

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

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# **TRACEFINDER™ SOFTWARE OVERVIEW**

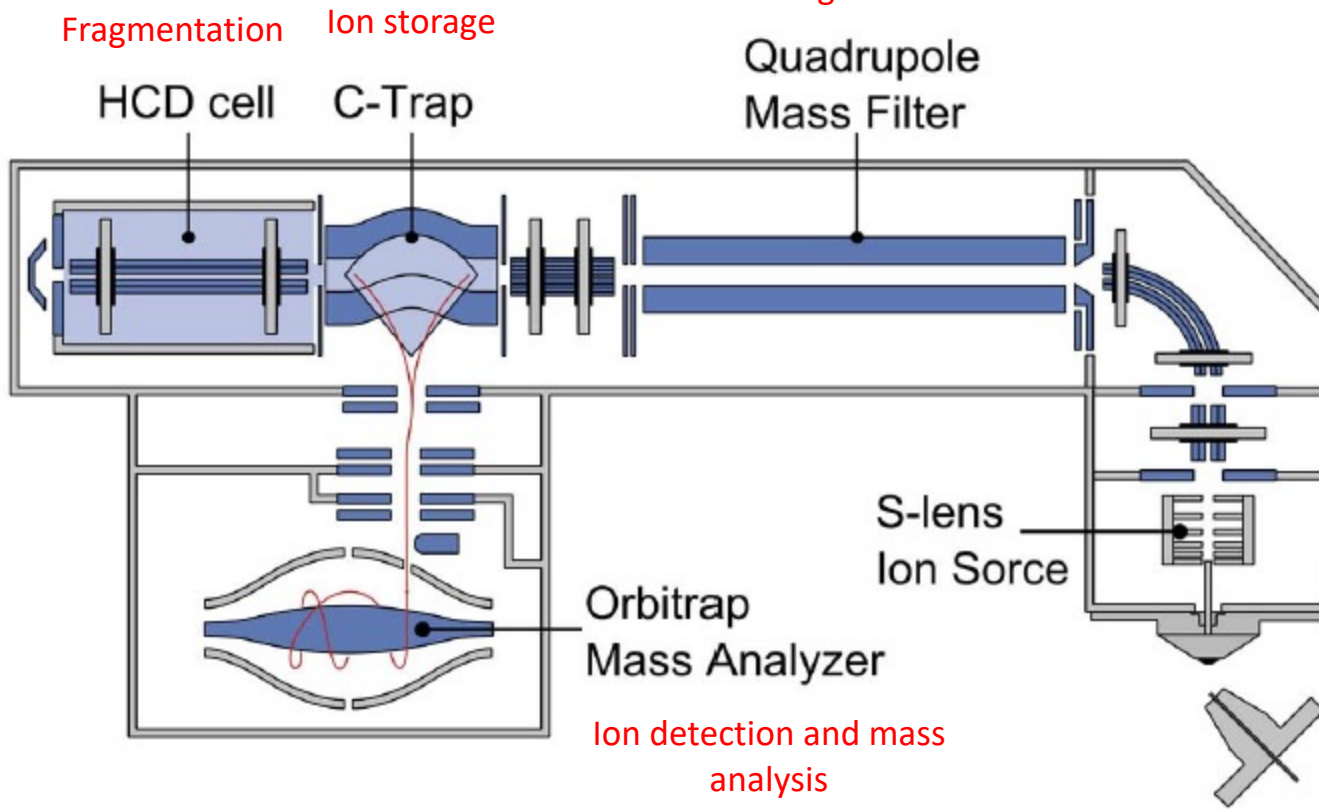
# Overview

- Q Exactive<sup>TM</sup> Hybrid Quadrupole-Orbitrap<sup>TM</sup>
- 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



# How to run a sample?

## 1. Method Set Up

1.1 Infusion AS

1.2 Master Method

1.3 Instrument  
Method

## 2. Analysis

2.1 Acquisition

2.2 Raw data

## 3. Processing

3.1 Library

3.2 Run sample

3.3 Sample  
processing

# 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

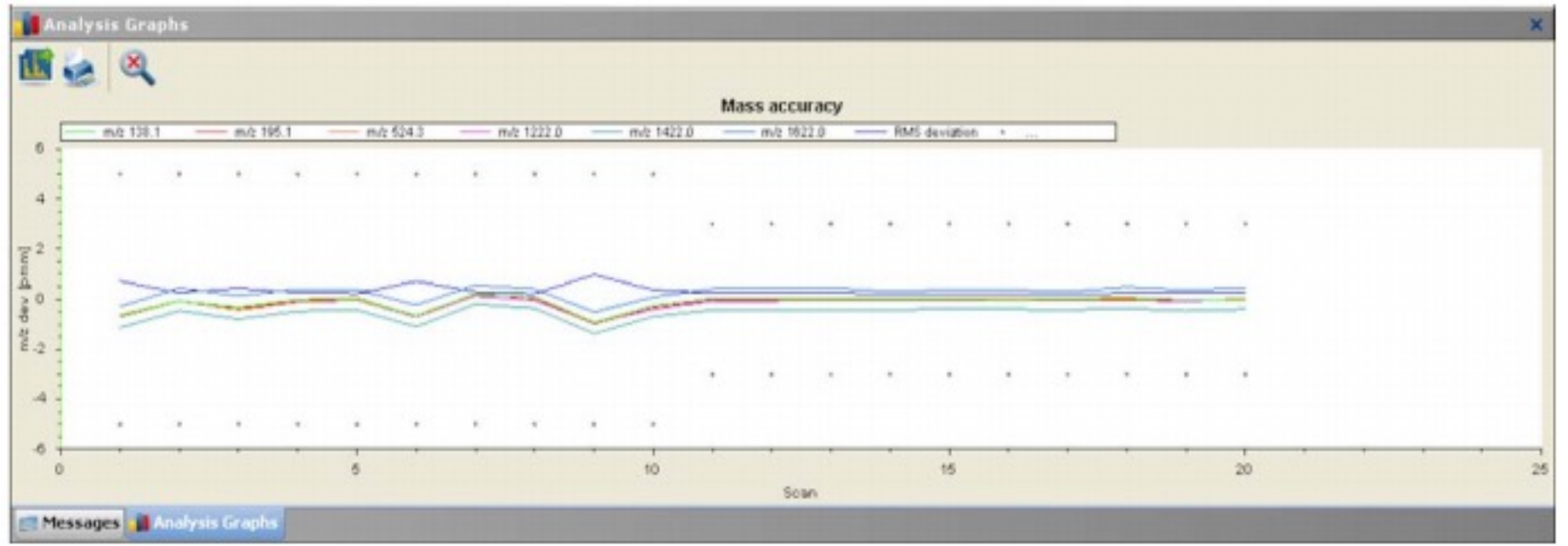
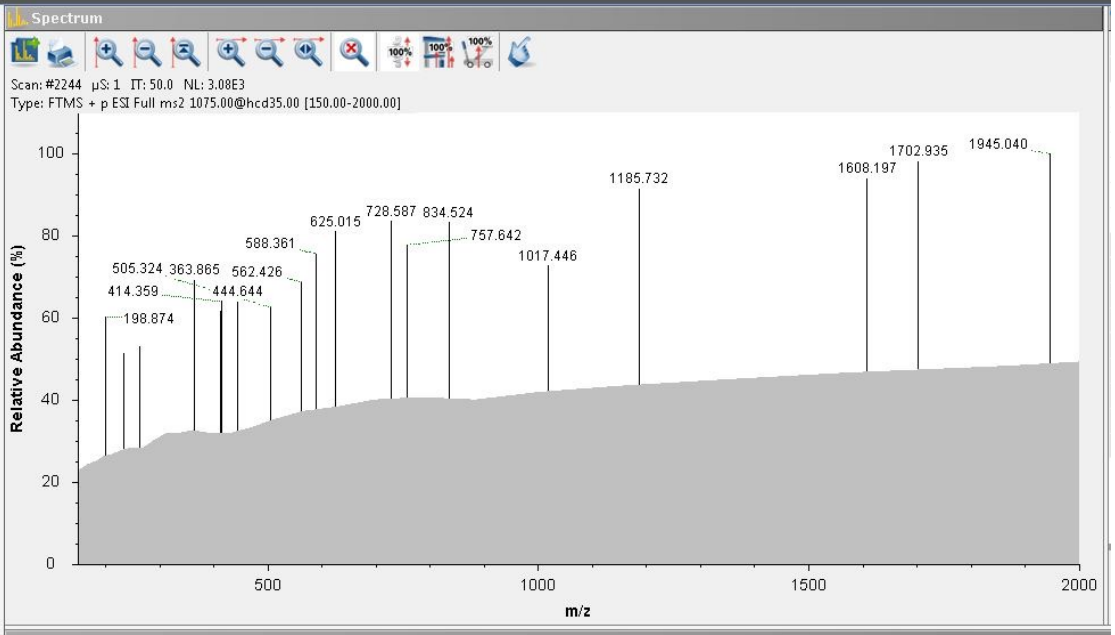
#### Scan parameters

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
Aux gas flow rate	0
Sweep gas flow rate	0
Spray voltage ( [kV] )	3.80
Spray current ( μA )	7.30
Capillary temp. ( °C )	320
S-lens RF level	50.0



# 1. Method Setup

- Master method

**Create Master Method**

There are multiple ways to create a master method.  
Select the technique you want to use.

<input type="radio"/> Use Method Forge	<b>Method Forge</b> Performs peak detection against a raw data file. Performs library lookup if requested.
<input type="radio"/> Import Xcalibur Processing Method	<b>Import Xcalibur Processing Method</b> Imports a previously created processing method, finding configured compounds and reference spectra.
<input type="radio"/> Create blank quantitation method	<b>Create blank method</b> Associate a raw data file and manually select peaks.
<input type="radio"/> Select compounds from CDB	<b>Select Compounds from a compound database</b> Creates a blank master method and displays the configured compound database, allowing compound selection.
<input checked="" type="radio"/> Create screening method	<b>Create a screening method</b>

OK Cancel

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

The screenshot displays the 'Compound Database - Adulterants\_TRF' interface. On the left is a 'Tree View Pane' showing a hierarchical list of compounds, including '10,11-Dihydrodibenzene (b,f) (1,4) oxazepin-11-one', 'SF-ADB', 'SF-MDMB-PICA', and '6-MAM'. The main area is the 'Peak View Pane', which contains a table of peak data. The table has columns for Compound Name, Peak Label, Peak Workflow, Associated Target Peak, Chemical Formula, MS Order, Precursor m/z, Product m/z, m/z, Adduct, and Polarity. The table lists various peaks, with peak 8 (SF-ADB, T1: 378.21875) highlighted as the 'TargetPeak'. Below the table is the 'Compound Details Pane' for the selected compound 'SF-ADB', showing its ionization method (ESI), chemical formula (C20H28FN3O3), neutral mass (377.21146984), and other parameters.

Compound Name	Peak Label	Peak Workflow	Associated Target Peak	Chemical Formula	MS Order	Precursor m/z	Product m/z	m/z	Adduct	Polarity
1 *	T1: 0.000	TargetPeak	-	-	ms1	0.000	0.000	0.000	None	Positiv
2	10,11-Dihydrodibenzene (b,f) (1,4) oxazepin-11-one	T1: 212.07061	TargetPeak	-	ms1	212.07061	212.07061	212.07061	Hydrogen	Positiv
3	10,11-Dihydrodibenzene (b,f) (1,4) oxazepin-11-one	T1F1: 212.07061->106	Fragment	-	ms2	212.07061	166.0649	166.0649	Hydrogen	Positiv
4	10,11-Dihydrodibenzene (b,f) (1,4) oxazepin-11-one	T1F2: 212.07061->156	Fragment	-	ms2	212.07061	156.0806	156.0806	Hydrogen	Positiv
5	10,11-Dihydrodibenzene (b,f) (1,4) oxazepin-11-one	T1F3: 212.07061->136	Fragment	-	ms2	212.07061	139.0541	139.0541	Hydrogen	Positiv
6	10,11-Dihydrodibenzene (b,f) (1,4) oxazepin-11-one	T1F4: 212.07061->95	Fragment	-	ms2	212.07061	95.0495	95.0495	Hydrogen	Positiv
7	10,11-Dihydrodibenzene (b,f) (1,4) oxazepin-11-one	T1F5: 212.07061->65	Fragment	-	ms2	212.07061	65.0393	65.0393	Hydrogen	Positiv
8	SF-ADB	T1: 378.21875	TargetPeak	-	ms1	378.21875	378.21875	378.21875	Hydrogen	Positiv
9	SF-ADB	T1F1: 378.21875->233	Fragment	-	ms2	378.21875	233.1091	233.1091	Hydrogen	Positiv
10	SF-ADB	T1F2: 378.21875->318	Fragment	-	ms2	378.21875	318.1974	318.1974	Hydrogen	Positiv
11	SF-ADB	T1F3: 378.21875->213	Fragment	-	ms2	378.21875	213.1026	213.1026	Hydrogen	Positiv
12	SF-ADB	T1F4: 378.21875->177	Fragment	-	ms2	378.21875	177.0462	177.0462	Hydrogen	Positiv
13	SF-ADB	T1F5: 378.21875->145	Fragment	-	ms2	378.21875	145.0397	145.0397	Hydrogen	Positiv
14	SF-ADB	T1F6: 378.21875->346	Fragment	-	ms2	378.21875	346.1943	346.1943	Hydrogen	Positiv
15	SF-ADB	T1F7: 378.21875->251	Fragment	-	ms2	378.21875	251.1191	251.1191	Hydrogen	Positiv
16	SF-ADB	T1F8: 378.21875->69	Fragment	-	ms2	378.21875	69.0707	69.0707	Hydrogen	Positiv
17	SF-ADB	T1F9: 378.21875->163	Fragment	-	ms2	378.21875	163.0504	163.0504	Hydrogen	Positiv
18	SF-ADB	T1F10: 378.21875->117	Fragment	-	ms2	378.21875	117.0462	117.0462	Hydrogen	Positiv
19	SF-ADB	T1F11: 378.21875->90	Fragment	-	ms2	378.21875	90.0345	90.0345	Hydrogen	Positiv
20	SF-MDMB-PICA	T1: 377.2235	TargetPeak	-	ms1	377.2235	377.2235	377.2235	Hydrogen	Positiv
21	SF-MDMB-PICA	T1F1: 377.2235->232	Fragment	-	ms2	377.2235	232.113	232.113	Hydrogen	Positiv
22	SF-MDMB-PICA	T1F2: 377.2235->144	Fragment	-	ms2	377.2235	144.0431	144.0431	Hydrogen	Positiv
23	SF-MDMB-PICA	T1F3: 377.2235->130	Fragment	-	ms2	377.2235	130.06512	130.06512	Hydrogen	Positiv

# 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

Method Development ▾

Method View >

- Acquisition
- Target Screening
- Processing**
- Peak Detection
- Reports

Compound Database

Instrument View

Method View - Anticoagulant Screening

Settings

Peak Filter Settings

Use RT Limits  Search from  minutes to  minutes

Use Matrix Blank  Amplifier

Chromatogram View Width  minutes

Use Source CID Scans

Show all compounds

Unknown Screening

Include Unknown Screening

Target Screening Settings

Compound Databases

Enabled	Database Name	
<input checked="" type="checkbox"/>	Superwarfarin	<a href="#">open</a>
<input type="checkbox"/>	Adulterants_TRF	<a href="#">open</a>
<input type="checkbox"/>	Adulterants_TRF_04-09-19	<a href="#">open</a>
<input type="checkbox"/>	Clin_Tox_Endura_SRM	<a href="#">open</a>
<input type="checkbox"/>	Clin_Tox_Quantiva_SRM	<a href="#">open</a>
<input type="checkbox"/>	DefaultGC	<a href="#">open</a>
<input type="checkbox"/>	DefaultLC	<a href="#">open</a>
<input type="checkbox"/>	EFS_Database	<a href="#">open</a>
<input type="checkbox"/>	EFS_HRAM_Compound_Database	<a href="#">open</a>
<input type="checkbox"/>	GCMSMS Pesticide Analyzer 1001	<a href="#">open</a>
<input type="checkbox"/>	Metabolite_Database	<a href="#">open</a>
<input type="checkbox"/>	Metabolite_Database_HILIC_01	<a href="#">open</a>
<input type="checkbox"/>	Toxicology_HRAM_Compound_Database_v1	<a href="#">open</a>

Identification and Confirmation Settings

Peaks  *m/z* Threshold Override

S/N Ratio Threshold

Mass Tolerance:  ppm

Retention Time  Identify  Confirm Ignore if Not Defined

Window Override (sec)

Fragment Ions  Identify  Confirm Ignore if Not Defined

Min. # of Fragments

Intensity Threshold

Mass tolerance  ppm

MS Order

Isotopic Pattern  Identify  Confirm Fit Threshold (%)

Allowed Mass Deviation (ppm)

Allowed Intensity Deviation (%)

Use Internal Mass Calibration

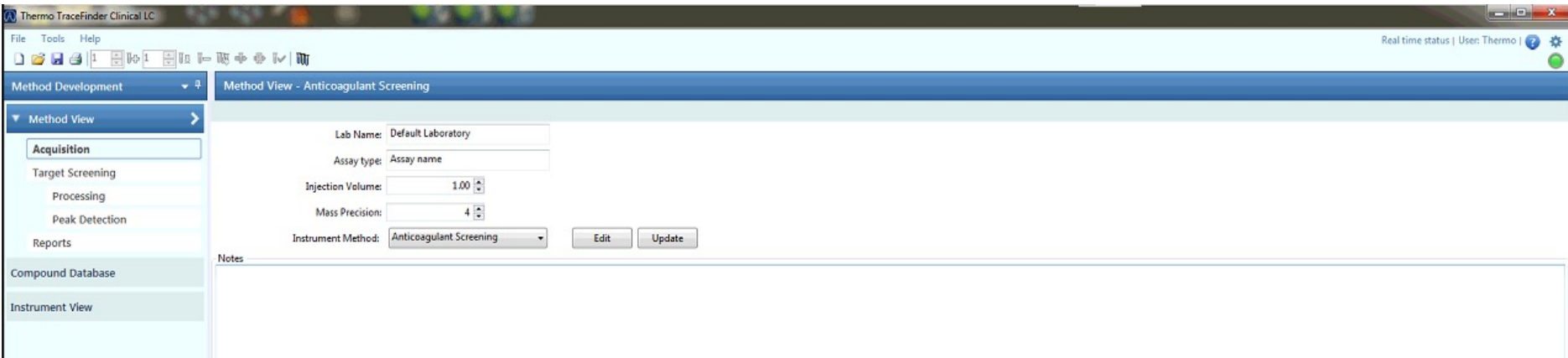
Acquisition

Analysis

Method Development

# 1. Method Setup

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



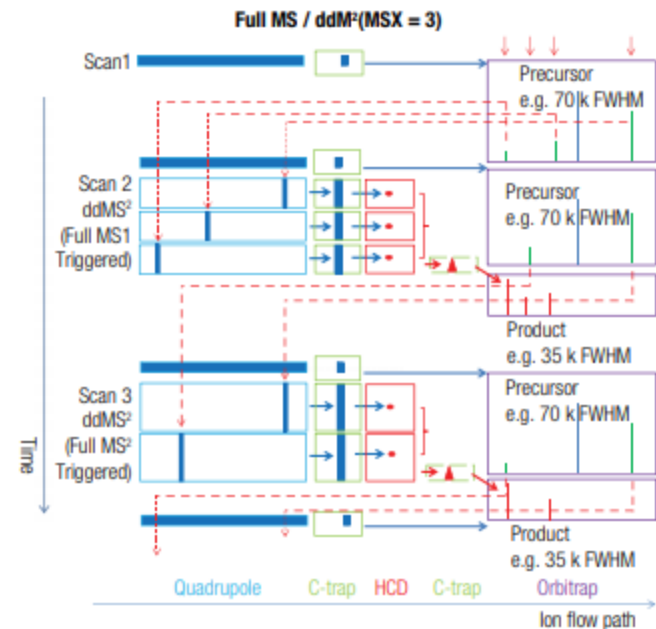
# 1. Method Setup

- Instrument method
  - Types of experiments:

The screenshot displays the Thermo Xcalibur Instrument Setup interface for the method 'Anticoagulant Screening.meth'. The main window features a central timeline from 0 to 8.5 minutes. Two 'Top5' scan events are scheduled: one from 0 to 4.2 minutes and another from 4.2 to 7.0 minutes. Below the timeline, two experimental setups are visualized as boxes. Each box contains a 'Full MS' event followed by a 'ddMS<sup>2</sup>' event, with a yellow arrow pointing from the Full MS to the ddMS<sup>2</sup> event. The left sidebar lists various experiment types, including 'Full MS - SIM', 'AIF', 'Full MS / AIF', 'Full MS / dd-MS<sup>2</sup> (TopN)', 'Targeted-SIM', 'PRM', 'Targeted-SIM / dd-MS<sup>2</sup>', 'Full MS / AIF / NL dd-MS<sup>2</sup>', and 'DIA'. The right sidebar contains 'Properties' for the method and 'Properties of 2 scan events'. The 'Properties of the method' section includes 'Use lock mass: best', 'Chrom. peak wi 6 s', and 'Time' with a 'Method duration 8.50 min'. The 'Properties of 2 scan events' section is divided into 'dd Settings' (Minimum AGC t: 8.00e3, Intensity thresh: 1.6e5, Apex trigger: —, Charge exclusio: —, Peptide match, Exclude isotopes: on, Dynamic exclus) and 'dd-MS<sup>2</sup> / dd-SIM' (Resolution: 17,500, AGC target: 1e5, Maximum IT: 50 ms, Loop count: 5, TopN: 5, Isolation window, Fixed first mass: —, (N)CE / stepped). The 'Full MS' section includes 'Resolution: 70,000', 'AGC target', 'Maximum IT', and 'Scan range: 50 to 750 m/z'. The 'General' section includes 'Runtime', 'Polarity', 'Default charge', and 'Inclusion'. At the bottom, the 'Runtime' section shows 'Data acquisition start time and end time for selected MS experiment [min] (0.00 ...'. The status bar at the bottom left indicates 'Ready'.

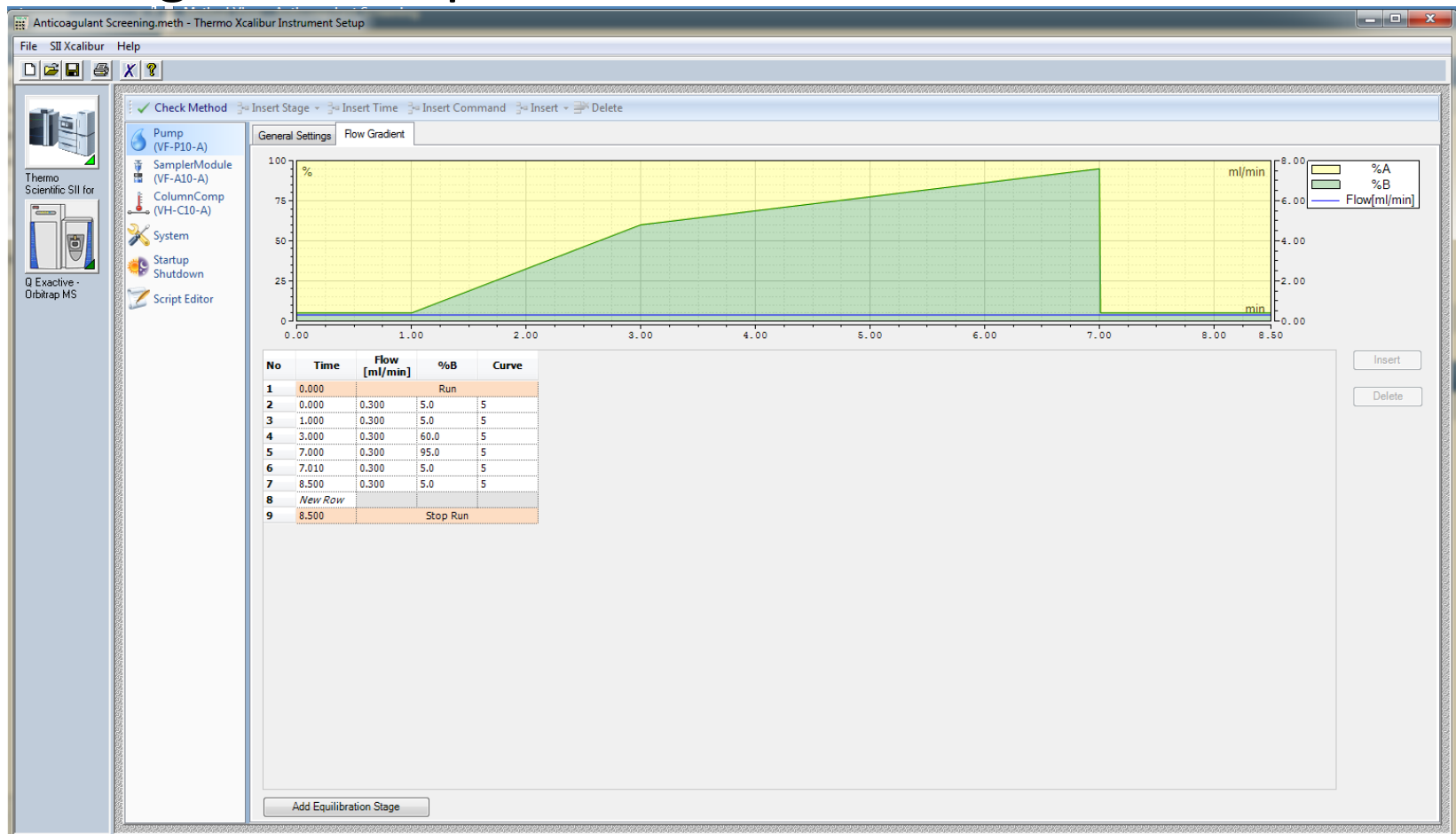
# 1. Method Setup

- Instrument method
  - Full MS-SIM
  - AIF
  - Full MS/AIF
  - Full MS/ddMS<sup>2</sup>
  - Targeted-SIM
  - PRM
  - Targeted-SIM/ddMS<sup>2</sup>
  - Full MS/AIF/ddMS<sup>2</sup>
  - DIA



# 1. Method Setup

- Instrument method
  - As general as possible to start:

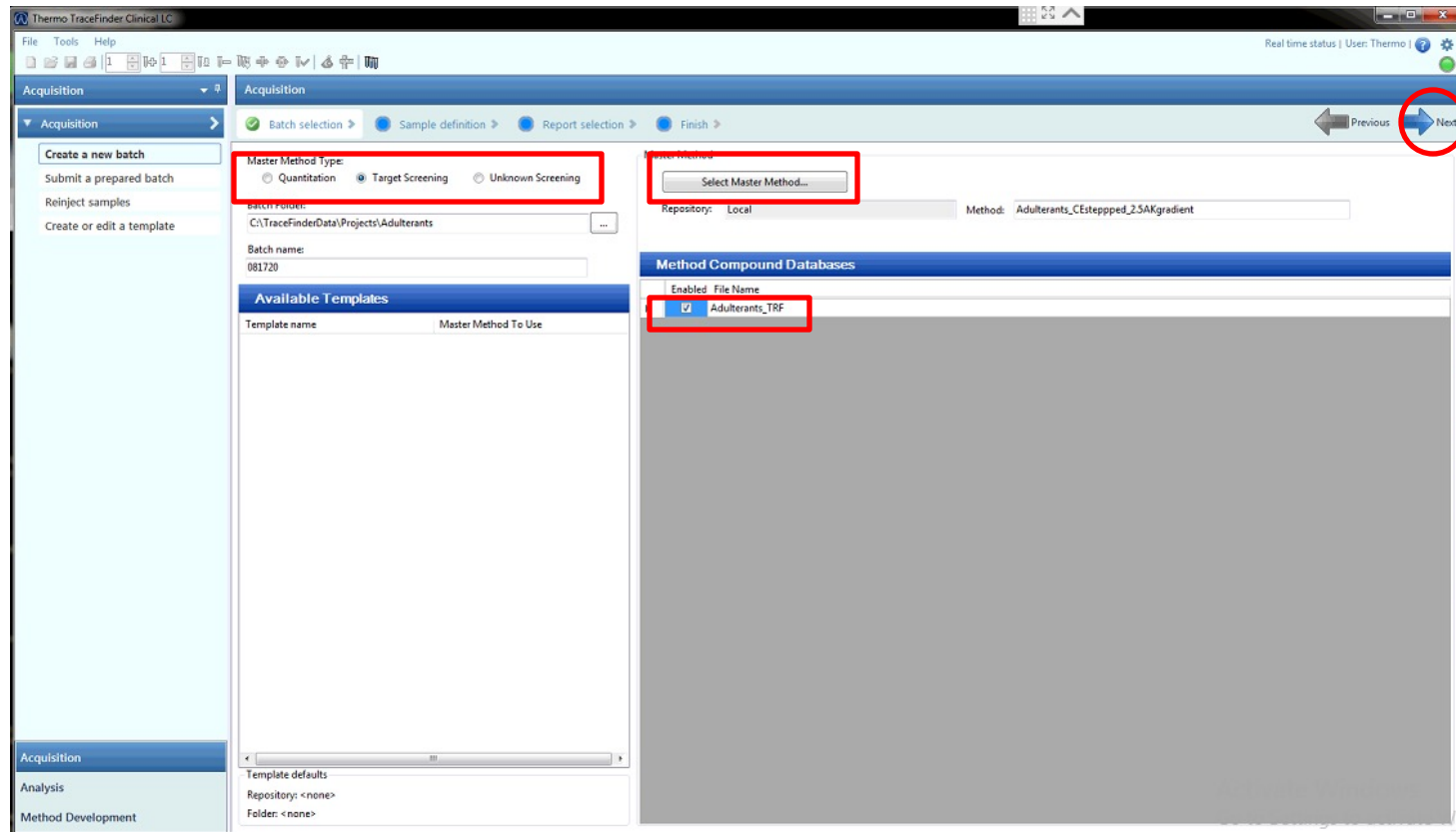






# 2. Analysis

- Acquisition







# 2. Analysis

- Acquisition

The screenshot displays the Thermo TraceFinder Clinical L.C. software interface. The main window is titled "Acquisition" and shows a progress bar with four steps: "Batch selection", "Sample definition", "Report selection", and "Finish", all of which are completed. The "Samples" table is the central focus, listing 30 samples with columns for Status, Filename, Sample type, Groups, Blank Subtraction, Level, Sample ID, Sample name, Comment, Vial position, Injection volume, Conversion Factor, and Barcode Expected. The table shows a sequence of specimens with varying vial positions and injection volumes. At the bottom, there are "Sample Controls" buttons for "Add", "Insert", and "Import".

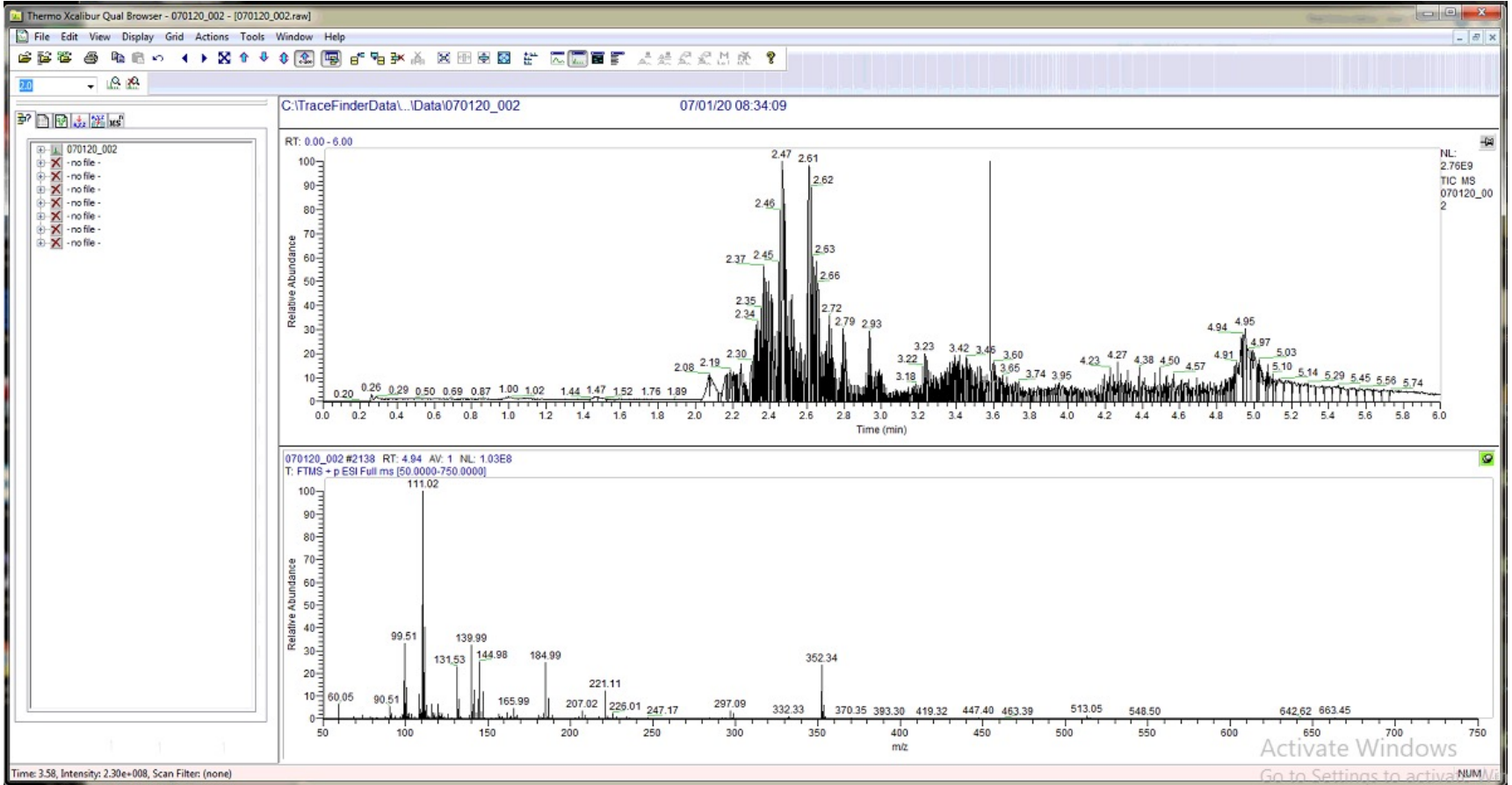
Sample	Status	Filename	Sample type	Groups	Blank Subtraction	Level	Sample ID	Sample name	Comment	Vial position	Injection volume	Conversion Factor	Barcode Expected
1	●	070620_001	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
2	●	070620_002	Specimen		<input type="checkbox"/>		1			G:A2	1.000	1.000	
3	●	070620_003	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
4	●	070620_004	Specimen		<input type="checkbox"/>		2			G:A3	1.000	1.000	
5	●	070620_005	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
6	●	070620_006	Specimen		<input type="checkbox"/>		3			G:A4	1.000	1.000	
7	●	070620_007	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
8	●	070620_008	Specimen		<input type="checkbox"/>		5			G:A5	1.000	1.000	
9	●	070620_009	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
10	●	070620_010	Specimen		<input type="checkbox"/>		7			G:A6	1.000	1.000	
11	●	070620_011	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
12	●	070620_012	Specimen		<input type="checkbox"/>		8			G:A7	1.000	1.000	
13	●	070620_013	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
14	●	070620_014	Specimen		<input type="checkbox"/>		9			G:A8	1.000	1.000	
15	●	070620_015	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
16	●	070620_016	Specimen		<input type="checkbox"/>		10			G:A9	1.000	1.000	
17	●	070620_017	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
18	●	070620_018	Specimen		<input type="checkbox"/>		11			G:B1	1.000	1.000	
19	●	070620_019	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
20	●	070620_020	Specimen		<input type="checkbox"/>		13			G:B2	1.000	1.000	
21	●	070620_021	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
22	●	070620_022	Specimen		<input type="checkbox"/>		14			G:B3	1.000	1.000	
23	●	070620_023	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
24	●	070620_024	Specimen		<input type="checkbox"/>		15			G:B4	1.000	1.000	
25	●	070620_025	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
26	●	070620_026	Specimen		<input type="checkbox"/>		16			G:B5	1.000	1.000	
27	●	070620_027	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
28	●	070620_028	Specimen		<input type="checkbox"/>		17			G:B6	1.000	1.000	
29	●	070620_029	Specimen		<input type="checkbox"/>		MP			G:A1	1.000	1.000	
30	●	070620_030	Specimen		<input type="checkbox"/>		18			G:B7	1.000	1.000	

## 2. Analysis

- Acquisition
  - Batch view: status indicator show the current status of each sample during the acquisition and processing
    -  Sample is not acquired.
    -  Sample is acquired but not processed.
    -  Sample is acquired and processed.
    -  Sample is currently acquiring.

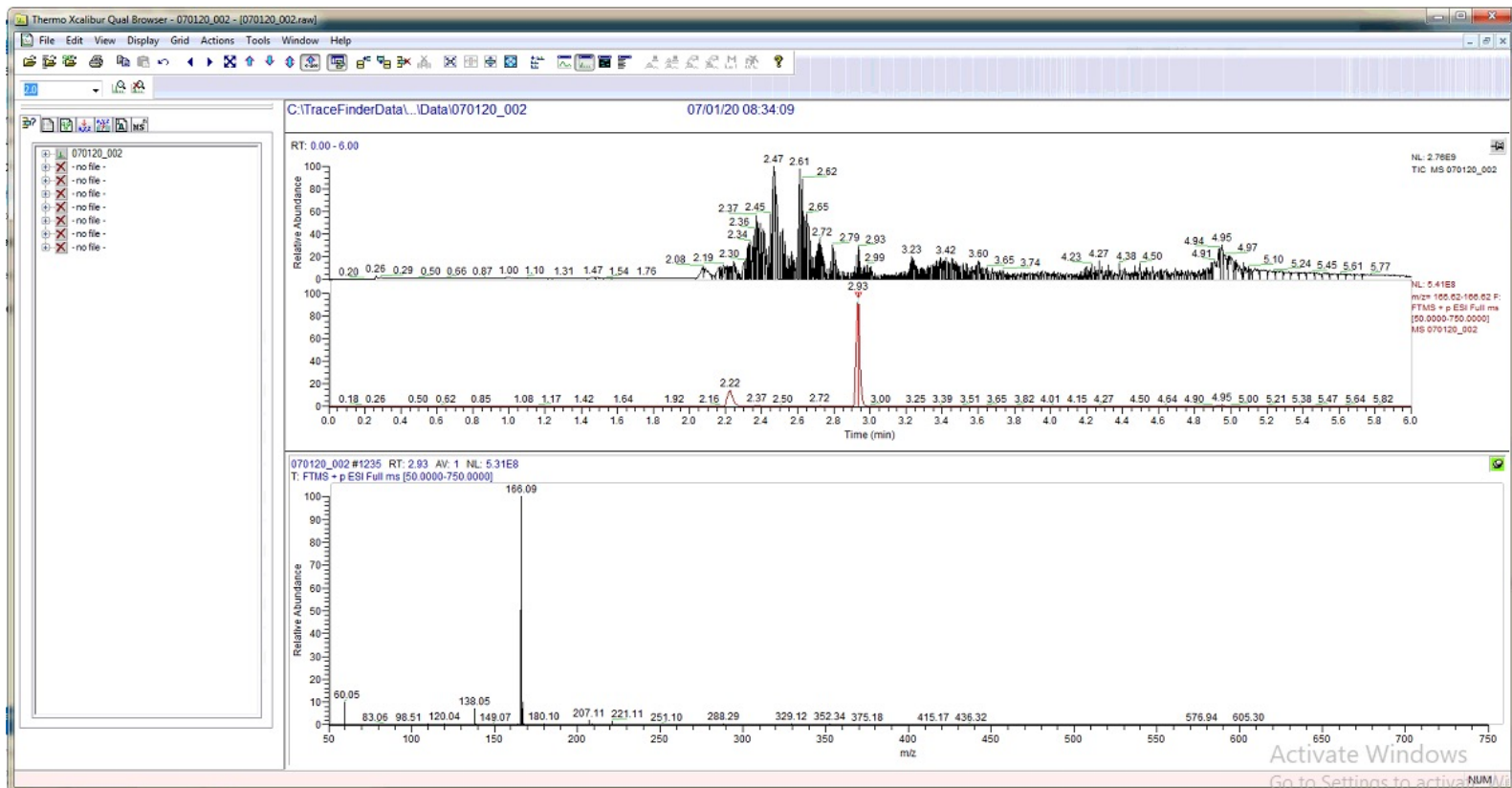
# 2. Analysis

- Raw Data



# 2. Analysis

- Raw Data



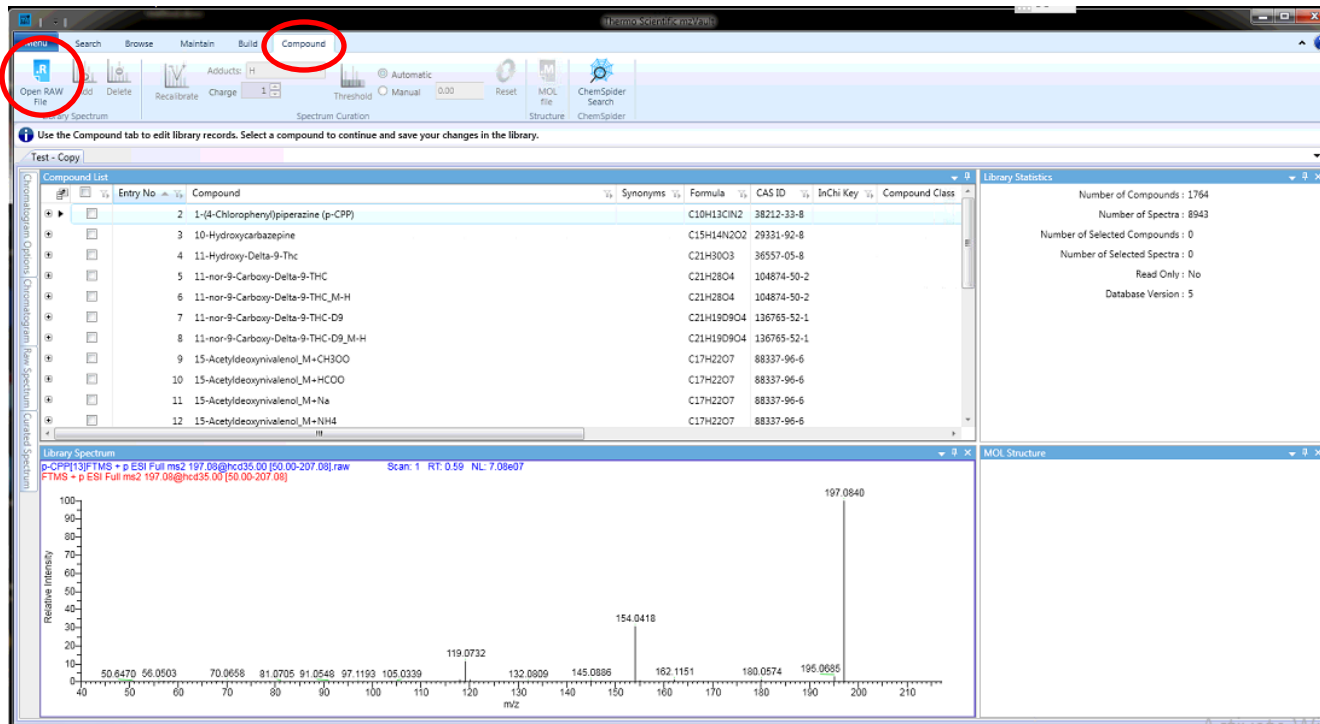
# 2. Analysis

- Raw Data
  - mzCloud Database

The screenshot displays the mzCloud database interface. The left sidebar contains navigation options: Views (Standard, Compare, Structures), Libraries (Reference Library, Autoprocessed Library), Search, Search Results, and Tools. The main content area shows search results for Benzocaine (No: 1803, Monoisotopic Mass: 165.07898) and other compounds like Miconazole, Methyseleno-L-cystein, Flunarizine, Fluticasone propionate, and Albendazole. A mass spectrum plot is shown, titled 'Recalibrated Spectrum' (FTMS = ESI ms [116.09-216.09]). The x-axis represents m/z from 0 to 180, and the y-axis represents relative intensity from 0 to 100. A prominent peak is labeled at m/z 166.08626 (MS<sup>1</sup>). A precursor structure is shown on the right, which is the chemical structure of Benzocaine: CCOC(=O)c1ccc(N)cc1. The interface also includes a search bar, a navigation menu, and a footer with copyright information and an 'Activate Windows' watermark.

# 3. Processing

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



# 3. Processing

- Library
- Filter mass > select mass spec > add

The screenshot displays the ChemSpider software interface. The 'Add' button in the top toolbar is circled in red. The 'Compound List' table is visible, listing compounds such as 1-(4-Chlorophenyl)piperazine (p-CPP) and 10-Hydroxycarbazine. The 'Chromatogram' plot shows a peak at 5.16 minutes, which is also circled in red. The 'Raw Spectrum' plot shows the mass spectrum of this peak, with the base peak at 90.0086 m/z. The 'Curated Spectrum' plot shows the resulting mass spectrum after filtering, with peaks at 65.0132, 74.0235, 90.0086, 96.0079, 116.0244, and 125.6789 m/z. The 'Filter' field in the 'Chromatogram Options' is set to 'FTMS - p ESI d Full ms2 116.0242@hcd', which is also circled in red. The 'Library Statistics' panel on the right shows the number of compounds (1764) and spectra (8943).

Entry No.	Compound	Formula	CAS ID	InChi Key	Compound Class	ChemSpider ID
2	1-(4-Chlorophenyl)piperazine (p-CPP)	C10H13ClN2	38212-33-8			
3	10-Hydroxycarbazine	C15H14N2O2	29331-92-8			
4	11-Hydroxy-Delta-9-Thc	C21H30O3	36557-05-8			
5	11-nor-9-Carboxy-Delta-9-THC	C21H28O4	104874-50-2			
6	11-nor-9-Carboxy-Delta-9-THC_M-H	C21H28O4	104874-50-2			
7	11-nor-9-Carboxy-Delta-9-THC-D9	C21H19O9O4	136765-52-1			
8	11-nor-9-Carboxy-Delta-9-THC-D9_M-H	C21H19O9O4	136765-52-1			
9	15-Acetyldioxyvalenol_M+CH3OO	C17H22O7	88337-96-6			
10	15-Acetyldioxyvalenol_M+HCOO	C17H22O7	88337-96-6			
11	15-Acetyldioxyvalenol_M+Na	C17H22O7	88337-96-6			
12	15-Acetyldioxyvalenol_M+NH4	C17H22O7	88337-96-6			



# 3. Processing

- Library
- Build > Name compound > Save

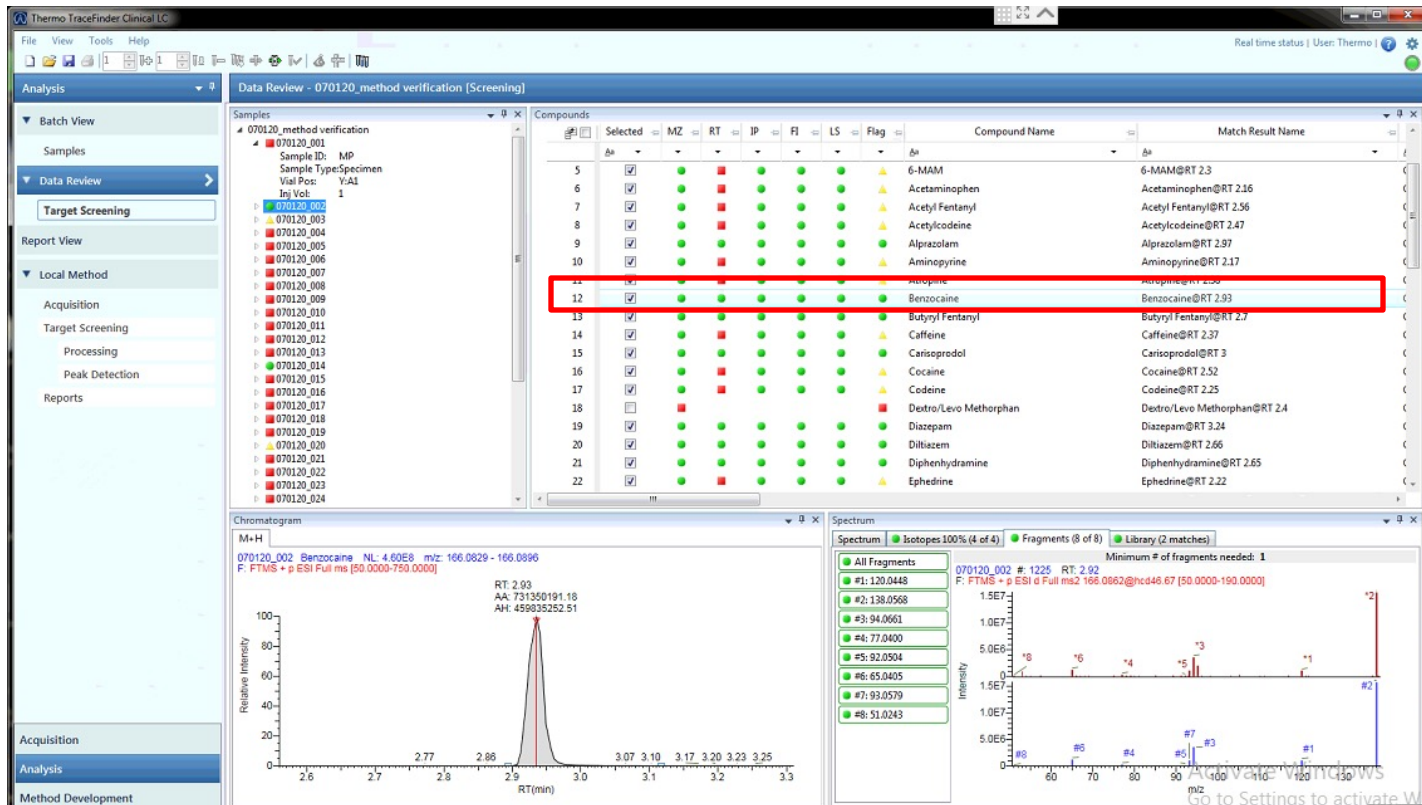
The screenshot displays the Thermo Scientific Vanos software interface. The 'Build' menu is highlighted with a red circle, and the 'Save' option is also highlighted with a red box. The 'Compound List' table shows a list of compounds, with '1767 Benzocaine' selected and highlighted in blue. The 'Library Spectrum' plot shows a mass spectrum with relative intensity on the y-axis (0 to 100) and m/z on the x-axis (40 to 150). The base peak is at m/z 90.0086. Other significant peaks are labeled at m/z 65.0132, 74.0235, 89.0133, 115.0291, 116.0244, and 117.0198.

Entry No.	Compound	Synonyms	Formula	CAS ID	InChi Key	Compound Class	ChemSpider ID	HMC
1759	Modafinil							
1760	N-Ethylamphetamine							
1761	5F-ADB							
1762	FUB AMB/MMB FUBINACA							
1763	N-Isopropylamphetamine							
1764	10,11-Dihydrodibenzene (b,f) (1,4) oxazepin-11-one							
1765	5F-MDMB-PICA							
1766	Benzocaine							
1767	Compound							

Spectrum	Compound ID	Scan Filter	Retention Time	Scan Number	Precursor m/z	Neutral Mass	Collision Energy	Polarity	Fragmentation M
6946	1767	FTMS - p ESI d Full ms2 116.0243@hcd30.00 [50.0000-140.0000]	5.156	1116	116.0243	0.0000	30.00	-	HCD

# 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

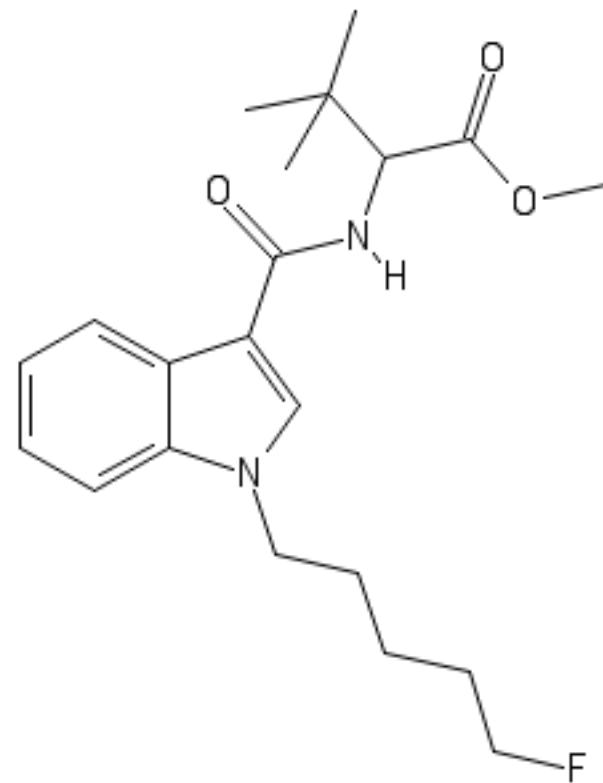
# 5F-MDMB- PICA

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

5F-MDMB-2201

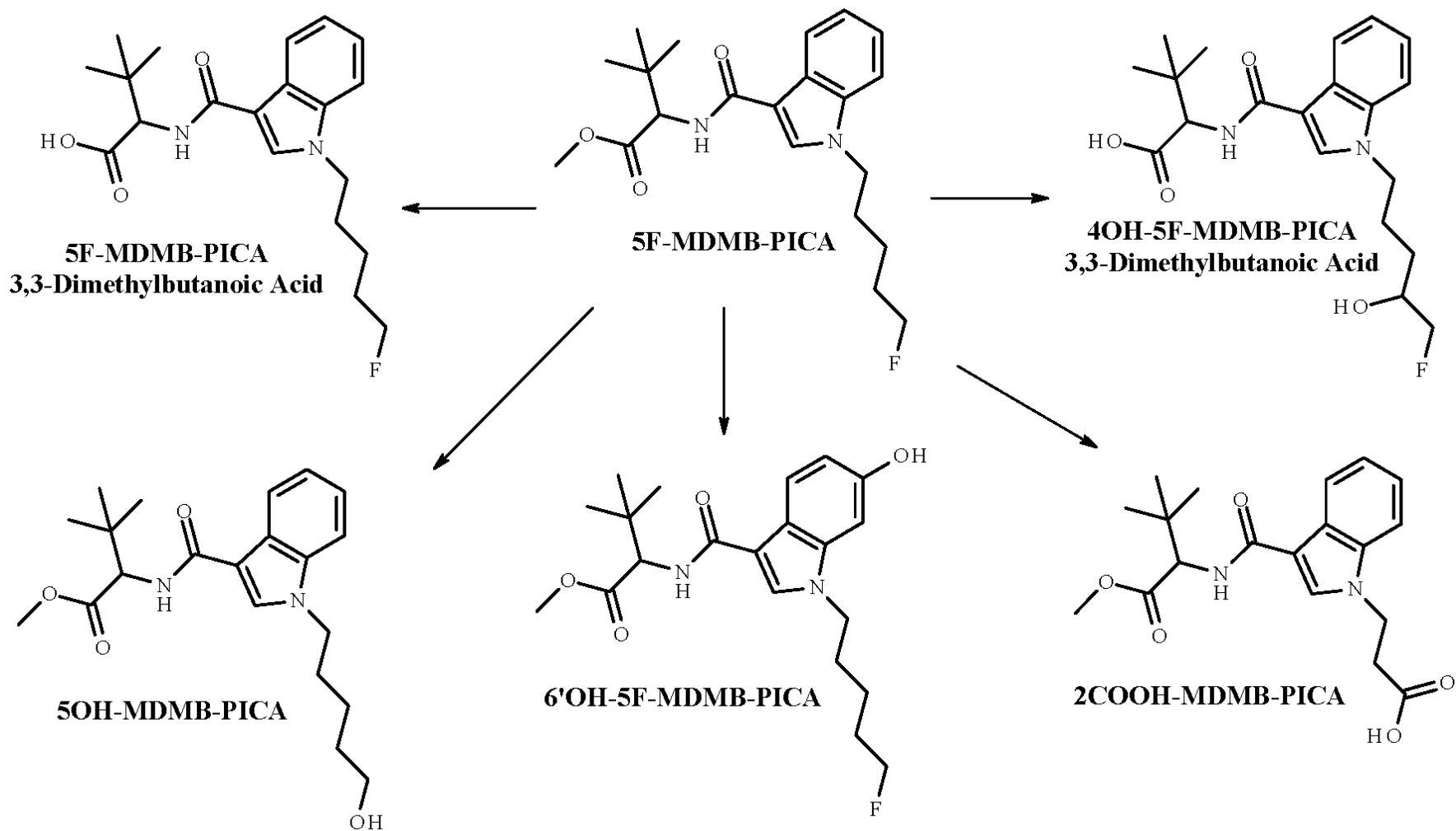
## 5F-MDMB-PICA

- 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



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## CONSIDERATIONS



Acquisition  
Parameters



Mobile Phase  
Gradient



Extraction technique  
and optimization



Calibration curve

1

0.5 mL  
blank blood  
fortified  
with  
working  
std. and 50  
µL of ISTD

2

Add 1 mL  
of 5%  
phosphoric  
acid in  
water and  
3 mL of  
extraction  
solvent

3

Rotate 15  
min then  
centrifuge  
at 4600  
rpm for 15  
min.

4

Store in -  
80°C  
freezer for  
15 min  
then  
transfer  
organic  
layer to a  
new tube

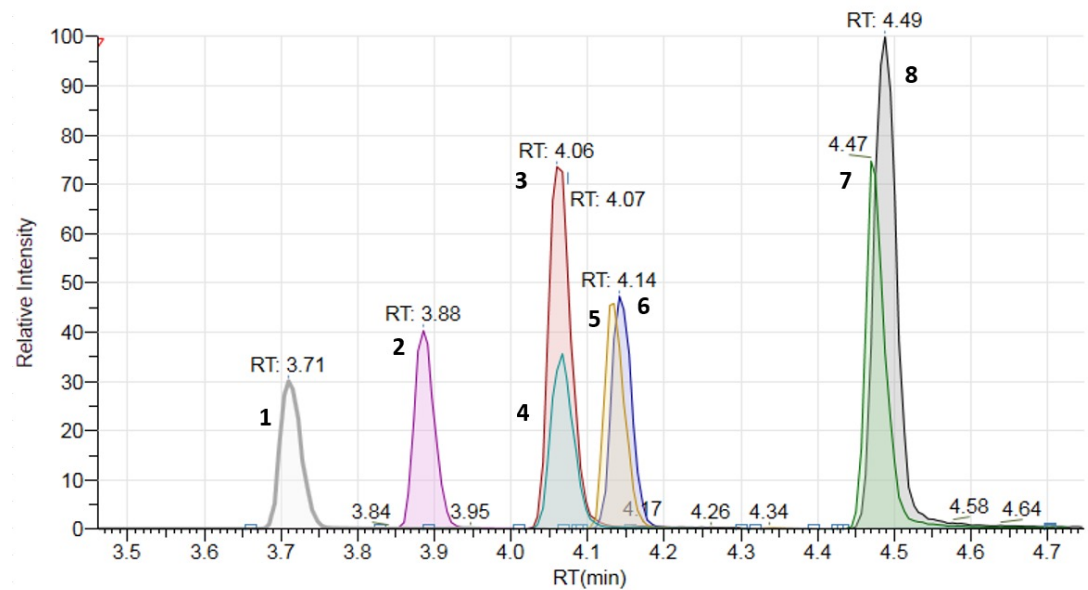
5


Dry down  
at 40°C for  
30 min and  
reconstitute  
in 90:10  
(MPA:MPB)

## 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-dimethylbutanoic 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  
 $LOD/LOQ = 0.5 \text{ ng/mL}$

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**

