TraceFinder™ Software Overview and Implementation of a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer for the Identification and Quantification of Synthetic Cannabinoids in Postmortem Forensic Investigations

The Center for Forensic Science Research and Education

Taís Regina Fiorentin, PhD

Alex Krotulski, PhD

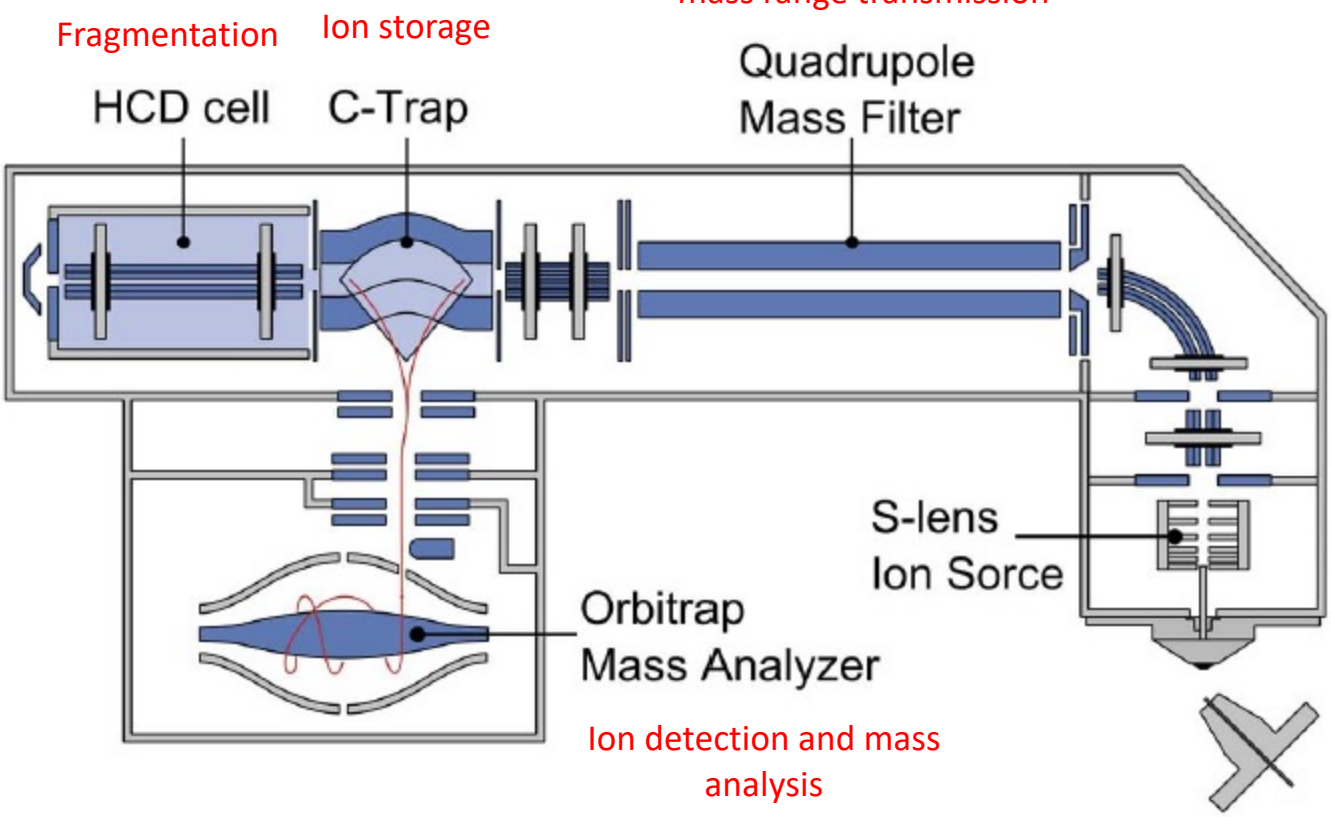
TRACEFINDER™ SOFTWARE OVERVIEW

Overview

- Q Exactive™ Hybrid Quadrupole-Orbitrap™
- Similar to triple quadrupole (QQQ) instruments
- Key differences:
  - Trapping
  - Selectivity and sensitivity
  - Mass resolution

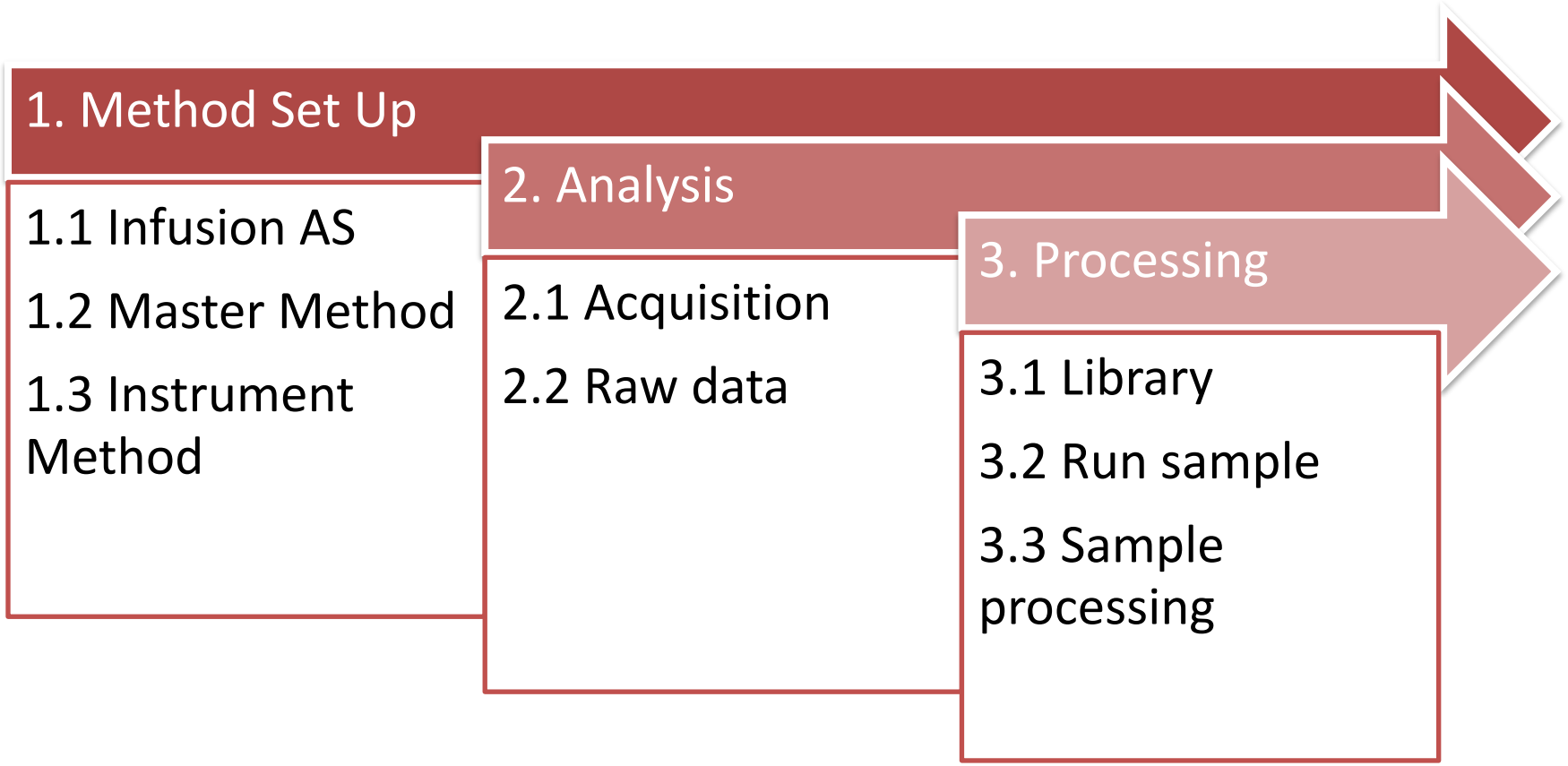
Overview

Either broad (10 to 100 Da) or narrow (0.4 to 2 Da) mass range transmission



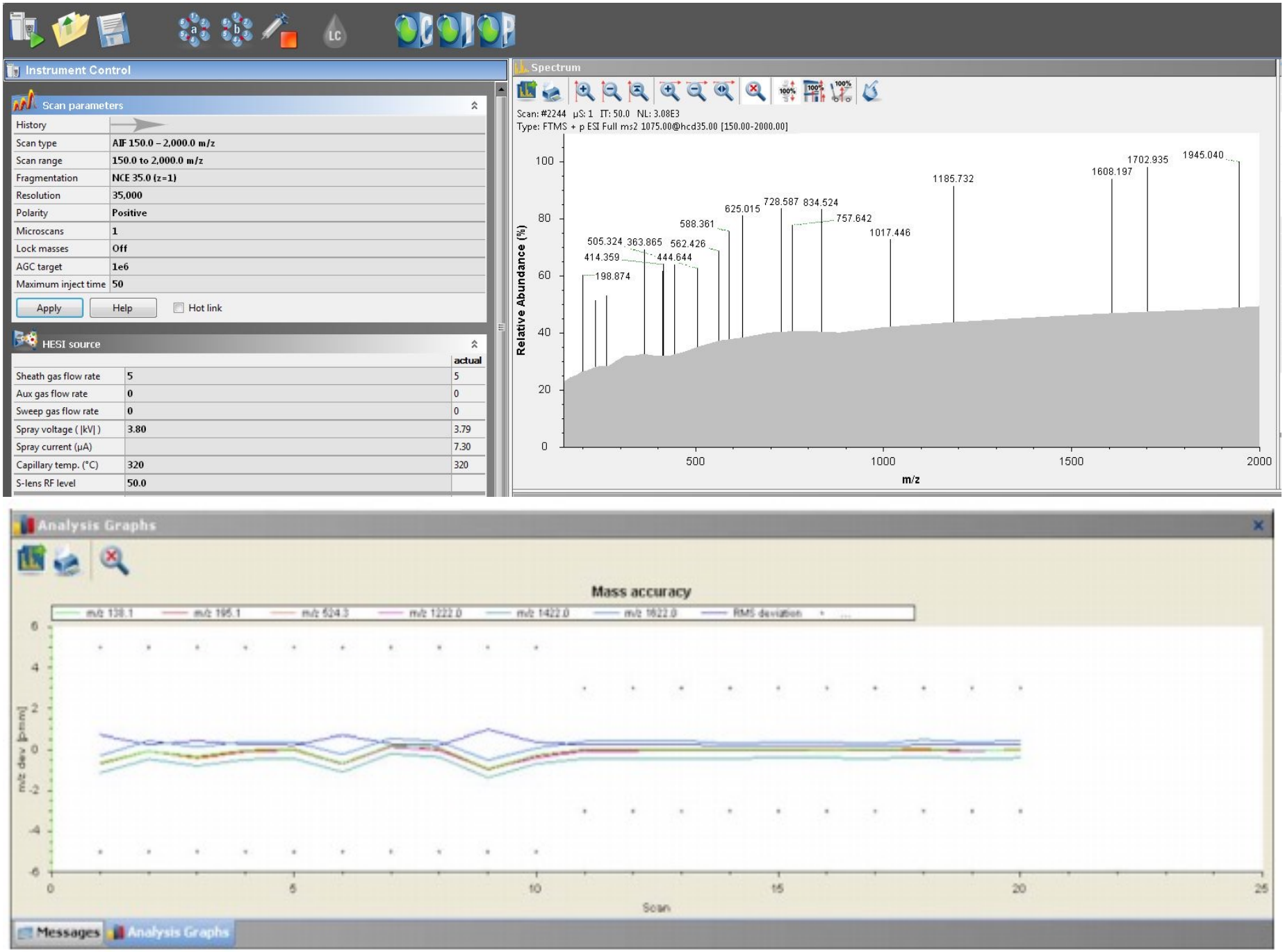
Ion detection and mass analysis

How to run a sample?



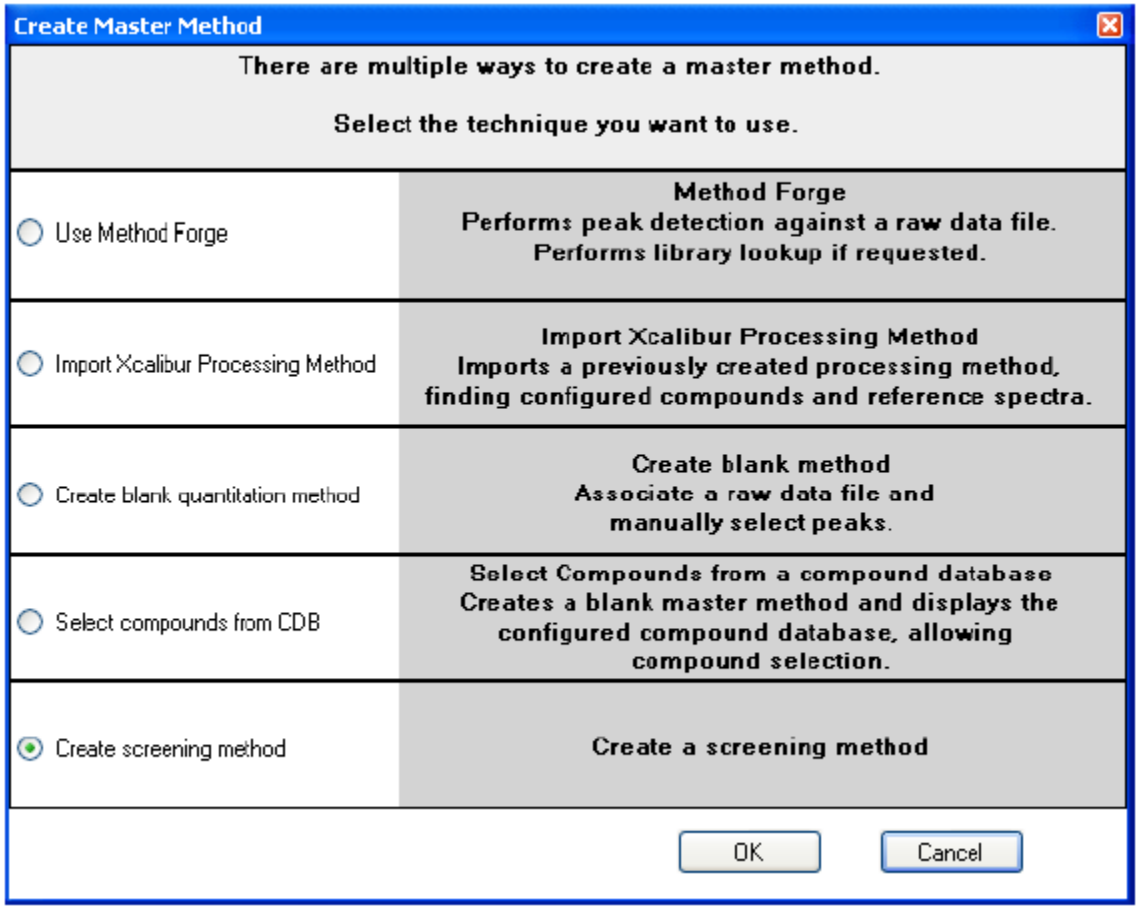
1. Method Setup

- Infusion
  - Direct injection in the MS module
  - Analytical standard of the target compound
  - Define polarity
  - Scan type: Full MS → AIF
  - Define collision energy


Instrument Control
Scan parameters
History
Scan type
AIF 150.0 – 2,000.0 m/z
Scan range
150.0 to 2,000.0 m/z
Fragmentation
NCE 35.0 (z=1)
Resolution
35,000
Polarity
Positive
Microscans
1
Lock masses
Off
AGC target
1e6
Maximum inject time
50
Apply
Help
Hot link
HESI source
actual
Sheath gas flow rate
5
5
Aux gas flow rate
0
0
Sweep gas flow rate
0
0
Spray voltage (|kV|)
3.80
3.79
Spray current (µA)
7.30
Capillary temp. (°C)
320
320
S-lens RF level
50.0
Spectrum
Scan: #2244 µS: 1 IT: 50.0 NL: 3.08E3
Type: FTMS + p ESI Full ms2 1075.00@hcd35.00 [150.00-2000.00]
Relative Abundance (%)
m/z
198.874
414.359
505.324
363.865
562.426
444.644
588.361
625.015
728.587
834.524
757.642
1017.446
1185.732
1608.197
1702.935
1945.040
Analysis Graphs
Mass accuracy
m/z dev [ppm]
Scan
Messages
Analysis Graphs

1. Method Setup

- Master method



Indicated for Full scan analysis with long list of compounds

Import from Xcalibur

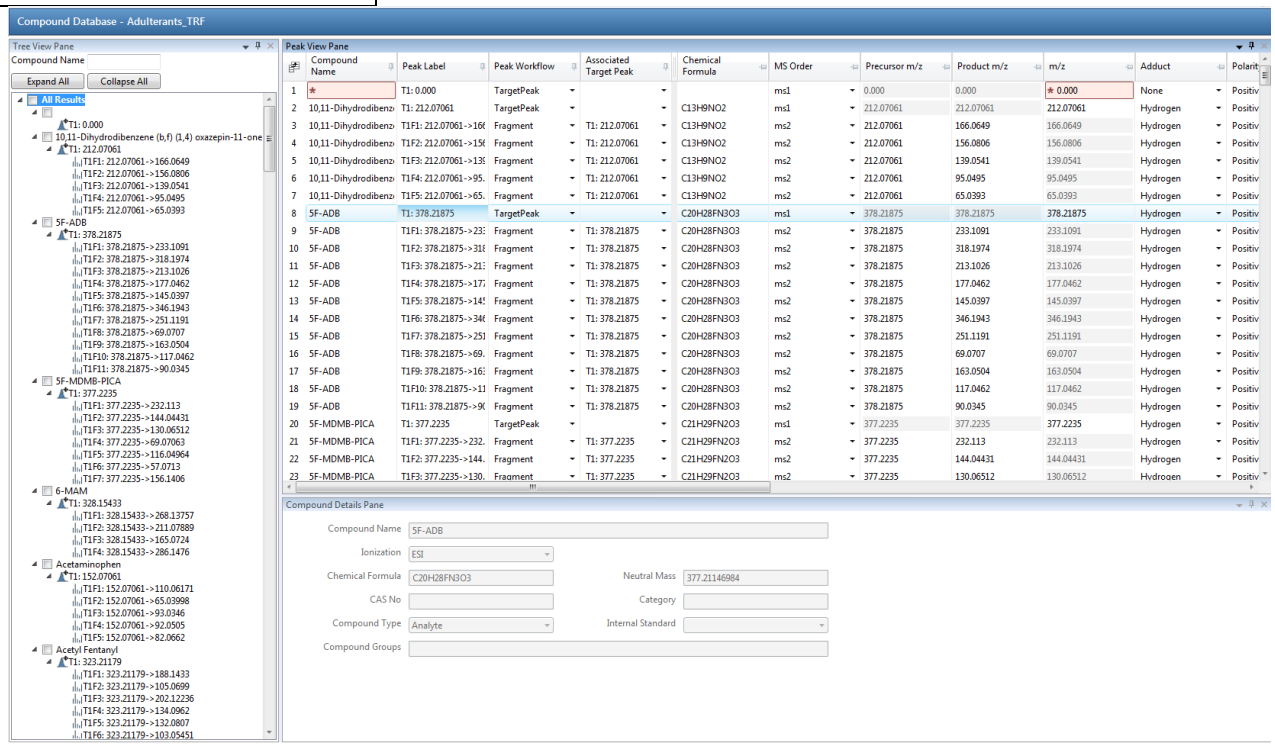
Requires more manual interaction with the software

Select MRM transitions from an available datastore

1. Method Setup

- Master method
  - Instrument Method
  - Compound Database
  - Processing Parameters

- Imported and exported as .xlsx files
- Easily transferred to other instruments in your network to minimize time-consuming method development activities



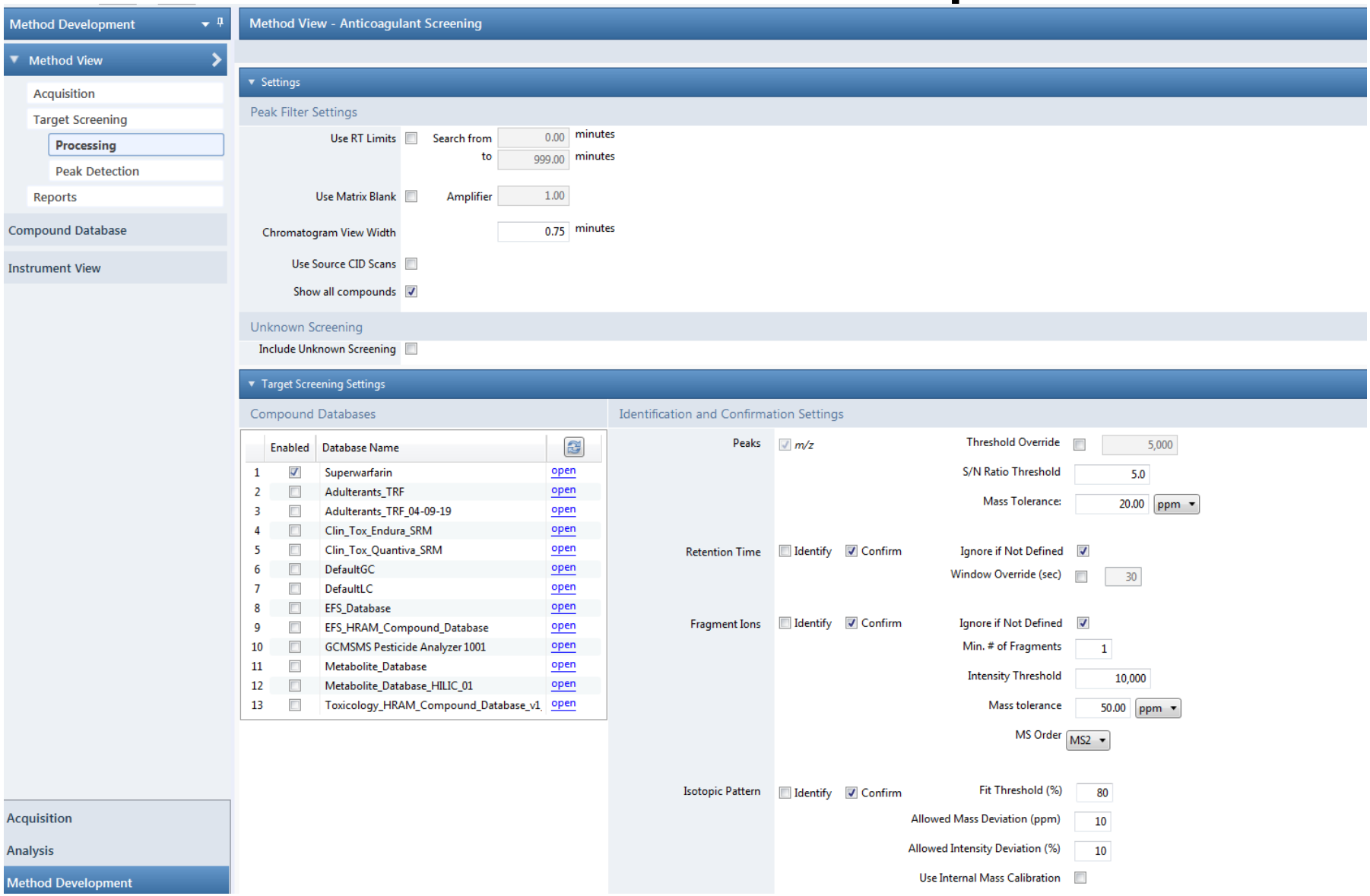
1. Method Setup

- Master method
  - Instrument Method
  - Compound Database
  - Processing Parameters

Define Thresholds for:

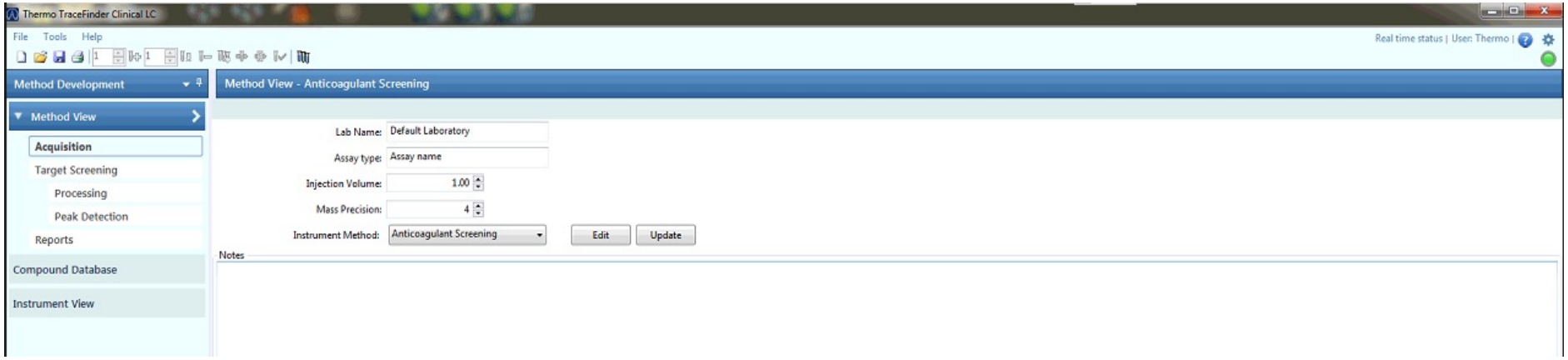
- Mass tolerance (ppm)
- Retention time window
- Fragment ions (intensity, number of fragments, mass tolerance)
- Isotopic Pattern (%)
- Library search

1. Method Setup



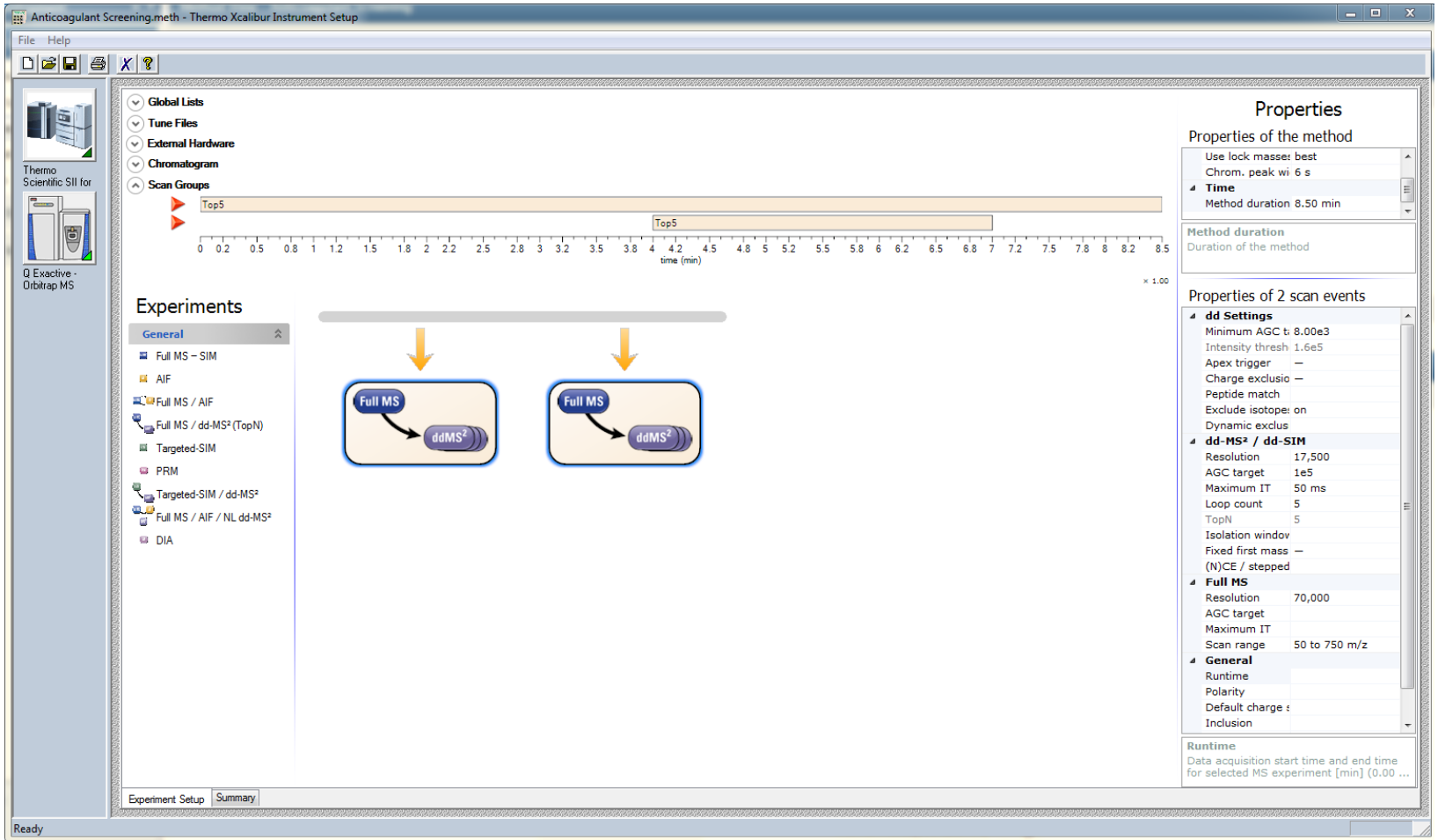
1. Method Setup

- Master method
  - Instrument Method
  - Compound Database
  - Processing Parameters



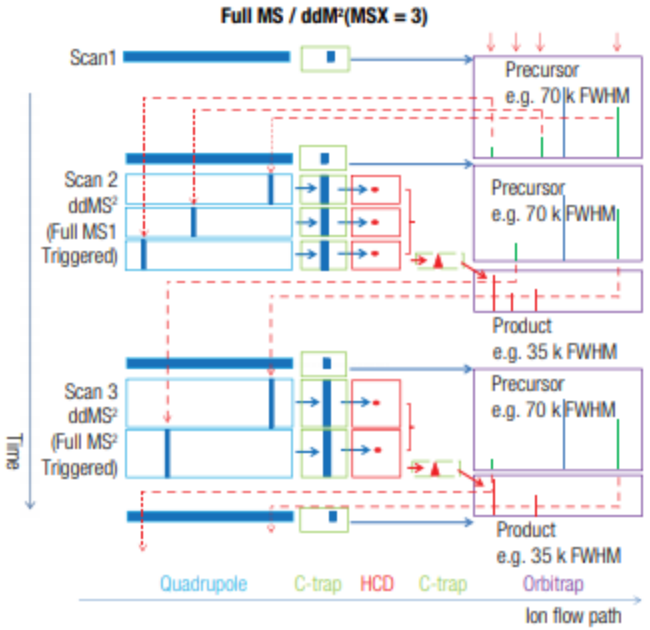
1. Method Setup

- Instrument method
  - Types of experiments:



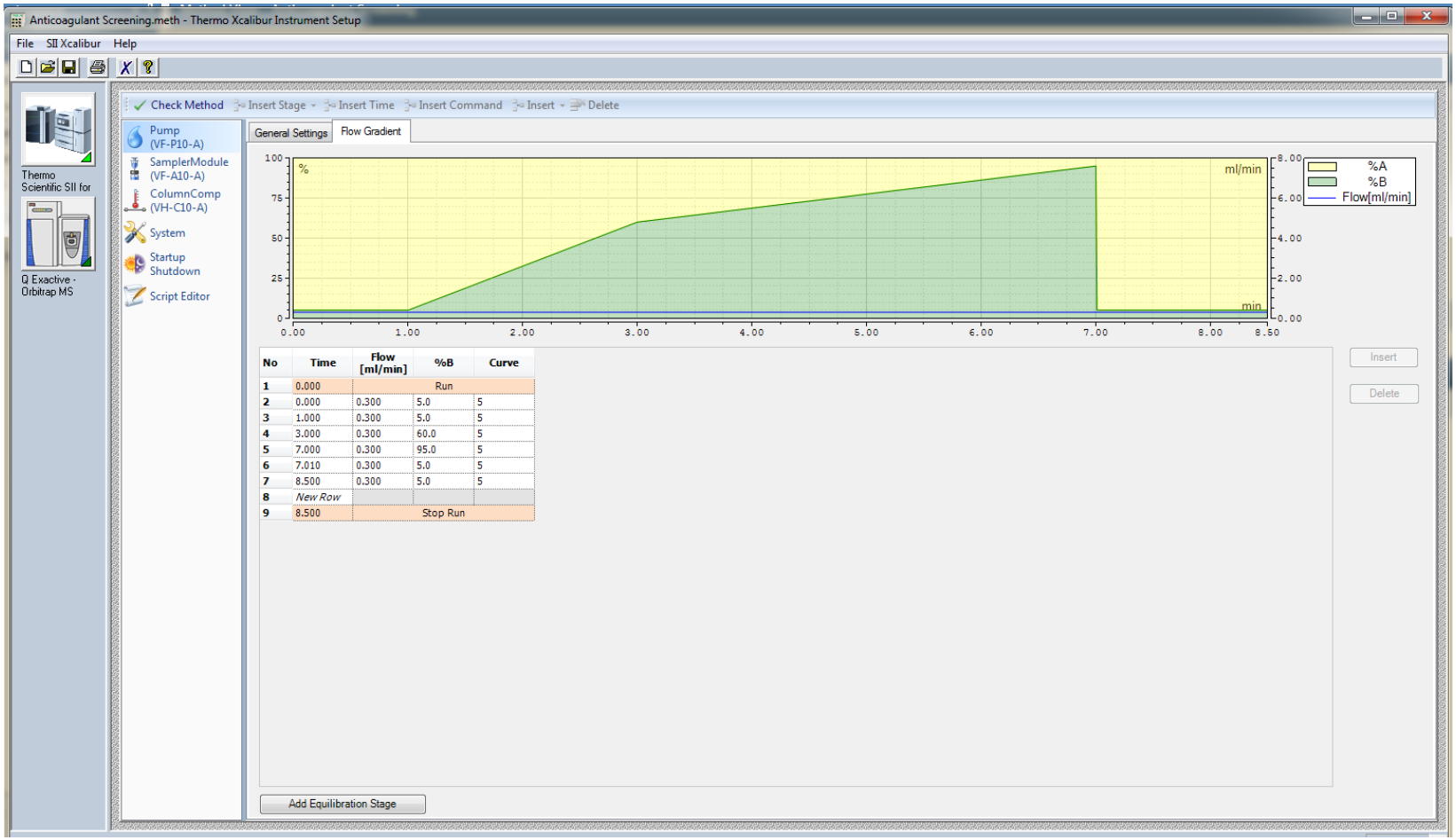
1. Method Setup

- Instrument method
  - Full MS-SIM
  - AIF
  - Full MS/AIF
  - Full MS/ddMS^2
  - Targeted-SIM
  - PRM
  - Targeted-SIM/ddMS^2
  - Full MS/AIF/ddMS^2
  - DIA



1. Method Setup

- Instrument method
  - As general as possible to start:



1. Method Setup

- Instrument method
  - Inclusion list:

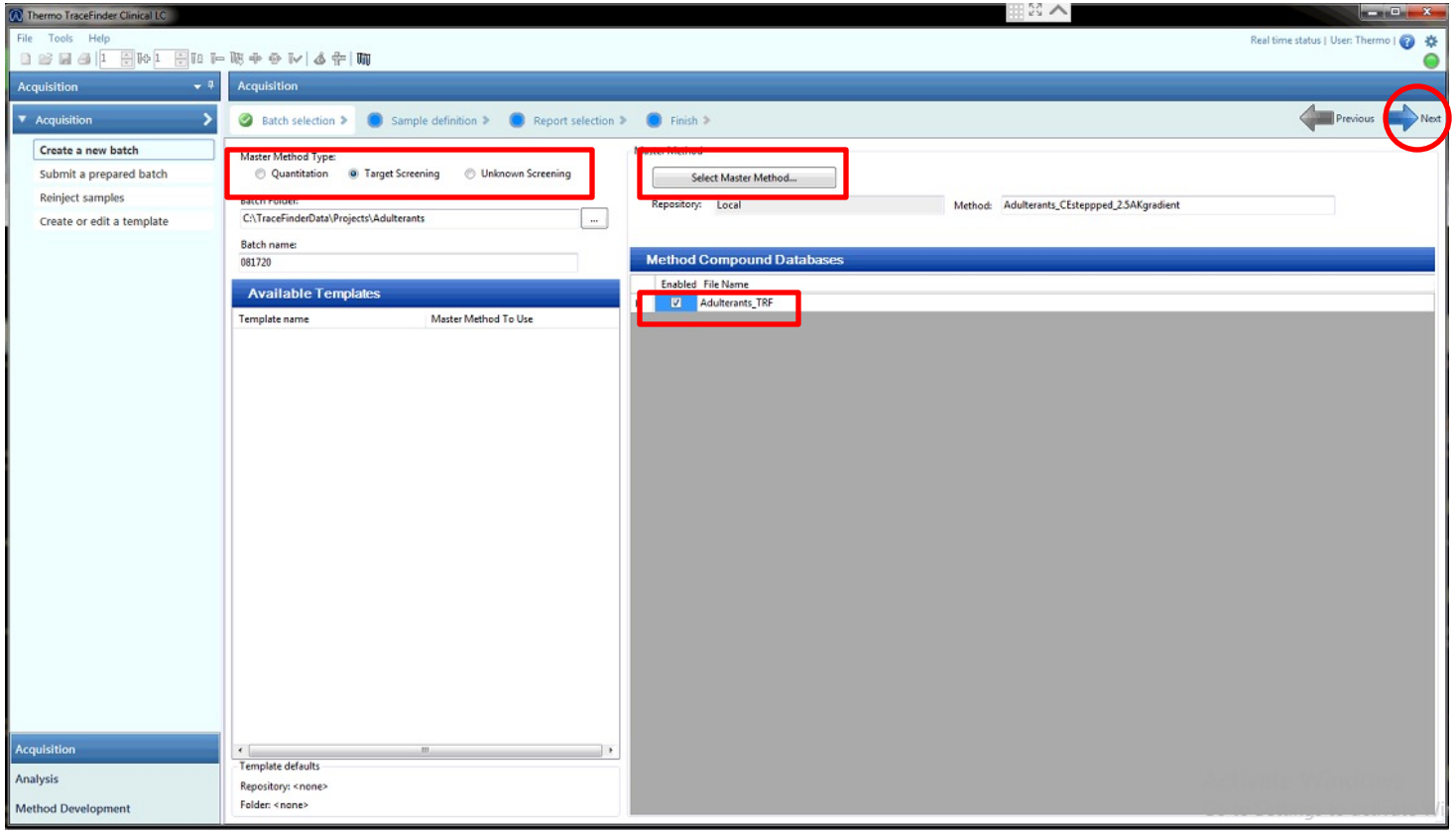
Method editor — Inclusion List

File Edit Help Done

	Mass [m/z]	Formula [M]	Species	CS [z]	Polarity	Start [min]	End [min]	(N)CE	MSX ID	Comment
1	445.17982	C31H24O3	+ H	1	Positive					Difenacoum
2	543.17777	C33H25F3O4	+ H	1	Positive					Flocoumafen
3	293.11722	C19H16O3	+ H	1	Positive					Coumatetralyl
4	231.10157	C14H14O3	+ H	1	Positive					Pindone
5	309.11214	C19H16O4	+ H	1	Positive					Warfarin
6	341.11722	C23H16O3	+ H	1	Positive					Diphacinone
7	523.09033	C31H23BrO3	+ H	1	Positive					Brodifacoum
8	539.06749	C31H23BrO2S	+ H	1	Positive					Difethialone
9	375.07825	C23H15ClO3	+ H	1	Positive					Chlorophacinone
10	525.07070	C30H23BrO4	- H	1	Negative					Bromadiolone
11	509.07578	C30H23BrO3	- H	1	Negative					Bromadiolone Breakdown
12	527.11544	C31H19D4Br...	+ H	1	Positive					Brodifacoum D4
13										

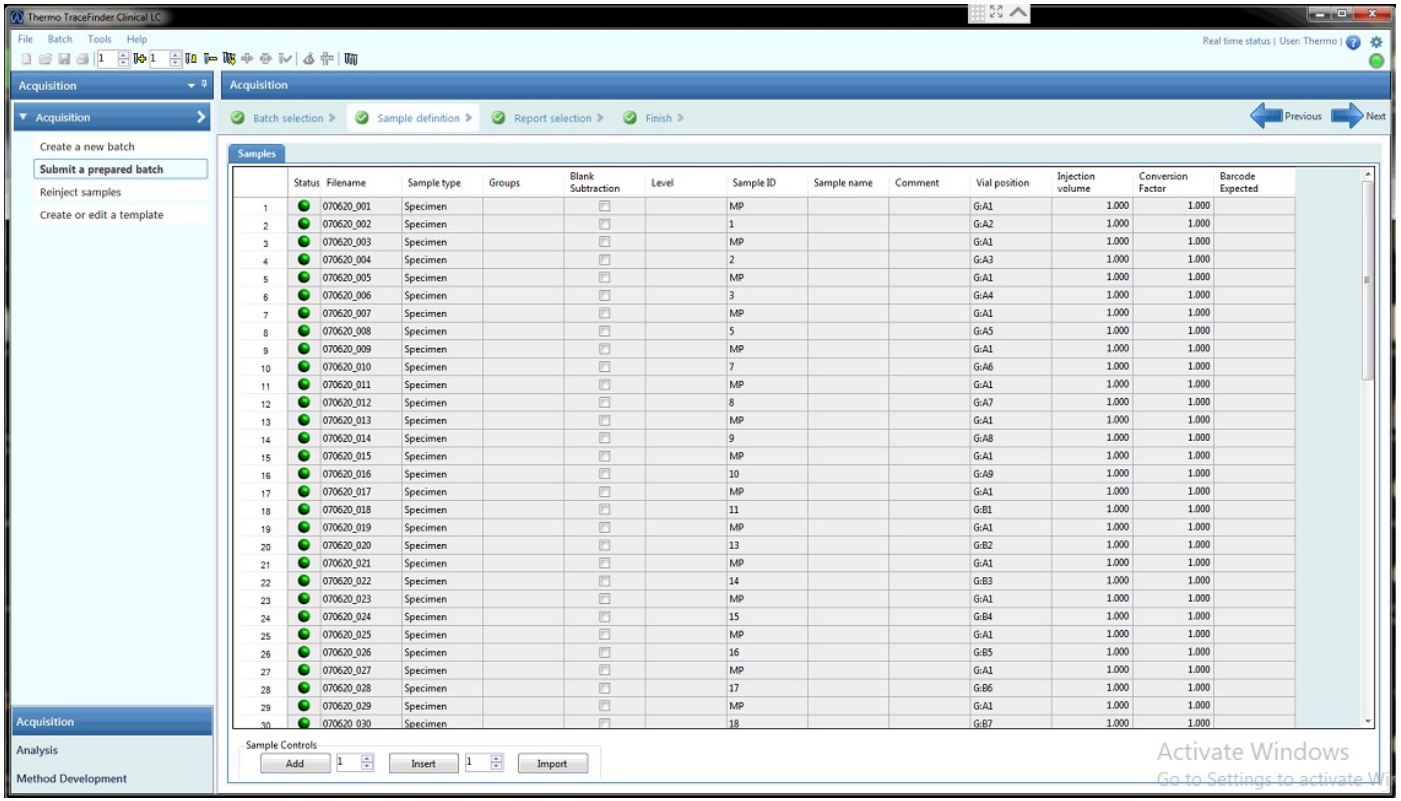
2. Analysis

- Acquisition



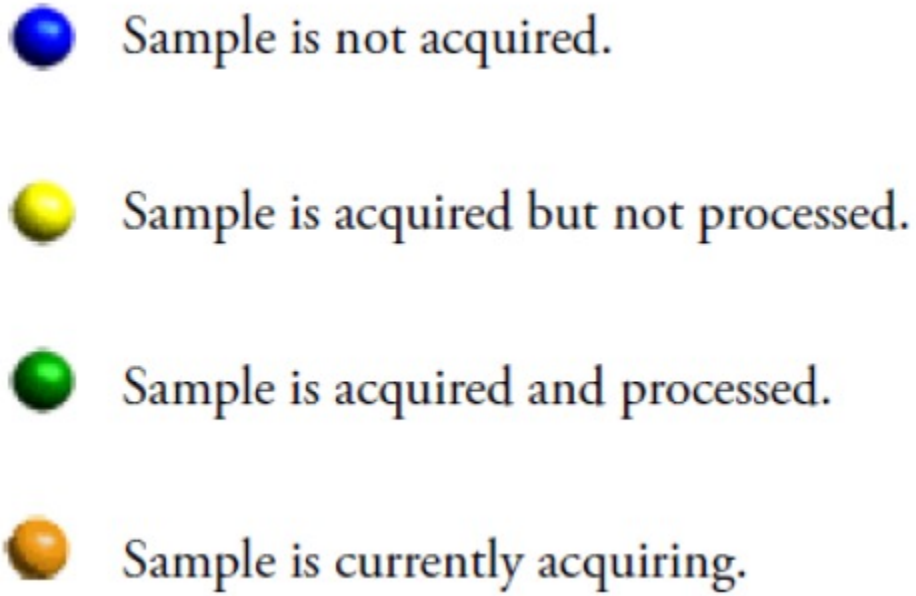
2. Analysis

- Acquisition



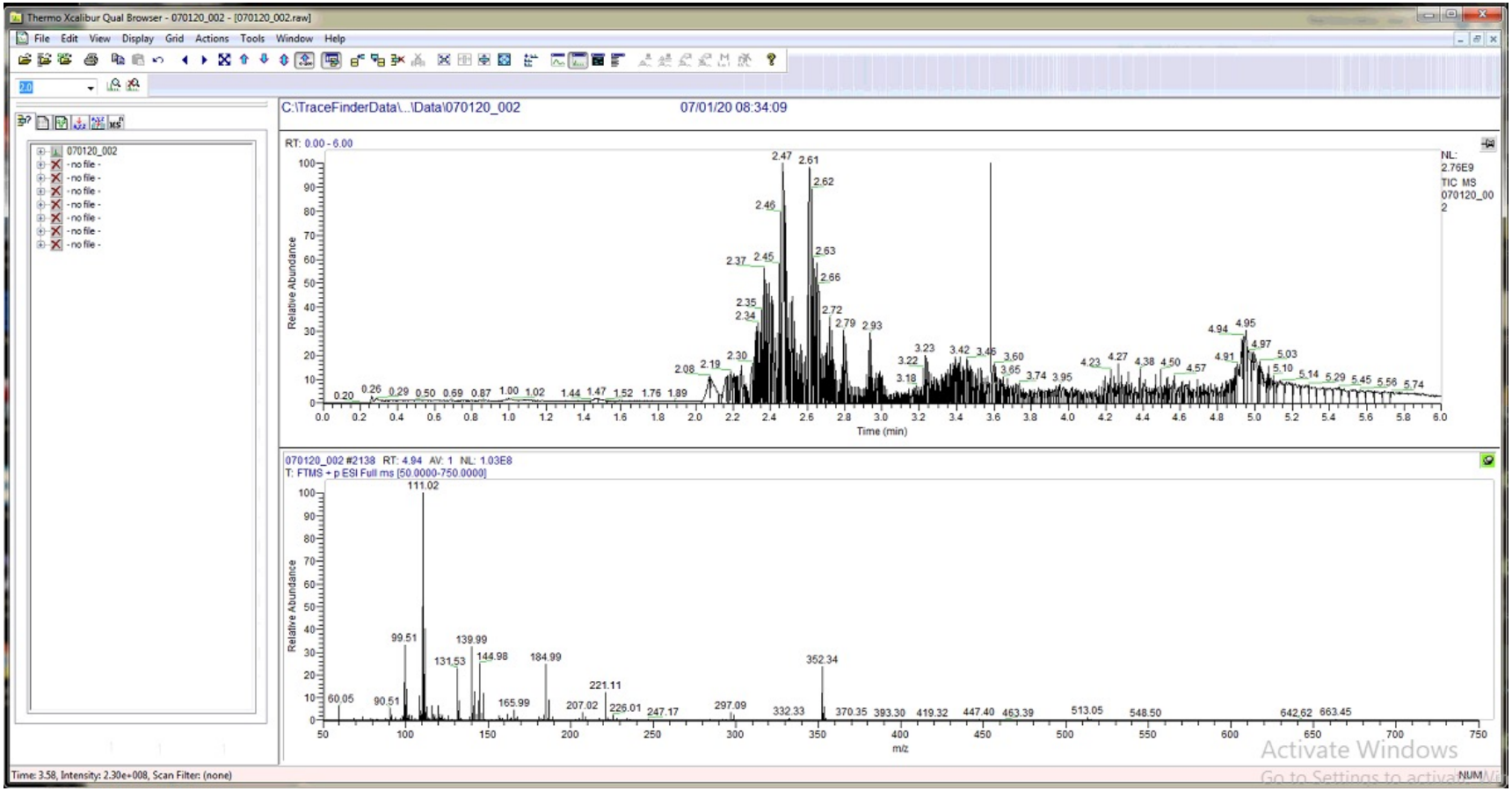
2. Analysis

- Acquisition
  - Batch view: status indicator show the current status of each sample during the acquisition and processing



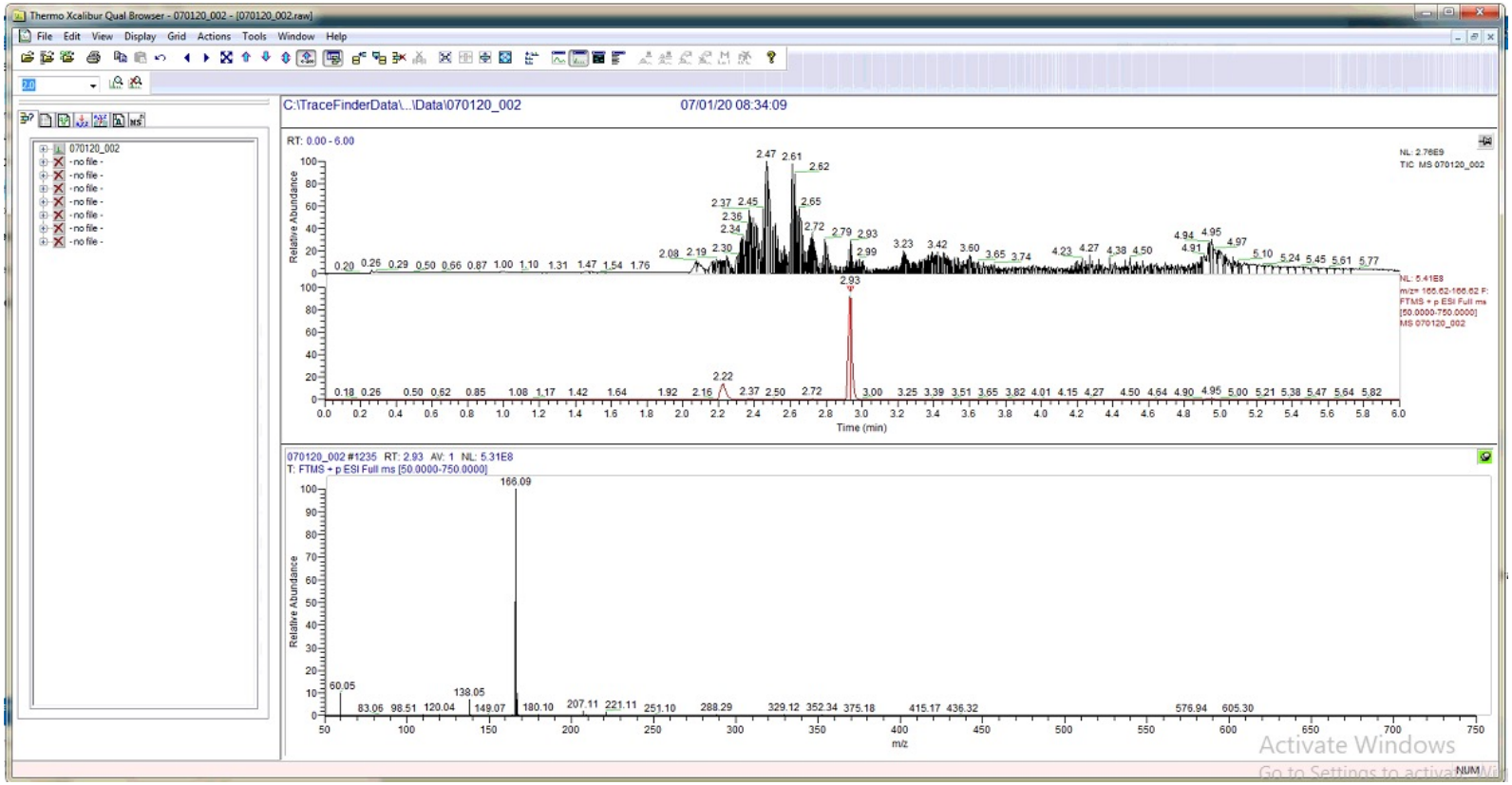
2. Analysis

- Raw Data



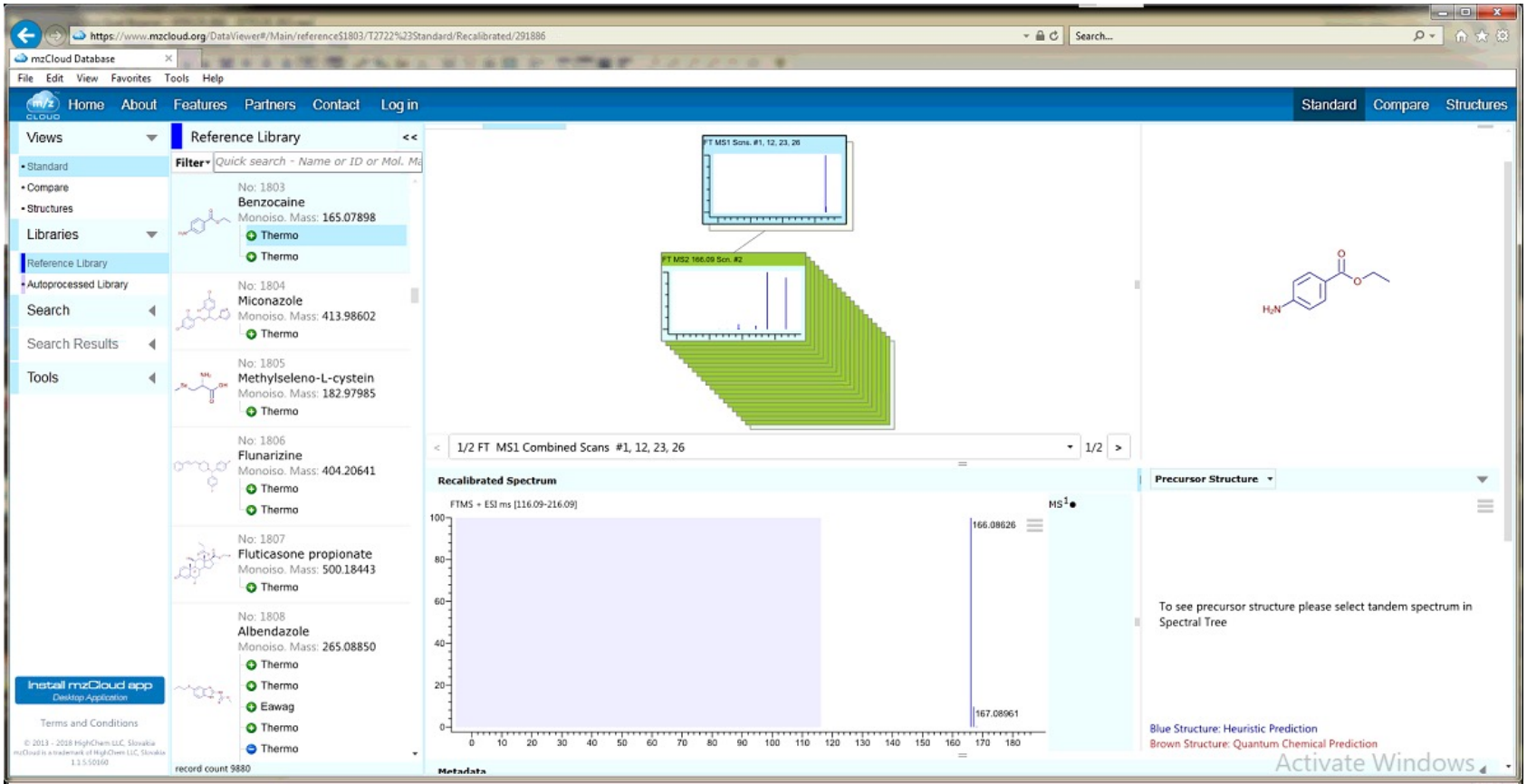
2. Analysis

- Raw Data



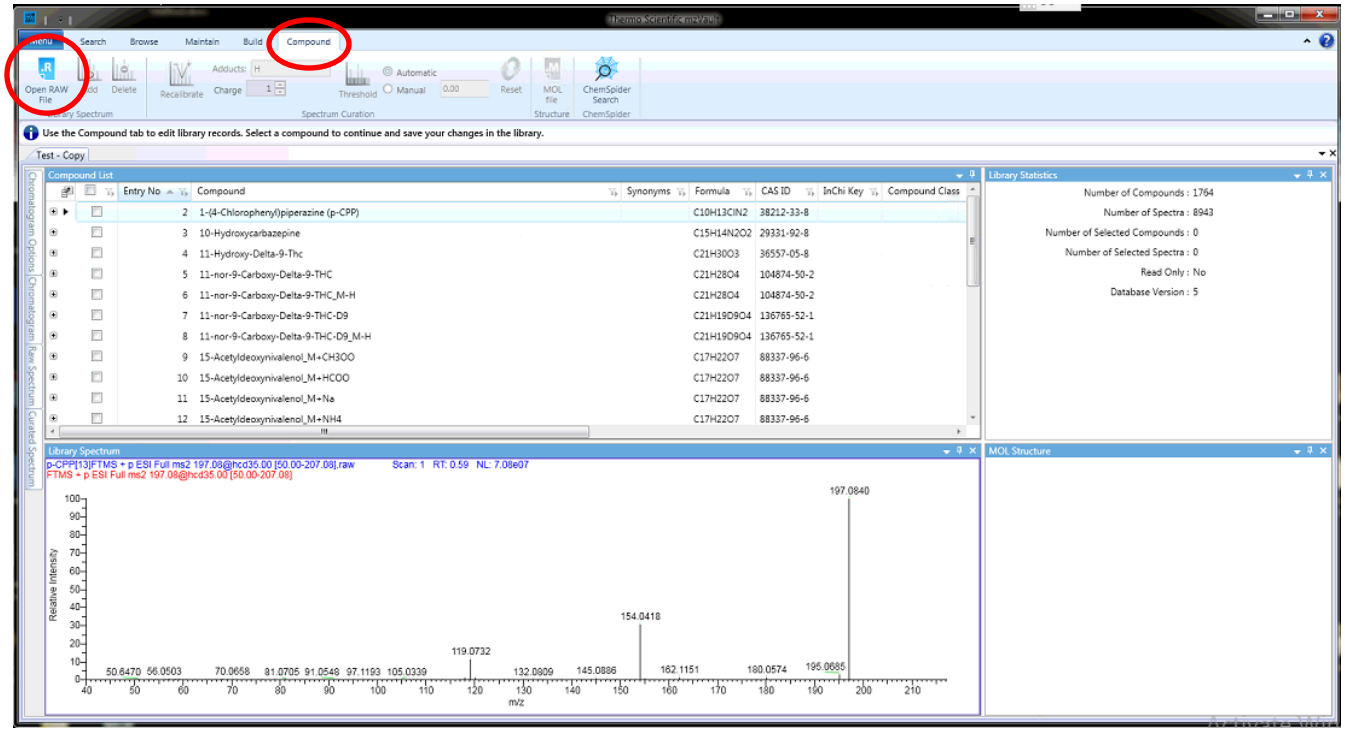
2. Analysis

- Raw Data
  - mzCloud Database



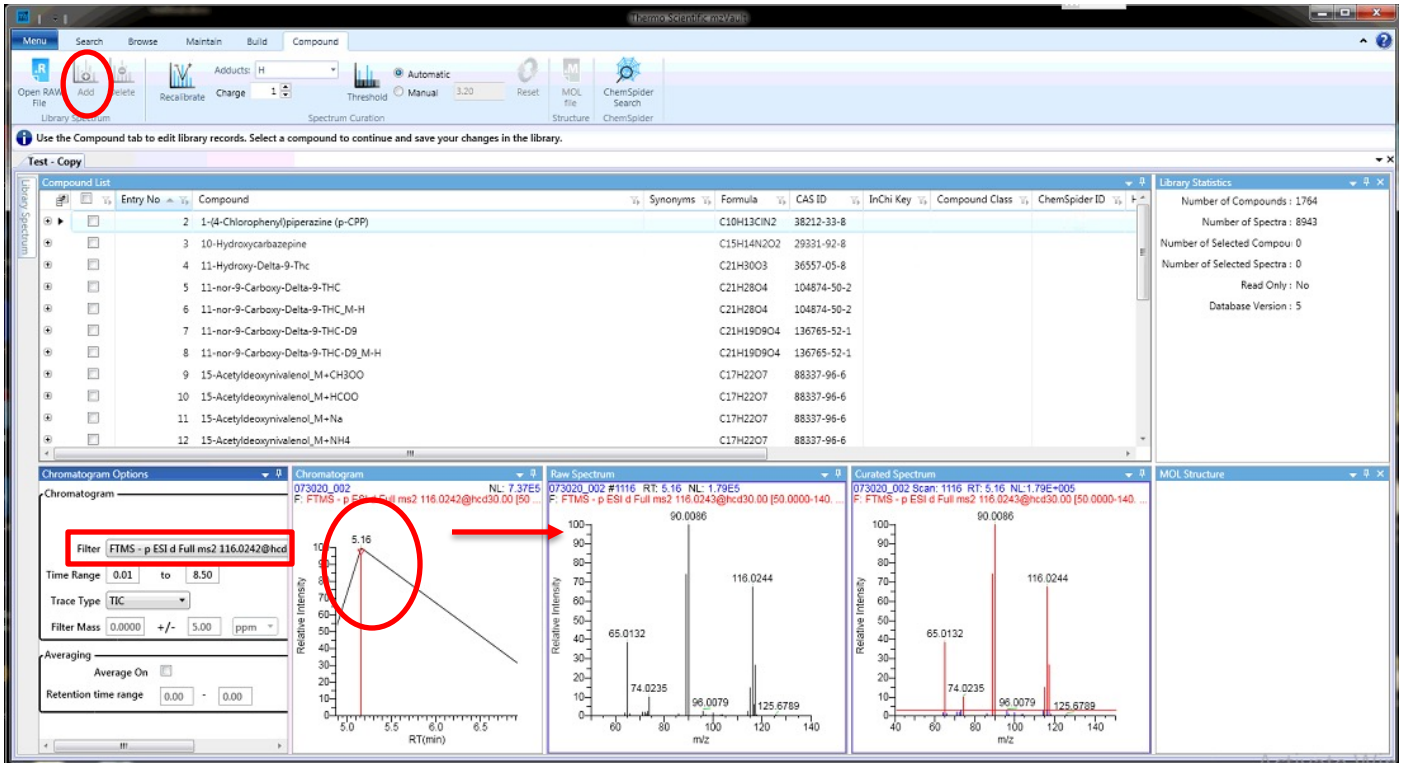
3. Processing

- Library
  - Open Thermo mz/Vault
  - Compound > open raw file



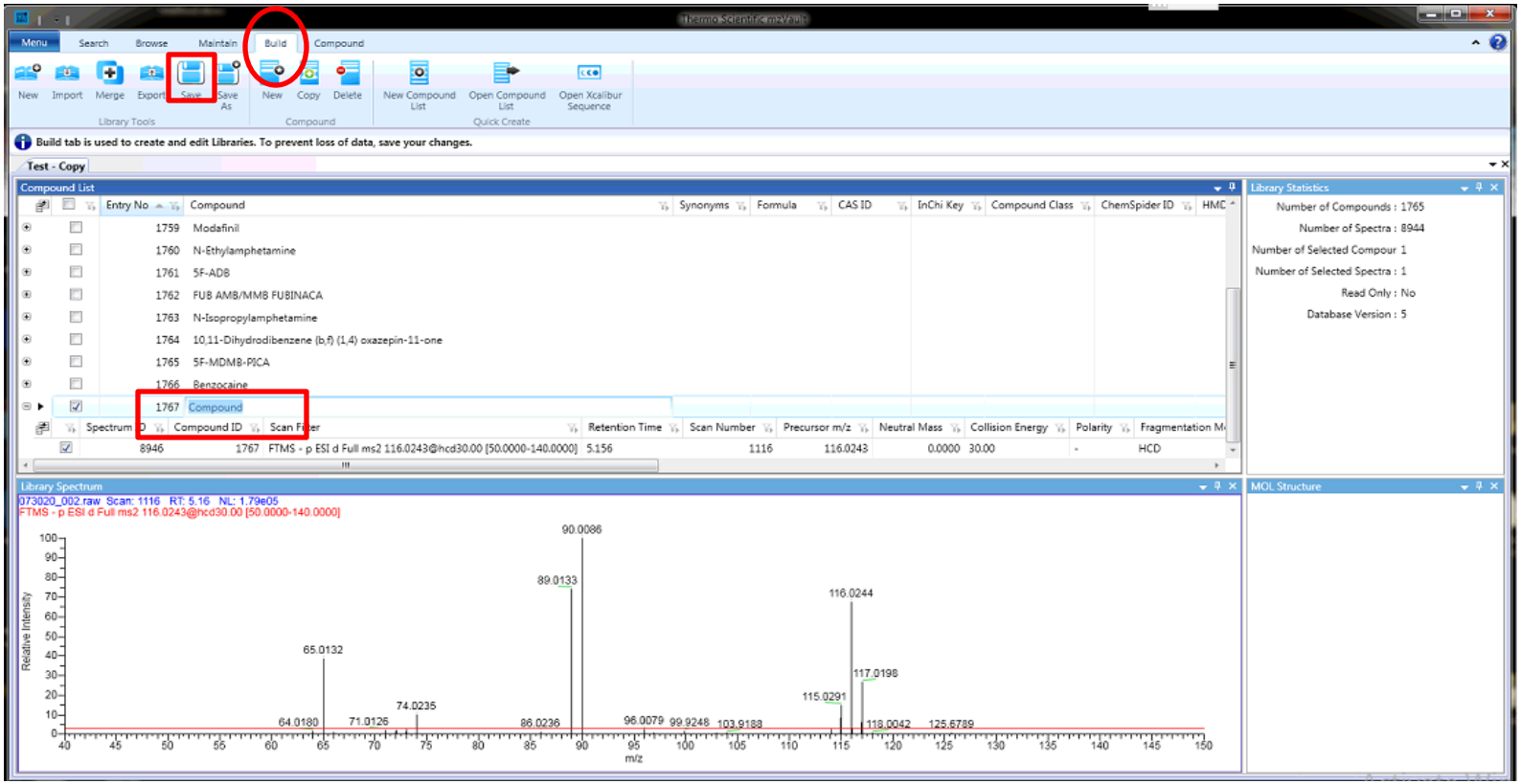
3. Processing

- Library
- Filter mass > select mass spec > add



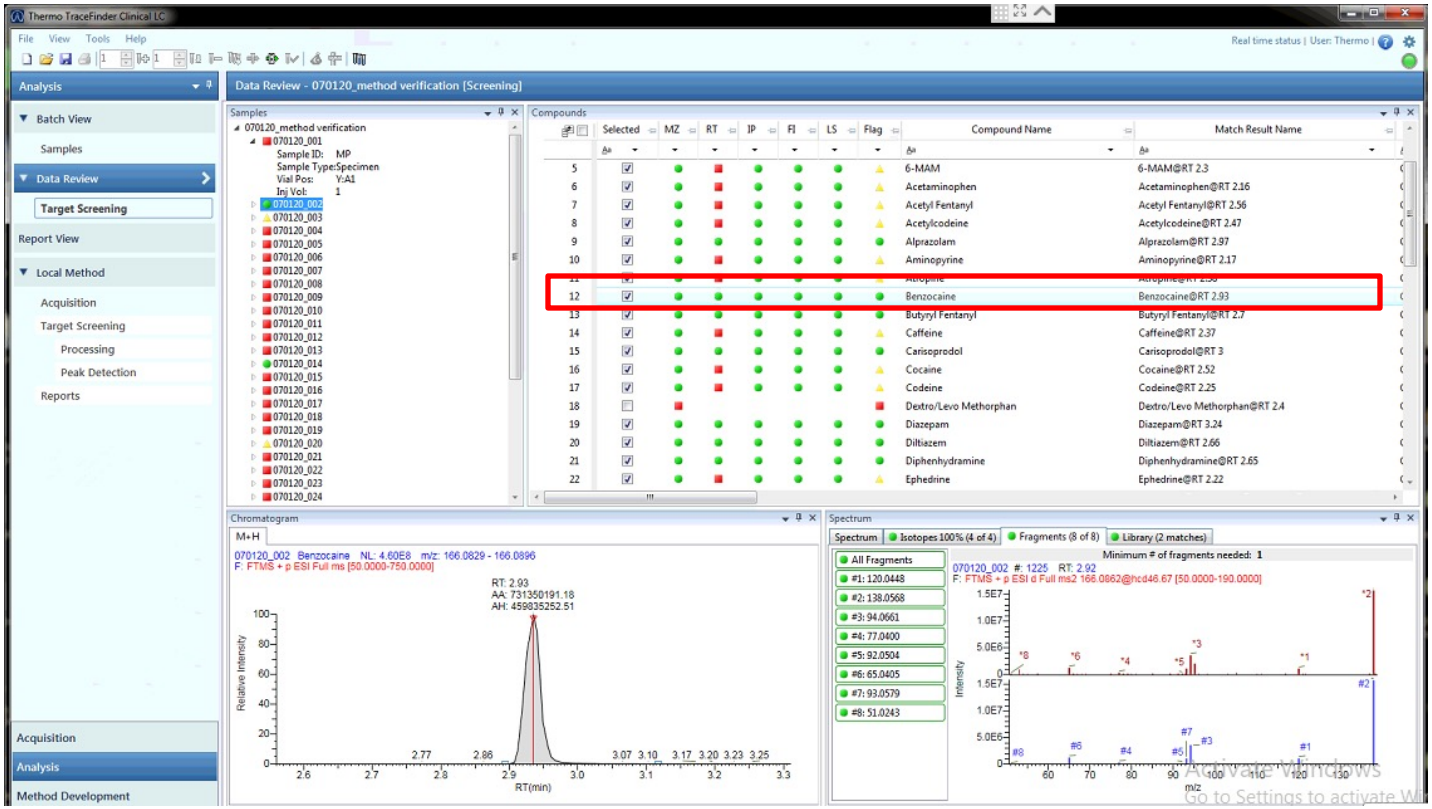
3. Processing

- Library
- Build > Name compound > Save



3. Processing

- Run/Sample processing



IMPLEMENTATION FOR SYNTHETIC CANNABINOIDS IN POSTMORTEM FORENSIC INVESTIGATIONS

WHAT ARE SYNTHETIC CANNABINOIDS (SC)?

- Man made compounds intended to research the interactions between cannabinoids and their receptors and possible therapeutic value
- These compounds are pharmacologically modeled after the main psychoactive constituent of delta-9-tetrahydrocannabinol (THC)
- In the early 2000's, SC were detected in plant material commonly sold as 'herbal incense' and it became known that they were being used as novel psychoactive substances (NPS)

PREVALENCE OF SC

- SC have become one of the largest groups of NPS monitored by the European Monitoring Center for Drugs and Drug Addiction (EMCDDA)
- As of 2017, 179 different SC compounds have been identified

WHY ARE THEY BAD?

- SC can often have an increased toxicity over THC
- Smoking THC can produce mild acute effects; however, it very rarely causes the adverse effects observed rather commonly with similar use of SC
- SC can produce multiple active metabolites, which increase the compounds overall toxicity
- The constant evolution of SC make them difficult to keep up with

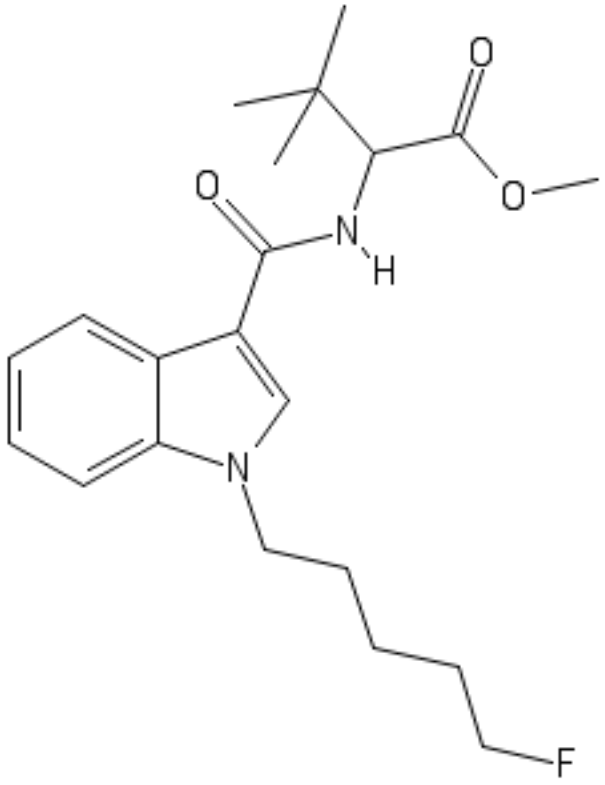
5F-MDMB-PICA

METHYL 2-[[1-(5-FLUOROPENTYL) INDOLE-3-CARBONYL]AMINO]-3,3- DIMETHYL-BUTANOATE

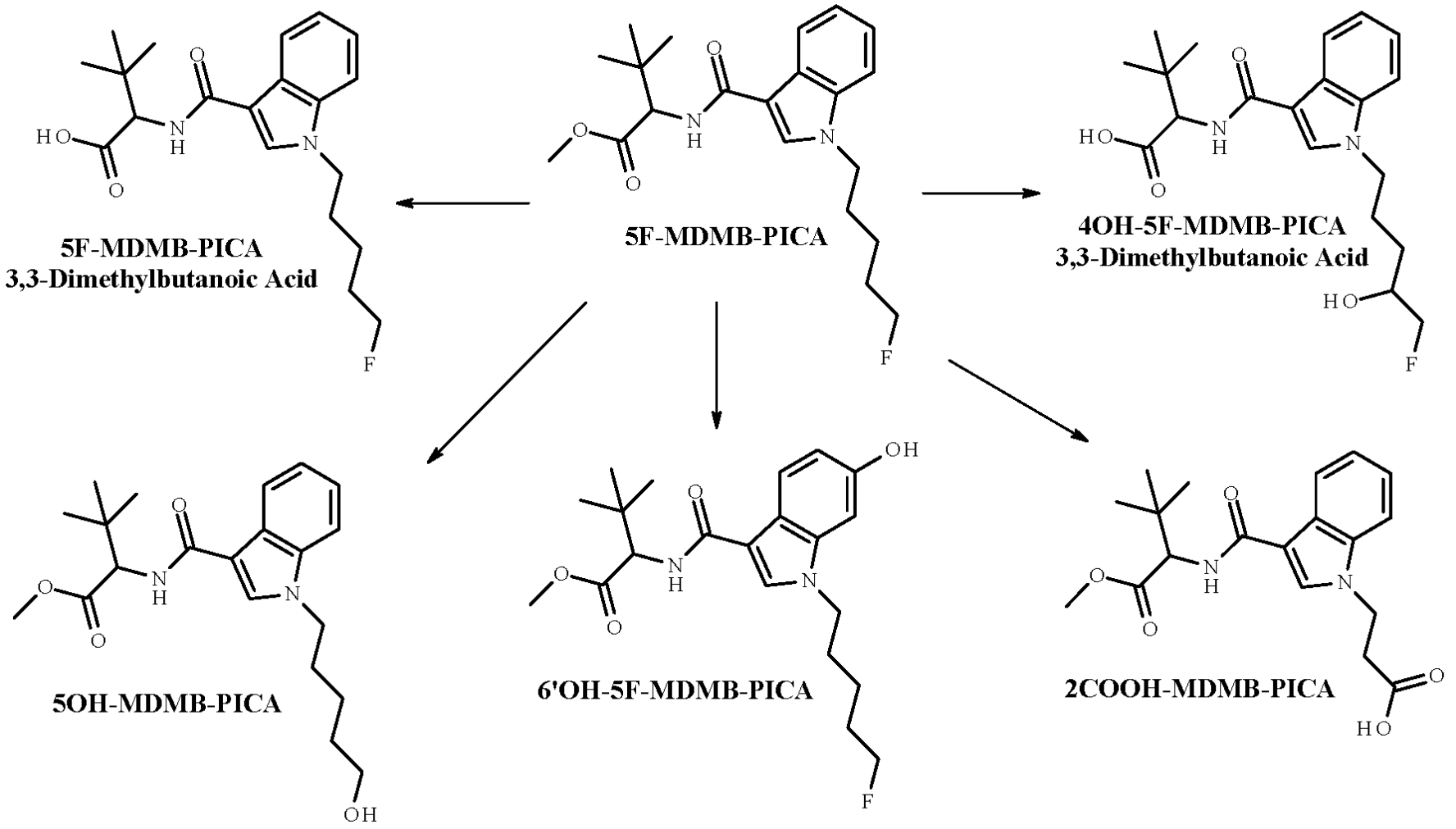
5F-MDMB-2201

5F-MDMB-PICA

- Synthesized while studying novel synthetic cannabinoids with similar structures in order to study efficacy and structure activity relationships
- First detected in as an ingredient in seized material in Belgium in November of 2016
- 5F-MDMB-PICA was identified in biological specimen by the Center for Forensic Science Research and Education (CFSRE) and the National Forensic Laboratory Information System (NFLIS) beginning in 2018
- It has since become the number one identified SC throughout 2019 and into 2020



METABOLITES



METHOD DEVELOPMENT

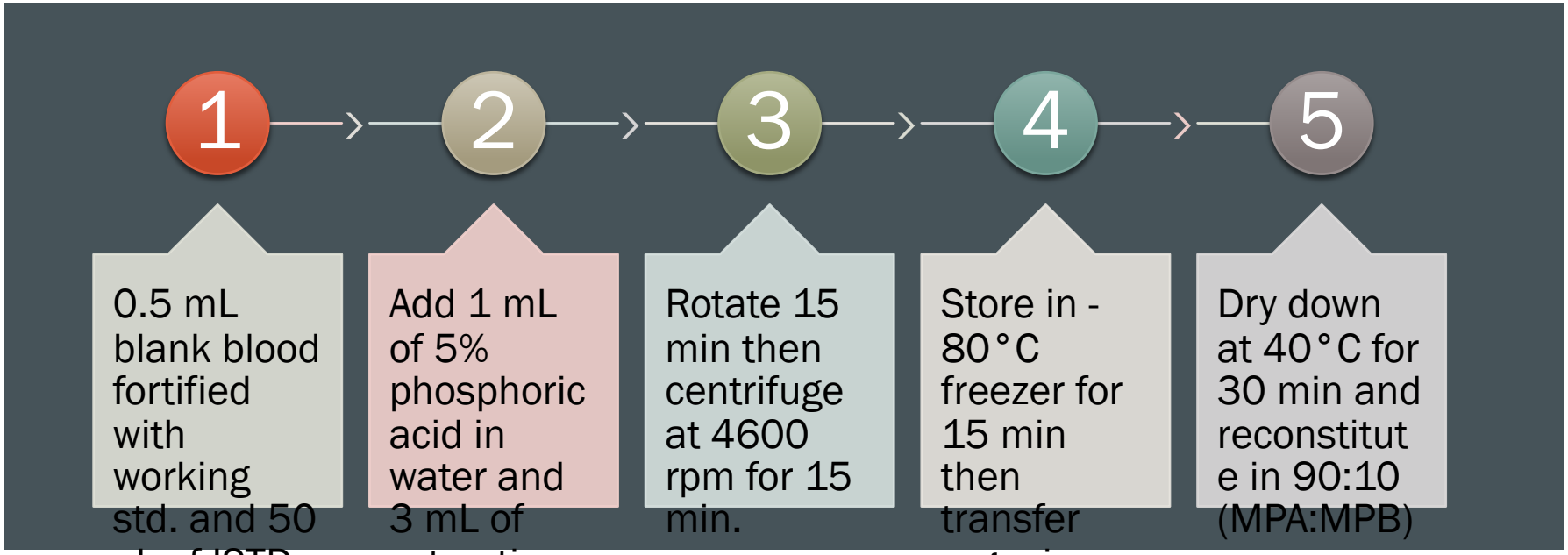
INSTRUMENTATION

- Thermo Scientific™ Vanquish™ UHPLC coupled with a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ mass spectrometer (LC-QE-MS)
- Data was evaluated using TraceFinder Clinical software version 4.1
- Column: Thermo Fisher Accu Van C18+ column (100 x 2.1mm, 2.6µm)
- Mobile Phase A: 0.1% formic acid in water
- Mobile Phase B: 0.1% formic acid in acetonitrile

CONSIDERATIONS

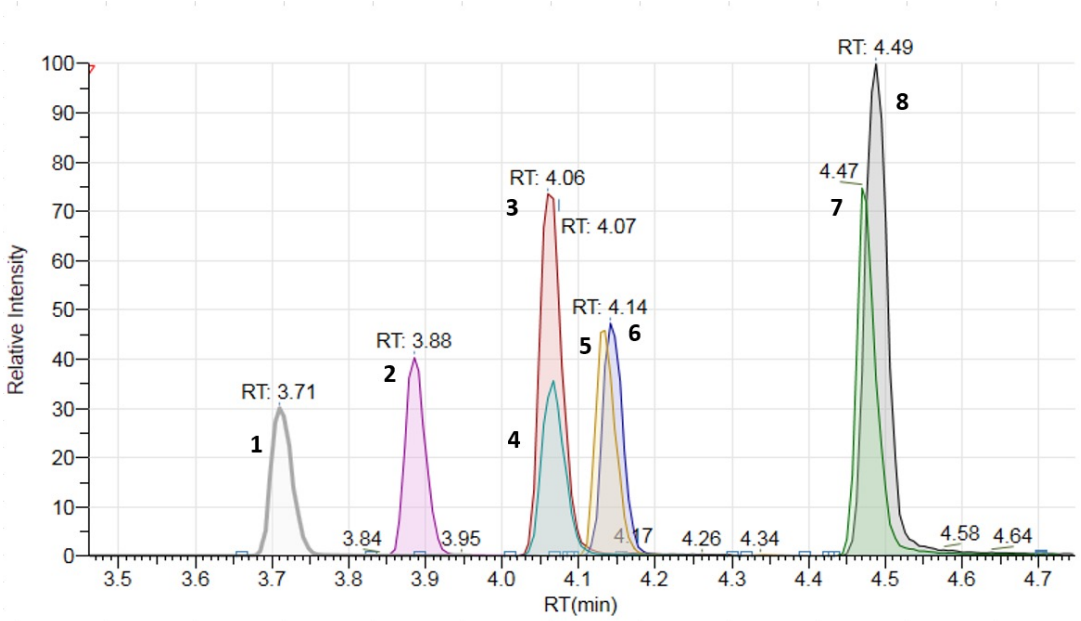
- Acquisition Parameters
- Mobile Phase Gradient
- Extraction technique and optimization
- Calibration curve

EXTRACTION PROCEDURE



FINAL CHROMATOGRAPHY

- From left to right 1: 4OH-5F-MDMB-PICA-3,3-dimethylbutanoic acid, 2: 2-COOH-MDMB-PICA, 3: 5OH-MDMB-PICA, 4: 6'OH-5F-MDMB-PICA, 5: 5F-MDMB-PICA-3,3-dimethylbutanoic acid-d5, 6: 5F-MDMB-PICA-3,3-dimehtylbutanoic acid, 7: 5F-MDMB-PICA-d5, 8: 5F-MDMB-PICA.



METHOD VALIDATION

VALIDATION METHOD

The method developed was validated in accordance with the AAFS Standards Board (ASB) Standard Practices for Method Validation in Forensic Toxicology

Testing included:

- Bias/ Precision
- Limit of detection (LOD)
- Limit of quantitation (LOQ)
- Carryover
- Interferences
- Dilution integrity
- Processed sample stability
- Ionization suppression/ enhancement
- Recovery

RESULTS

- Bias, precision, ionization suppression, and the calibration model were acceptable
- LOD/LOQ = 0.5 ng/mL
- Carryover limit = 200 ng/mL
- No interferences detected
- Samples were stable for 4 days after extraction*
- Dilution integrity did not have acceptable results for all analytes
- Recovery was acceptable (>70%) for 4 of the 6 analytes

APPLICATION TO AUTHENTIC SAMPLES