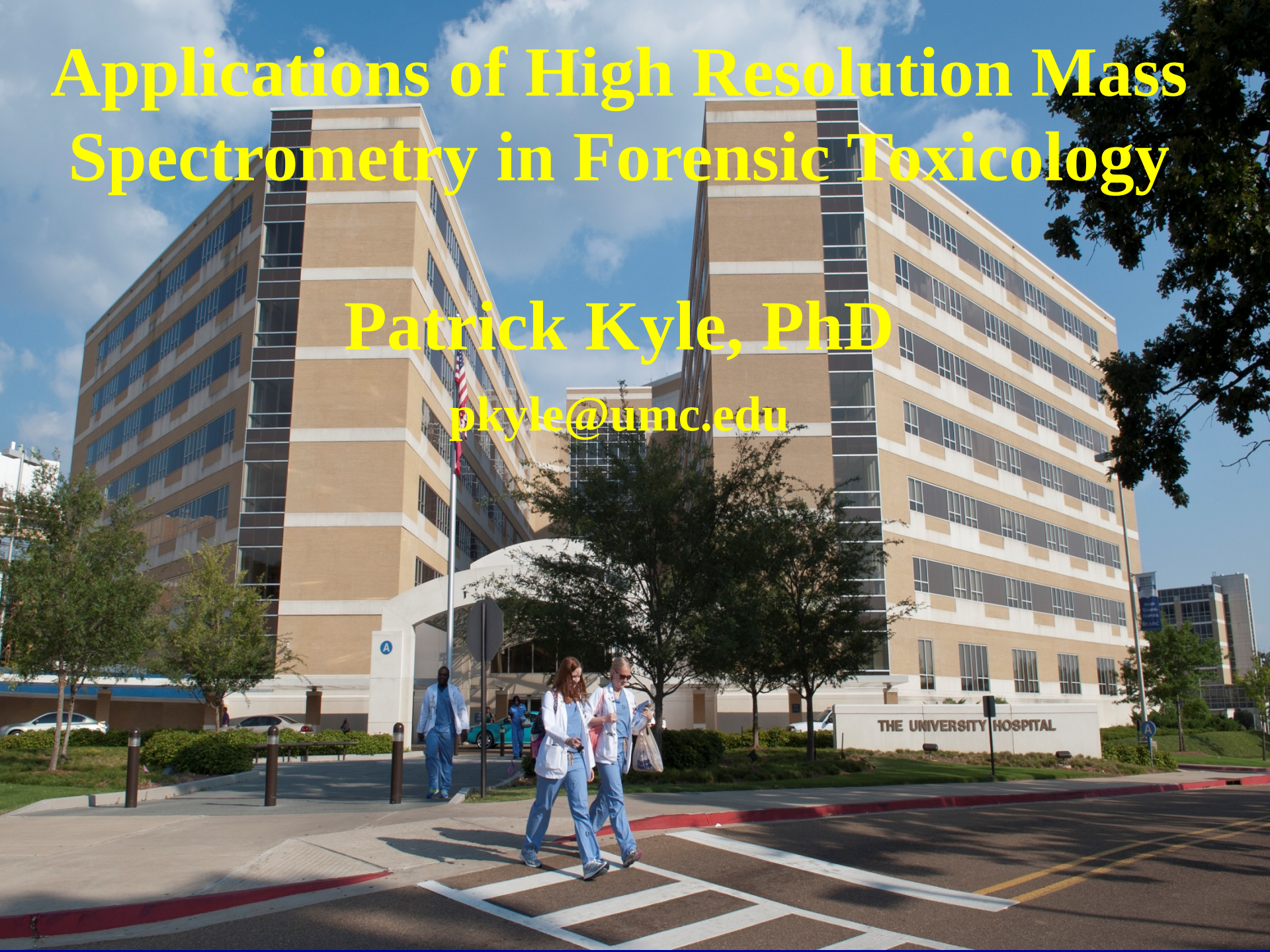
Applications of High Resolution Mass Spectrometry in Forensic Toxicology



Patrick Kyle, PhD

pkyle@umc.edu

Financial Disclosures

- Grant/Research Support: None
- Salary/Consultant Fees: None
- Board/Committee/Advisory Board:

  -Clinical and Laboratory Standards Institute (CLSI)

  -American Board of Clinical Chemistry
- Stocks/Bonds: None
- Expenses: Thermo Fisher Scientific
- Intellectual Property/Royalties: None

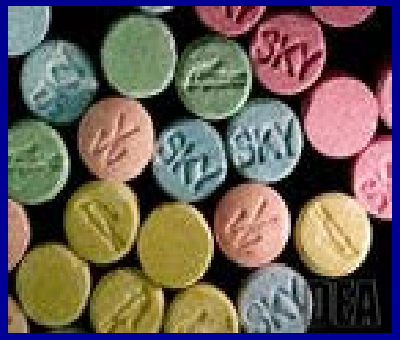
Expected Outcomes

Participants will be able to:

- Cite common applications of High Resolution LC-MS
- Relate advantages of Full Scan Spectra acquisition during Toxicology Screening
- Identify utilities of mzCloud™ software for Unknown Toxicology Screening

Forensic Toxicology
Drugs of Abuse

- Prevalent in Society
- Commonly used by Teenagers and Adults



THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Drug Abuse

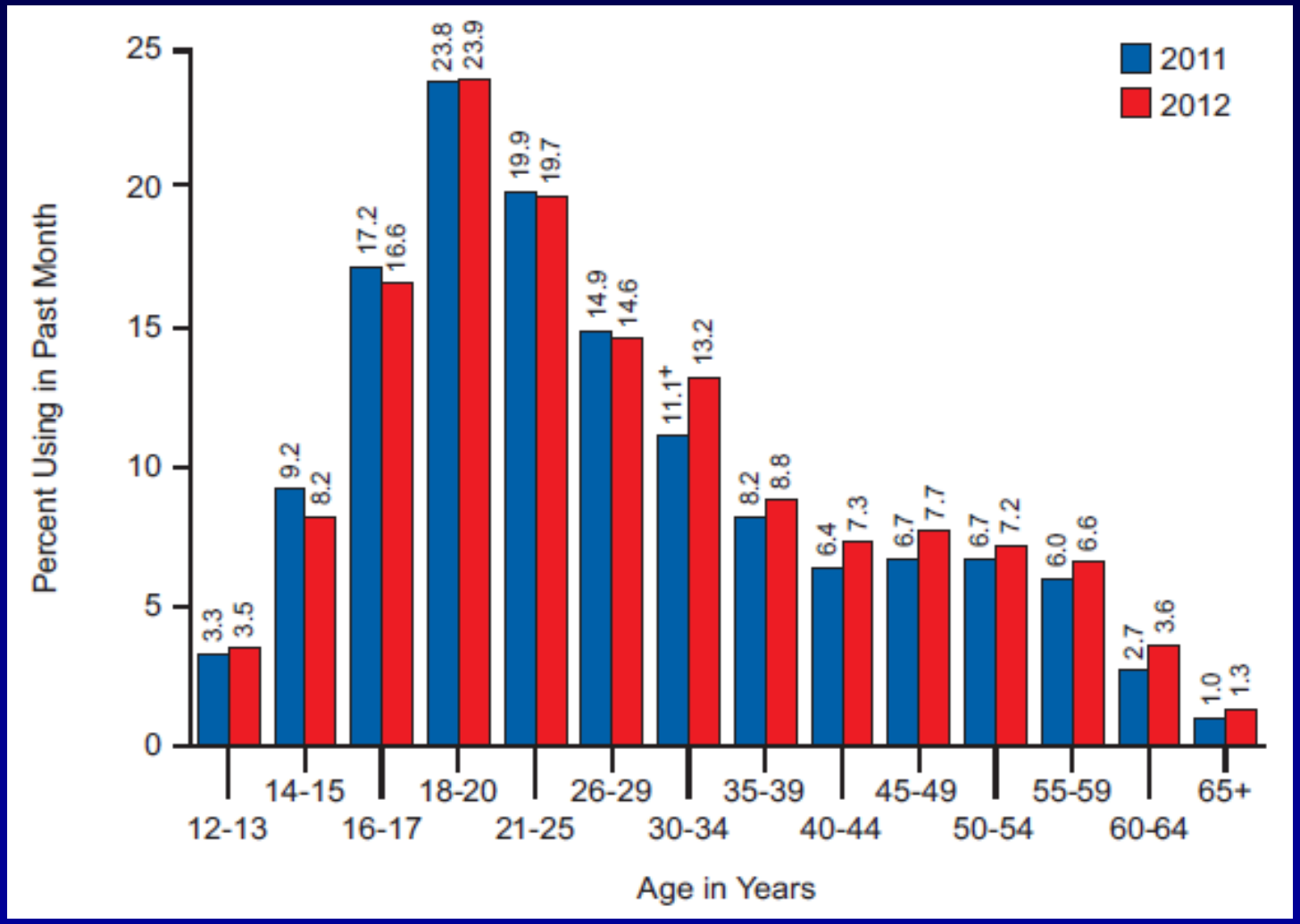
Dangers:

- Decreased Mental Capacity / Test Scores
- Miscarriage during Pregnancy
- Decreased Immune Response, ↑ Illness
- Risky Behavior → Accidents, Disease
- Tolerance → Addiction → Overdose → Death

Factoids

- The United States consumes 99% of the World's hydrocodone production, and 80% of the World's opiate production
- Hydrocodone is the most highly prescribed drug in the United States (for past 8-9 years)
- Use of PCP increased 400% in the USA from 2005-2011
- Of 22.5 million Americans surveyed, 8.7 self-reported past month illicit drug use (SAMHSA 2012)
- Abuse of synthetic designer drugs has increased 500% since 2010

Current Illicit Drug Use

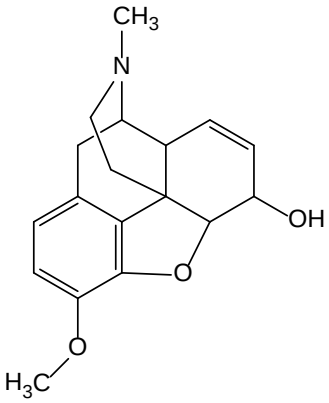


SAMHSA 2013

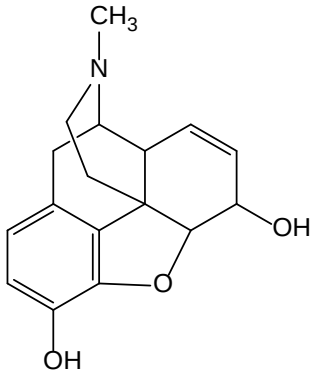
Drugs of Abuse

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

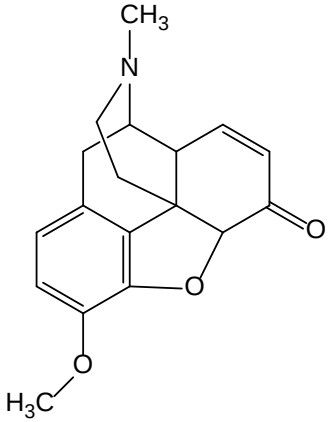
Immunoassay Screening



Codeine



Morphine



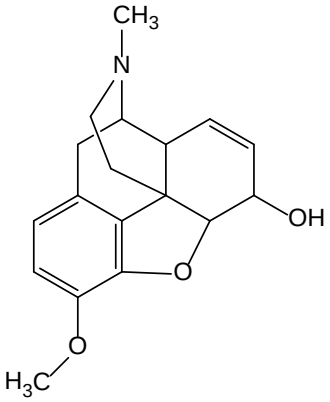
Hydrocodone

False-negatives: Concentration Below Cutoff
Dilute Urine
New Drug Formulations

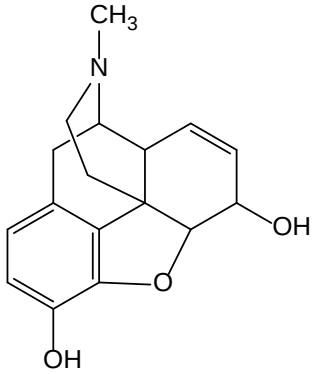
For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

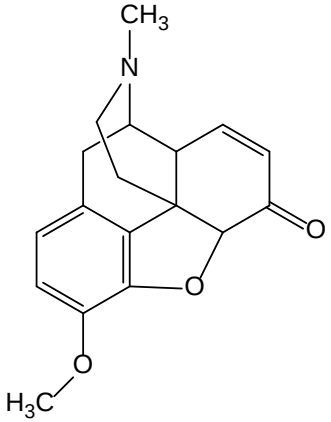
Immunoassay Screening



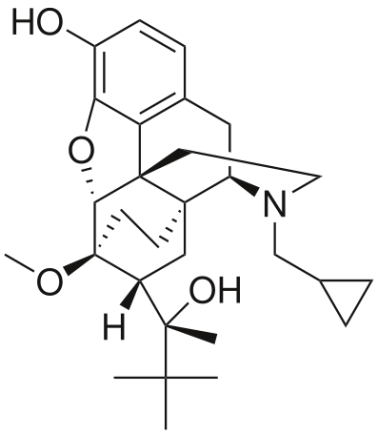
Codeine



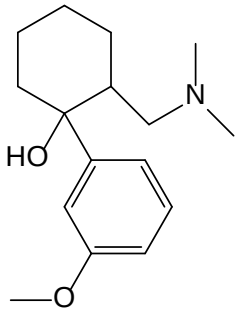
Morphine



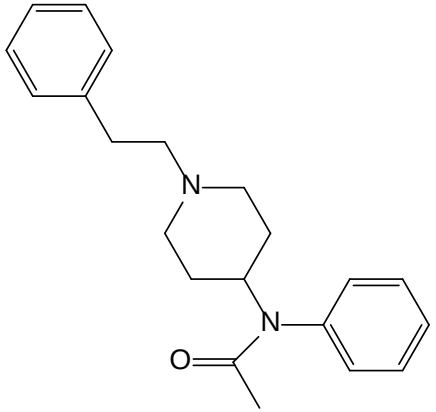
Hydrocodone



Buprenorphine



Tramadol



Fentanyl

Immunoassay Screening

Compound	Siemens	Roche
6-Acetylmorphine	435	386
Morphine	300	300
Codeine	306	224
Dihydrocodeine	291	510
Hydrocodone	247	1,086
Hydromorphone	498	1,425
Meperidine	> 15,000	> 100,000
Nalbuphine	> 100,000	?
Oxycodone	1,500	> 75,000
Tramadol	> 100,000	?

*Siemens EMIT II Plus Opiate Assay using 300 ng/mL cutoff.
**Roche Cobas Opiates II assay using 300 ng/mL cutoff.

For forensic use only.

Why HRAM LC-MS/MS ?

- Drug/Analyte extraction into solvent not required
- Accurate mass (0.0001) is more specific
- Orbitrap™ Technology (Thermo Scientific™)
  - Supports Spectral Matching (like EI GC-MS)
  - Allows Retrospective Analysis of Previously-injected Samples

Opiates Confirmation using HRAM LC-MS/MS

Mobile Phase: A) 0.1% Formic Acid (in water)

B) 0.1% Formic Acid in Acetonitrile

1) Initial: 5% Acetonitrile
2) Increase to 50% Acetonitrile over 5.5 min
3) Increase to 90% Acetonitrile over 0.5 min
4) Hold at 90% Acetonitrile for 4 min
5) Return to 5% Acetonitrile over 2 min
6) Re-equilibrate with 5% Acetonitrile for 2 min

For forensic use only.

Opiates Confirmation

Sample Preparation

- Combine: 0.5 mL urine, β-glucuronidase, 2 M Sodium Acetate (pH 4.5), D^2-Internal Standard Mix
- Incubate for 30 minutes at 65°C
- Centrifuge for 5 minutes at 8000 rpm (in microfuge)
- Dilute 1/10
- Inject 10 µL onto LC-MS/MS

Analytical Column: 2.1x100 mm Phenyl

For forensic use only.

Opiates Confirmation

Mass Transitions @ 35% CE

6-MAM: **328.1543** → 165.0697, 211.0754, 193.0646

Codeine: **300.1594** → 215.1064, 199.0751, 183.0802

Dihydrocodeine: **302.1750** → 199.0751, 227.1063

Hydrocodone: **300.1594** → 199.0759, 241.0865, 213.0917

Hydromorphone: **286.1437** → 185.0597, 227.0704, 157.0648

Morphine: **286.1437** → 201.0914, 185.0601, 165.0701

Norcodeine: **286.1467** → 165.0701, 181.0654

Oxycodone: **316.1543** → 241.1091, 256.1328, 298.1433

Oxymorphone: **302.1386** → 227.0937, 242.1173, 284.1277

For forensic use only.

Opiates Confirmation

Mass Transitions @ 35% CE

6-MAM: **328.1543** → 165.0697, 211.0754, 193.0646

Codeine: **300.1594** → 215.1064, 199.0759, 183.0802

Dihydrocodeine: **302.1750** → 199.0751, 227.1063

Hydrocodone: **300.1594** → 199.0759, 241.0865, 213.0917

Hydromorphone: **286.1437** → 185.0601, 227.0704, 157.0648

Morphine: **286.1437** → 201.0914, 185.0601, 165.0701

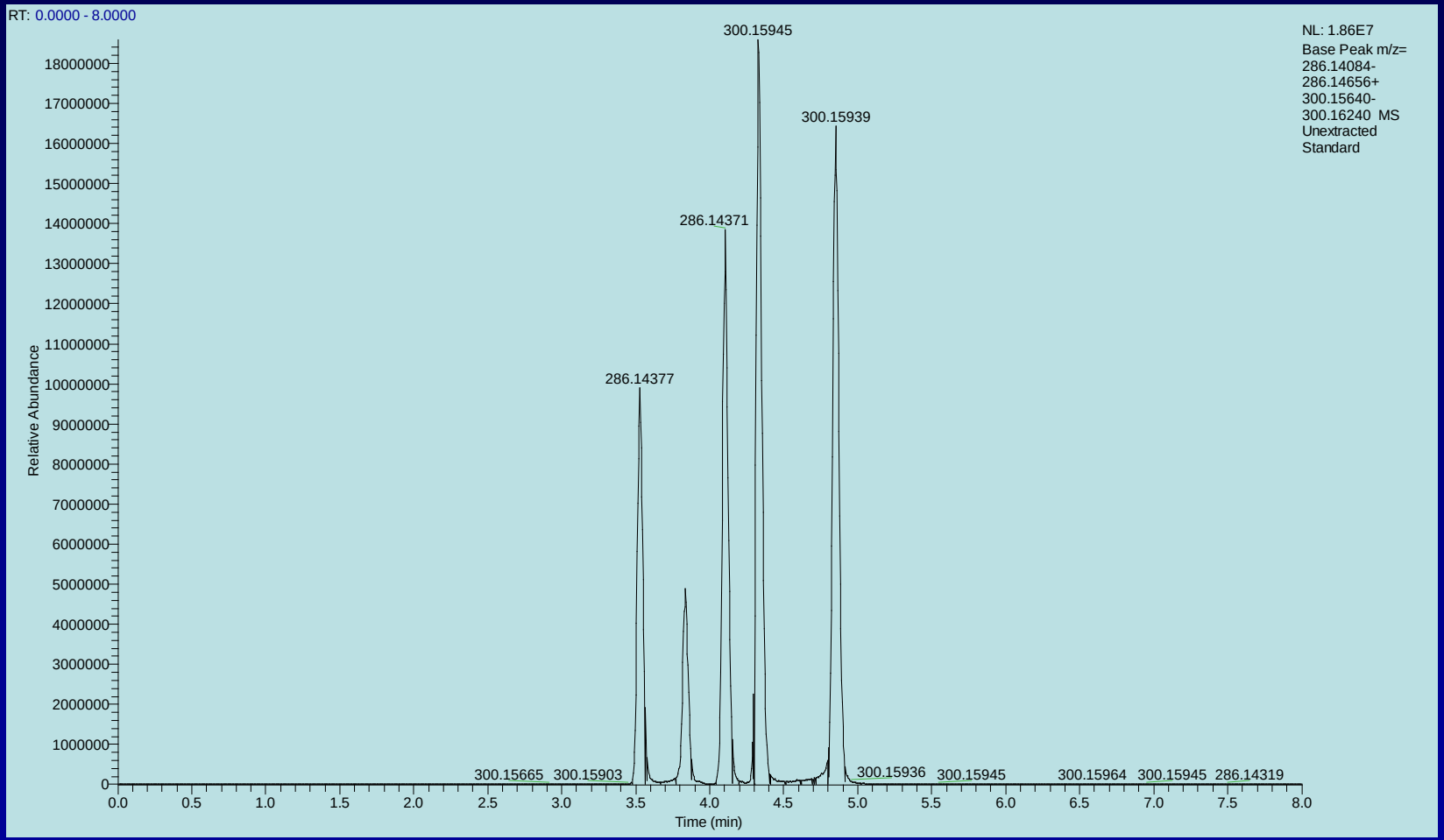
Norcodeine: **286.1467** → 165.0701, 181.0654

Oxycodone: **316.1543** → 241.1091, 256.1328, 298.1433

Oxymorphone: **302.1386** → 227.0937, 242.1173, 284.1277

For forensic use only.

Opiates Confirmation



For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Opiates Confirmation

- LLOD = 3 ng/mL
- LLOQ = 10 ng/mL
- Specific (HRMS fragments)
- Complex Precision (2x/day for 10 days)

    Morphine 4.6 CV%

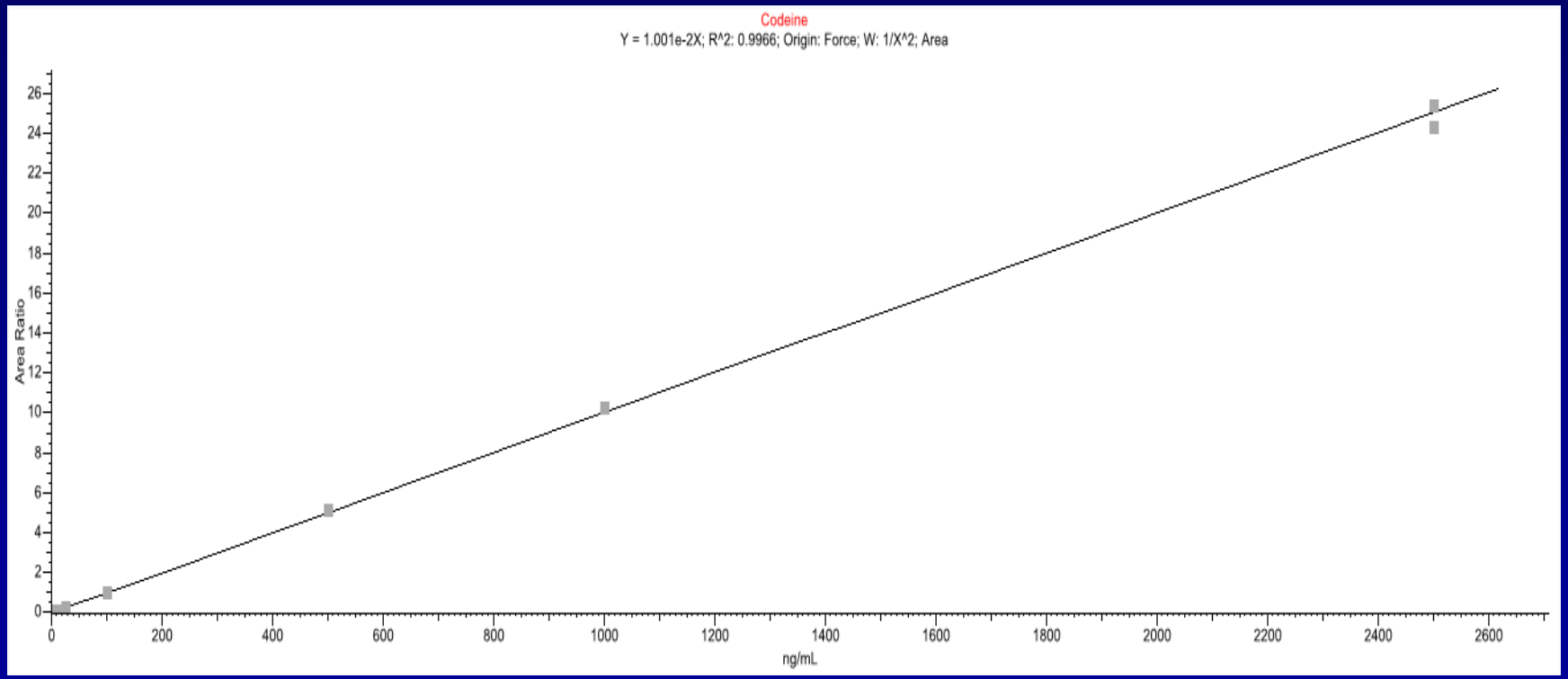
    Codeine 11.6 CV%

Linear Response 10 - 2500 ng/mL (tested)

For forensic use only.

Opiates Confirmation

Codeine
Triplicate Cal Curves
10 - 2,500 ng/mL (R2 = 0.9966)



For forensic use only.

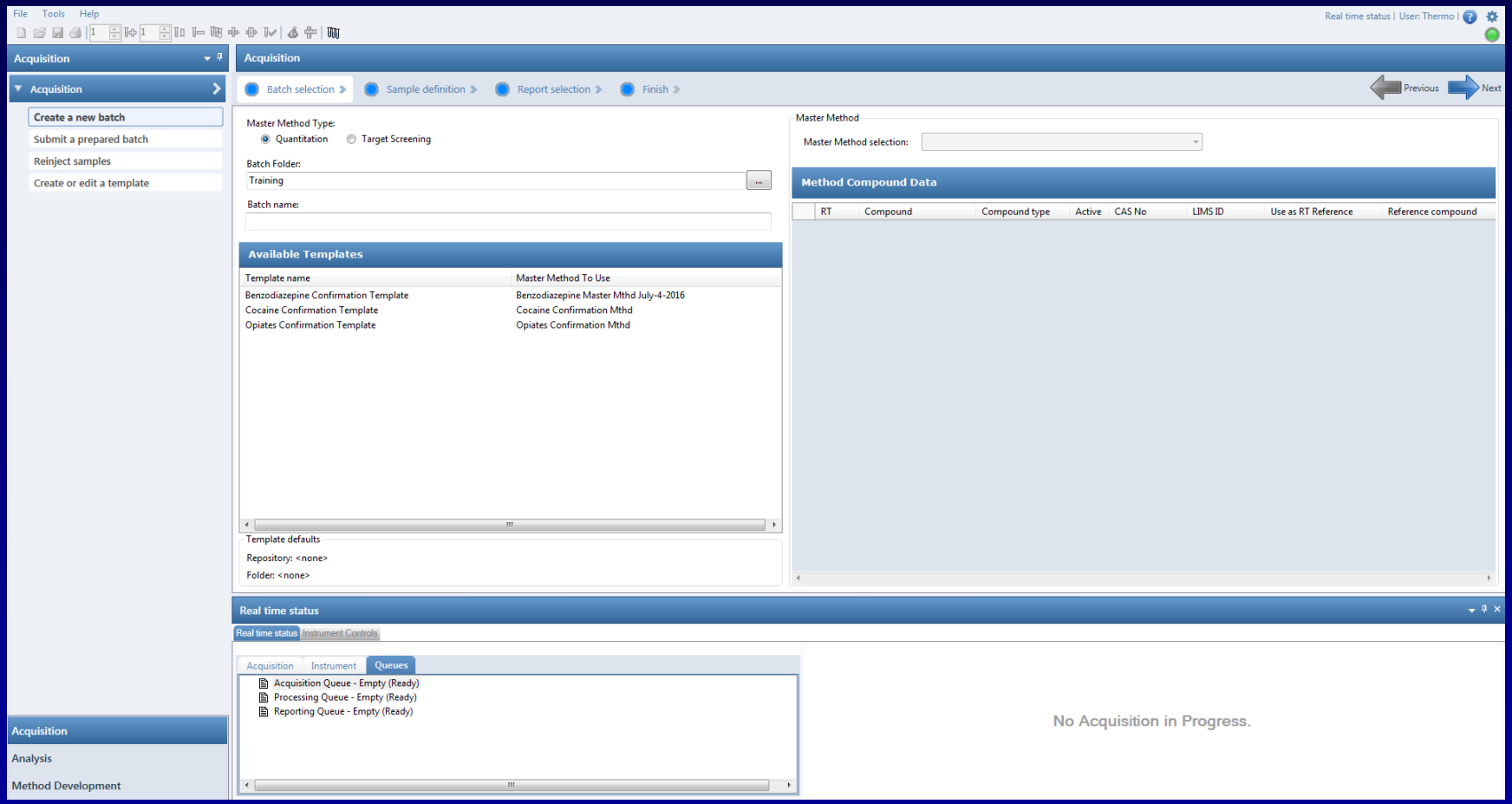
THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Benzodiazepines Confirmation using HRAM LC-MS/MS

- 7-aminoflunitrazepam
- Alprazolam
- α-Hydroxyalprazolam
- α-Hyroxymidazolam
- Clonazepam
- Desalkylflurazepam
- Diazepam
- Estazolam
- Etizolam
- Flunitrazepam
- Flurazepam
- Lorazepam
- Midazolam
- Nordiazepam
- Oxazepam
- Temazepam
- Triazolam
- Zaleplon
- Zolpidem
- Zopiclone

For forensic use only.

TraceFinder™ Software

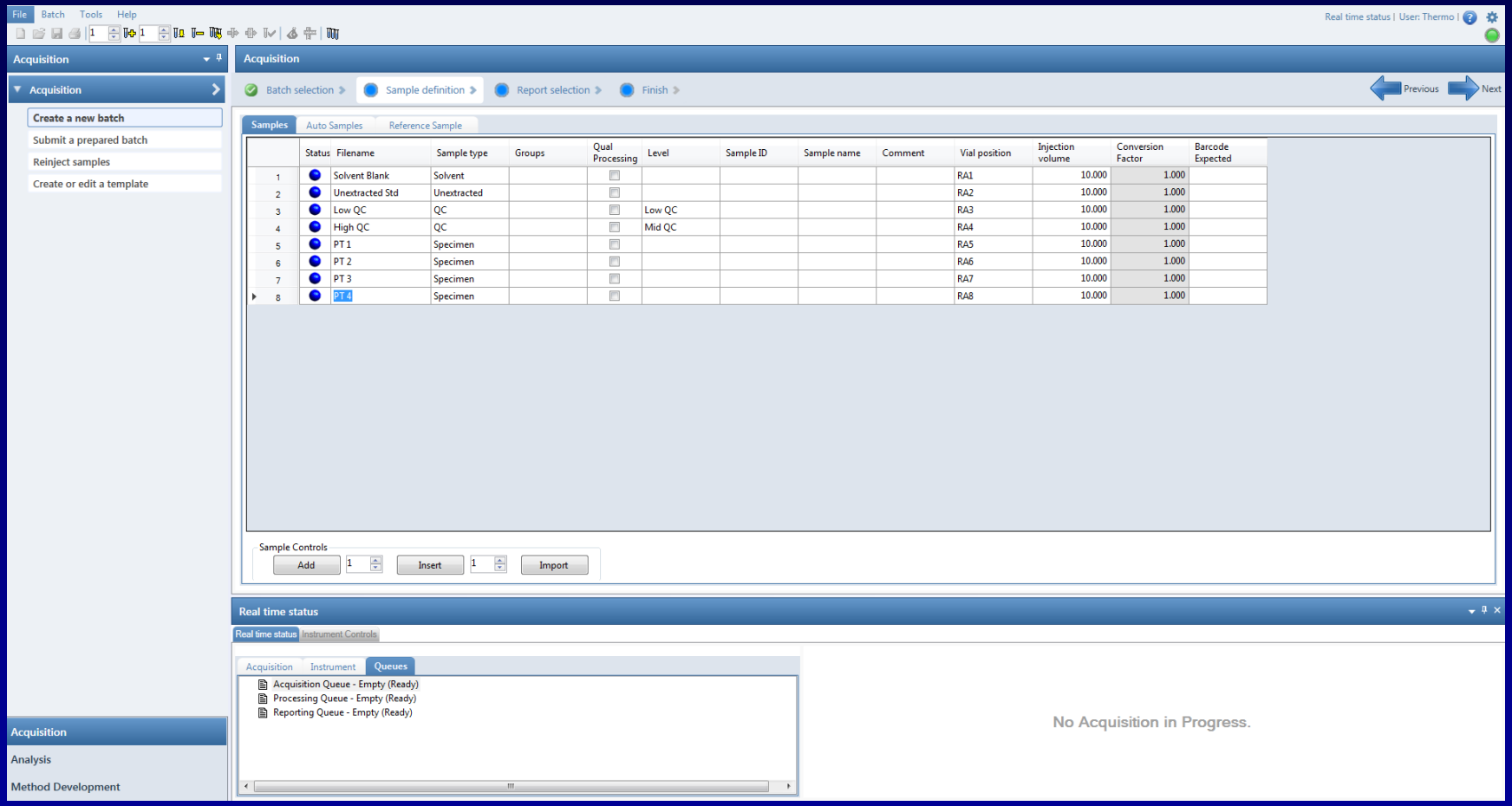


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

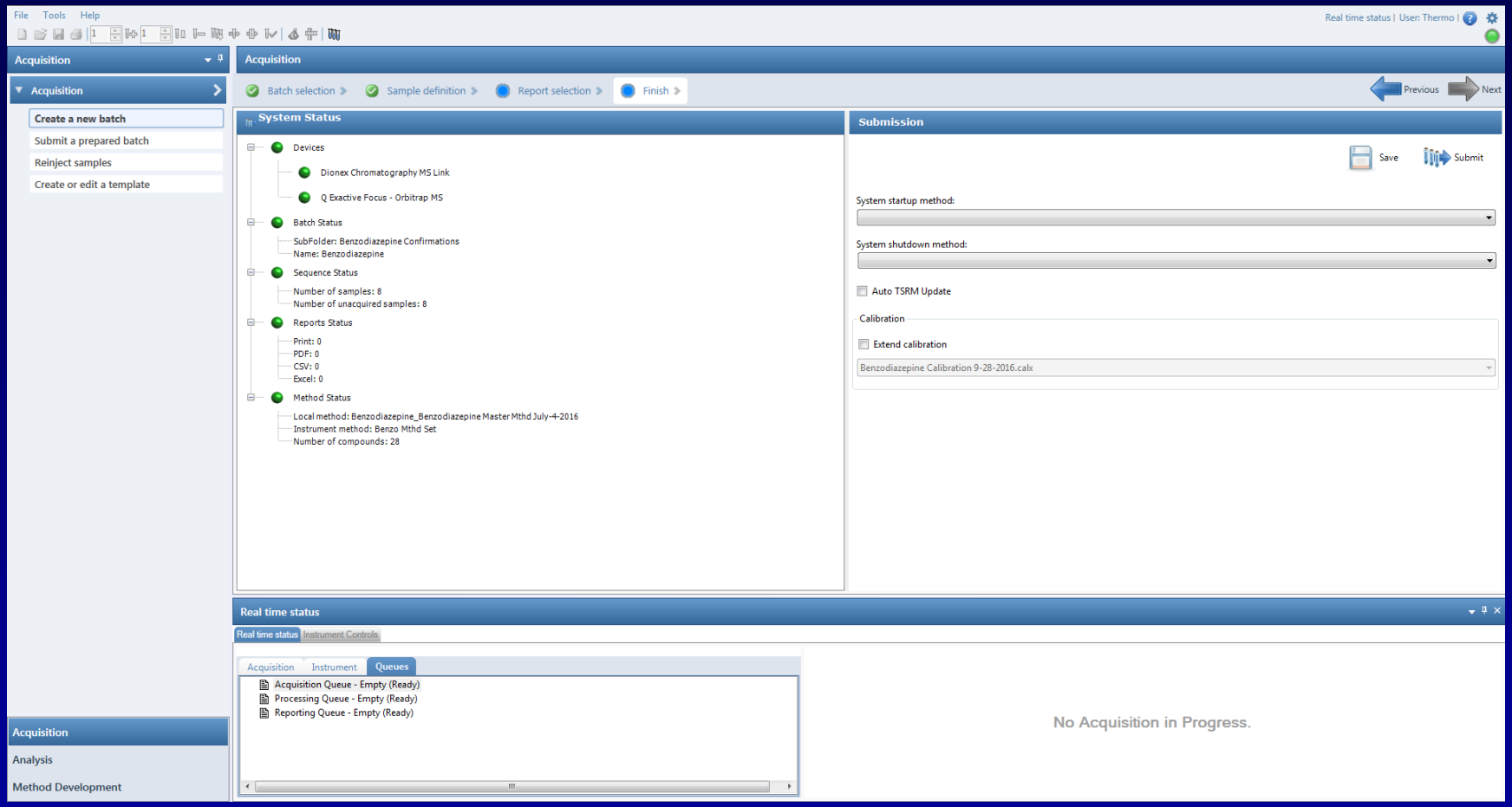


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

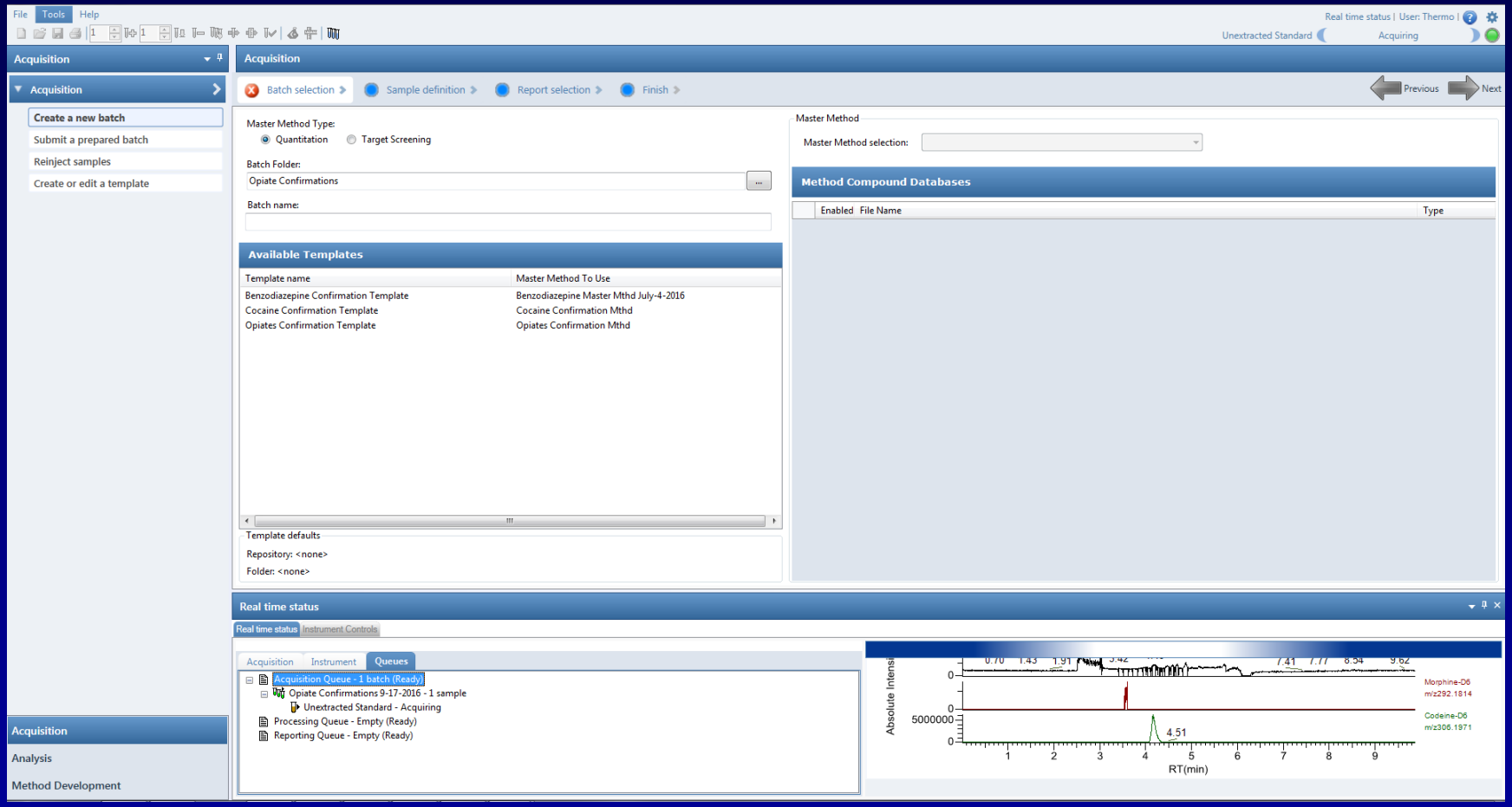


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

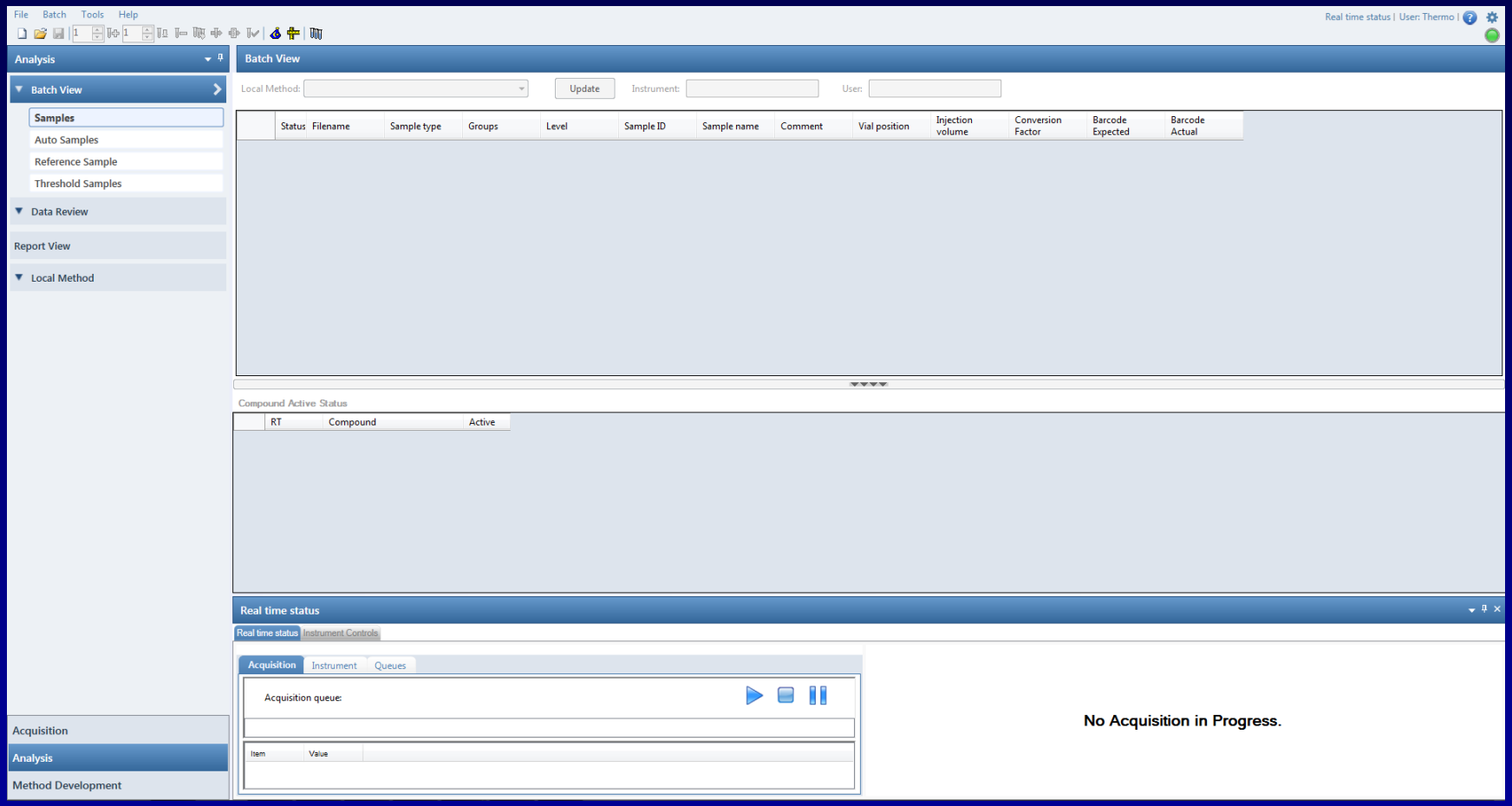


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

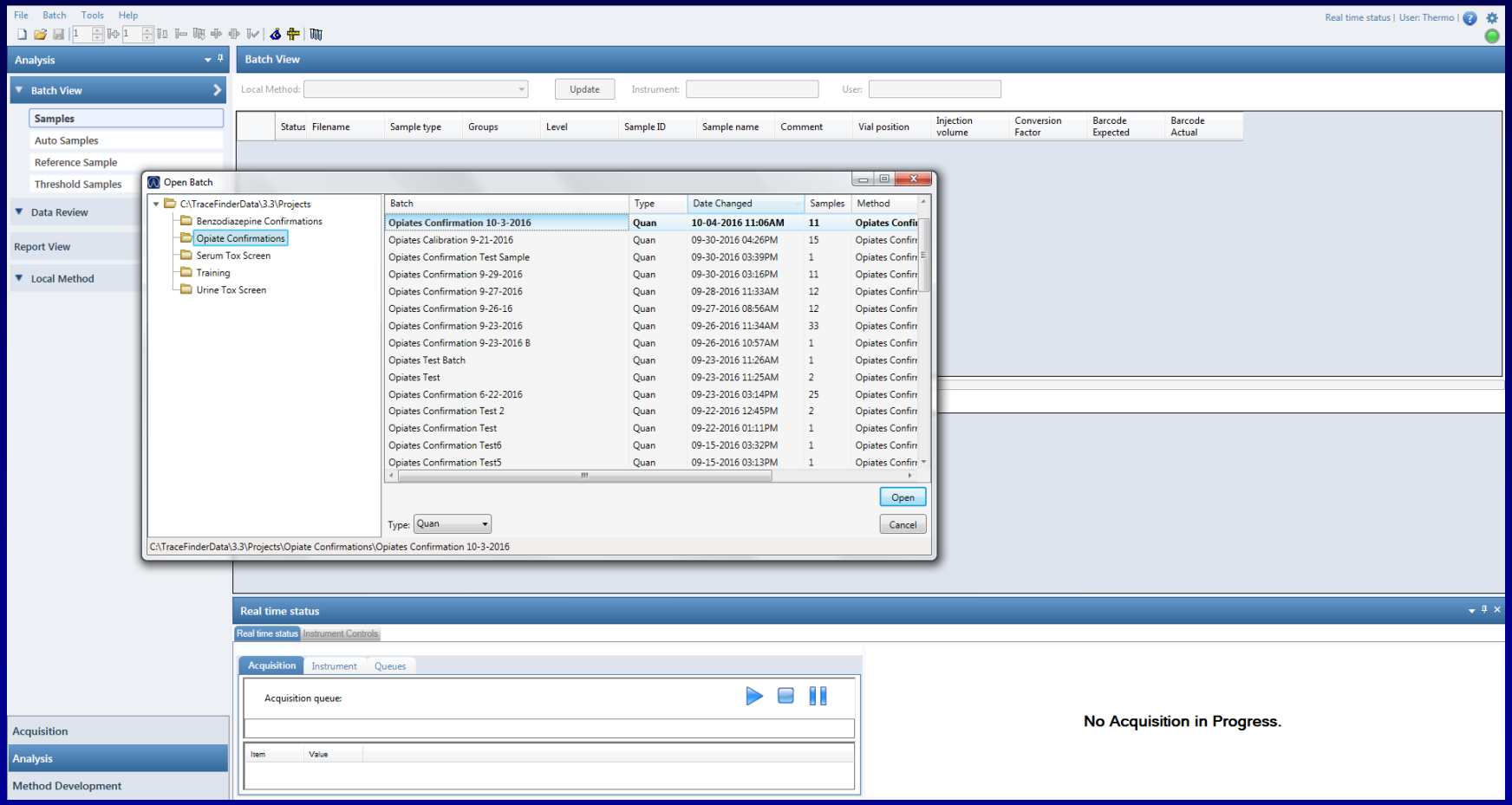


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

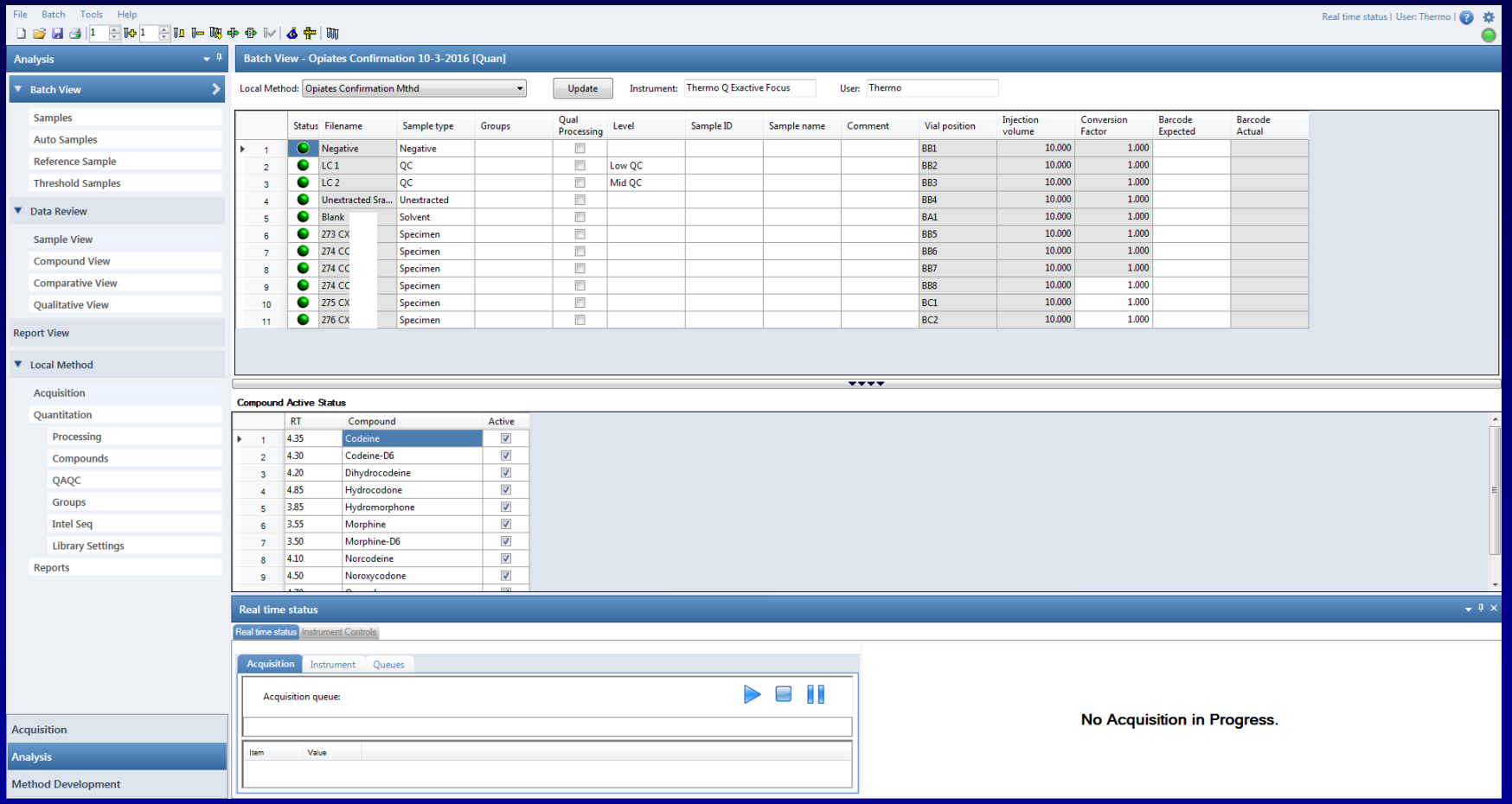


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

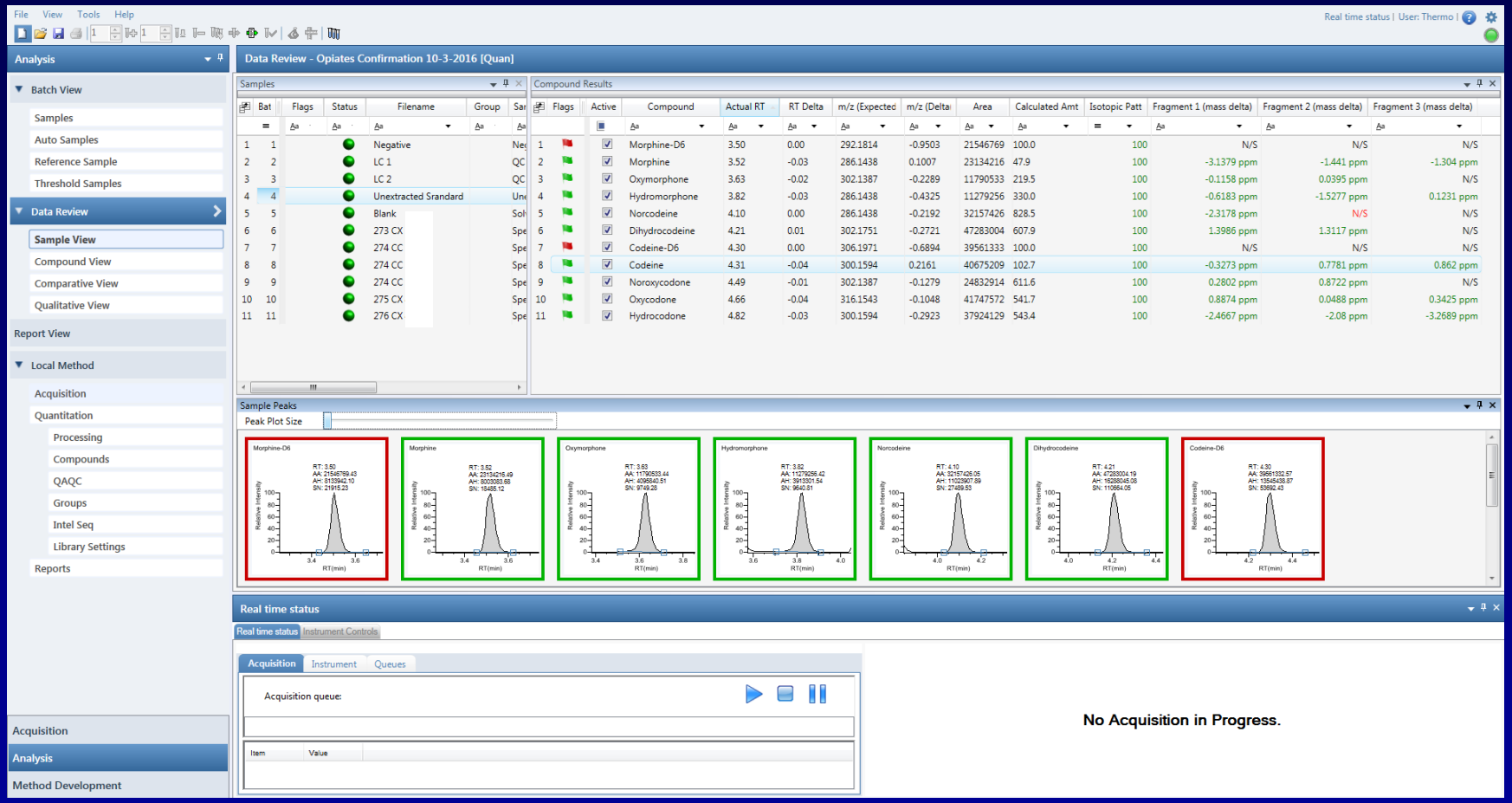


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

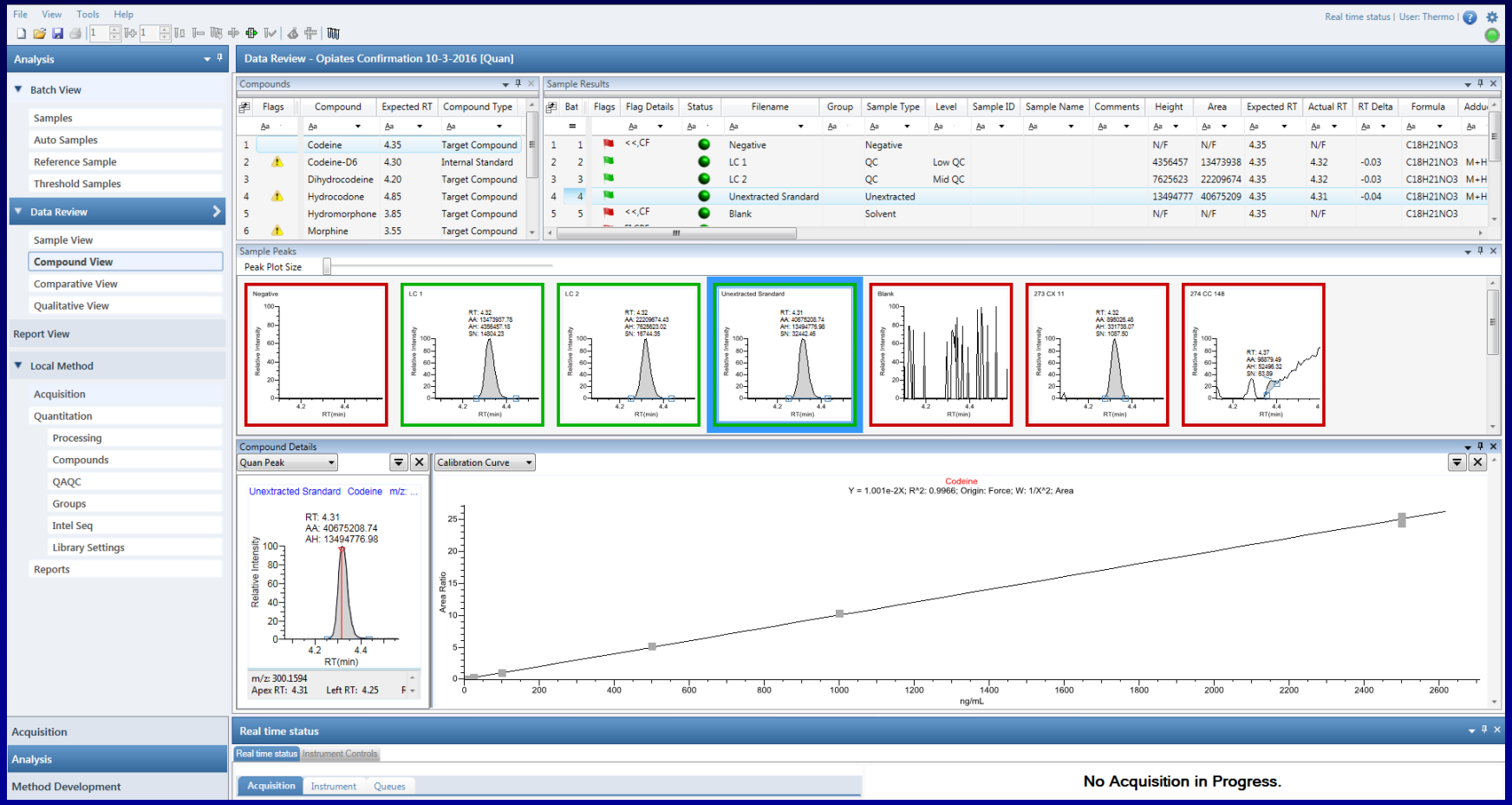


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

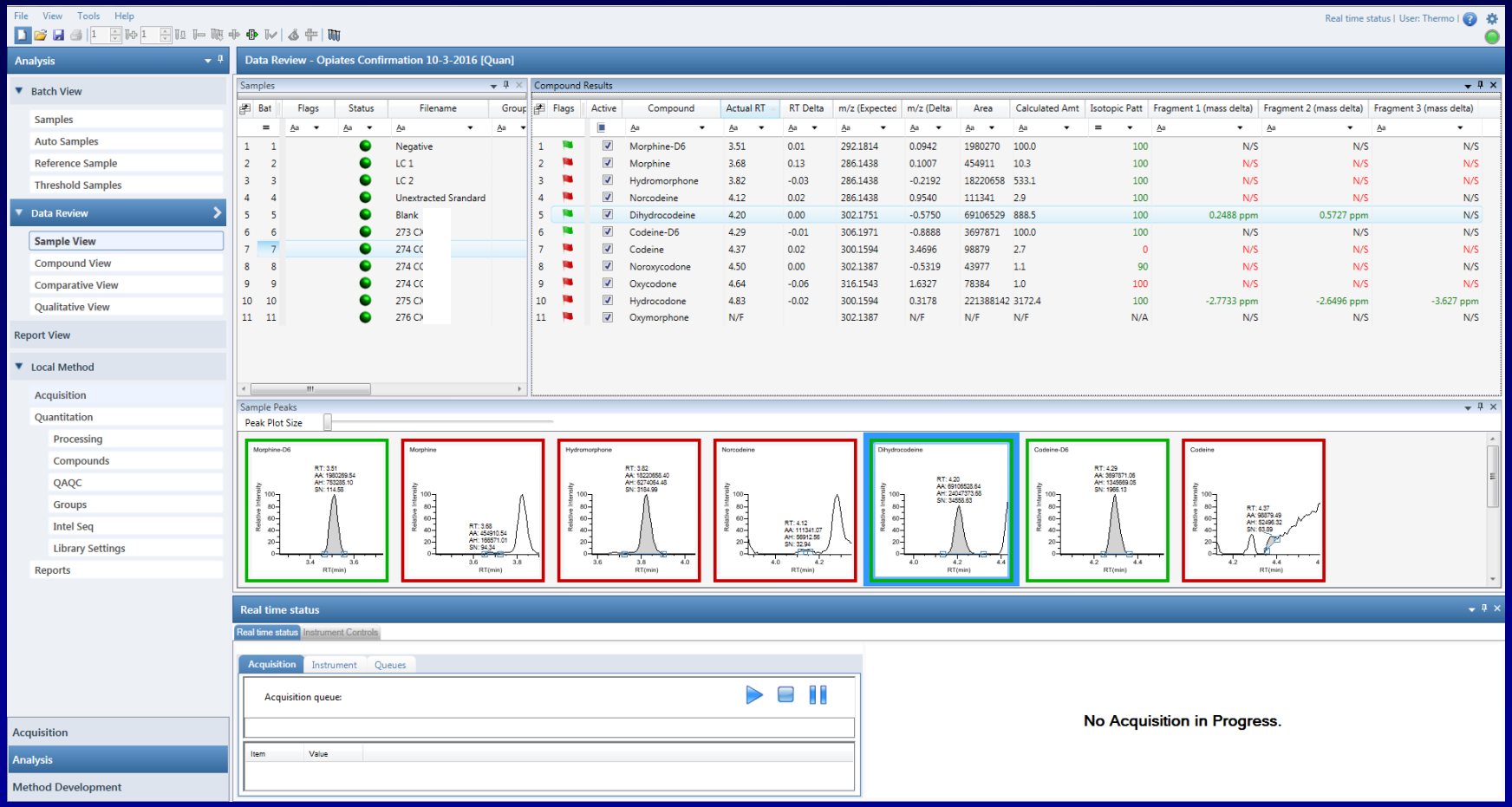


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software



Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Recent Illicit Drugs seized in Mississippi

1. Marijuana +/- PCP
2. Opiates/Opiods

   Hydrocodone, Heroin, Oxycontin
3. MDMA (Ecstasy, Molly)
4. Synthetic Cannabinoids (K2, Spice)
5. Carisoprodal/Meprobamate

Synthetic Cannabinoids

‘Spice’ overdose cases now up to 45 at UMMC, another 40 overdose cases treated at other Mississippi Hospitals April 9, 2015

Synthetic marijuana leads to nationwide spike in hospitalizations May 8, 2015

33 Suspected of Overdosing on Synthetic Marijuana in Brooklyn NY July 12, 2016

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Synthetic Cannabinoids

- Compounds often sprayed onto plant material and then smoked.
- Vape Pens & eCigarettes recently used
- Can produce 10x intense effects than THC because many analogues are full CB1 agonists (THC is a partial agonist)
- JWH-018 = 5x activity of THC

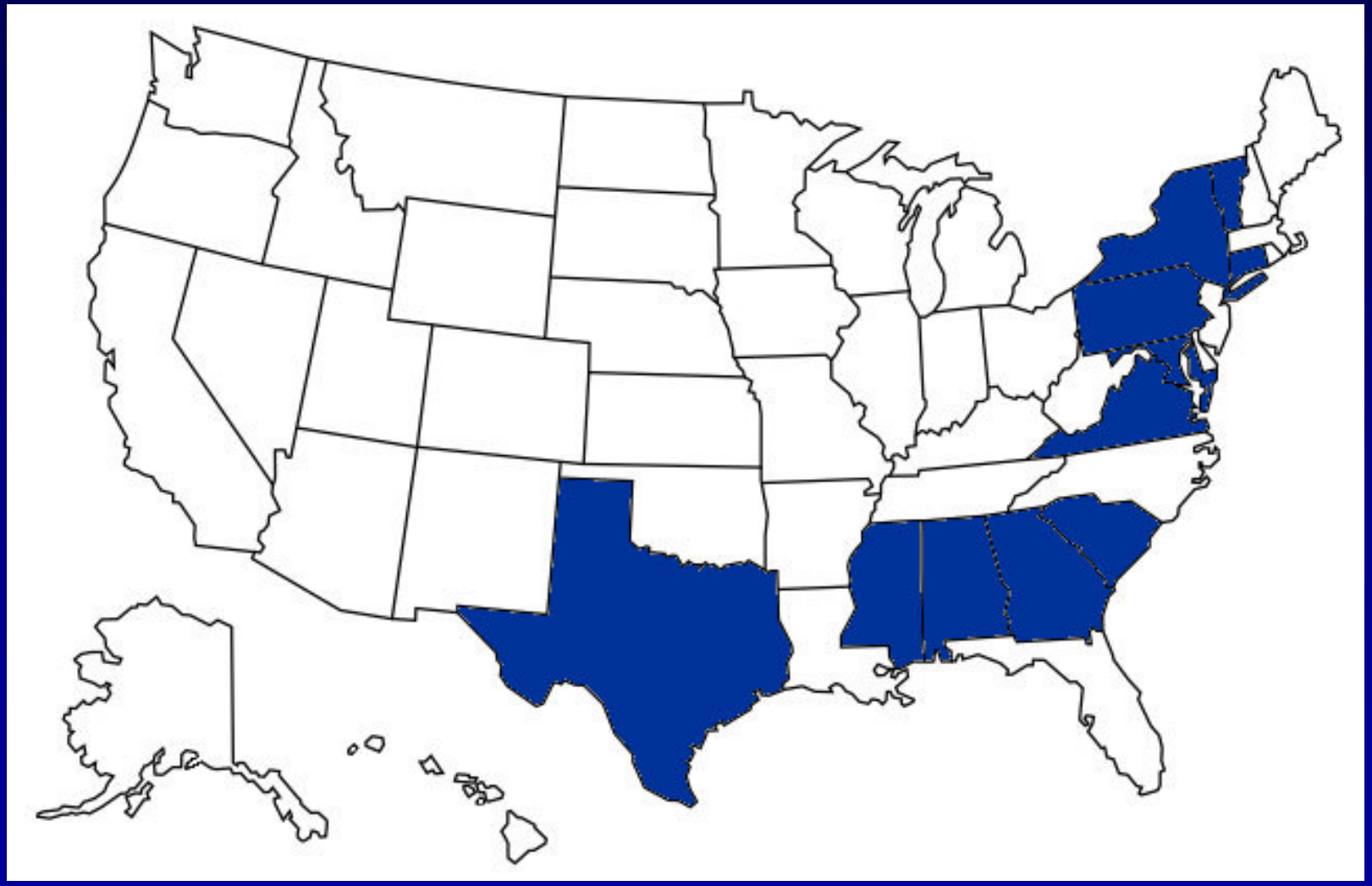
Synthetic Cannabinoids

$^{\Delta}$9-THC

JWH-018

MAB-CHMINACA

2015 MAB-CHMINACA



Synthetic Cannabinoids

Physiology/Symptoms

- **GABA inhibition** → paranoia, agitation, anxiety, seizures
- **B1 Agonist** → hypertension, tachycardia, palpitations
- Psychosis reported in some individuals
- Renal failure in some individuals

Synthetic Cannabinoids

- No formal Toxicity Testing performed
- Some 5x – 10x more potent than THC
- No Quality Control
- Compounds Sprayed -or- Soaked onto leaf material → Variable Concentrations
- Multiple compounds often in a single batch
- Attractive packaging may lure youngsters

Synthetic Cannabinoids

Synthetic Cannabinoids

Use of HRAM LC-MS/MS

Advantages

- Less Sample Preparation than GC-MS
- Differentiate isobaric compounds via chromatography and/or fragmentation
- Linear response
- Full Scan MS/MS Spectral Matching
- Identify new Synthetic Designer Drugs

Targeted Screening using HRAM LC-MS/MS

Instrument: Q-Exactive Focus (Thermo Scientific™)

Detection: ddMS/MS (90-600 m/z)

Library: HRMS Maurer/Wissenbach/Weber (Wiley)

Also Incorporated:

- >150 most commonly prescribed medications
- >30 Synthetic Cannabinoids
- >25 “Bath Salts”
- >20 Other abused Drugs (Mytraginine, etc)

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Targeted Screening using HRAM LC-MS/MS

Sample Preparation

- Add internal standard mix to 0.5 mL urine
- Centrifuge for 5 minutes at 8000 rpm (in microfuge)
- Dilute 1/20 into 0.1% Formic Acid
- Inject 10 µL onto LC-MS/MS

Mobile Phase: 5% B to 90% B over 15 minutes

Total Analytical Run Time: 20 minutes

For forensic use only.

Targeted Screening

- 27 y/o Male transported to ED after “running in traffic, barking at cars” and assaulting a pedestrian. Altered mental status noted.
- DOA Immunoassays: THC Positive
- THC Confirmation (GC-MS): Positive for THC-COOH

Targeted Screening

GC-MS

Caffeine

Theobromine

PCP

LC-MS/MS

Caffeine

Cotinine

Nicotine

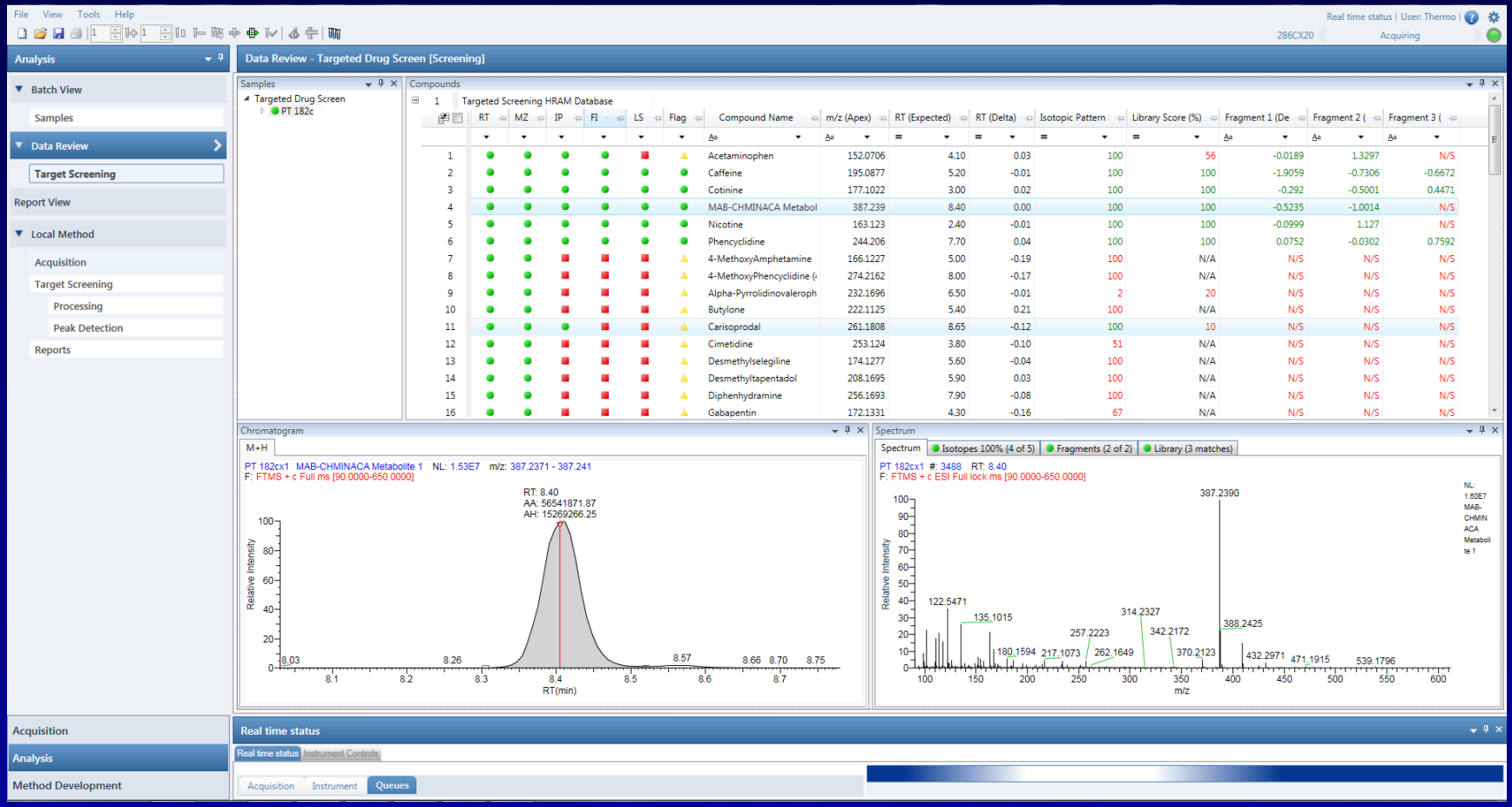
Theobromine

PCP

MAB-CHMINACA - Metabolite

For forensic use only.

TraceFinder™ Software

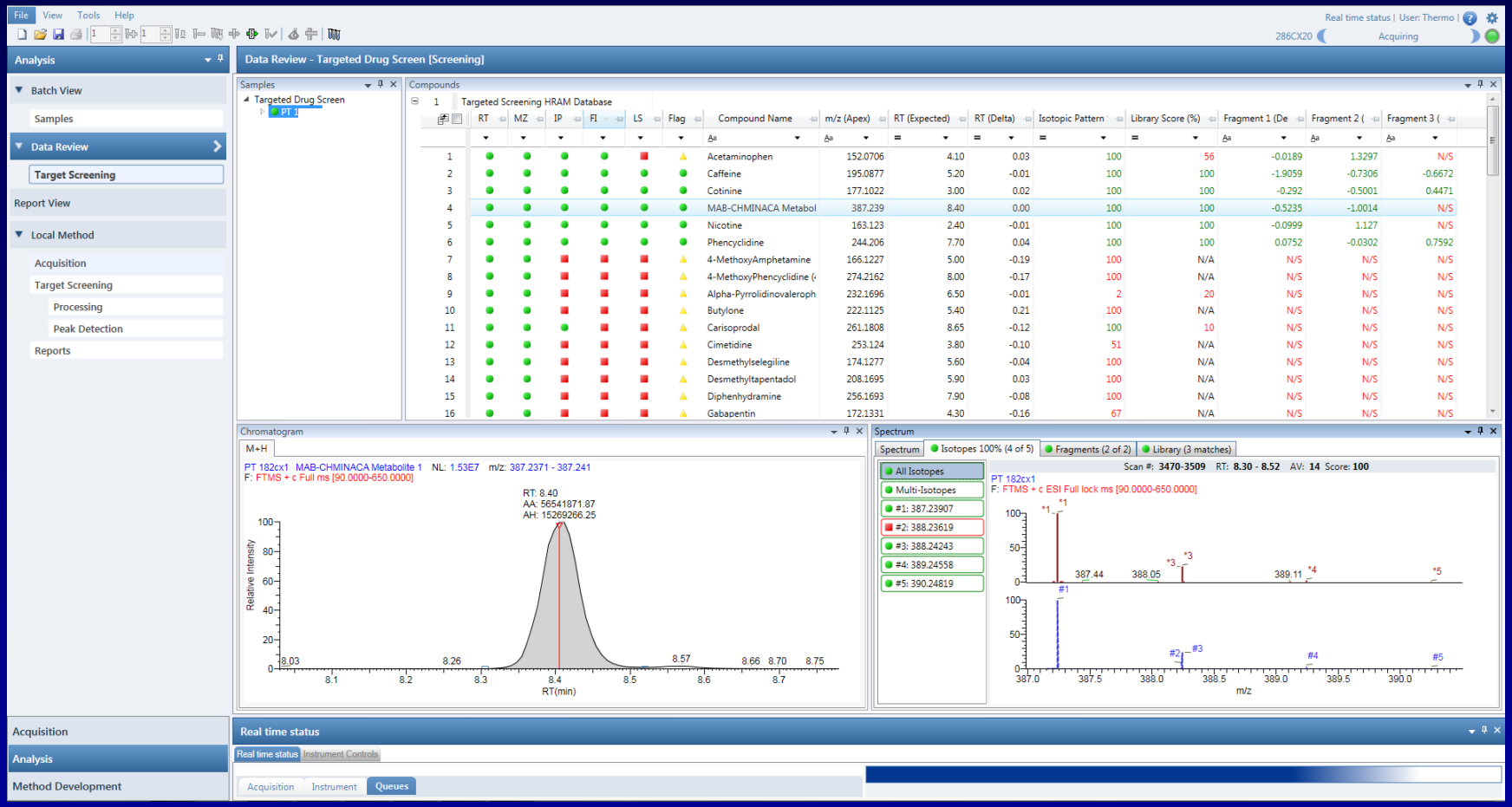


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

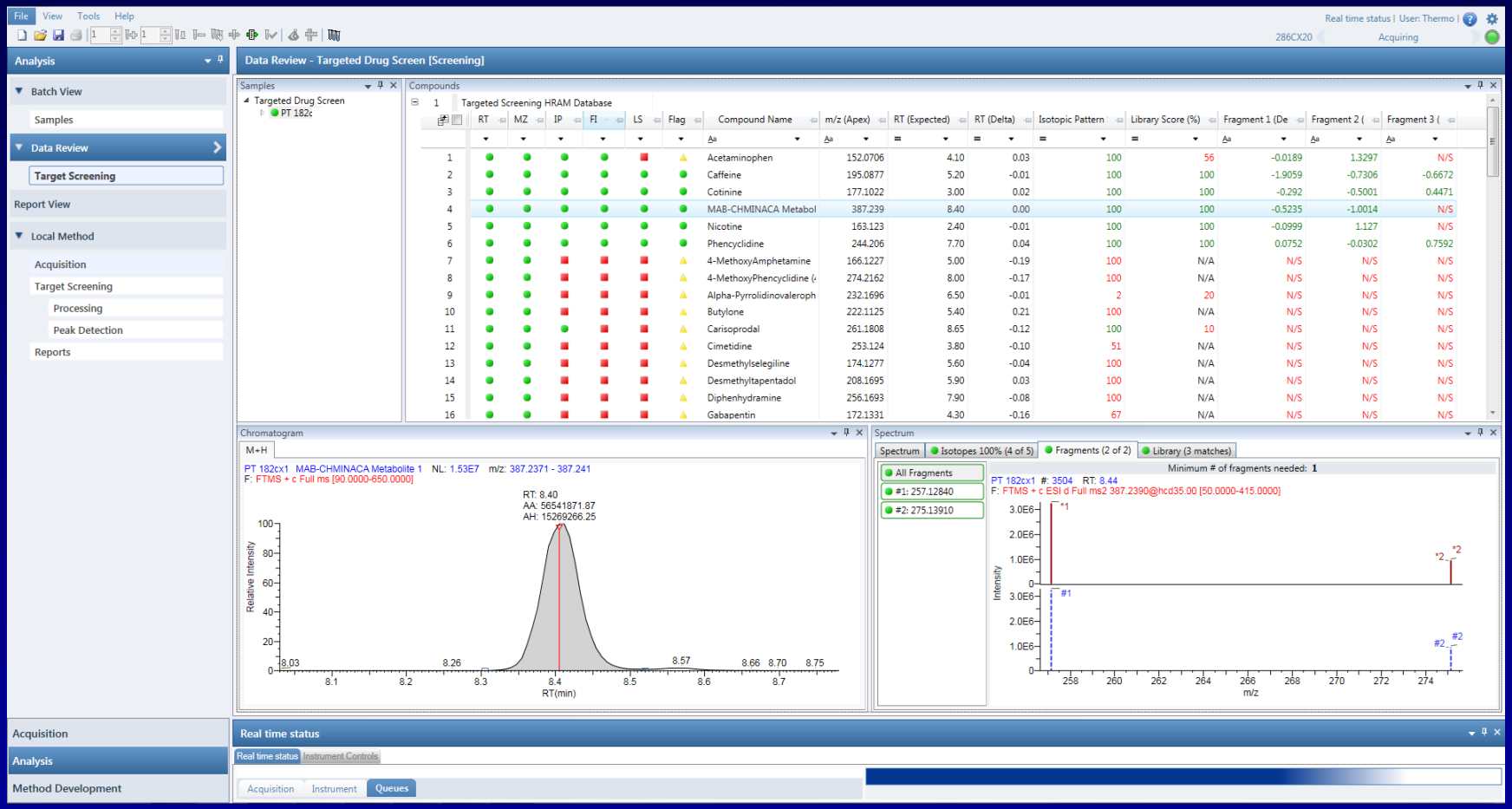


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software

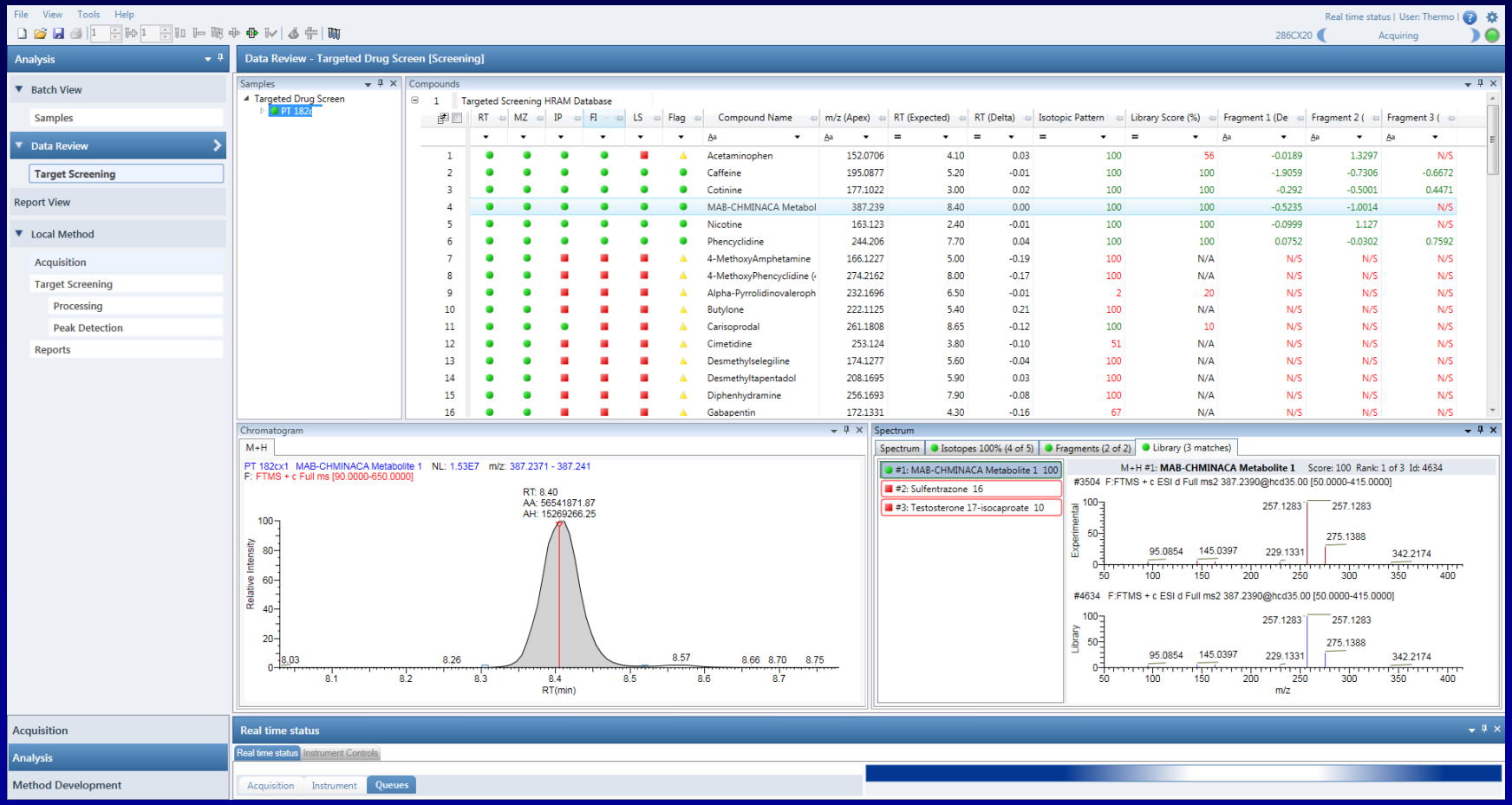


Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

TraceFinder™ Software



Thermo Scientific™ TraceFinder™ software

For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Targeted Screening

- 37 y/o Female jumped from her attic and attacked family members, then found naked on the scene by police. History of cocaine, methamphetamine, and ecstasy (MDMA) use.
- BP 128/113 (↑), Resp 20, Temp 98.9
- Ethanol: Negative

Targeted Screening

GC-MS	LC-MS/MS
Nicotine	Nicotine
Cotinine	Cotinine
Cocaine	Cocaine
Benzoylecgonine	Benzoylecgonine
Ecgonine Methylester	Levamisole
Levamisole	Naproxen
PCP	PCP
Tramadol	Tramadol
Tramadol Metabolite	Ziprasidone

Targeted Screening

- A 19 y/o Male presented to the PER with altered mental status (delusions, hallucinations).
- Ethanol: Neg
- DOA Immunoassays: Amphetamines – Pos
  Benzodiazepines - Pos

Targeted Screening

GC-MS	LC-MS/MS
Caffeine	Caffeine
Nicotine	Nicotine
Cotinine	Cotinine
Diphenhydramine	Diphenhydramine
Diphenhydramine Metab	Alprazolam + Metab
Methamphetamine	Ketamine
Olanzapine	Norketamine
	Methamphetamine
	Olanzapine

Unknown Screening using m/zCloud™ Software

- WWW.mzCloud.org
- Online MS searches in the Cloud
- Database updated daily
- Other ID Functions:
  - -Structure
  - -Tree (Molecular Fragments)
  - -MSn

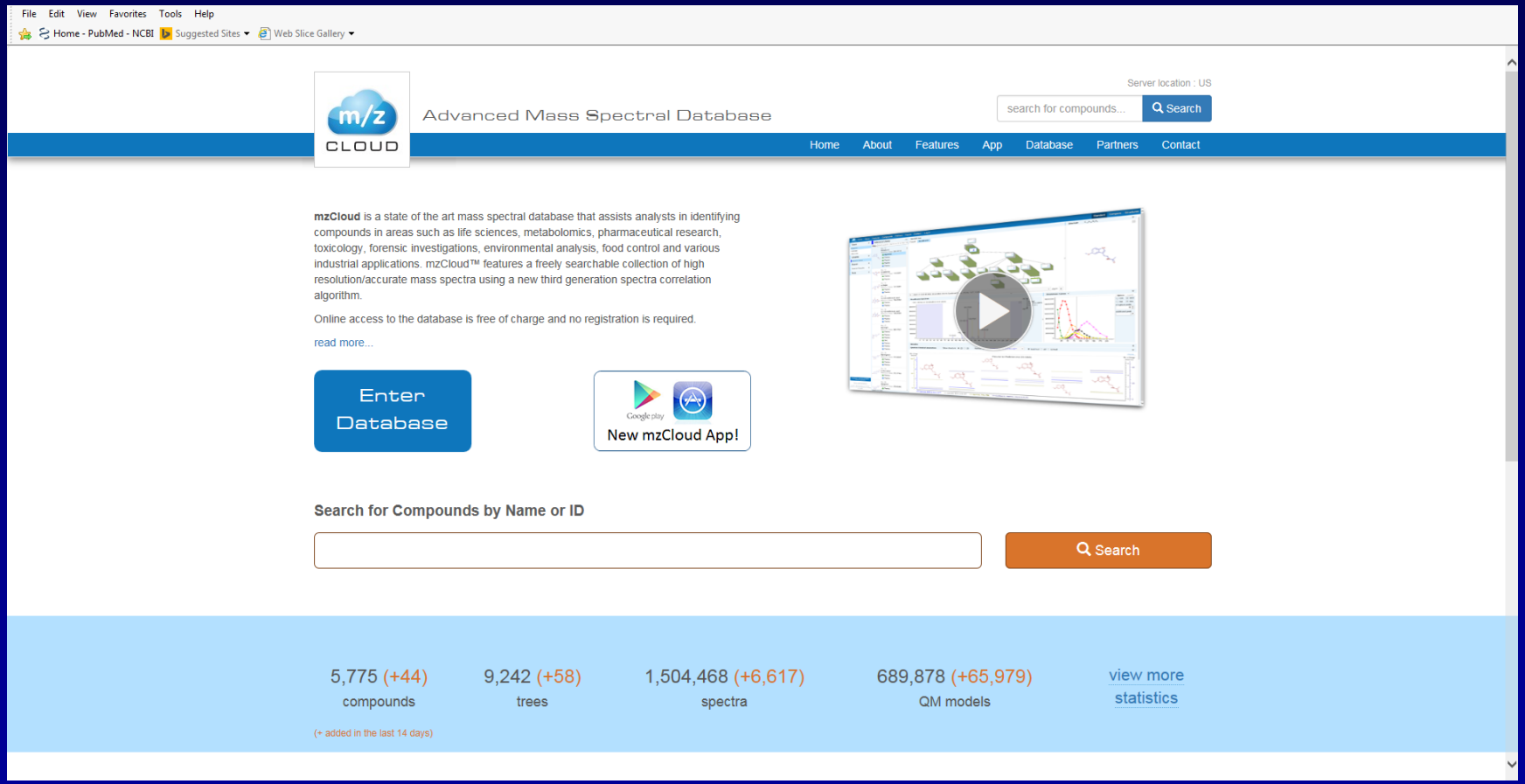
For forensic use only.

Unknown Screening using m/zCloud™ Software

- 21 y/o Male presented to the ED with incessant vomiting. Admitted smoking “Spice”
- Leafy material found in bag in pocket.
- GC-MS (methanolic extraction): Δ9-THC, MAB-CHMINACA, and large unknown Peak

For forensic use only.

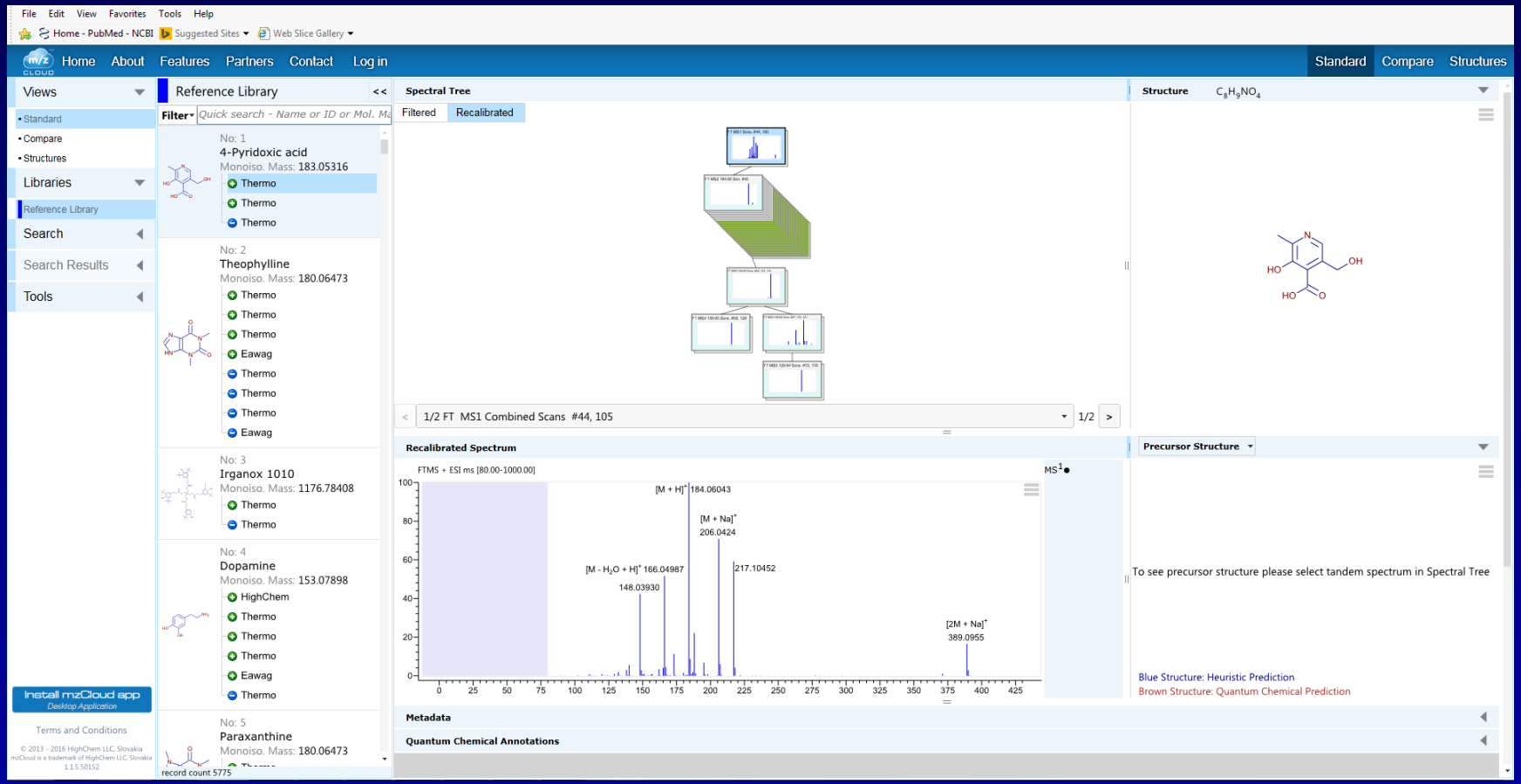
m/zCloud™ Software



For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

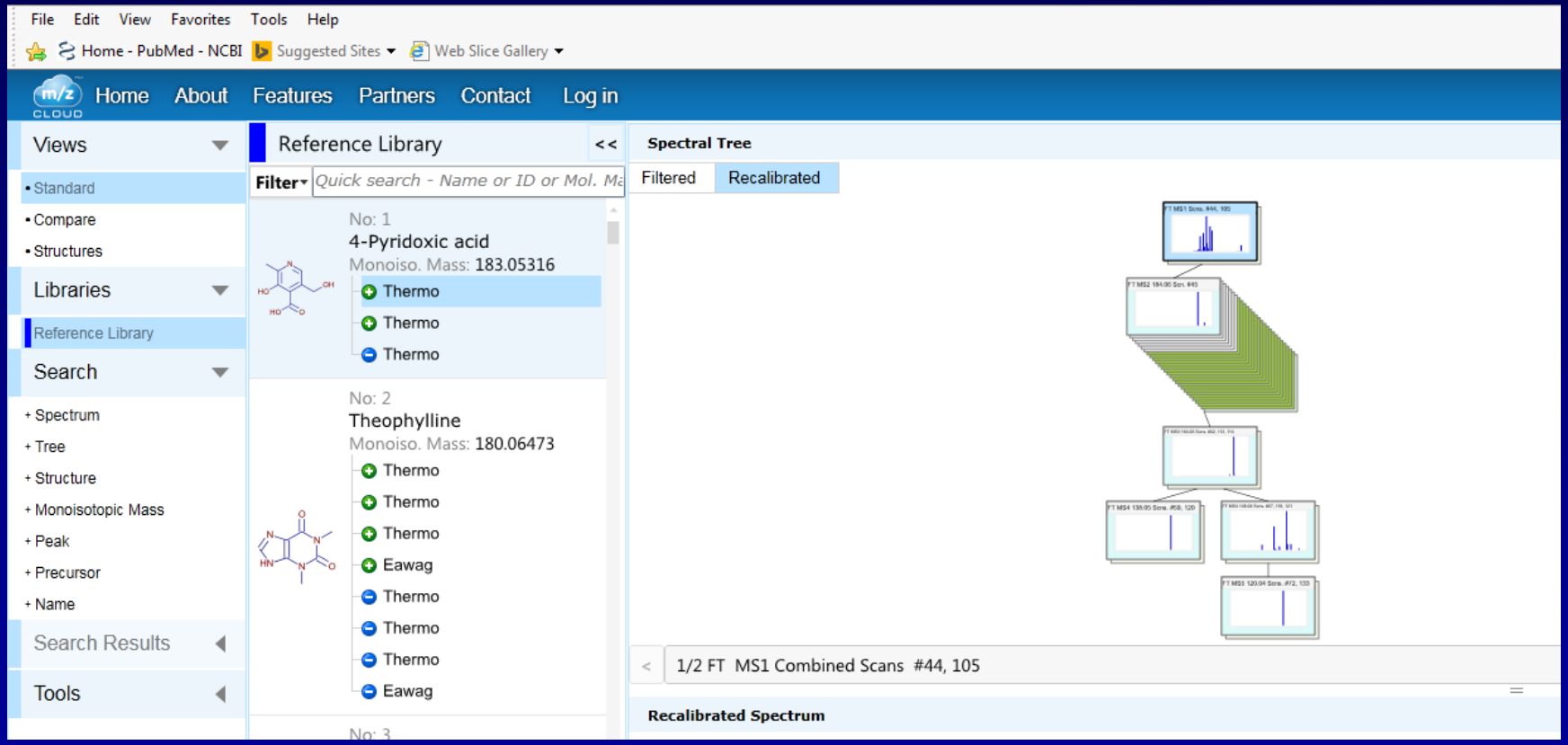
m/zCloud™ Software



For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

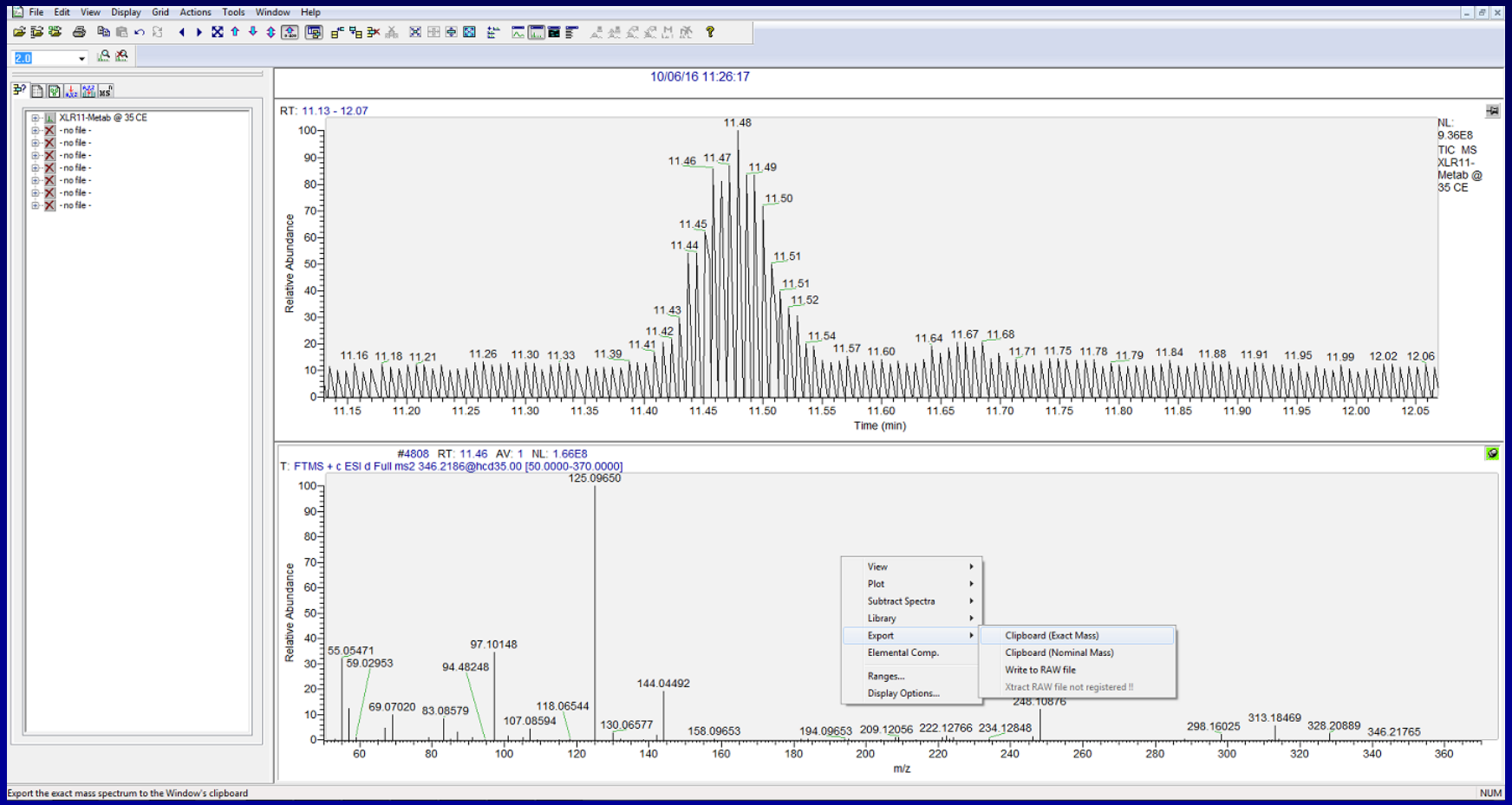
m/zCloud™ Software



For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

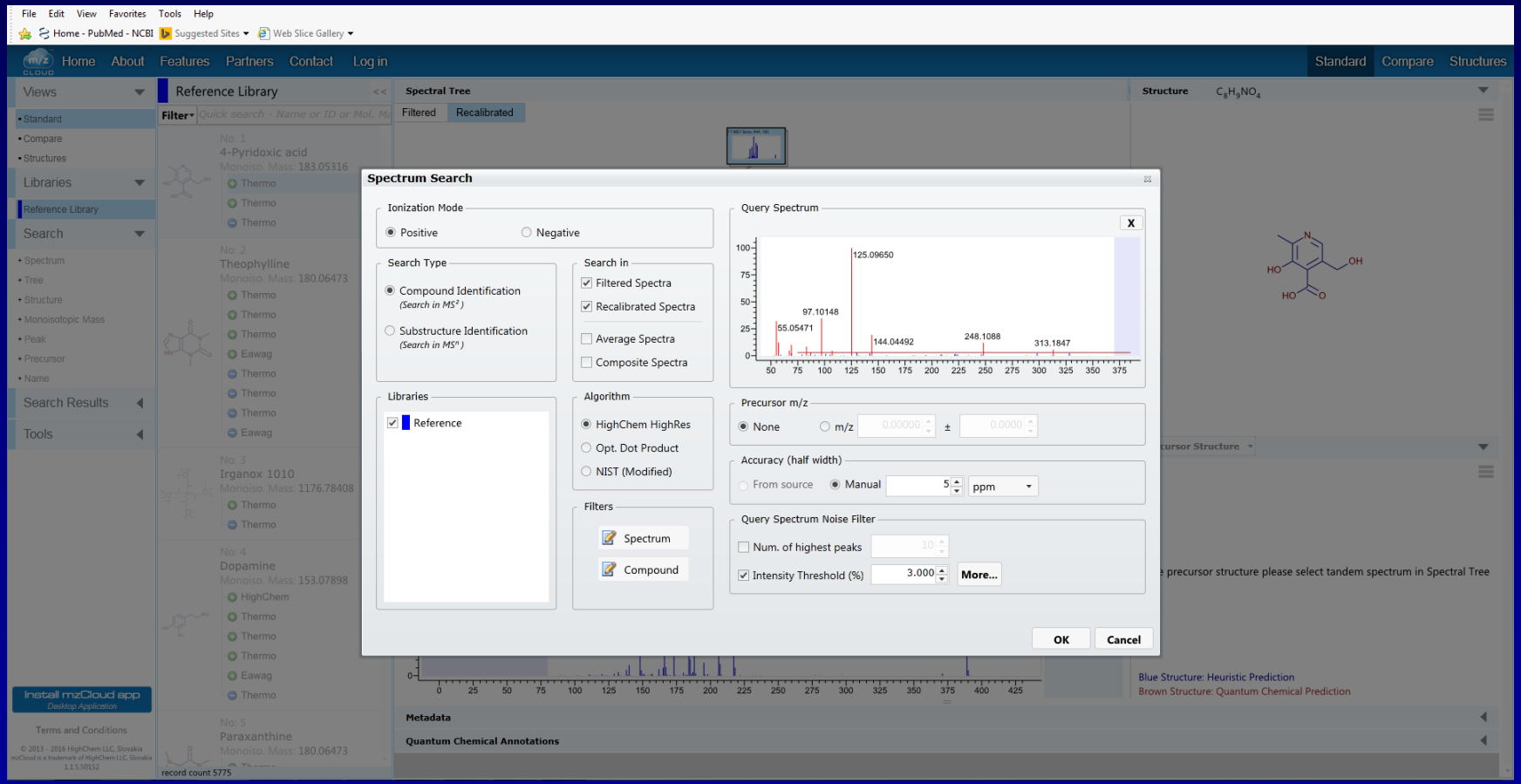
m/zCloud™ Software



For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

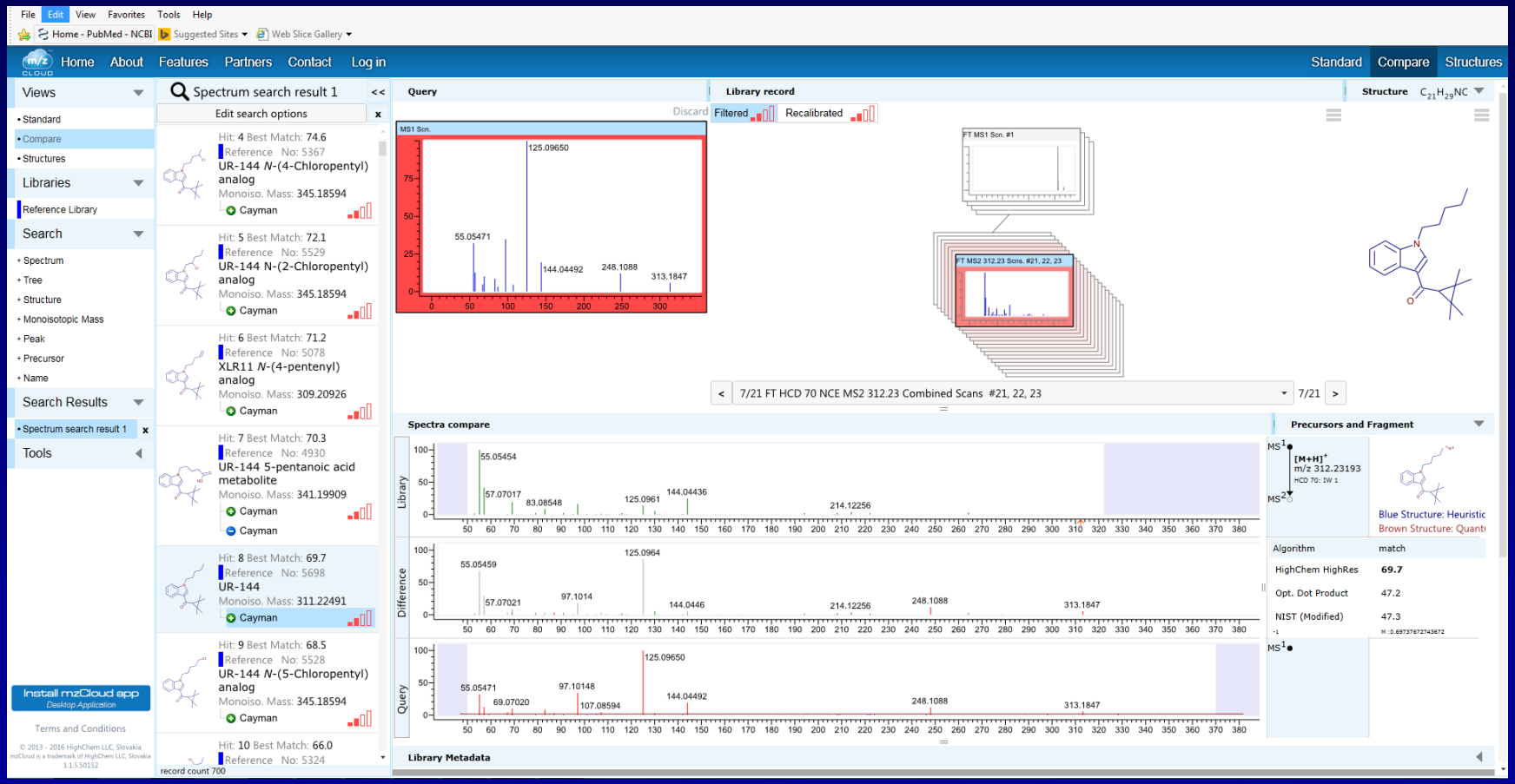
m/zCloud™ Software



For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

m/zCloud™ Software



For forensic use only.

THE UNIVERSITY OF MISSISSIPPI MEDICAL CENTER

Summary

Advantages of HRMS LC-MS/MS Analysis

- Only one HRMS fragment required for drug confirmation
- Less Sample Preparation vs GC-MS
- Specific Compound Identification
- HRMS MS/MS Full Scan spectra allow users to identify compounds using multiple databases (User's Library, Commercial Libraries, m/zCloud™, m/zVolt™)

For forensic use only.

Questions?

The University of Mississippi Medical Center

THE UNIVERSITY OF MISSISSIPPI
MEDICAL CENTER