



**HPTN**

HIV Prevention  
Trials Network

# **Drug Screening in Biological Samples using High Resolution Mass Spectrometry**

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# Disclosures

- Grant/Research Support: Thermo Fisher, Shimadzu, Nova Biomedical, Instrumentation Laboratories, miDiagnostics
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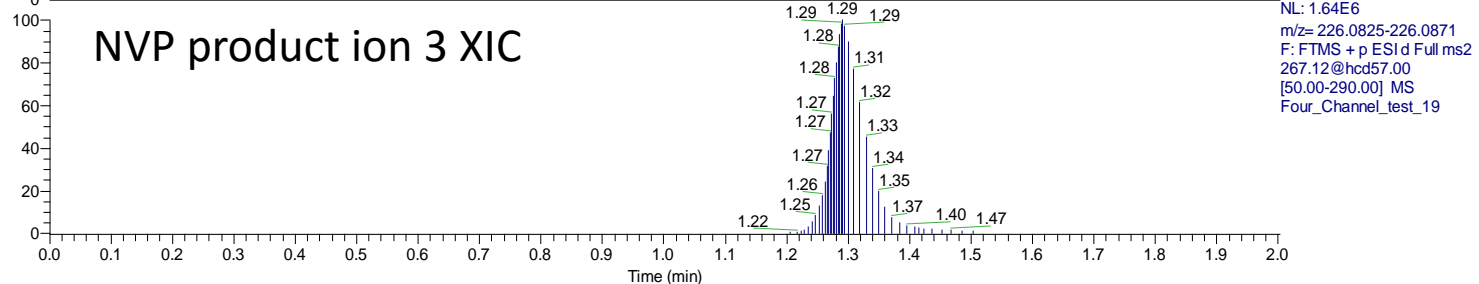
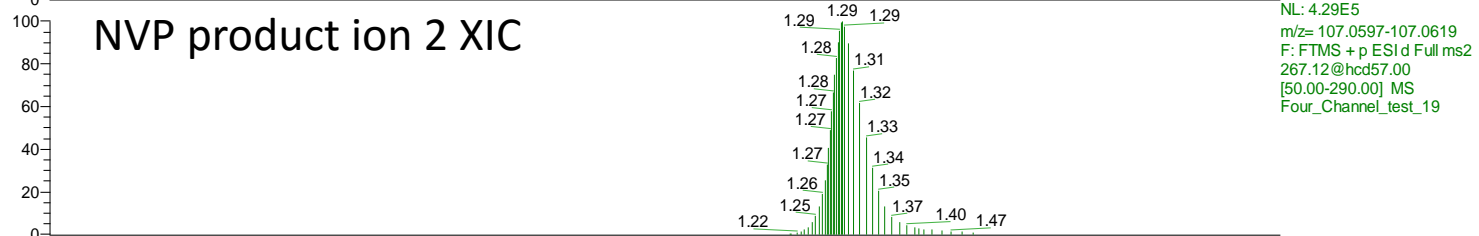
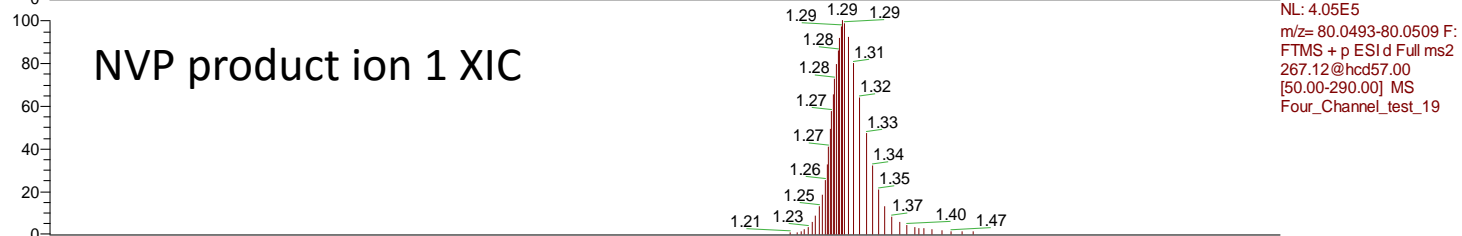
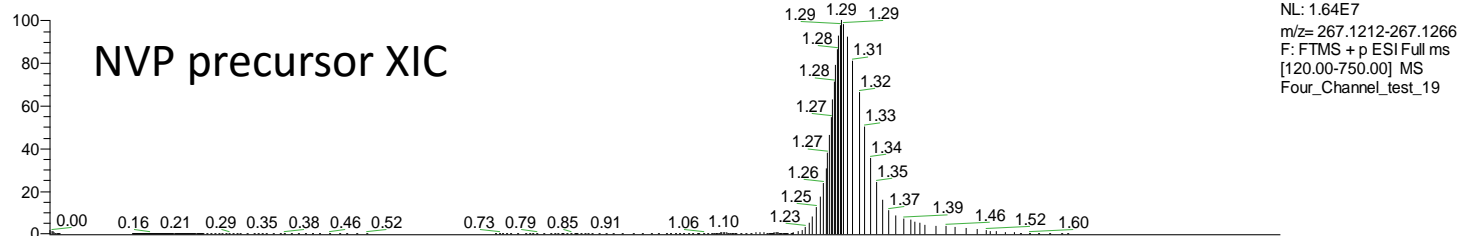
# **RAPID ANTIRETROVIRAL (ARV) SCREENING**

# Rapid Analysis with Q Exactive and LC-MS

- Preparation: protein precipitation plates on a Tecan Evo robotic station
- Two minute chromatographic method for 20 compounds
- Full scan MS-data dependent MS2 (ddMS2): fragmentation is triggered if a compound of interest is detected above a threshold; exact mass for analysis of fragments
  - Positive mode electrospray ionization; resolution = 17.5K at  $m/z$  200
- Detection utilizes 1-3 product ions per compound; verification possible through data query for precursor exact mass

# Nevirapine (NVP) 20 ng/mL in serum

RT: 0.00 - 2.00 SM: 11G



# High Throughput Screening Assay

- Automated sample preparation
  - 30 min/96-well plate (active run time); 0.3 min/sample
- 4 min to first result by LC-MS
  - 2 min sample to sample
- Approx. 3h/plate (172 min for subjects + QC)
- Overnight runs (18h) = 6 plates per instrument
- 2 instruments = 972 specimens/day
- LOD = 2-20 ng/mL for all ARV drugs

# Applications of the multi-drug assay in HPTN studies

## Discrepant HIV diagnostic test results

**HPTN 043:** Most HIV-infected adults with discordant rapid tests were virally suppressed without ARV drugs

Fogel et al. J AIDS. 2015; 69:446

## Cross-sectional HIV incidence (as part of a multi-assay algorithm)

**HPTN 043:** 6.7% of MAA-positive individuals had ARV drugs and were excluded from incidence assessments

Laeyendecker et al. PLoS One. 2013; 8:e68349

# Applications of the multi-drug assay in HPTN studies

## Transmitted HIV drug resistance

**HPTN 061:** Analysis of ARV drug resistance in seroconverters; estimation of transmitted drug resistance was reduced (23%→12%) after accounting for ARV drug use

Chen et al. J AIDS 2015; 69:446



# Applications of the multi-drug assay in HPTN studies

## Undisclosed ART use among HIV-infected participants

**HPTN 052:** 45 (46.9%) of 96 “ARV naïve” index participants who had a VL<400 at enrollment were on ART; many continued off-study ART after enrollment

Fogel et al. J Infect Dis. 2013; 208:1624

## Undisclosed knowledge of HIV status

**HPTN 061:** >40% of 155 men initially characterized as “newly diagnosed, ARV naïve” were on ART at enrollment; many had unusual patterns of ARV drugs detected

Marzinke et al. Clin Infect Dis. 2014; 58:117

# Applications of the multi-drug assay in HPTN studies

## Use of ARV drugs in HIV-uninfected cohorts

**HPTN 064:** 2% of 1,806 HIV-uninfected women had ARV drugs detected at enrollment (15% in Baltimore; 5% in Bronx; NNRTIs and PIs; 1-4 drugs/sample)

Chen et al. PLoS One 2015; 10:e0140074

**HPTN 068:** None of >2,000 HIV-uninfected young women had ARV drugs detected at enrollment

Zhang, Sivay et al. Manuscript in preparation

**HPTN 073:** Two of 208 HIV-uninfected Black MSM were taking off-study TDF/FTC at enrollment

Zhang, Manuscript submitted

# Applications of the multi-drug assay in HPTN studies

## Population-level ARV drug use

**HPTN 043:** ARV drug use was analyzed in a large community-randomized clinical trial; ARV drug use was associated with sex (women>men), pregnancy, older age, and study site; increased ARV drug use was associated with reduced HIV incidence at one study site

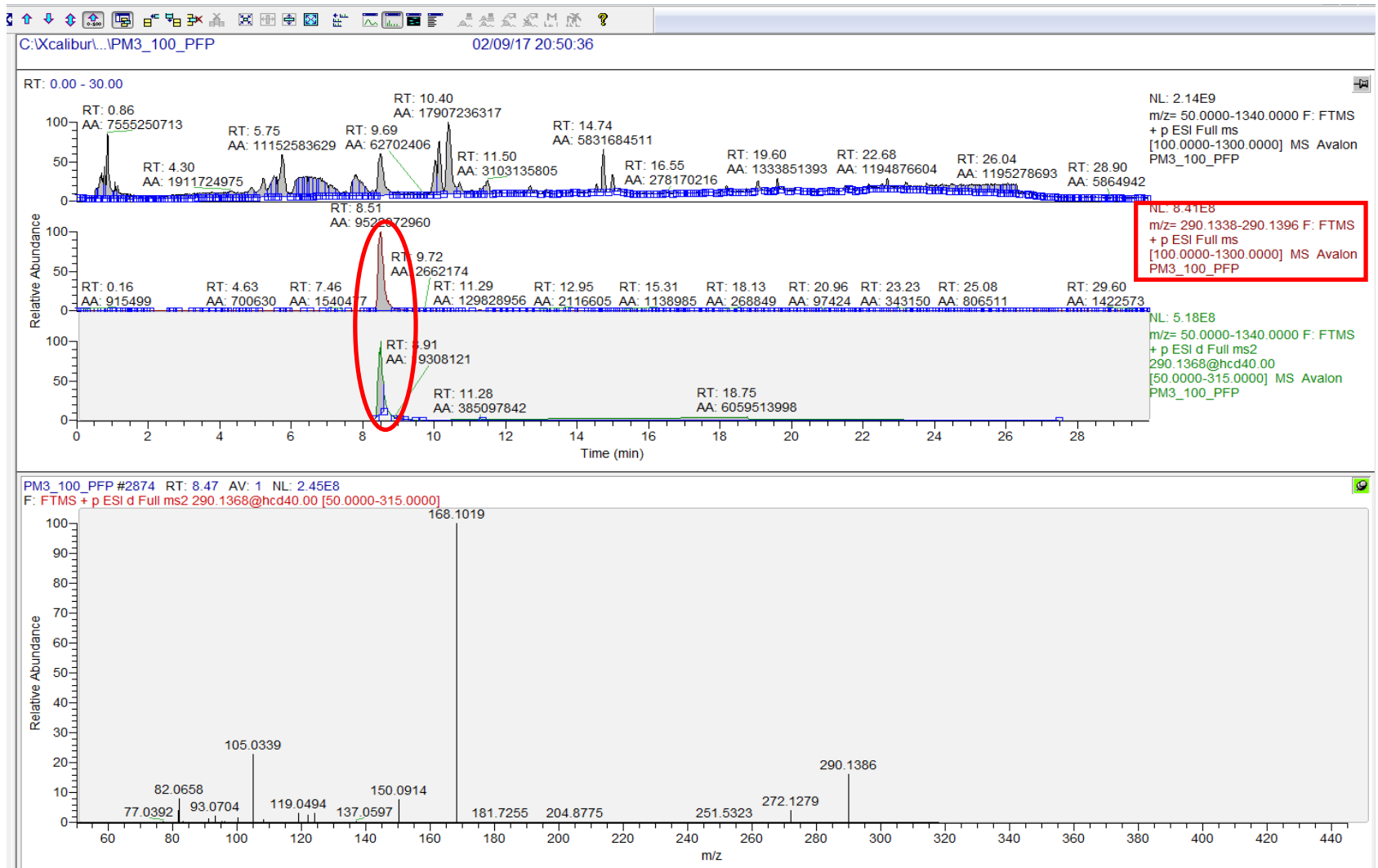
Fogel et al. J AIDS. 2017; 74:158

# UNTARGETED TOXICOLOGY SCREENING

# Untargeted LC-HRMS Screening

- Samples prepared by simple protein precipitation and dilution; 30-minute mixed mode chromatography
- Mass peaks are selected based on minimum intensity threshold (ion current in quadrupole)
- Selected peaks are fragmented and analyzed by high-resolution orbitrap
  - Data-dependent fragmentation and analysis
- Resulting pattern matched to stored mass spectra patterns
  - Curated spectra: MZ cloud
  - Theoretical spectra: ChemSpider

# Analysis of known cocaine positive specimen



# Analysis of known cocaine positive specimen

Features Partners Contact Log in Standard Compare Structures

Spectrum search result 2 << Query Library record Structure C<sub>16</sub>H<sub>19</sub>NO<sub>2</sub>

Edit search options x Discard Filtered Recalibrated

Hit: 1 Best Match: 98.9  
Reference No: 1001  
**Benzoylcegonine**  
Monois. Mass: 289.13741  
+ Eawag  
+ Cayman

Hit: 2 Best Match: 73.6  
Reference No: 845  
**Norcocaine**  
Monois. Mass: 289.13741  
+ Thermo  
+ Thermo

Spectra compare

Precursors and Fragment

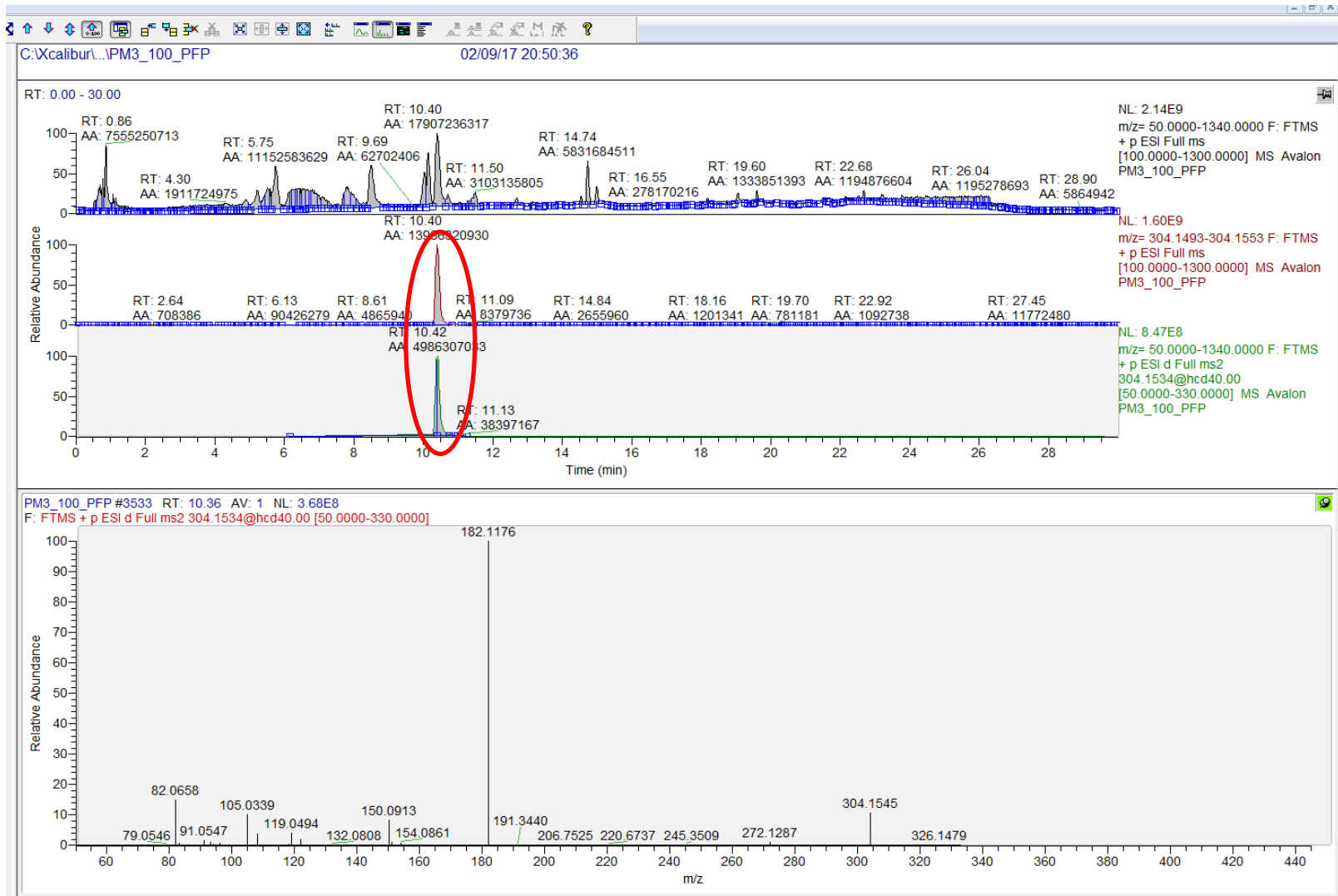
Blue Structure: Heuristic Prediction  
Brown Structure: Quantum Chemical Predict

Algorithm	match
HighChem HighRes	98.9
Opt. Dot Product	99.9
NIST (Modified)	93.7

M: 0.9885109882101828

record count 2

# Analysis of known cocaine positive specimen





# Analysis of known cocaine positive specimen

Features Partners Contact Log in Standard Compare Structures

Q Spectrum search result 3 << Query Library record Structure C<sub>17</sub>H<sub>21</sub>NO<sub>2</sub>

Edit search options x

Hit: 1 Best Match: 98.2  
Reference No: 1016  
**Cocaine**  
Monoiso. Mass: 303.14706  
+ Eawag  
+ Cayman  
+ Cayman

Hit: 2 Best Match: 33.7  
Reference No: 595  
**Scopolamine**  
Monoiso. Mass: 303.14706  
+ Thermo  
+ Thermo

Hit: 3 Best Match: 30.7  
Reference No: 4516  
**Hydromorphenol**  
Monoiso. Mass: 303.14706  
+ HighChem

Hit: 4 Best Match: 30.5  
Reference No: 2107  
**Fenoterol**  
Monoiso. Mass: 303.14706  
+ Thermo

Hit: 5 Best Match: 19.1  
Reference No: 5689  
**Ethyl 1-(2-thienylsulfonyl)-4-piperidinecarboxylate**  
Monoiso. Mass: 303.05990  
+ Thermo

Hit: 6 Best Match: 18.3  
Reference No: 5578  
**N-(8-Methyl-8-azabicyclo[3.2.1]oct-3-ylidene)-N'-(4-nitrobenzyl)oxy]amine**  
Monoiso. Mass: 303.12191  
+ Thermo

Hit: 7 Best Match: 10.8  
Reference No: 5800

record count 12

MS1 Scn.

FT MS1 Scns. #556, 571, 586

FT MS2 304.15 Scns. #560, 566, 576, 581, 590, 596

< 4/8 FT HCD 45 NCE, 27 eV MS2 304.15 Com1 4/8 >

Spectra compare

Precursors and Fragment

MS<sup>1</sup> [M+H]<sup>+</sup> m/z 304.15433 HCD 45: IW 1.5

MS<sup>2</sup>

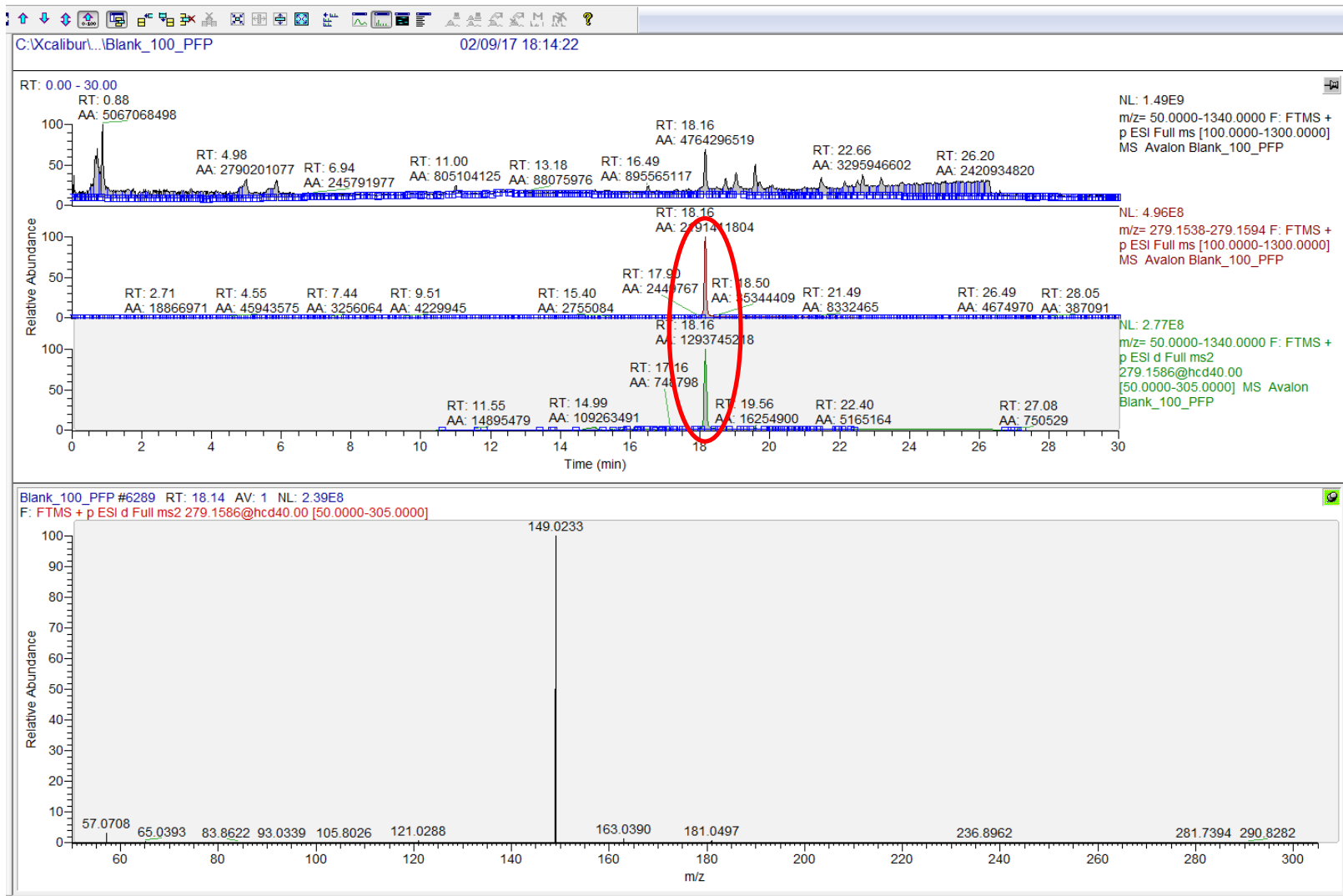
Blue Structure: Heuristic Prediction  
Brown Structure: Quantum Chemical Prediction

Algorithm	match
HighChem HighRes	98.2
Opt. Dot Product	99.8
NIST (Modified)	94.4

M : 0.981638039469162

Library Metadata

# Analysis of urine toxicology negative control



# Analysis of urine toxicology negative control

Features Partners Contact Log in

Spectrum search result 4 << Query Library record Structure  $C_{16}H_{22}O_4$  Standard Compare Structures

Edit search options x

Hit: 1 Best Match: 92.0  
Reference No: 32  
**Dibutyl phthalate**  
Monoiso. Mass: 278.15381

- Thermo
- Thermo
- Thermo
- Thermo
- Eawag
- Thermo
- Eawag

Hit: 2 Best Match: 90.7  
Reference No: 2033  
**Diisobutylphthalate**  
Monoiso. Mass: 278.15381

- Thermo
- Thermo

Hit: 3 Best Match: 87.8  
Reference No: 2914  
**Mono(2-ethylhexyl) phthalate (MEHP)**  
Monoiso. Mass: 278.15381

- Thermo
- Thermo

MS1 Scn. 149.0233 57.0708 163.0390

Discard Filtered Recalibrated

FT MS1 Scns. #23, 34, 45, 56

FT MS2 279.16 Scns. #27, 38, 49, 60

< 4/11 FT HCD 40 NCE, 22.333 eV MS2 279.16 Combined 4/11 >

Spectra compare

Library

100 50 0

57.07075 149.02333

50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320

Difference

100 50 0

150.02662

50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320

Query

100 50 0

149.0233

50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300 310 320

Library Metadata

Precursors and Fragment

MS<sup>1</sup> [M+H]<sup>+</sup> m/z 279.15909 HCD 40: IW 4

MS<sup>2</sup>

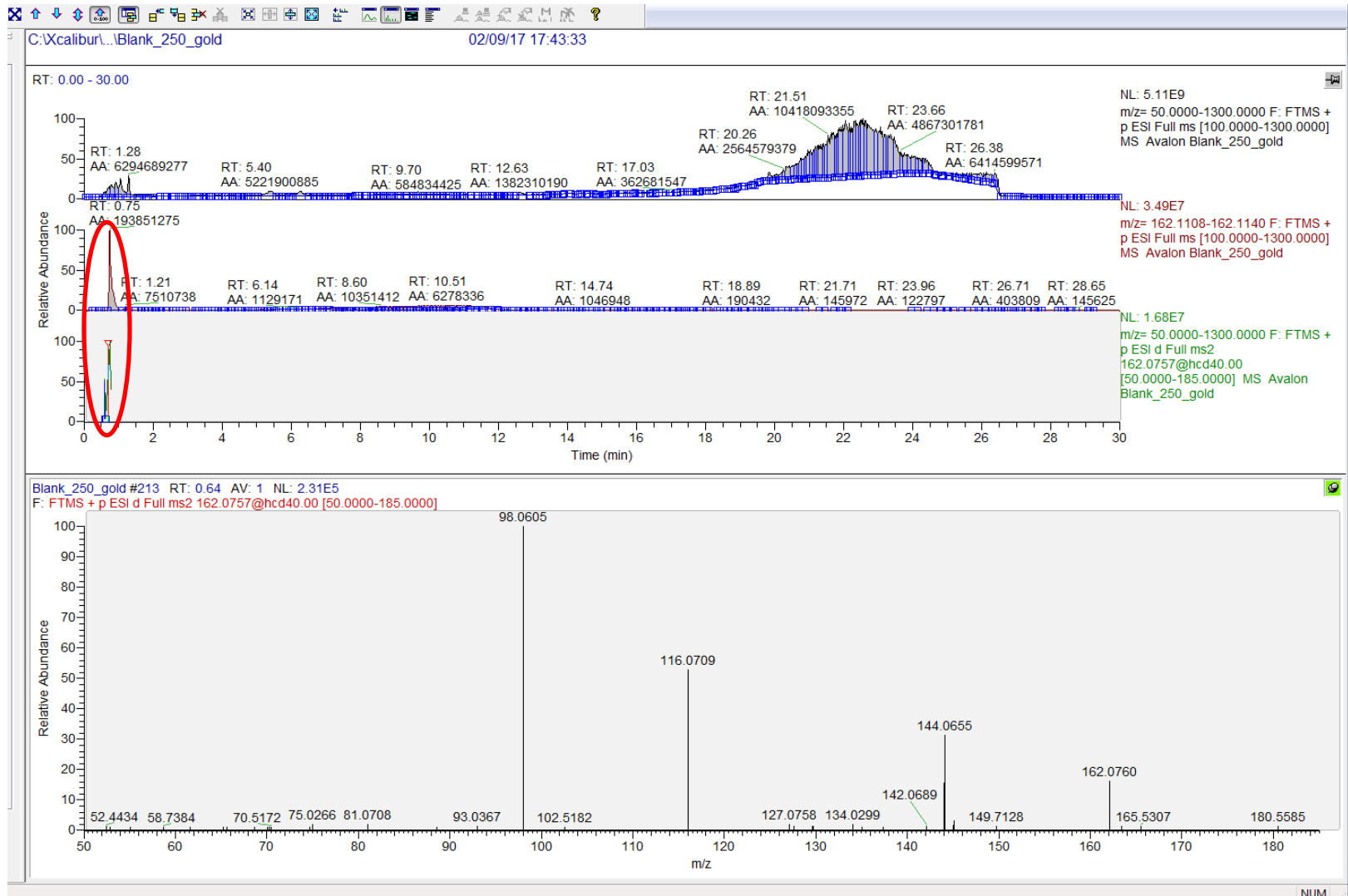
Blue Structure: Heuristic Prediction  
Brown Structure: Quantum Chemical Predict

Algorithm	match
HighChem HighRes	85.3
Opt. Dot Product	99.5
NIST (Modified)	87.7

MS<sup>1</sup> •

M: 0.853284601934797

# Analysis of urine toxicology negative control



# Analysis of urine toxicology negative control

Features Partners Contact Log in

Spectrum search result 8 << Edit search options x

Hit: 1 Best Match: 99.3  
Reference No: 881  
DL-Carnitine  
Monoiso. Mass: 162.1124  
+ Thermo  
+ Thermo  
+ Thermo

Hit: 2 Best Match: 98.8  
Reference No: 1837  
L(-)-Carnitine  
Monoiso. Mass: 162.1124  
+ Thermo

Query MS1 Scan

Library record Discard Filtered Recalibrated

Structure  $C_7H_{16}NO_3^+$

2/26 FT CID 15 NCE MS2 162.11 Combined Scans #16, 17, 18 2/26

Spectra compare

Library

Difference

Query

Precursors and Fragment

MS<sup>1</sup> M<sup>+</sup> m/z 162.11205 CID 15: IW 1

MS<sup>2</sup>

Blue Structure: Heuristic Prediction  
Brown Structure: Quantum Chemical Pr

Algorithm	match
HighChem HighRes	99.3
Opt. Dot Product	90.8
NIST (Modified)	68.6

MS<sup>1</sup>

Library Metadata

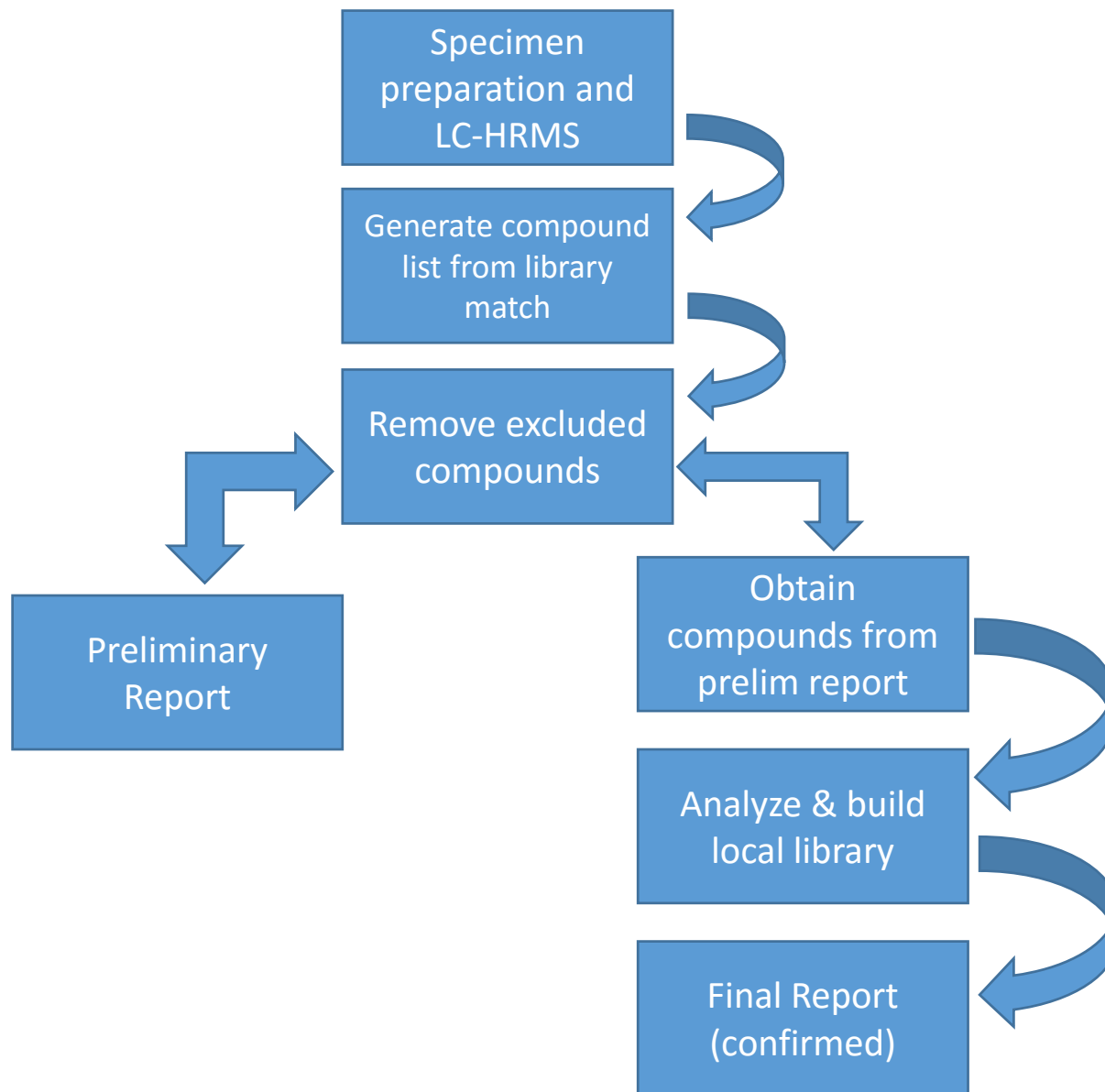
record count 2

# Next Steps

- Post-analysis data processing is necessary to exclude endogenous metabolites
- Analyze “drug-free” urines to set exception list
- Analyze known pain management samples to optimize algorithm
- Analyze blinded samples from external reference lab

# Next Steps

- Repeat workflow optimization with serum samples
- Analyze known serum/plasma TDM samples
- Analyze serum toxicology samples (collaboration with Medical Examiner?)
- Validation of both urine and serum workflows and sample preparation





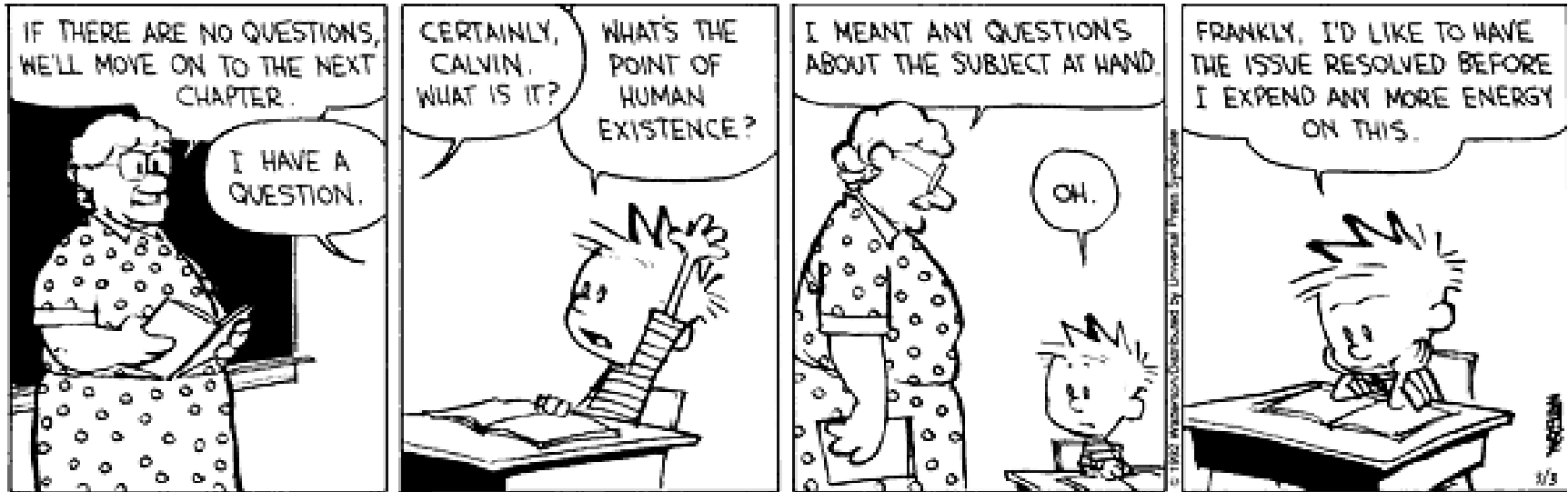
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# QUESTIONS??



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