Towards Routine NTA – Metrics of Data Quality and Acceptance

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Outline

- Why Do We Need Non-Targeted Analysis (NTA)?
- What is NTA and its purpose?
- NTA Quality Metrics to Increase Data Confidence
- Typical NTA Toolbox
- Conclusion – Future of Data Quality in NTA

Targeted methods alone cannot protect human health and the environment.

Why NTA?
To Let Contaminants Emerge

New contaminants are generated at significantly much faster rate than we can develop targeted methods to detect contaminants in the environment

Properly inform environmental occurrence (should be part of UCMR)

Emerging contaminants come forth at a rate proportional to our concern (philosophical nature of CECs)

Public prefers pro-active approaches over reactive approaches

Why NTA?
To Tackle PFAS problem

There are more PFAS present in environmental samples than are being targeted by current methods – even the largest custom lists.

NTA using High Res Mass Spec (HRMS) allows the use of specialized tools to provide more info (eg: Compound classes, compound discovery)

To inform decision makers (regulators) on what chemicals are commonly occurring in samples for the purpose of prioritization

What is NTA?
Non-Targeted Analysis

BP4NTA - The characterization of the chemical composition of any given sample without the use of a priori knowledge regarding the sample's chemical content. (see oral Tues 9:30am)

The **framework** by which a defined **chemical space** is investigated within a sample without a priori knowledge for the primary purpose of **chemical discovery**.

What is NTA?
Generalized Non-Targeted Analysis

- Capture the largest chemical space possible (see oral Tues. 10:30am)
- Sufficiently sensitive (Solid Phase extraction + Mass Spec)
- Sufficiently selective (High Resolution MS = Orbitrap or QTOF and may Ion mobility)
- No single NTA workflow can capture 100% of sample chemical space
  - LC + Electrospray provides a broad coverage of historically missed chemical space

What is NTA?
Data Processing (Workflow)

- Must find the unknown peaks (features) without knowing the m/z
- Must find isotopically labeled standards (pre/post extraction)
- Strategy attempts to form a consensus using NTA Toolbox:
  - MS/MS spectral libraries or Denovo MS2 Deconvolution
  - m/z to Chemical Formula
  - Chemical Database searches
  - And much more

Specific and meaningful data quality metrics provide insight into the reliability of the NTA data.

Typical Quality Controls Still Useful

Targeted Analysis

- Blanks (prevent false positives best case)
- Blank Spikes (prevent false negatives best case)
- Matrix Spikes (prevent false negatives worst case)
- Blank Spike Duplicate (precision best case)
- Matrix Spike Duplicate (precision worst case)
- Low Level Blank Spike (sensitivity best case)

Non-Targeted Analysis

- Blanks
- Blank Spikes
- Matrix Spikes
- Blank Spike Duplicate
- Matrix Spike Duplicate
- Low Level Blank Spike (DW)

Typical Quality Controls Still Useful

Targeted Analysis

- CCV standards
- Retention Time stability
- Surrogates (extracted Isotopes)
- Instrument Performance Standards (post extraction isotopes)

Non-Targeted Analysis

- CCV standards
- Retention Time stability
- Surrogates (extracted Isotopes)
- Instrument Performance Standards (post extraction isotopes)

What other Quality Controls are needed for NTA?

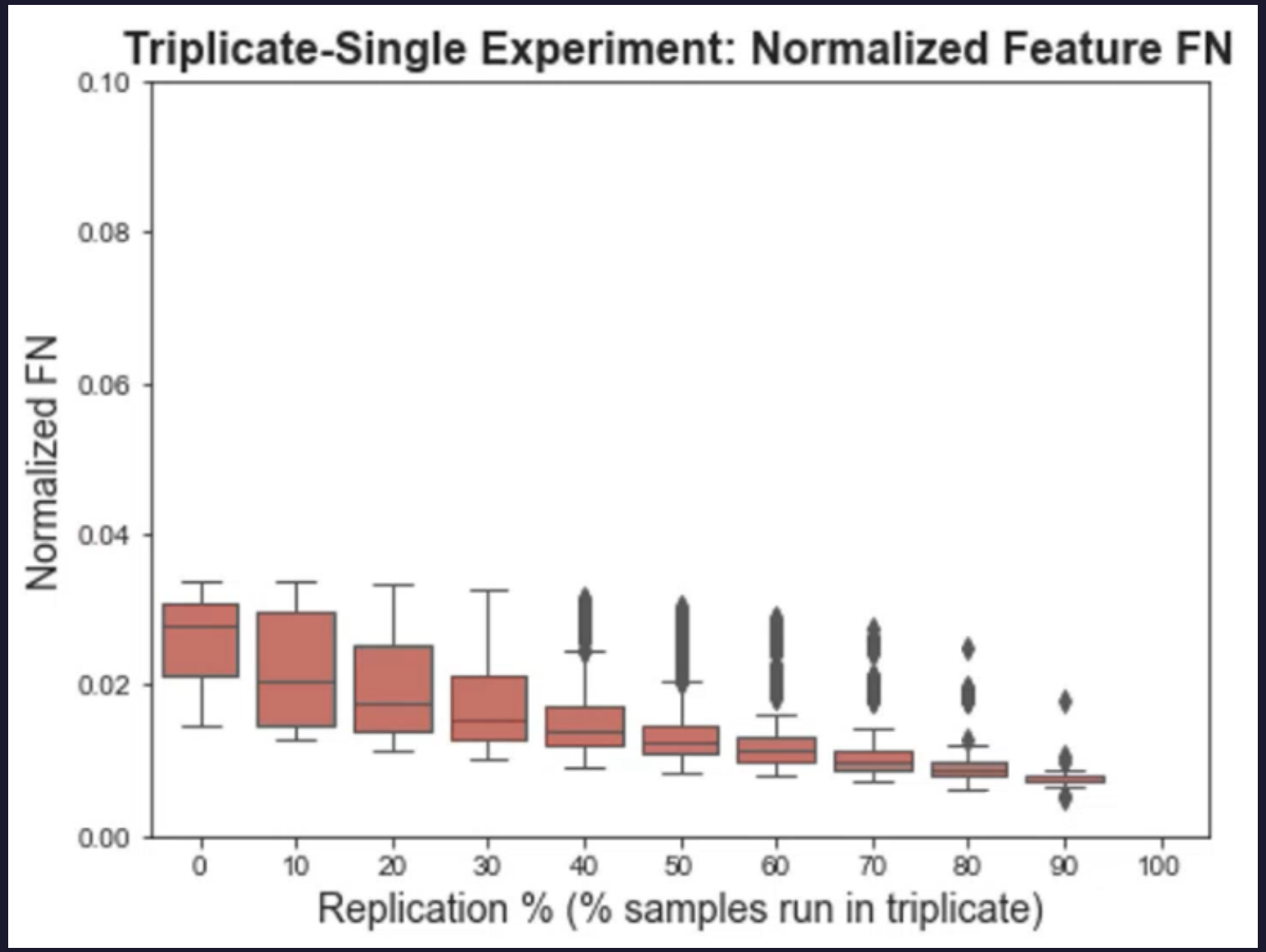
NTA Quality Controls

Technical Replicates (n=3)

- Prevent false **negatives**: Running a sample for NTA once risks missing a feature/chemical (either the MS or the data processing)
- Prevent false **positives**: A single detection of a feature/chemical in a sample for NTA once risks reporting a random analytical anomaly as a compound
- Statistical significance – Allows ANOVA tests to determine sample ratios against blanks.
- Percent CV – Between replicates must be < 125%

False Negatives

Cost of replication yields decrease in False negatives



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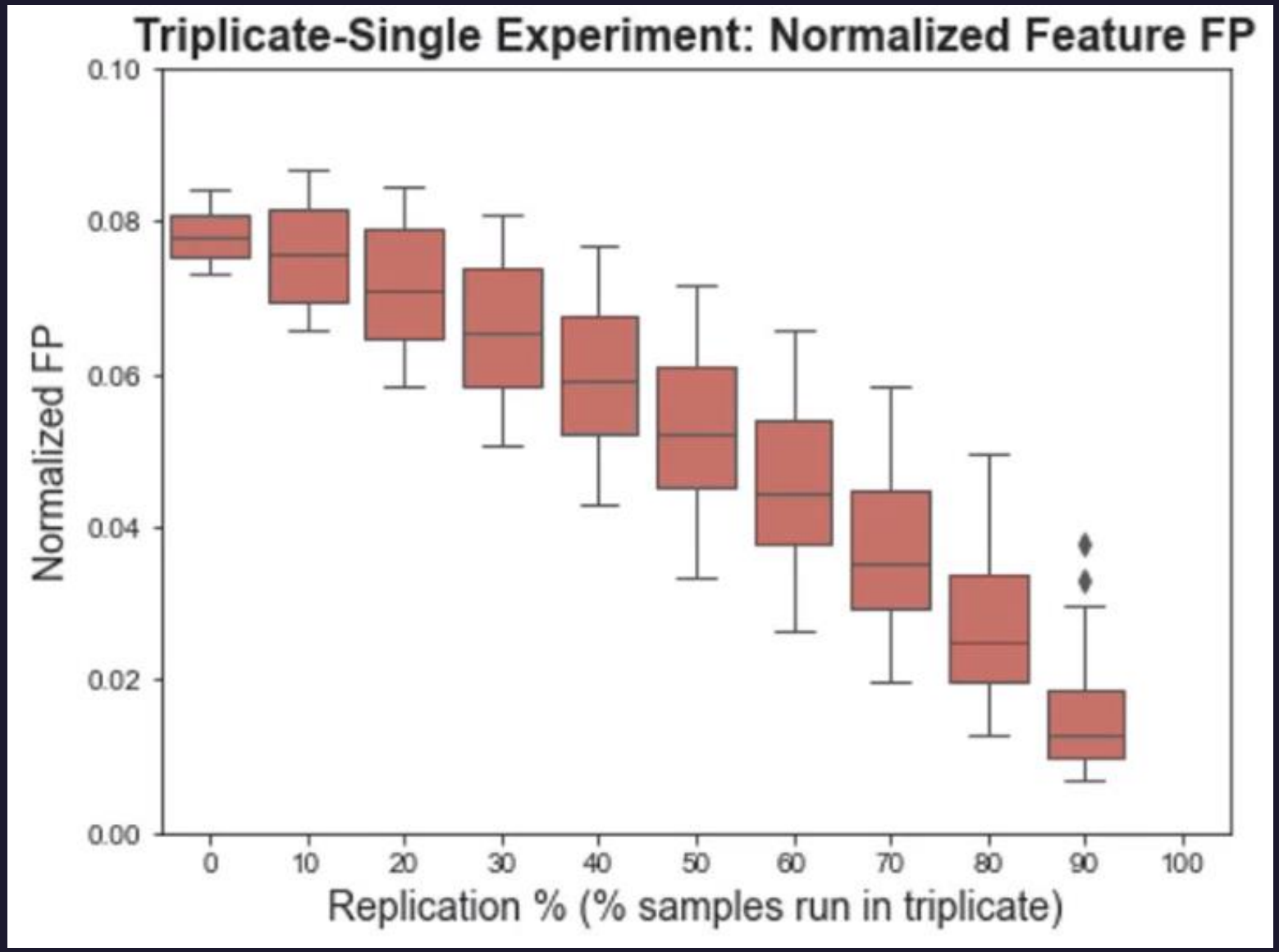
Raw Data from Babcock Labs

False Positives

Cost of replication yields decrease in False positives



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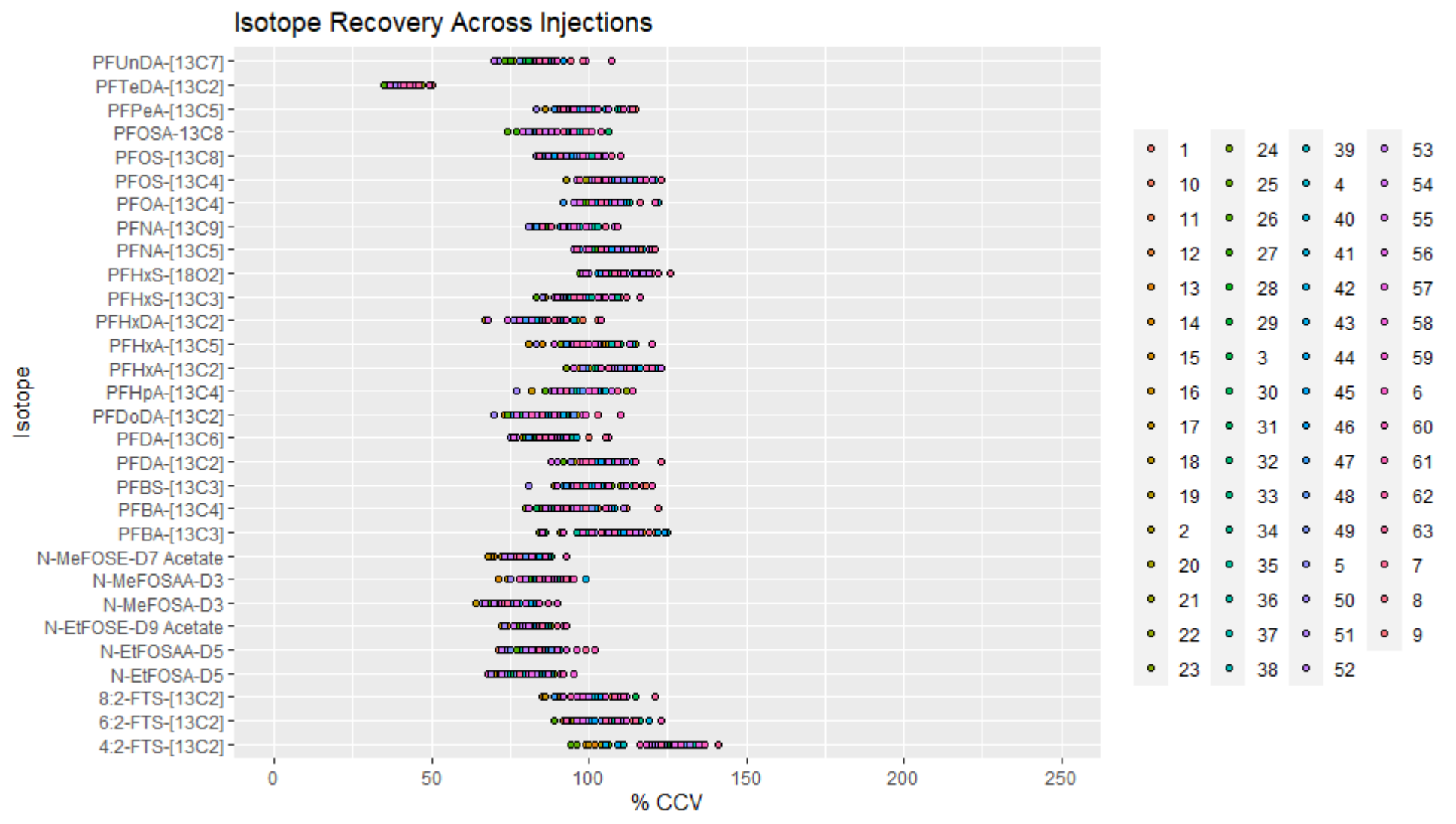
NTA Quality Controls

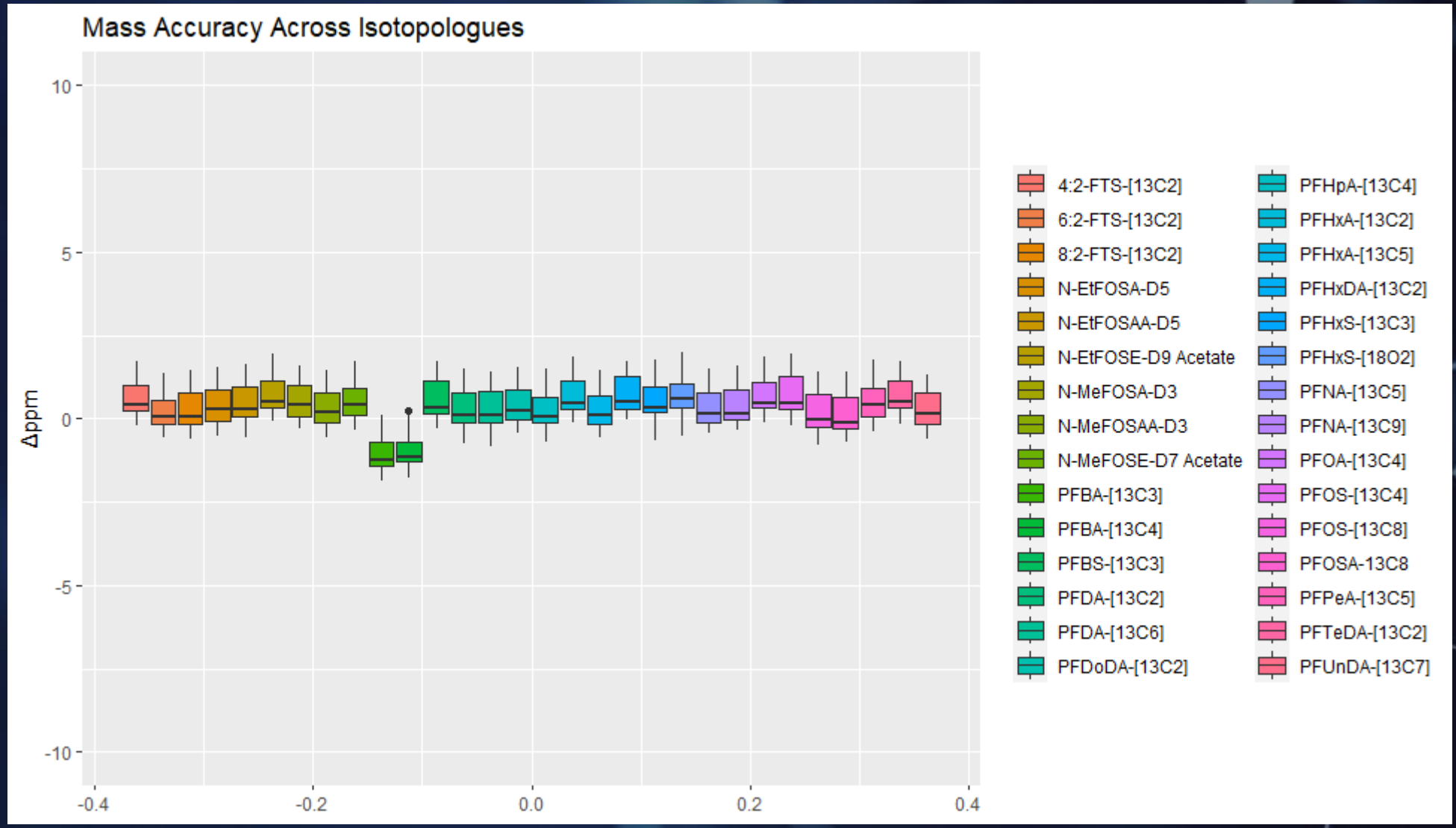
General

- Mass calibration must be performed prior to analysis.
- Mass Error for identified features must be <10 ppm (0.0001%) expected
  - QTOF must be set wider (20 ppm)
- Must detect isotopologues in all batch QC and samples at least 2/3 replicates (typically 3/3 observed)
- Must detect 90% of the isotopologues in QC/samples
- Feature must be detected 2/3 replicates
- Quasi MDLs taken from 2 blanks run in triplicate (units of Area)

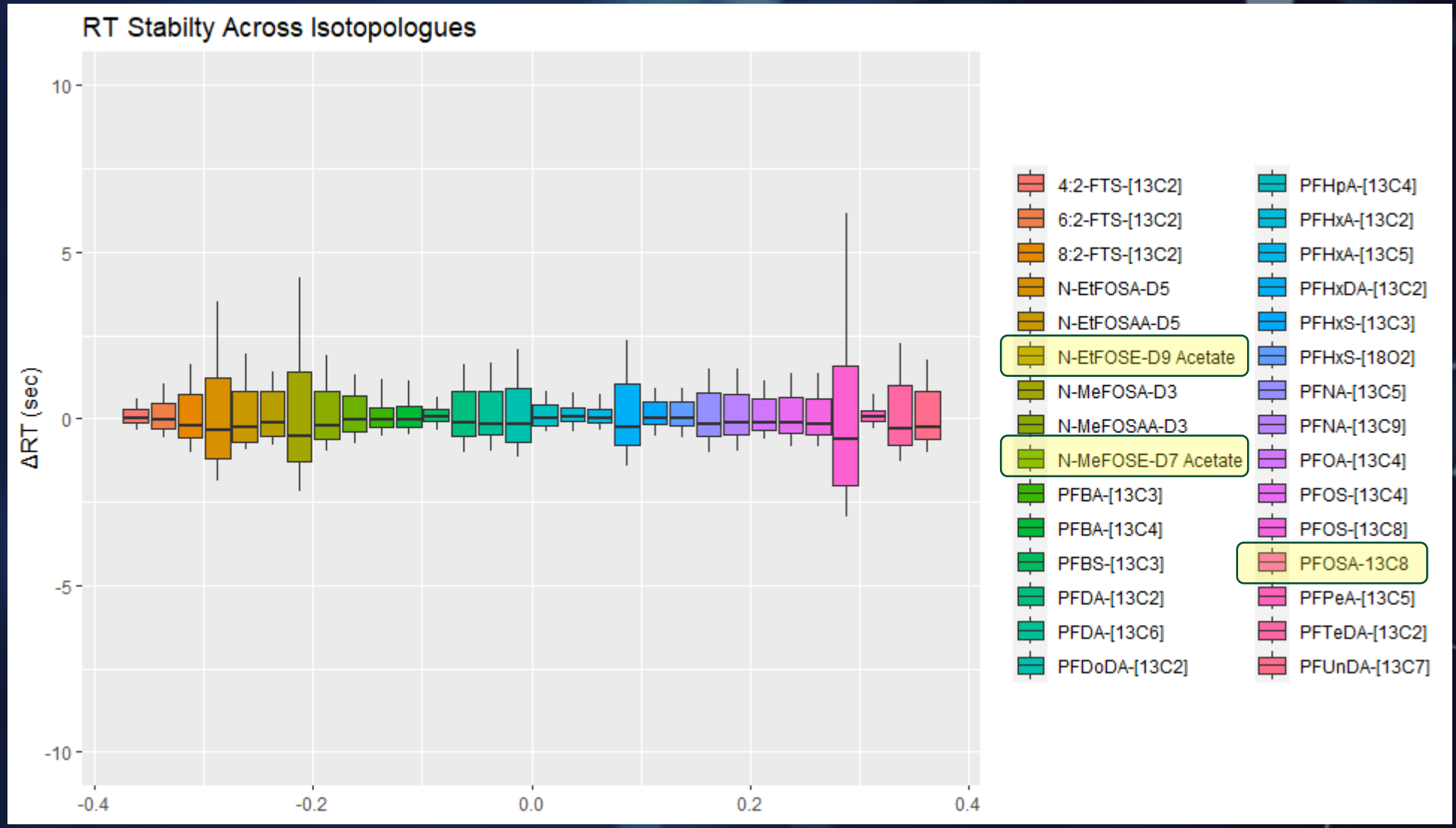
Isotopologue Recovery



Mass Accuracy/Stability



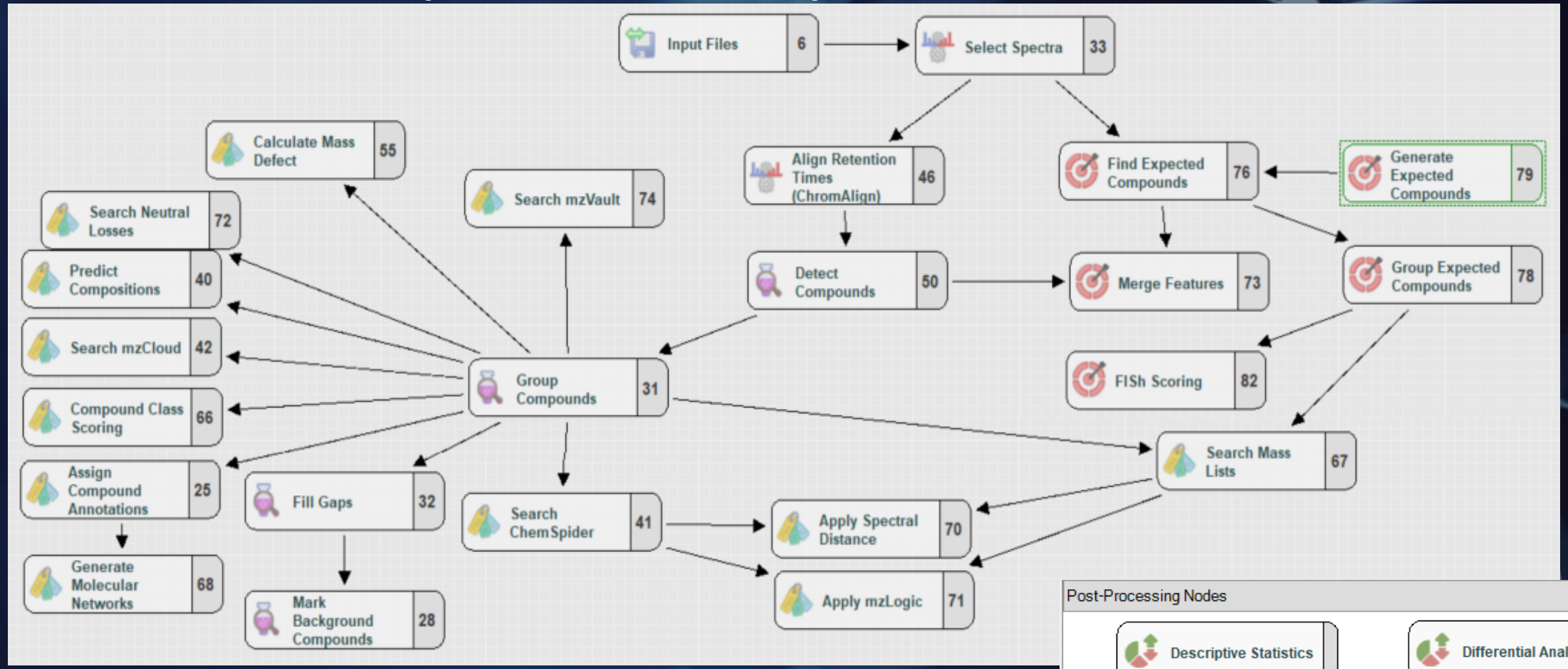
Retention Time Stability



The NTA Toolbox

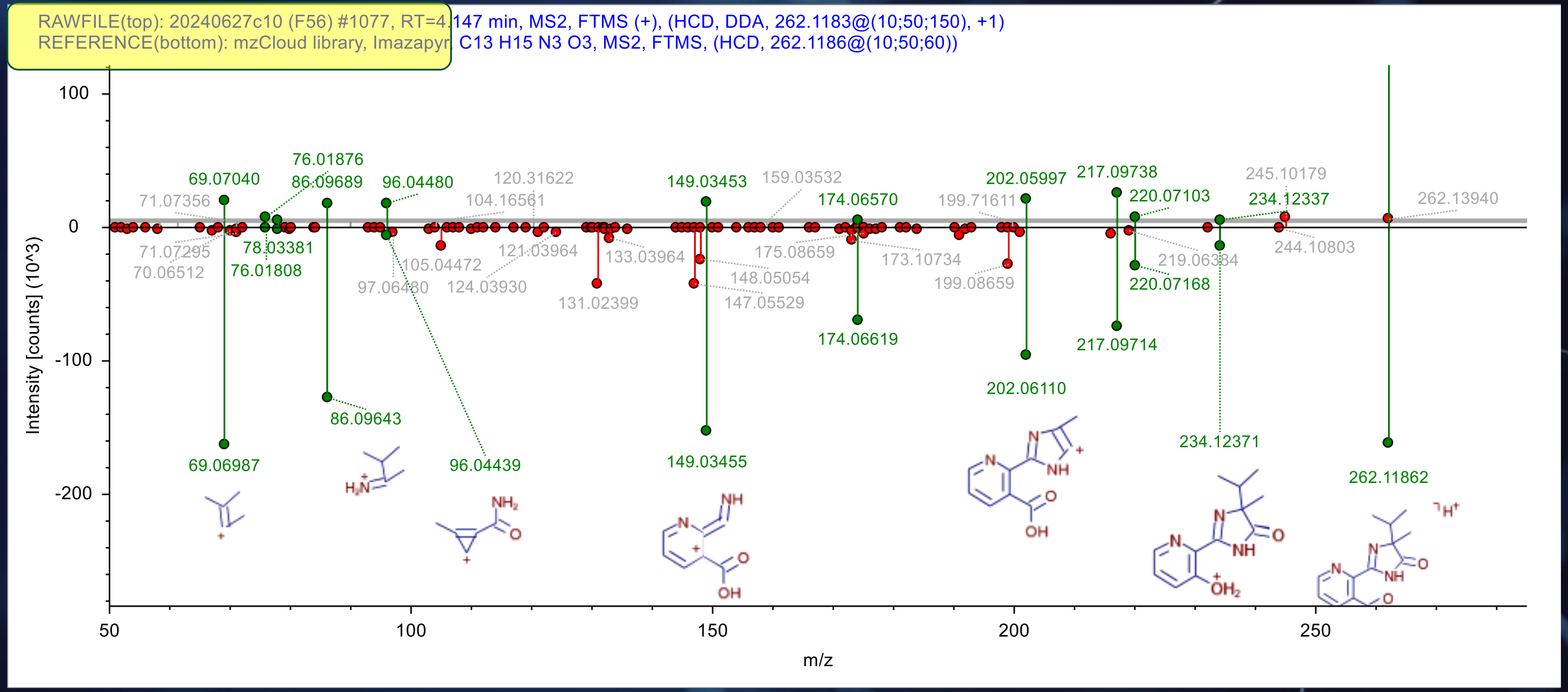
Complete NTA Workflow

Example – Thermo Compound Discoverer 3.3



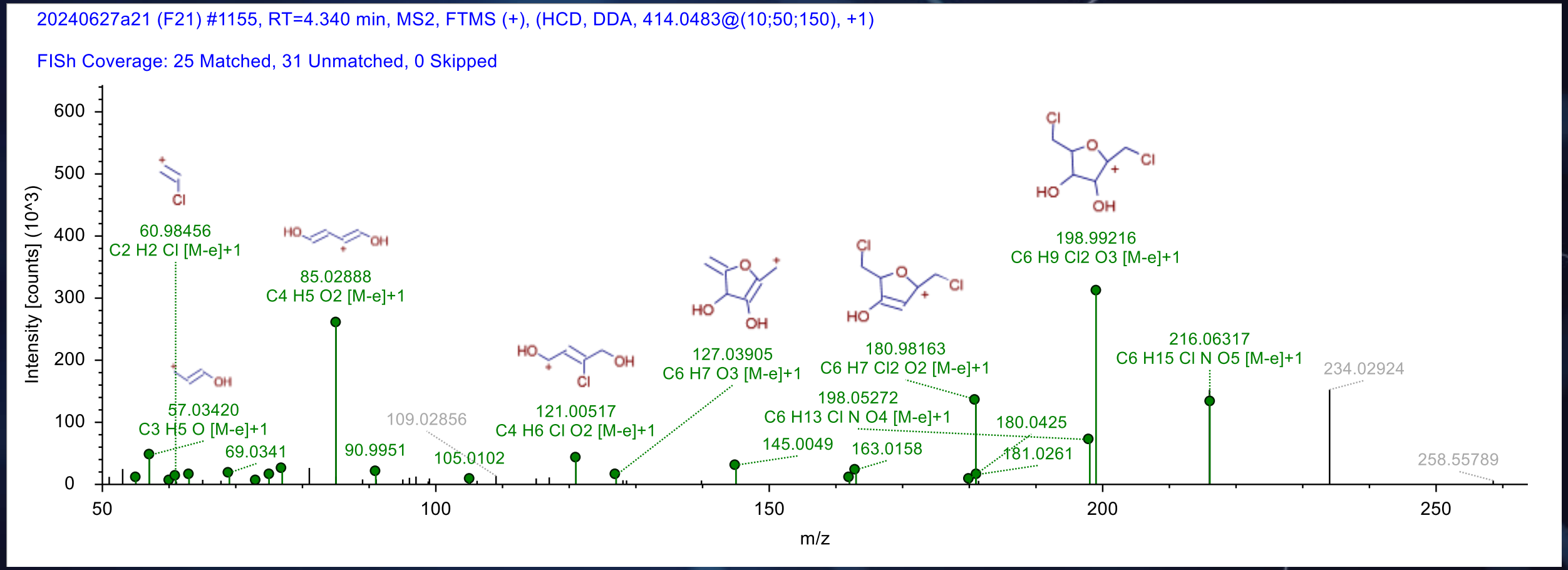
MS2 Spectra

Spectral Library Matches (Thermo mzCloud)



Complete NTA Workflow

MS2 Deconvolution – DeNovo methods



Annotation – Feature Identification

Annotation by Confidence Scale

- Each ID not JUST a chemical name – has an annotation similar to a qualifier
- Includes Confidence Scale
  - Schymanski (1 to 5) – straightforward but sometimes not as specific
  - Charbonnet (1(a – b), 2 (a – c), 3 (a – d), 4 (a – b), 5. Specific for PFAS or homologous series
  - Koelmel (A, A-, B+, B, B-, C+, C, C-, D+ D)
- Feature ID may be a class containing structural info (C2 benzenesulfonic acid)
- Feature ID may simply be a molecular formula (C20H40O2)
- Feature ID may only be a m/z value (obvious level 5)

Current NTA Improvements/Trends

- New quantitative models to estimate concentration
  - Estimate lowest/highest concentrations possible for risk-based assessments (EPA ORD).
  - Simple models for ionization efficiency (Quantem Analytics)
- Computational toxicology data becoming more readily available as models improve.
- Larger MS/MS libraries, more in-house libraries developed, more in-silico libraries
- Deconvolution of MS/MS data using machine learning or AI (ref?)
- Cross-vendor mass spectral libraries more characterized (Hoang et al)
- Retention time prediction models have been improving (we use Artificial Neural Net)
- Consensus based approaches to identifications has improved ID accuracy.
- Development of bodies (BP4NTA) to guide best practices for NTA for confidence scales
- Automate Annotation based on decision trees

Thank you

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