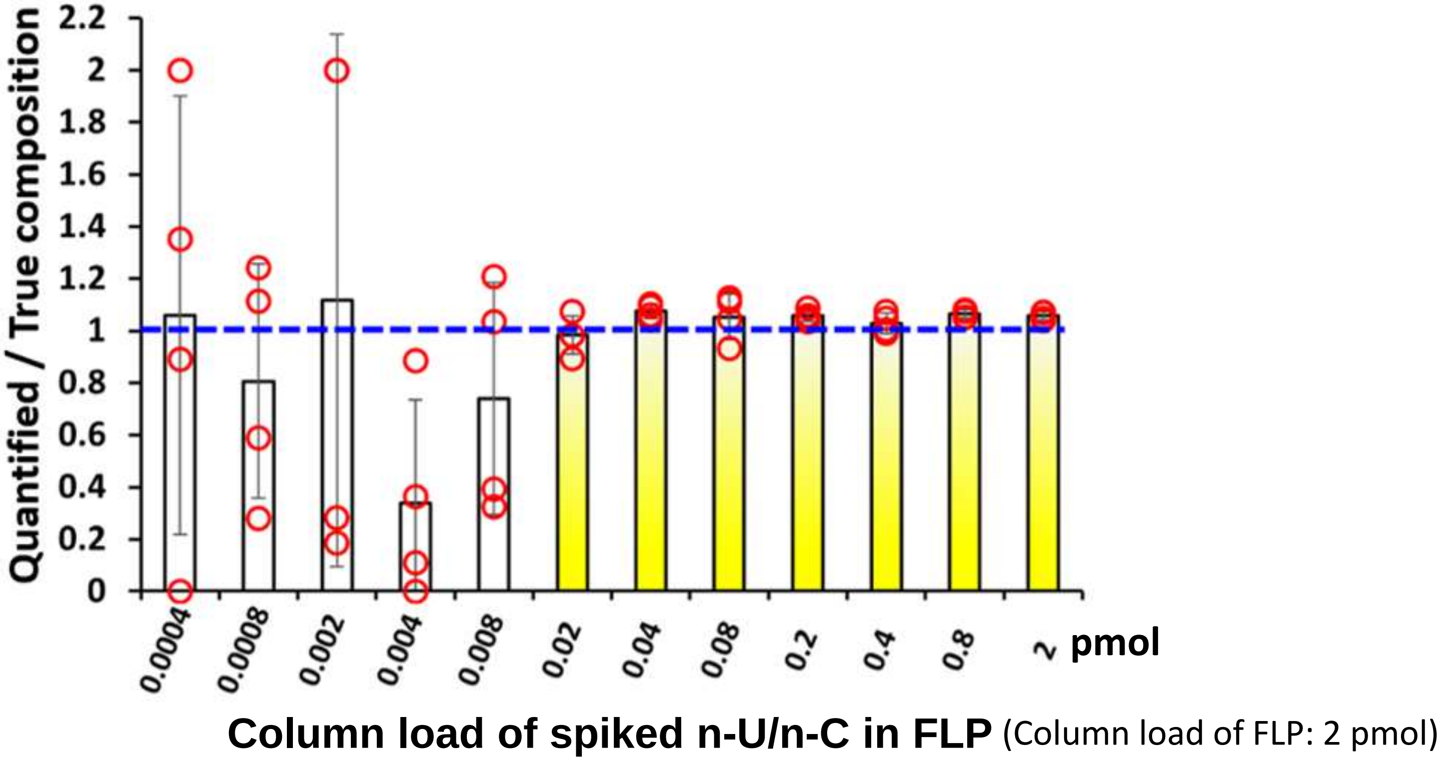
Quantification of isobaric composition of n-U/n-C mixtures

(covering 5%/95% n-U/n-C to 95%/5% n-U/n-C)



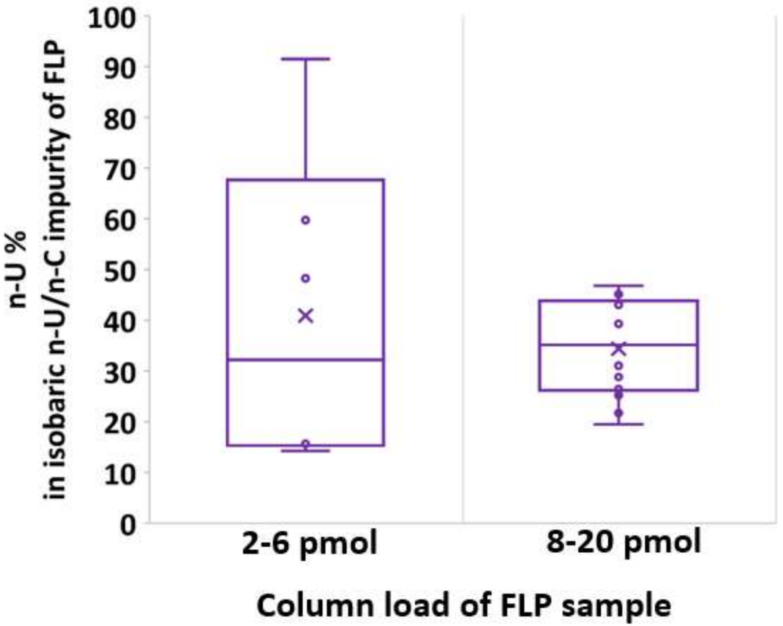
Unpublished data: A.M. Abdullah, C. Sommers, J. Rodriguez, D. Zhang, D. Kozak, J. Hawes, M. Sapru, and K. Yang. Manuscript under revision.

Quantification of isobaric composition of the n-U/n-C standard ***spiked*** in FLP



Unpublished data: A.M. Abdullah, C. Sommers, J. Rodriguez, D. Zhang, D. Kozak, J. Hawes, M. Sapru, and K. Yang. Manuscript under revision.

Quantification of isobaric composition of the n-U/n-C impurity ***present*** in FLP



On-column n-U/n-C

< 0.02 pmol **> 0.02 pmol**

FLP:

Custom synthesized by vendor:

- Synthesis – Solid phase phosphoramidite synthesis
- Purification method – RP HPLC

Total n-U/n-C in FLP quantified at 0.27±0.02% of FLP

Unpublished data: A.M. Abdullah, C. Sommers, J. Rodriguez, D. Zhang, D. Kozak, J. Hawes, M. Sapru, and K. Yang. Manuscript under revision.

High vs low resolution MS

	HRMS	Unit- or low-resolution MS
Isotopic peak-resolved envelope	Yes	No
Identification of coexisting n-U and n-C (isobaric)	Yes	No (n-U/n-C)
Quantitation of coexisting n-U/n-C ratio	Yes	No (sum of n-U/n-C)

Summary

- Synthetic oligonucleotides, as an evolving class of therapeutics, pose unique scientific and regulatory challenges.

- In-depth impurity assessment offers comprehensive impurity evaluation and is crucial for comparative impurity analysis in generic drug development.

- High resolution MS demonstrates advantages in resolving complex oligonucleotide impurities, such as isobaric sequence variants.

Acknowledgement

○ **Office of Testing and Research (OTR)**

- ✓ Oligo research team
  A.M. Abdullah
- ✓ Division of Complex Drug Analysis (DCDA)
- ✓ OTR Management
  Cindy Sommers
  Jason Rodriguez
  David Keire

○ **Office of Generic Drugs (OGD)**

Deyi Zhang

Darby Kozak

○ **FDA Critical-Path Research Grants (FY20, FY22)**

- ✓ PI: Kui Yang
- ✓ Collaboration offices:
  OPQ, OGD, OND, NCTR

○ **OTR-led Oligo SME team / working group**

- ✓ Participating offices:
  OPQ, OGD, OND, NCTR, OTS

Challenge Question #1

Which of the following statements is NOT correct for synthetic oligonucleotide therapeutics (ONTs)?

A. ONTs target a broad range of mRNAs.

B. Like mRNAs, ONTs are regulated as biologics by FDA.

C. Currently no ICH regulatory guidelines specifically address quality aspects for ONTs.

D. FDA published the first PSG draft guidance for ONTs in 2022.

Challenge Question #2

True or False?

In-depth impurity assessment by HRMS is recommended in PSG draft guidance for Nusinersen published in Feb 2022.

A. True

B. False

Questions?

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