Targeted LC-HRMS with Library Matching for Toxicology Screening

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HPTN Annual Meeting

Disclosures

- Grant/Research Support: NIH, Thermo Fisher Scientific, Werfen, Roche Diagnostics, BD, IBS Inc., Shimadzu, Radiometer
- Consultant/Advisory Board: Werfen, Radiometer, Roche Diagnostics, Lumira DX, Saladax Biomedical, Truvian Health, HepQuant, Red Abbey Labs
- Equity: None
- Honorarium/Expenses: None
- Intellectual Property/Royalty Income: Johns Hopkins University, Elsevier

Presentation Highlights

- Today's presentation will describe the use of high-resolution accurate mass (HRAM) mass spectrometry for toxicology screening

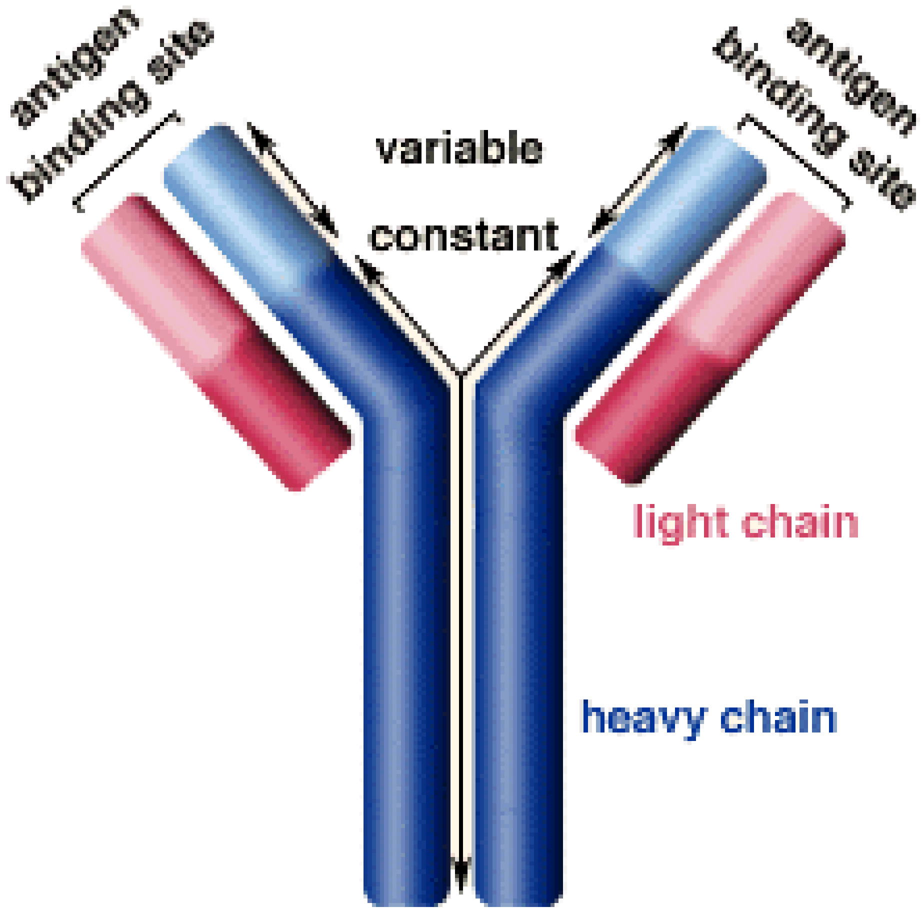
- Use of LC-HRMS allows us to identify specific drugs and metabolites in a sample, rather than class-based identification of related drugs

- Research with this technology allows us to identify specific patterns of substance use that will inform HIV prevention efforts in the context of substance use

Challenges in Toxicology Screening

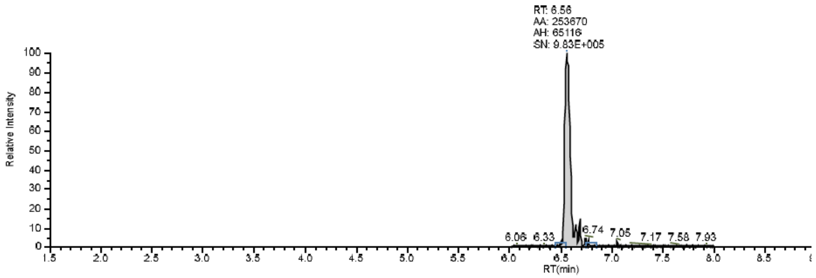
How Mass Spectrometry Meets Those Challenges

Immunoassa Limitations

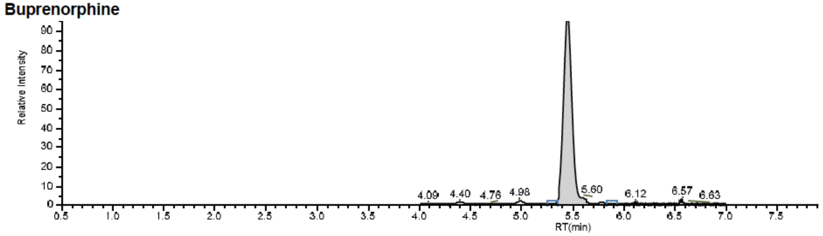


- Typically class-specific rather than compound specific
- Structurally similar epitopes can be displayed in unrelated compounds
- Cross-reactivity cannot be determined without additional testing
- May not be useful depending on the clinical or research question

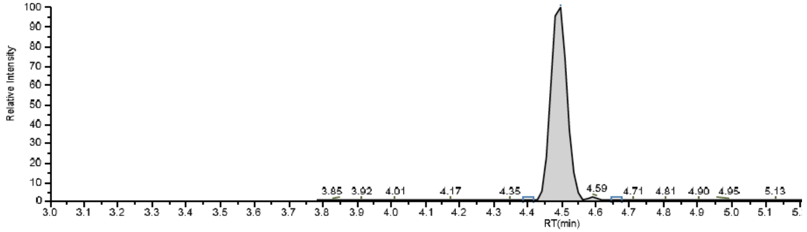
Buprenorphine Example



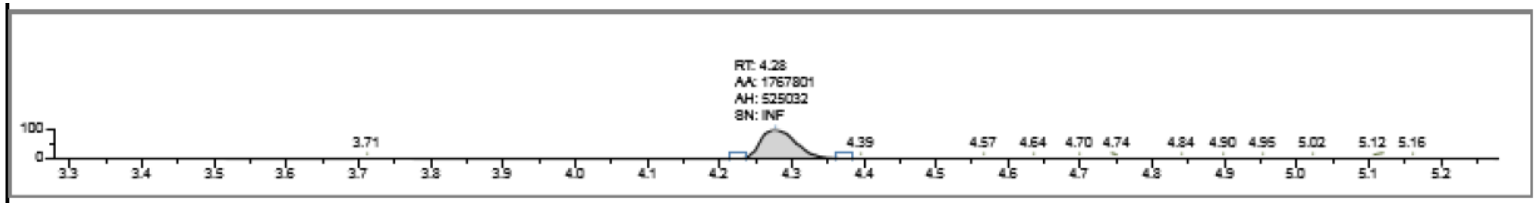
Buprenorphine



Norbuprenorphine



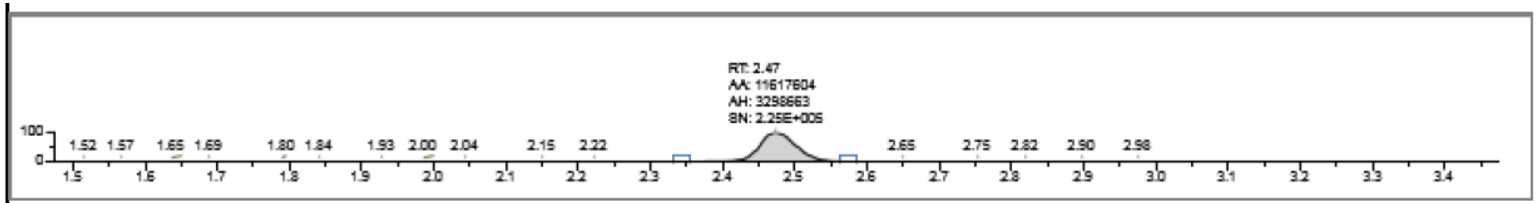
Buprenorphine-3beta-D-Glucuronide



Buprenorphine

Reference Flag: Positive

Concentration 41912.42

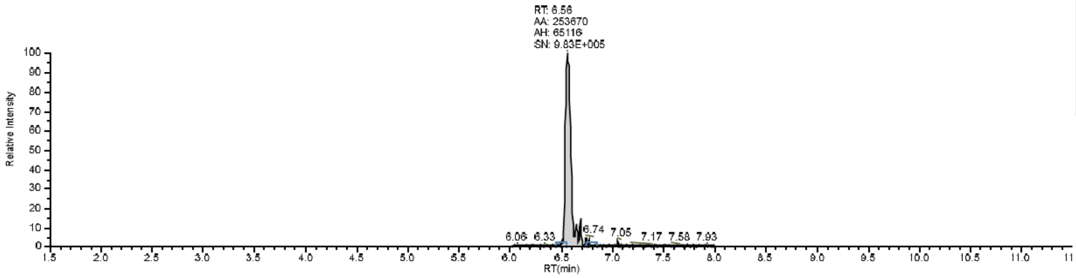


Naloxone

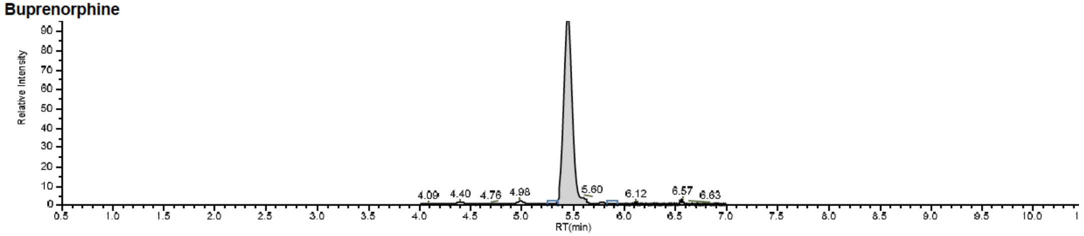
Reference Flag: Positive

Concentration 11818.71

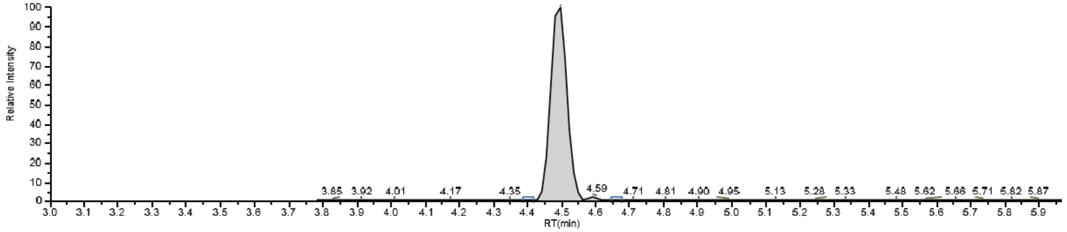
Buprenorphine Example



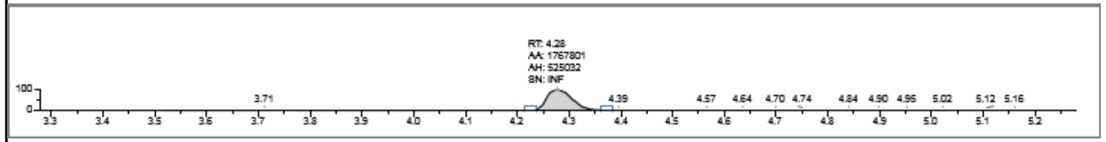
Buprenorphine



Norbuprenorphine



Buprenorphine-3beta-D-Glucuronide

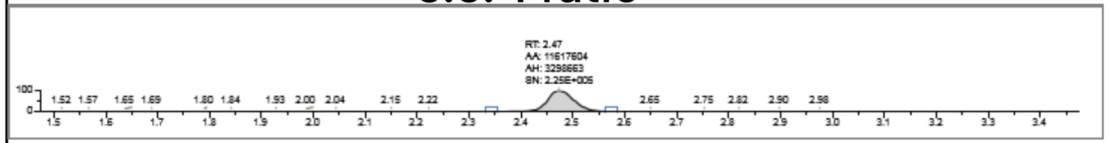


Buprenorphine

Reference Flag: Positive

Concentration 41912.42

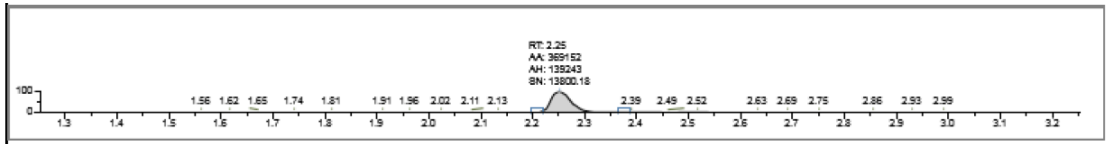
3.5: 1 ratio



Naloxone

Reference Flag: Positive

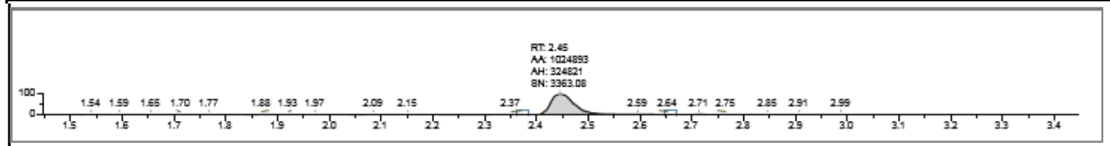
Concentration 11818.71



Benzoylecgonine

Reference Flag: Negative

Concentration 367.85



Cocaine

Reference Flag: Negative

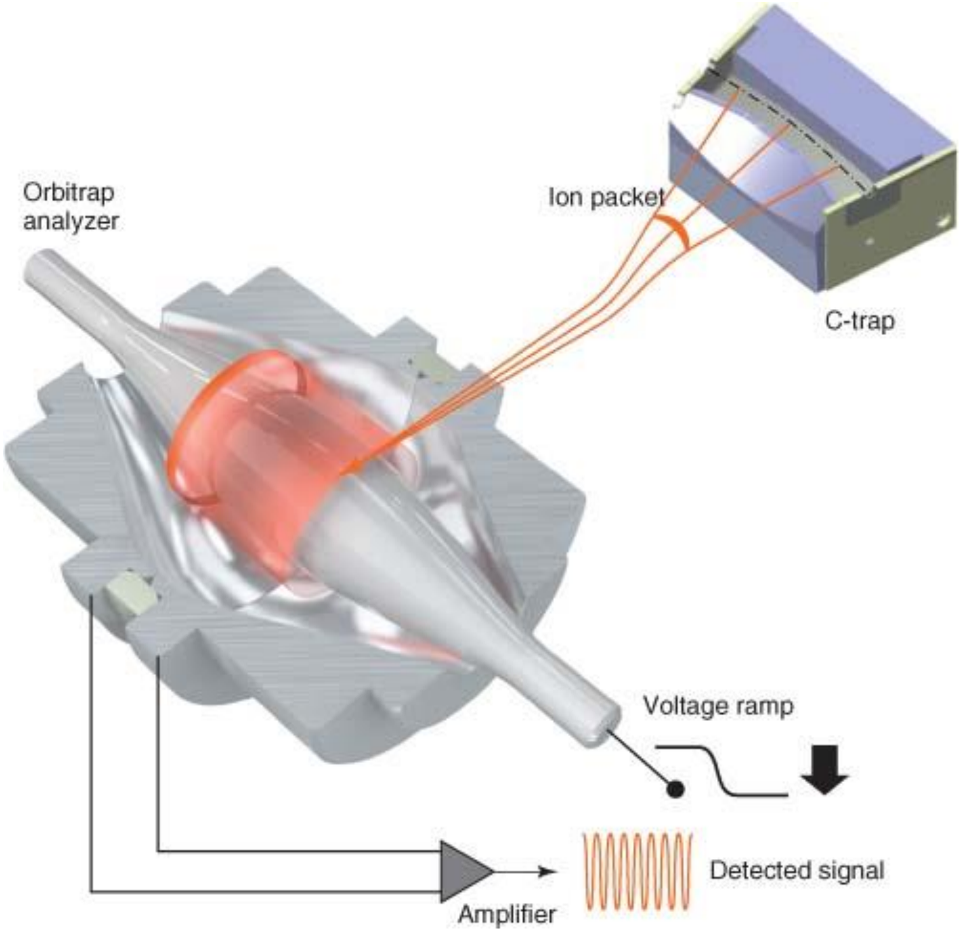
Concentration 644.86

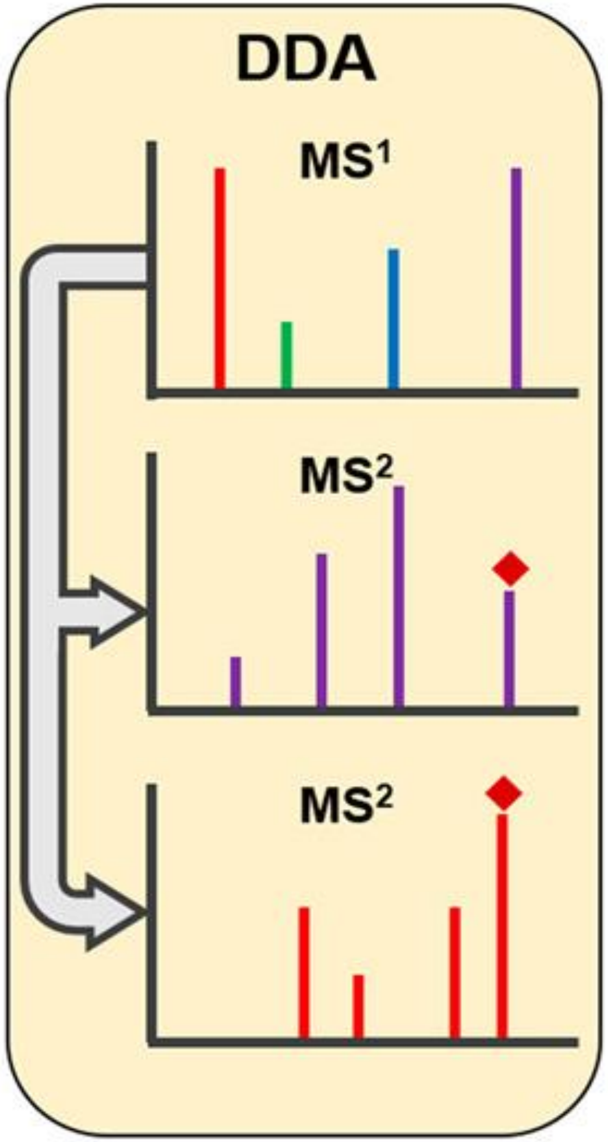
2

High Resolution Mass Spectrometry

Next Generation Tools for Mass Spectrometry-Based Toxicology Screening

HRMS Data Acquisition

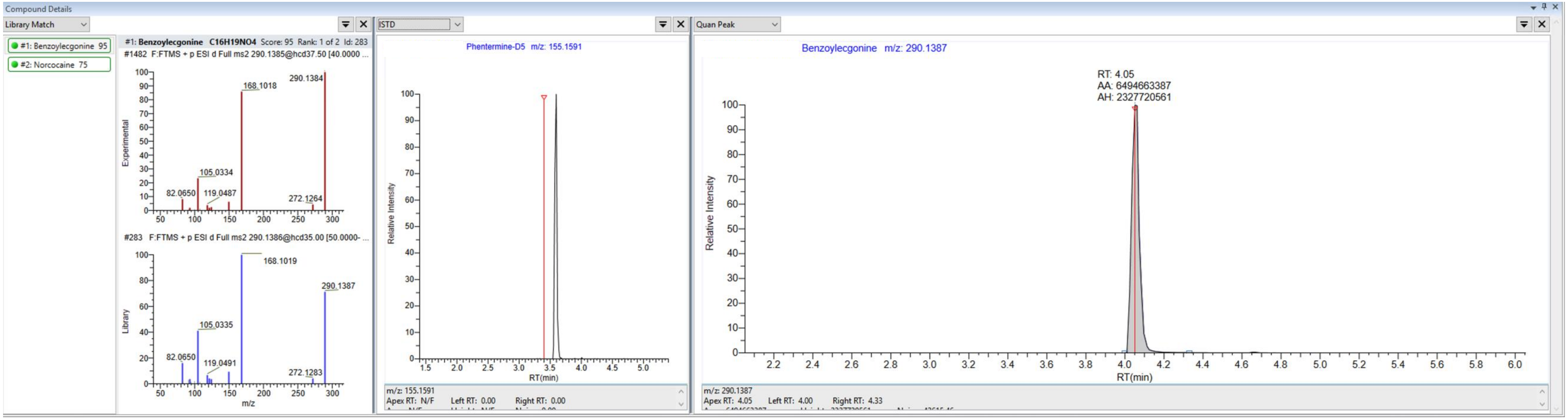




Library Matching

- MS2 (fragmentation) spectra are reduced from an array of m/z:intensity pairs to a vector in multi-dimensional space representing the entirety of the particular spectrum
- Each m/z value between the unknown spectrum and the reference spectrum are mass-aligned, and a dot-product algorithm is used to compare the 2 vectors
- The cosine of the angle between 2 vectors is calculated, and a probability score is generated to reflect the likelihood that the experimental and reference spectrum are from the same compound
- There are different approaches/algorithms for this type of comparison; often there are scoring penalties for sparse spectra (limited fragments)
- NOTE: MS2 spectra are dependent on the collision energy used to generate fragments; if the same CE is not used for the experimental and reference spectra generation, the closest energy matches are used

Interpretation



Compound Details

Library Match

#1: Benzoylecgonine 95

#2: Norcocaine 75

#1: **Benzoylecgonine** **C16H19NO4** Score: 95 Rank: 1 of 2 Id: 283

#1482 F:FTMS + p ESI d Full ms2 290.1385@hcd37.50 [40.0000 ...

#283 F:FTMS + p ESI d Full ms2 290.1386@hcd35.00 [50.0000- ...

ISTD

Phentermine-D5 m/z: 155.1591

m/z: 155.1591
Apex RT: N/F Left RT: 0.00 Right RT: 0.00

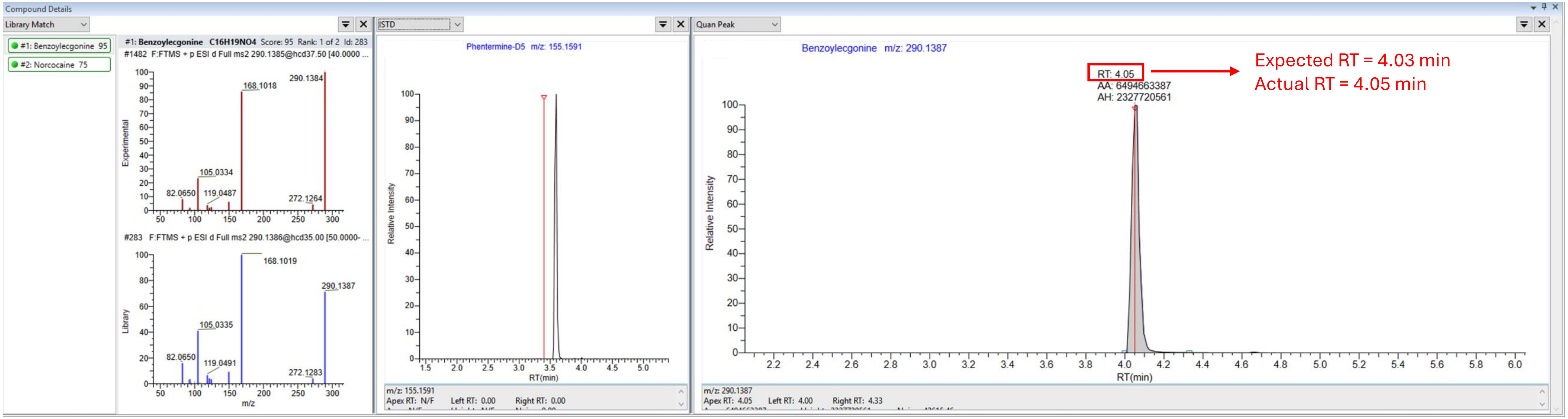
Quan Peak

Benzoylecgonine m/z: 290.1387

RT: 4.05
AA: 6494663387
AH: 2327720561

m/z: 290.1387
Apex RT: 4.05 Left RT: 4.00 Right RT: 4.33

Interpretation



Compound Details

Library Match

- #1: Benzoylecgonine 95
- #2: Norcocaine 75

#1: **Benzoylecgonine** **C16H19NO4** Score: 95 Rank: 1 of 2 Id: 283

#1482 F:FTMS + p ESI d Full ms2 290.1385@hcd37.50 [40.0000 ...

#283 F:FTMS + p ESI d Full ms2 290.1386@hcd35.00 [50.0000- ...

ISTD

Phentermine-D5 m/z: 155.1591

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Apex RT: N/F Left RT: 0.00 Right RT: 0.00

Quan Peak

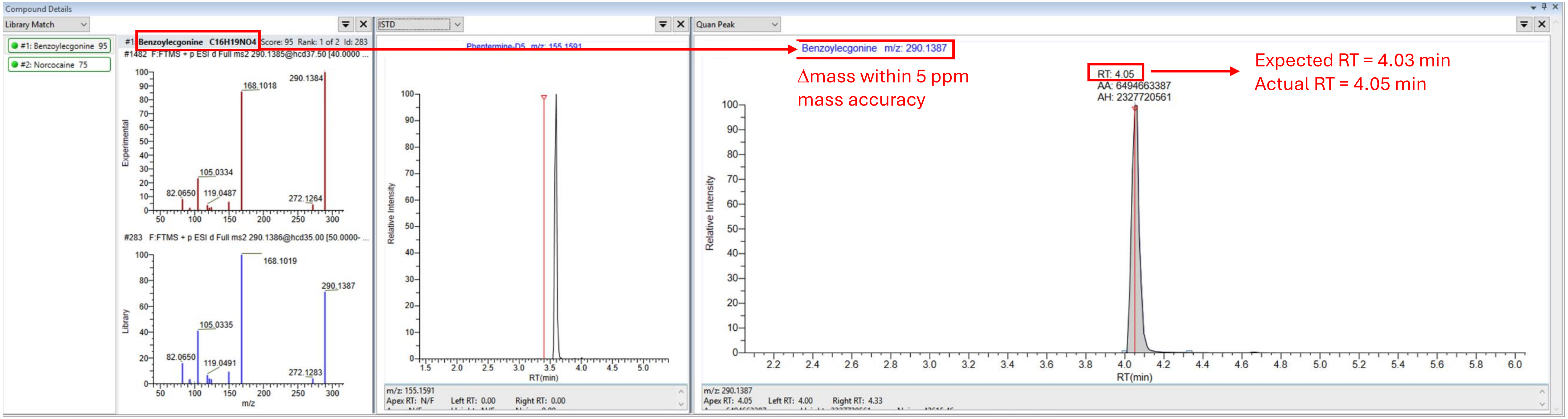
Benzoylecgonine m/z: 290.1387

RT: 4.05
AA: 6494663387
AH: 2327720561

m/z: 290.1387
Apex RT: 4.05 Left RT: 4.00 Right RT: 4.33

Expected RT = 4.03 min
Actual RT = 4.05 min

Interpretation



● #1: Benzoylecgonine 95

● #2: Norcocaine 75

#1: **Benzoylecgonine C16H19NO4** Score: 95 Rank: 1 of 2 Id: 283

#1482 F:FTMS + p ESI d Full ms2 290.1385@hcd37.50 [40.0000 ...

#283 F:FTMS + p ESI d Full ms2 290.1386@hcd35.00 [50.0000- ...

m/z: 155.1591
Apex RT: N/F Left RT: 0.00 Right RT: 0.00

m/z: 290.1387
Apex RT: 4.05 Left RT: 4.00 Right RT: 4.33

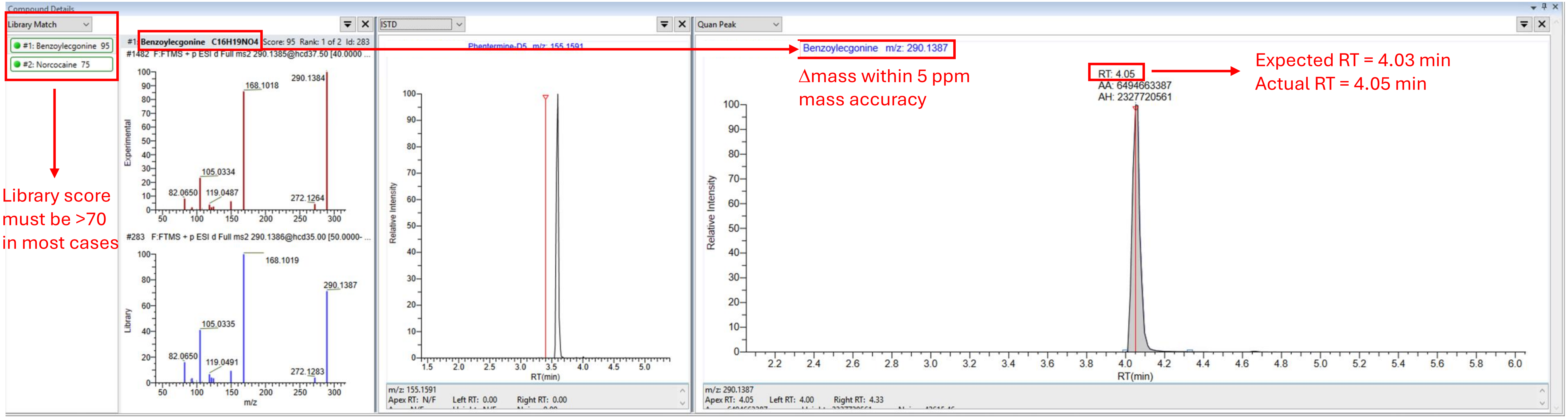
Benzoylecgonine m/z: 290.1387

Δmass within 5 ppm mass accuracy

RT: 4.05

Expected RT = 4.03 min
Actual RT = 4.05 min

Interpretation


Compound Details

Library Match

#1: Benzoylecgonine 95

#2: Norcocaine 75

#1: **Benzoylecgonine C16H19NO4** Score: 95 Rank: 1 of 2 Id: 283

#1482 F:FTMS + p ESI d Full ms2 290.1385@hcd37.50 [40.0000 ...

#283 F:FTMS + p ESI d Full ms2 290.1386@hcd35.00 [50.0000- ...

ISTD

Phentermine-D5 m/z: 155.1591

m/z: 155.1591
Apex RT: N/F Left RT: 0.00 Right RT: 0.00

Quan Peak

Benzoylecgonine m/z: 290.1387

RT: 4.05
AA: 6494663387
AH: 2327720561

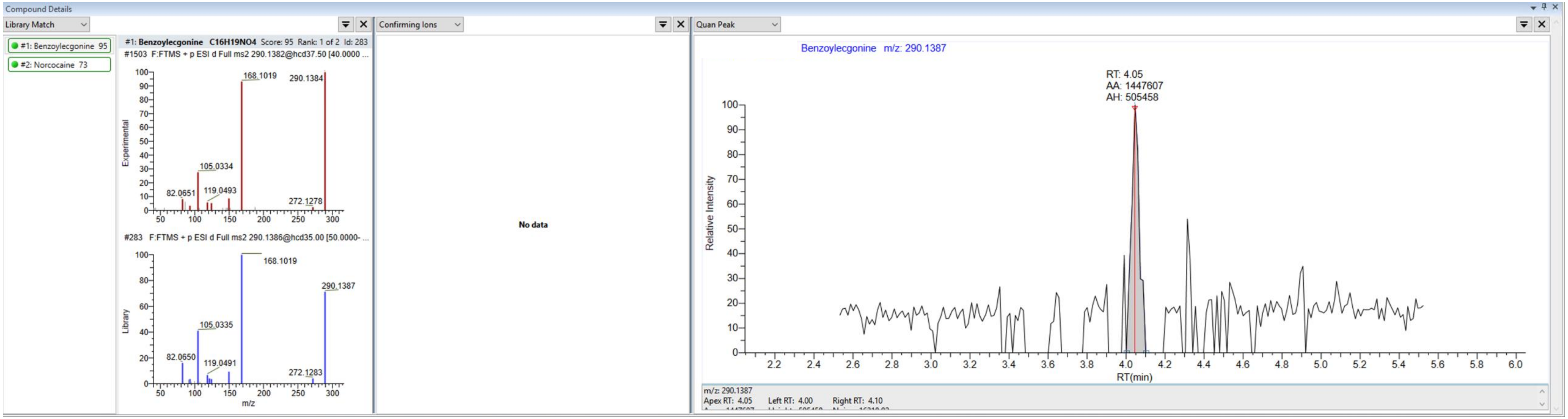
m/z: 290.1387
Apex RT: 4.05 Left RT: 4.00 Right RT: 4.33

Library score must be >70 in most cases

Δmass within 5 ppm mass accuracy

Expected RT = 4.03 min
Actual RT = 4.05 min

Low Signal Challenges



● #1: Benzoylecgonine 95

● #2: Norcocaine 73

#1: **Benzoylecgonine C16H19NO4** Score: 95 Rank: 1 of 2 Id: 283

#1503 F:FTMS + p ESI d Full ms2 290.1382@hcd37.50 [40.0000 ...

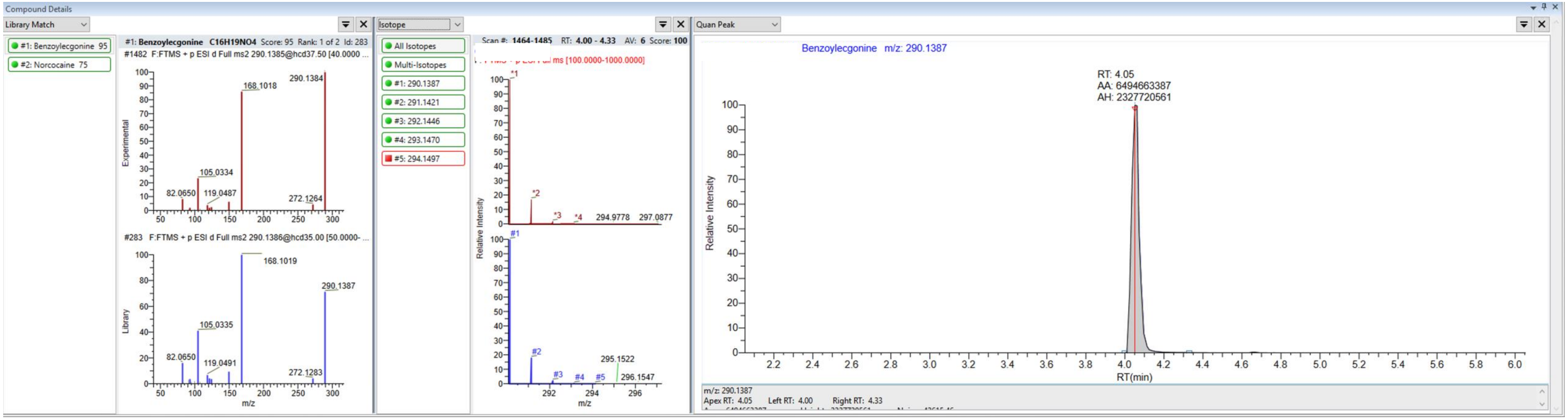
#283 F:FTMS + p ESI d Full ms2 290.1386@hcd35.00 [50.0000- ...

Benzoylecgonine m/z: 290.1387

RT: 4.05
AA: 1447607
AH: 505458

m/z: 290.1387
Apex RT: 4.05 Left RT: 4.00 Right RT: 4.10

Isotope Patterns



Compound Details

Library Match

● #1: Benzoylecgonine 95

● #2: Norcocaine 75

#1: **Benzoylecgonine C16H19NO4** Score: 95 Rank: 1 of 2 Id: 283

#1482 F:FTMS + p ESI d Full ms2 290.1385@hcd37.50 [40.0000 ...

#283 F:FTMS + p ESI d Full ms2 290.1386@hcd35.00 [50.0000- ...

Isotope

● All Isotopes

● Multi-Isotopes

● #1: 290.1387

● #2: 291.1421

● #3: 292.1446

● #4: 293.1470

🟥 #5: 294.1497

Scan #: **1464-1485** RT: **4.00 - 4.33** AV: **6** Score: **100**

Quan Peak

Benzoylecgonine m/z: 290.1387

RT: 4.05
AA: 6494663387
AH: 2327720561

m/z: 290.1387
Apex RT: 4.05 Left RT: 4.00 Right RT: 4.33

SUMMARY: Positive ID of Compounds

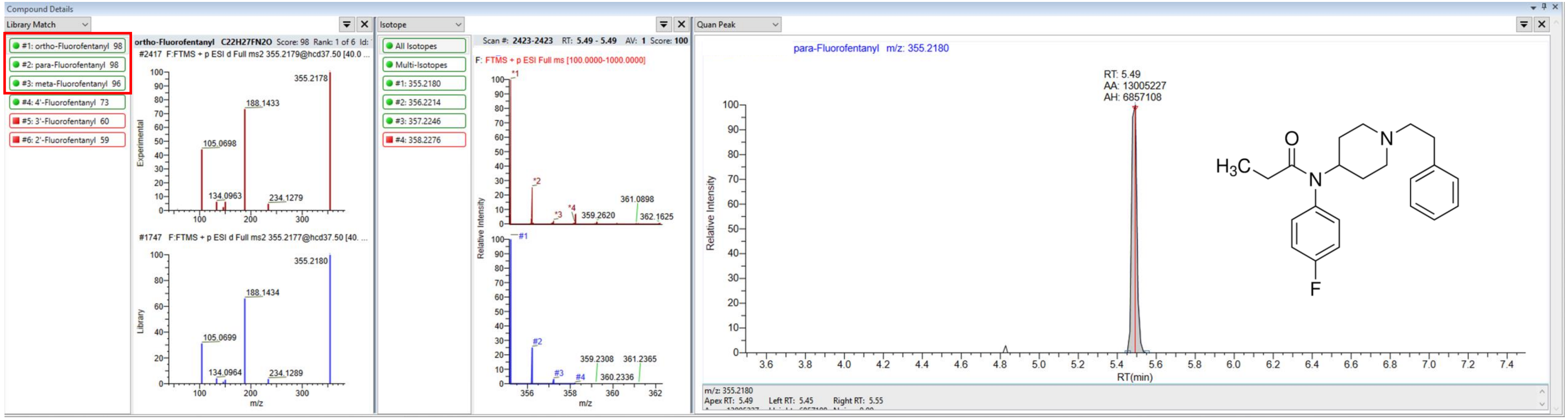
- Retention Time
- Exact Mass with 5 ppm tolerance
- Library Score match
- Visual inspection of peaks
- Isotope patterns can be used when there is low signal or questions

3

Practical Application of HRMS

Use of LC-HRMS in real clinical and research samples for substance use

Fluorofentanyl



Compound Details

- ● #1: ortho-Fluorofentanyl 98
- ● #2: para-Fluorofentanyl 98
- ● #3: meta-Fluorofentanyl 96
- ● #4: 4'-Fluorofentanyl 73
- 🟥 #5: 3'-Fluorofentanyl 60
- 🟥 #6: 2'-Fluorofentanyl 59

ortho-Fluorofentanyl **C22H27FN2O** Score: 98 Rank: 1 of 6 Id:

#2417 F:FTMS + p ESI d Full ms2 355.2179@hcd37.50 [40.0 ...

#1747 F:FTMS + p ESI d Full ms2 355.2177@hcd37.50 [40. ...

- ● All Isotopes
- ● Multi-Isotopes
- ● #1: 355.2180
- ● #2: 356.2214
- ● #3: 357.2246
- 🟥 #4: 358.2276

Scan #: **2423-2423** RT: **5.49 - 5.49** AV: **1** Score: **100**

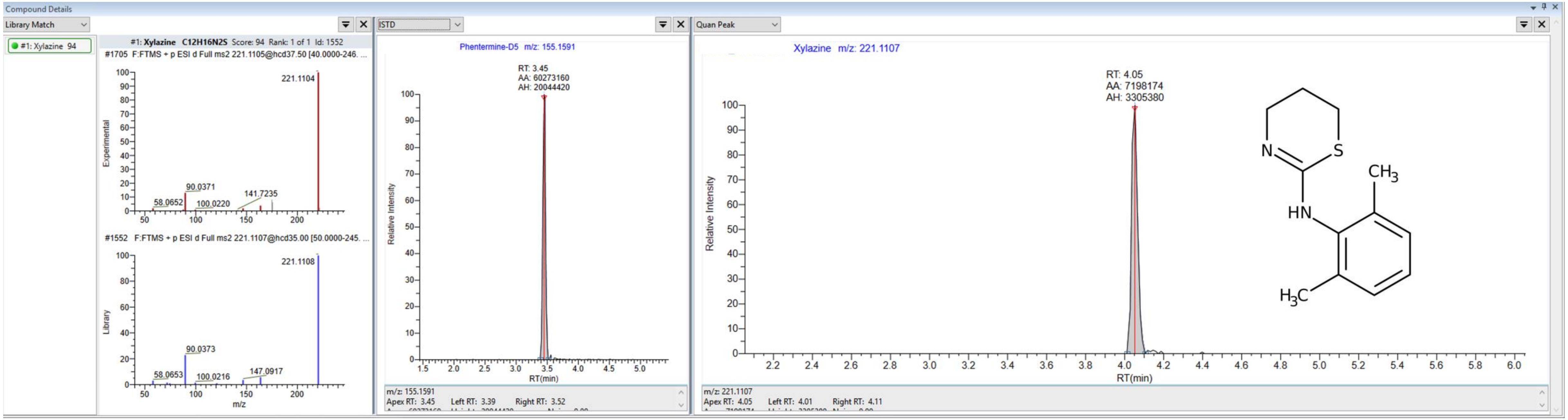
F: FTMS + p ESI Full ms [100.0000-1000.0000]

para-Fluorofentanyl m/z: 355.2180

RT: 5.49
AA: 13005227
AH: 6857108

m/z: 355.2180
Apex RT: 5.49 Left RT: 5.45 Right RT: 5.55

Xylazine



● #1: Xylazine 94

#1: **Xylazine** C12H16N2S Score: 94 Rank: 1 of 1 Id: 1552

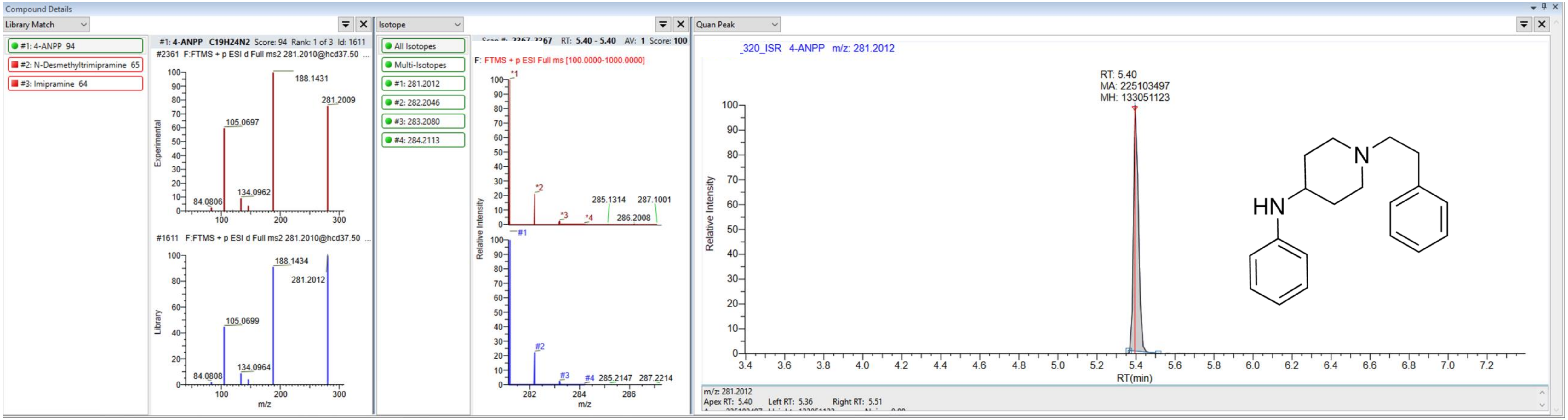
#1705 F:FTMS + p ESI d Full ms2 221.1105@hcd37.50 [40.0000-246. ...

#1552 F:FTMS + p ESI d Full ms2 221.1107@hcd35.00 [50.0000-245. ...

m/z: 155.1591
Apex RT: 3.45 Left RT: 3.39 Right RT: 3.52

m/z: 221.1107
Apex RT: 4.05 Left RT: 4.01 Right RT: 4.11

Study Subject 320



#1: 4-ANPP C19H24N2 Score: 94 Rank: 1 of 3 Id: 1611

#2361 F:FTMS + p ESI d Full ms2 281.2010@hcd37.50 ...

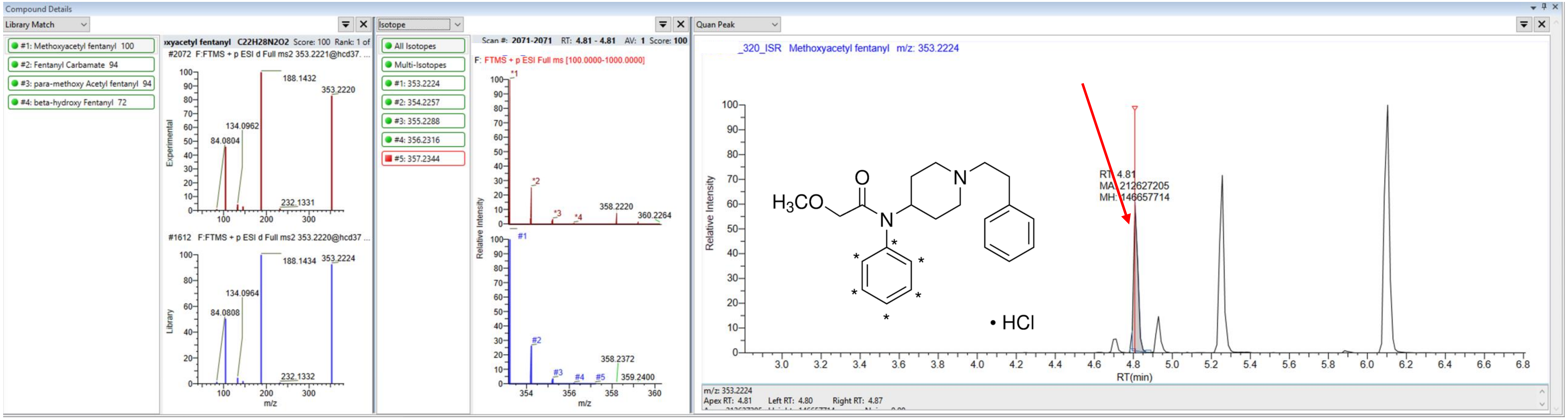
#1611 F:FTMS + p ESI d Full ms2 281.2010@hcd37.50 ...

Scan #: 2367-2367 RT: 5.40 - 5.40 AV: 1 Score: 100

m/z: 281.2012

Apex RT: 5.40 Left RT: 5.36 Right RT: 5.51

Study Subject 320



Compound Details

Library Match

- #1: Methoxyacetyl fentanyl 100
- #2: Fentanyl Carbamate 94
- #3: para-methoxy Acetyl fentanyl 94
- #4: beta-hydroxy Fentanyl 72

xyacetyl fentanyl C22H28N2O2 Score: 100 Rank: 1 of

#2072 F:FTMS + p ESI d Full ms2 353.2221@hcd37. ...

#1612 F:FTMS + p ESI d Full ms2 353.2220@hcd37 ...

Isotope

- All Isotopes
- Multi-Isotopes
- #1: 353.2224
- #2: 354.2257
- #3: 355.2288
- #4: 356.2316
- #5: 357.2344

Scan #: 2071-2071 RT: 4.81 - 4.81 AV: 1 Score: 100

F: FTMS + p ESI Full ms [100.0000-1000.0000]

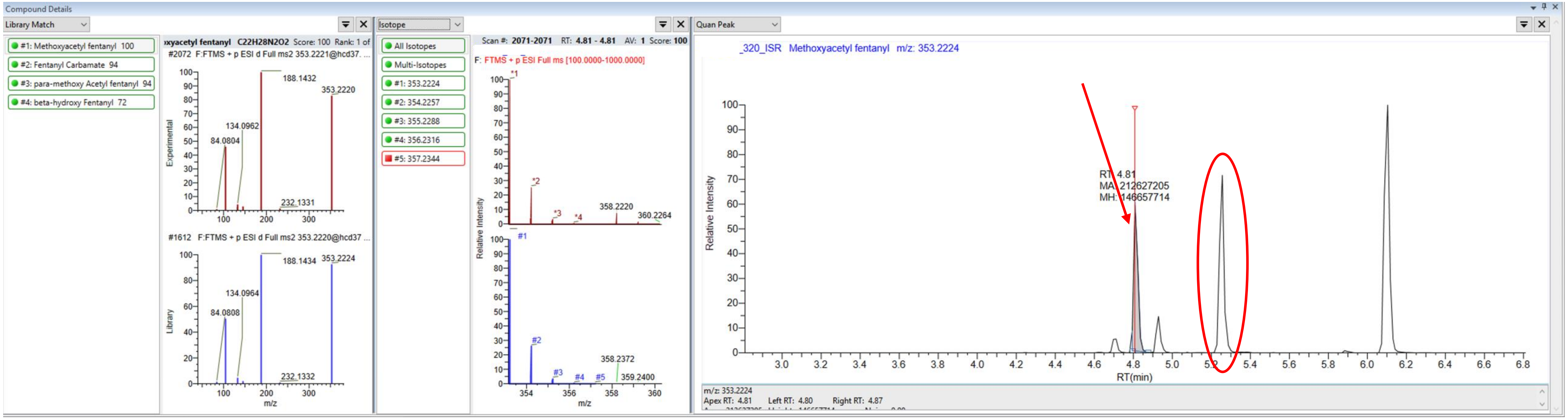
Quan Peak

_320_ISR Methoxyacetyl fentanyl m/z: 353.2224

RT 4.81
MA: 212627205
MH: 146657714

m/z: 353.2224
Apex RT: 4.81 Left RT: 4.80 Right RT: 4.87

Study Subject 320


Compound Details

Library Match

- #1: Methoxyacetyl fentanyl 100
- #2: Fentanyl Carbamate 94
- #3: para-methoxy Acetyl fentanyl 94
- #4: beta-hydroxy Fentanyl 72

xyacetyl fentanyl C22H28N2O2 Score: 100 Rank: 1 of

#2072 F:FTMS + p ESI d Full ms2 353.2221@hcd37. ...

#1612 F:FTMS + p ESI d Full ms2 353.2220@hcd37 ...

Isotope

- All Isotopes
- Multi-Isotopes
- #1: 353.2224
- #2: 354.2257
- #3: 355.2288
- #4: 356.2316
- #5: 357.2344

Scan #: 2071-2071 RT: 4.81 - 4.81 AV: 1 Score: 100

F: FTMS + p ESI Full ms [100.0000-1000.0000]

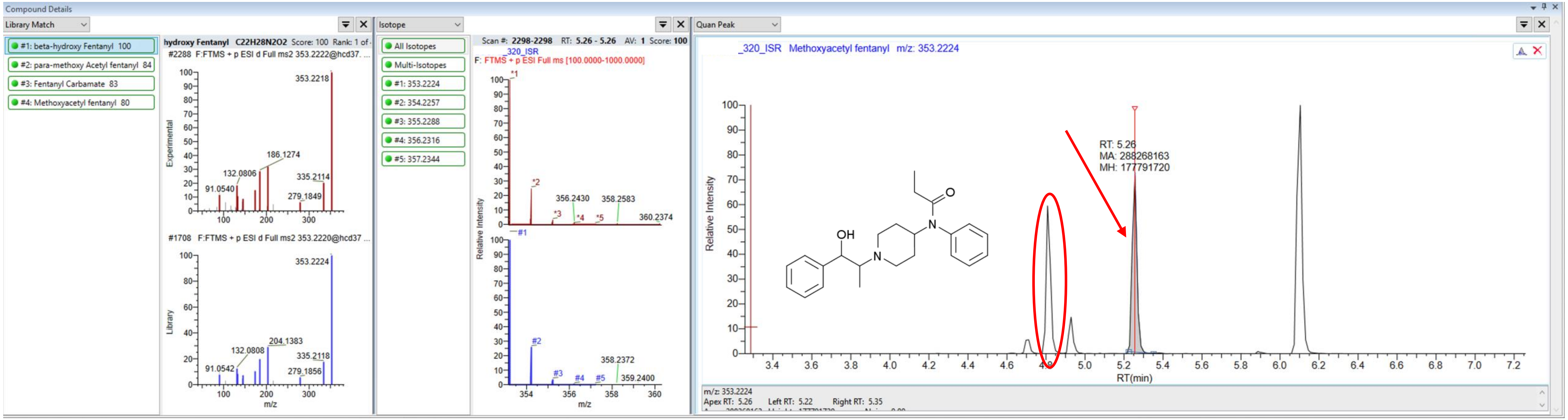
Quan Peak

_320_ISR Methoxyacetyl fentanyl m/z: 353.2224

RT: 4.81
MA: 212627205
MH: 146657714

m/z: 353.2224
Apex RT: 4.81 Left RT: 4.80 Right RT: 4.87

Study Subject 320



Compound Details

Library Match

- ● #1: beta-hydroxy Fentanyl 100
- ● #2: para-methoxy Acetyl fentanyl 84
- ● #3: Fentanyl Carbamate 83
- ● #4: Methoxyacetyl fentanyl 80

hydroxy Fentanyl C22H28N2O2 Score: 100 Rank: 1 of

#2288 F:FTMS + p ESI d Full ms2 353.2222@hcd37. ...

#1708 F:FTMS + p ESI d Full ms2 353.2220@hcd37 ...

Isotope

- ● All Isotopes
- ● Multi-Isotopes
- ● #1: 353.2224
- ● #2: 354.2257
- ● #3: 355.2288
- ● #4: 356.2316
- ● #5: 357.2344

Scan #: 2298-2298 RT: 5.26 - 5.26 AV: 1 Score: 100

_320_ISR

F: FTMS + p ESI Full ms [100.0000-1000.0000]

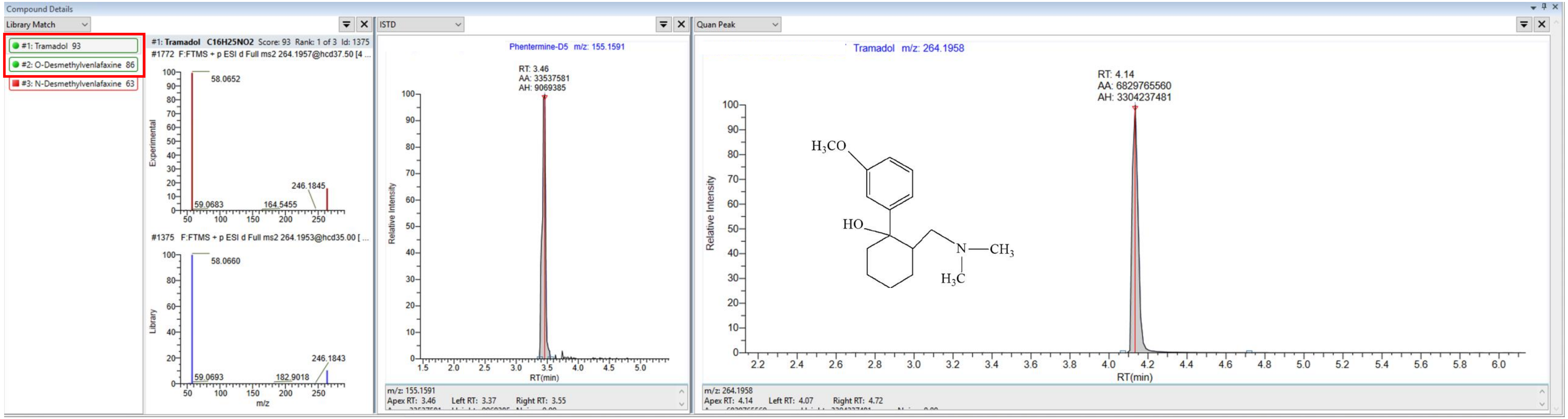
Quan Peak

_320_ISR Methoxyacetyl fentanyl m/z: 353.2224

RT: 5.26
MA: 288268163
MH: 177791720

m/z: 353.2224
Apex RT: 5.26 Left RT: 5.22 Right RT: 5.35

Tramadol



● #1: Tramadol 93
● #2: O-Desmethylvenlafaxine 86
■ #3: N-Desmethylvenlafaxine 63

#1: **Tramadol** **C16H25NO2** Score: 93 Rank: 1 of 3 Id: 1375
#1772 F:FTMS + p ESI d Full ms2 264.1957@hcd37.50 [4 ...
#1375 F:FTMS + p ESI d Full ms2 264.1953@hcd35.00 [...

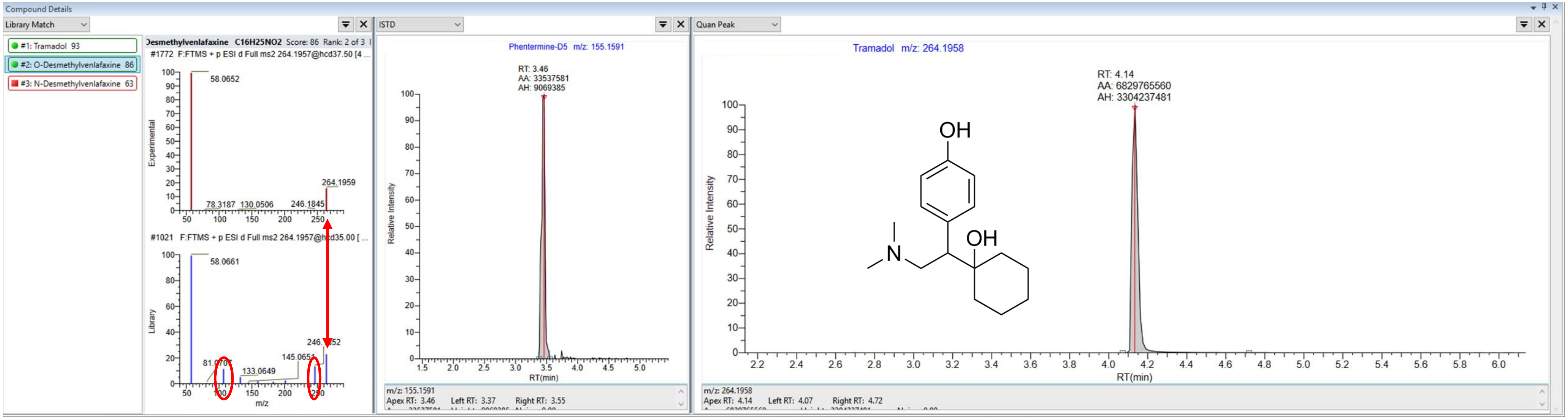
RT: 3.46
AA: 33537581
AH: 9069385

m/z: 155.1591
Apex RT: 3.46 Left RT: 3.37 Right RT: 3.55

RT: 4.14
AA: 6829765560
AH: 3304237481

m/z: 264.1958
Apex RT: 4.14 Left RT: 4.07 Right RT: 4.72

Tramadol-ODV Potential Interference



Compound Details

Library Match

- #1: Tramadol 93
- #2: O-Desmethylvenlafaxine 86
- #3: N-Desmethylvenlafaxine 63

Desmethylvenlafaxine C16H25NO2 Score: 86 Rank: 2 of 3

#1772 F:FTMS + p ESI d Full ms2 264.1957@hcd37.50 [4 ...

#1021 F:FTMS + p ESI d Full ms2 264.1957@hcd35.00 [...

ISTD

Phentermine-D5 m/z: 155.1591

RT: 3.46
AA: 33537581
AH: 9069385

m/z: 155.1591
Apex RT: 3.46 Left RT: 3.37 Right RT: 3.55

Quan Peak

Tramadol m/z: 264.1958

RT: 4.14
AA: 6829765560
AH: 3304237481

m/z: 264.1958
Apex RT: 4.14 Left RT: 4.07 Right RT: 4.72

Final Thoughts

- Use of LC-HRMS technology and library matching provides information rich data outputs that minimizes risk of false positives in toxicology screening
- Care must be taken due to high sensitivity of the technology – must put safeguards in place to avoid positive calls from system carryover or environmental sources
- This technology allows for rapid expansion of the testing menu when needed to adapt to the evolving substance use landscape
- In our experience, menu expansion allows us to identify emerging substance use patterns earlier to better inform our clinical and research partners

Acknowledgments

Johns Hopkins School of Medicine

- Katie Weaver
- Autumn Breaud
- Jonatan De Wree
- Susan Sherman

Thermo Fisher Scientific

- Tim Stratton
- Jingshu Guo
- Kerry Hassell

Acknowledgments

- Overall support for the HIV Prevention Trials Network (HPTN) is provided by the National Institute of Allergy and Infectious Diseases (NIAID), Office of the Director (OD), National Institutes of Health (NIH), National Institute on Drug Abuse (NIDA), the National Institute of Mental Health (NIMH), and the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) under Award Numbers UM1AI068619-17 (HPTN Leadership and Operations Center), UM1AI068617-17 (HPTN Statistical and Data Management Center), and UM1AI068613-17 (HPTN Laboratory Center).

- The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Thank you

QUESTIONS??

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HPTN
HIV Prevention
Trials Network