

# **Mass Spectrometry in Forensic Science**

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**Alabama Department of Forensic Sciences**  
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## **Overview**

- Introduction to forensic sciences
- Traditional techniques in the lab
- New technology in the lab
- Why expand the capabilities beyond what is currently accepted?

## Introduction to Forensic Sciences

Forensic Sciences is defined as:

the application of a broad spectrum of **sciences** to answer questions of interest to the legal system.



## Introduction to Forensic Sciences

Typical analytical sections within a forensic science laboratory:

**Drug Chemistry** – Analysis of pills, powders, liquids, plant materials, and other suspicious items for illegal drug content

**Toxicology** – Analysis of biological samples for alcohol, prescription medication, drugs of abuse, and other chemicals that are not naturally occurring in the body

**Biology** – Extraction and amplification of DNA from biological fluids for identification

**Firearms** – Microscopic examination of uniquely identifying marks left behind in the tooling process

**Fire Debris** -- Identification of ignitable liquids residues in fire debris samples

## Standards for Accepting the Scientific Validity of a Procedure, Technique, and Principle

### Alabama

#### – Frye standard:

- the court must decide if the questioned procedure, technique, and principles are “generally accepted” by a relevant community

#### – Federal Rule 702:

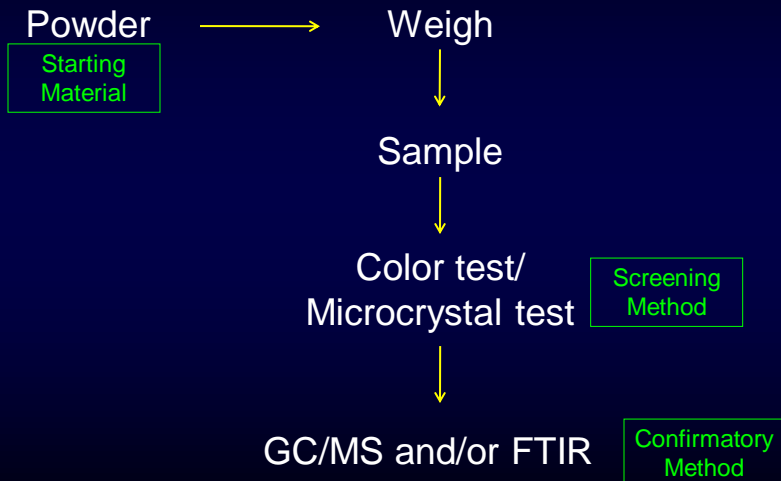
- a witness qualified as an expert may testify in the form of an opinion

### Federal

#### – Daubert:

- Has it been tested?
- Has it been published and peer reviewed?
- Potential rate of error
- Existence and maintenance of standards controlling the techniques operation
- Accepted in the relevant scientific community

## Typical Flow Chart for Case Analysis



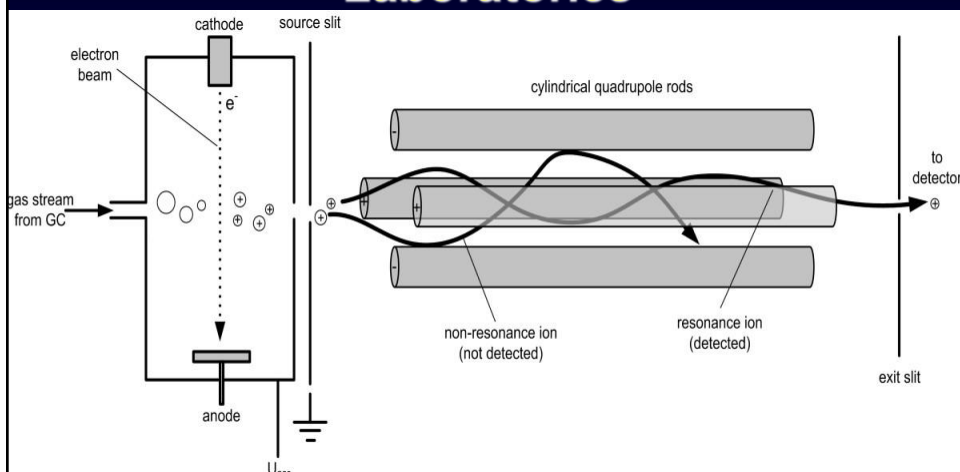
## Mass Spectrometry in Forensic Science

Traditionally, a gas chromatograph with a mass spectrometer is the final tool used in the analysis of drug chemistry and toxicology samples for identification and confirmation.

The Hoover Laboratory has 40 analysts, 12 of which work in either chemistry or toxicology. There are 9 GC-MS in the lab.

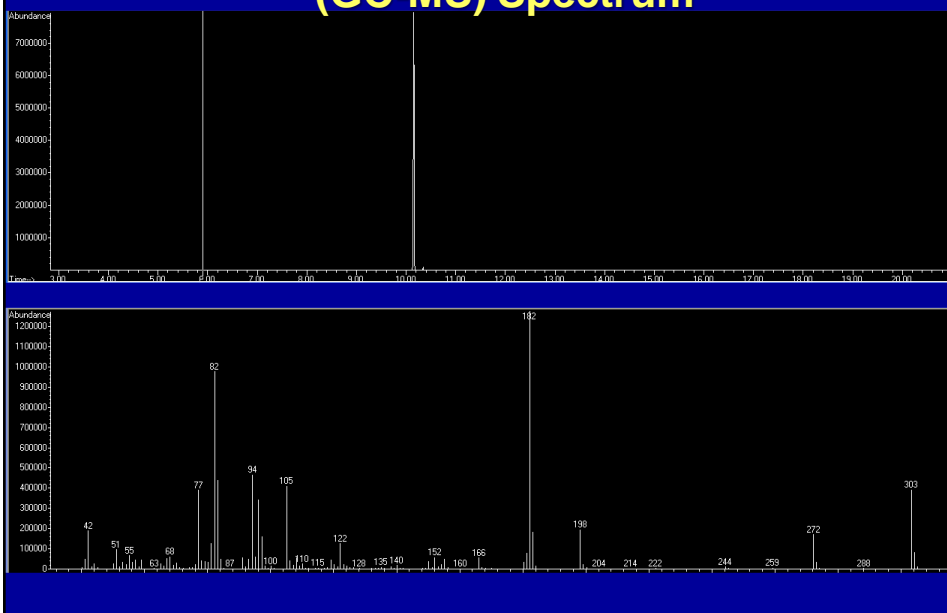


## Typical forms of Mass Spectrometry in Most Forensic Science Laboratories

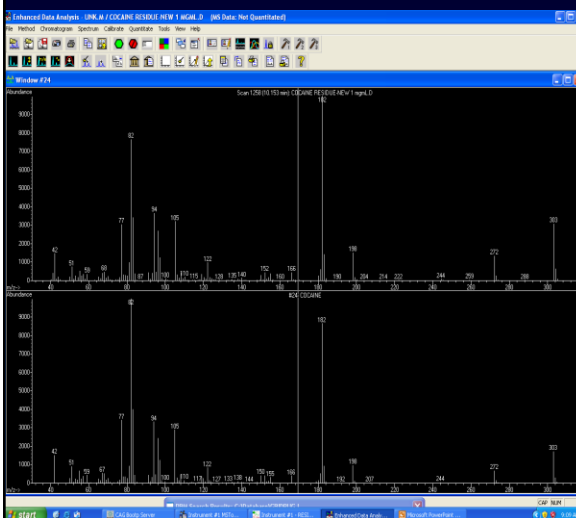


<http://www.microbialcellfactories.com/content/figures/1475-2859-6-6-4-1.jpg>

## Gas Chromatography-Mass Spectrometry (GC-MS) Spectrum



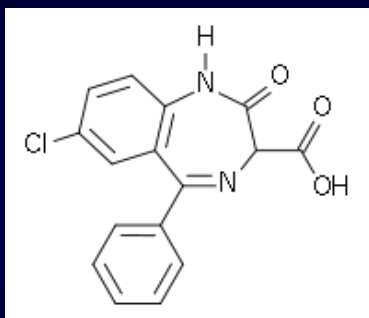
## Gas Chromatography-Mass Spectrometry (GC-MS) Spectrum



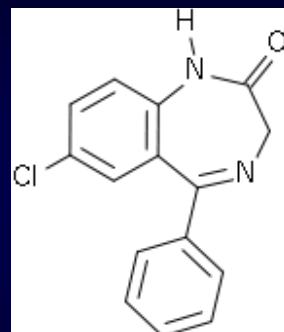
Spectra are searched against a library of known compounds in an effort to identify every peak in the Total Ion Chromatogram (TIC)

A standard is analyzed on the instrument to generate a known retention time and spectrum of the compound for that instrument

## Disadvantage of using GC/MS for chemical analysis



Clorazepate



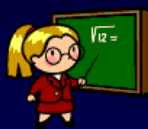
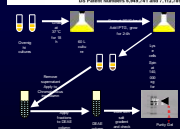
Nordiazepam

A heat labile compound could be misidentified as a related compound due to the necessary high temperature in GC-MS analysis

Example: Clorazepate to Nordiazepam

## Current Role in the Alabama Department of Forensic Science

Work with new technology/methods



Provide technical training

Conduct research independently and through collaborations



Troubleshoot existing methods and instrumentation



## New Technology at the Alabama Department of Forensic Science (ADFS)

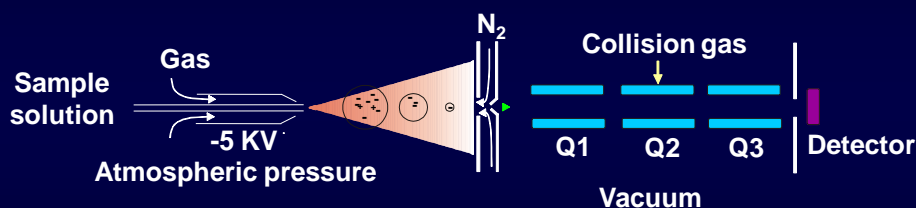
ADFS has four instruments that expand the capabilities beyond traditional methodologies.

1. AccuTOF-DART mass spectrometer
2. 3200 QTRAP mass spectrometer with LC
3. 3200 QTRAP mass spectrometer with DART
4. Head Space-GC-MSD



## Different forms of Mass Spectrometry

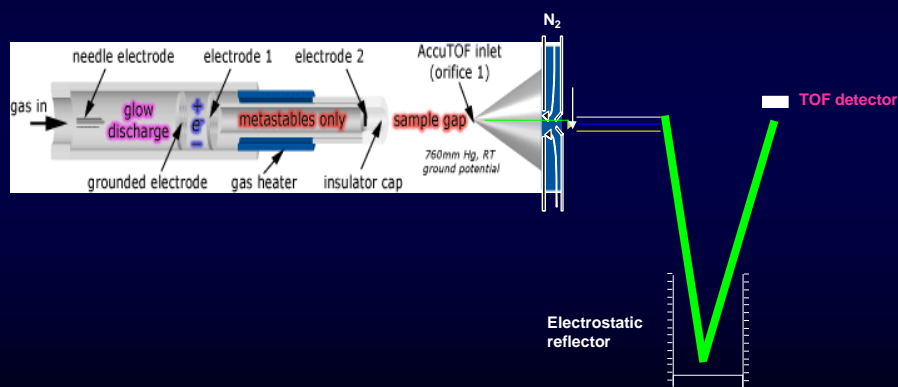
Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC-ESI-MS)



Courtesy of S. Barnes

## Different forms of Mass Spectrometry

### Direct Analysis in Real Time with Time of Flight Mass Spectrometry



Courtesy of S. Barnes

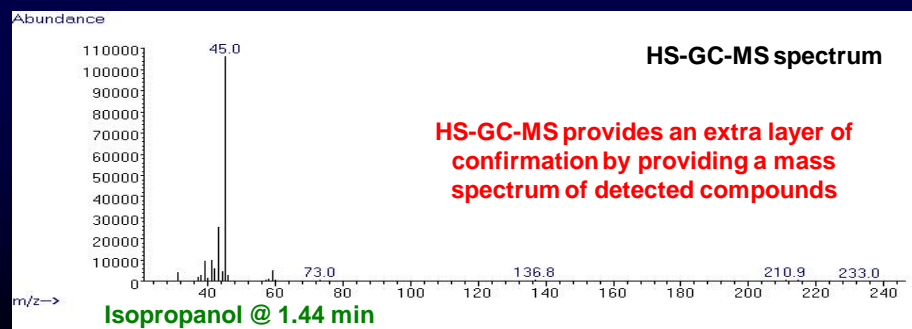
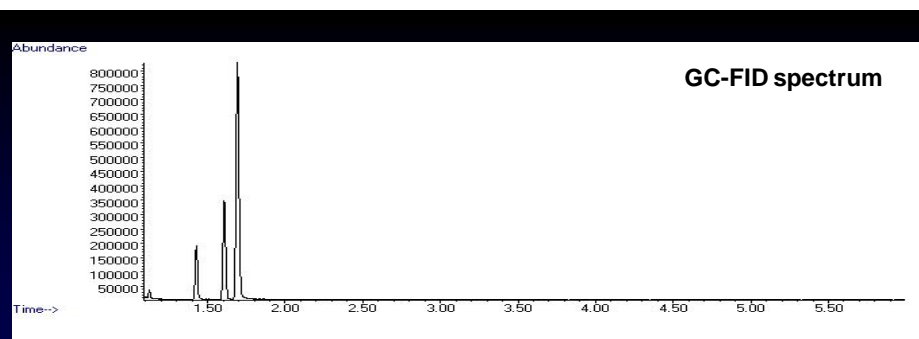
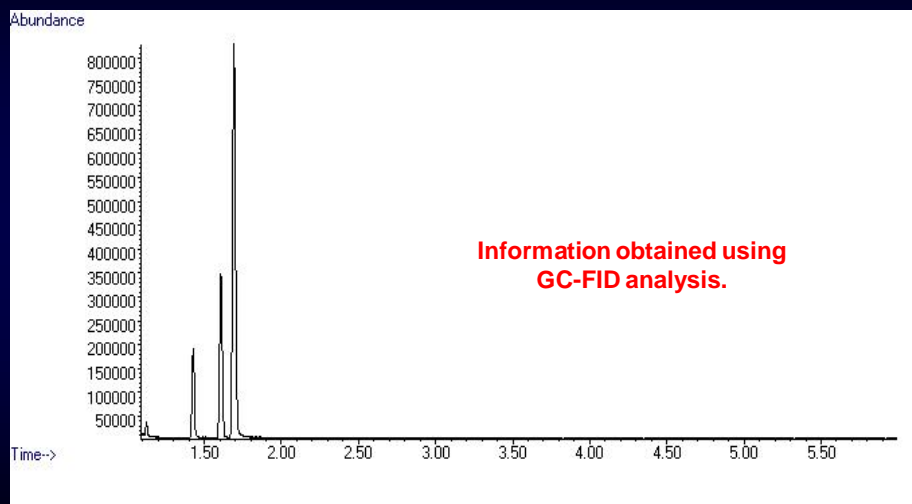
## HeadSpace-GC-MSD

- This instrument provides the opportunity for qualitative and quantitative identification of volatile compounds
- Traditionally volatile compounds are analyzed with a GC/FID instrument
- GC/FID instruments rely solely on retention time for identification
- HS-GC-MS instruments have the added benefit of including mass spectrometry data to the retention time data





## TIC of Volatiles Mix



## Why do we want to use this new technology?

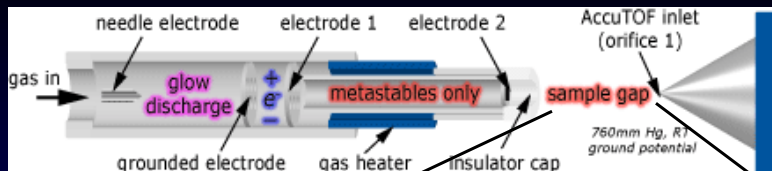
- GC-FID is widely accepted in the field for quantification of a variety of compounds
- HS-GC-MS provides a technique that uses a different detector, which increases the level of confidence in the results
  - Peaks at unknown retention times now have a corresponding mass spectrum for identification purposes
  - Currently know of only 1 other state forensic science lab with this technology

## AccuTOF-DART MS

- The DART is the first open air, ambient ion source for a mass spectrometer
- When coupled to a time of flight instrument, exact mass measurements can be used in the putative identification of compounds



## DART Ionization



- Penning ionization: energy is transferred from metastable ions ( $M^*$ )
- Positive ions:  $He^*$  ionizes water which transfers a proton to the sample
- Negative ions: Penning electrons are rapidly thermalized and captured by oxygen which ionizes the sample

<http://www.jeolusa.com/PRODUCTS/AnalyticalInstruments/MassSpectrometers/AccuTOFDART/AccuTOFDARTIonizationMechanisms/tabid/450/Default.aspx>

## Real Time Sample Analysis with the AccuTOF-DART MS

Efficient screening instrument because...

- Mass accuracy allows matches within 5 mmu of the theoretical mass of a compound when calibration is performed within the analysis
- No extraction is required for sample analysis
  - Raw samples are the preferred sample
- High-throughput
  - Typical analysis time for a sample is 1-2 min

## Types of Samples Analyzed with the AccuTOF-DART MS



[http://www.ecstasy2.com/img/ecstasy\\_pill\\_collage1.jpg](http://www.ecstasy2.com/img/ecstasy_pill_collage1.jpg)



## Sample Introduction with the AccuTOF-DART MS



Liquid samples are introduced with a glass capillary tube closed at one end

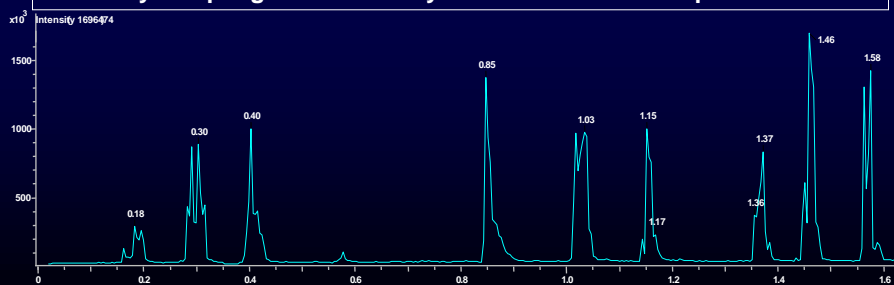


Solid samples are introduced into the stream with tweezers

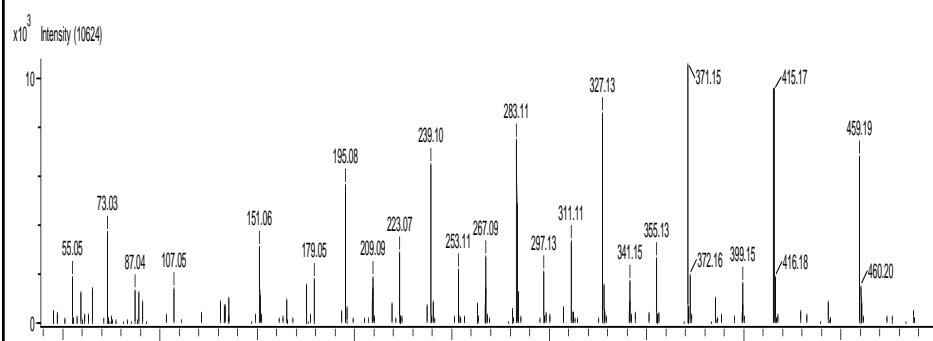
## Sample Introduction with the AccuTOF-DART MS



Every sampling device is analyzed for contamination prior to use.

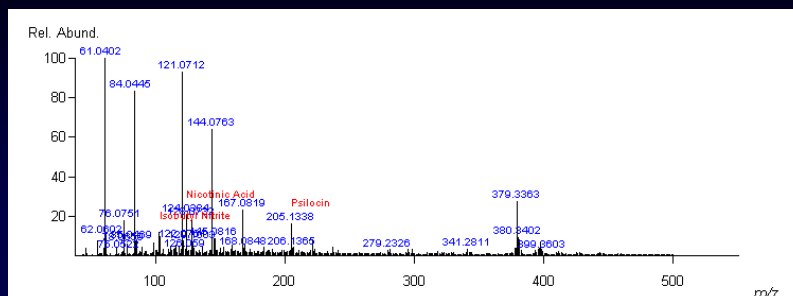
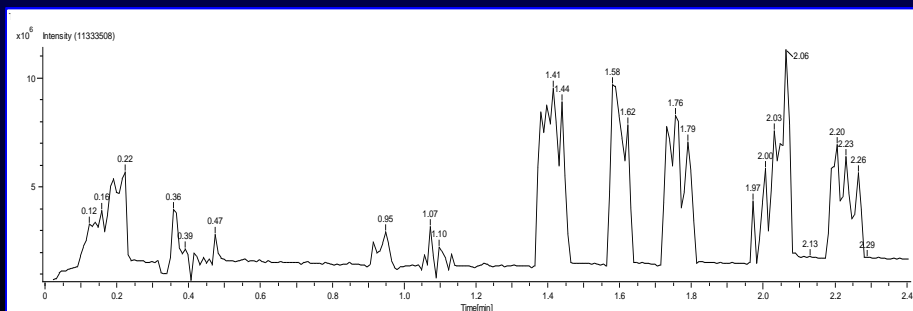
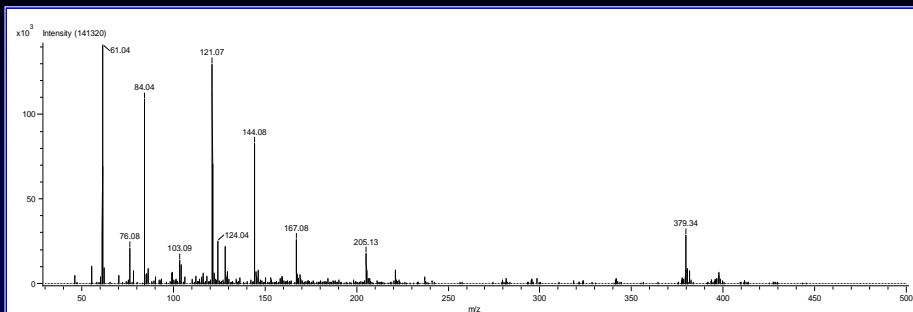


## Polyethylene Glycol (PEG)



Every spectrum undergoes internal calibration with polyethylene glycol.

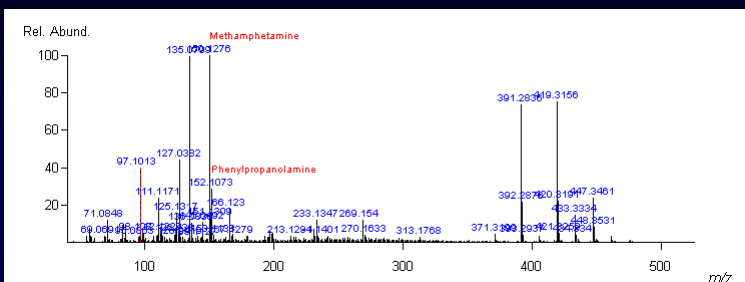
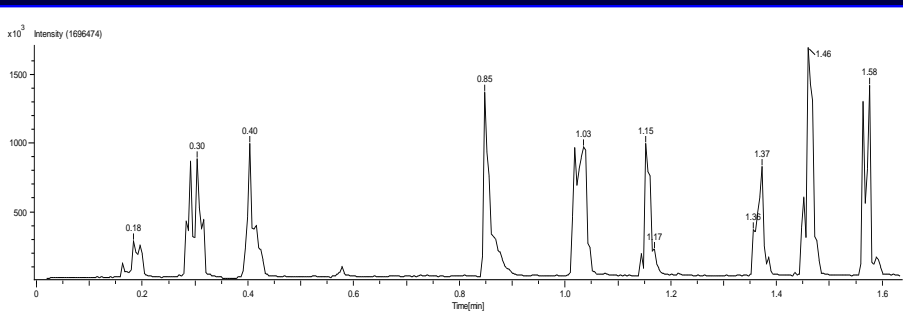
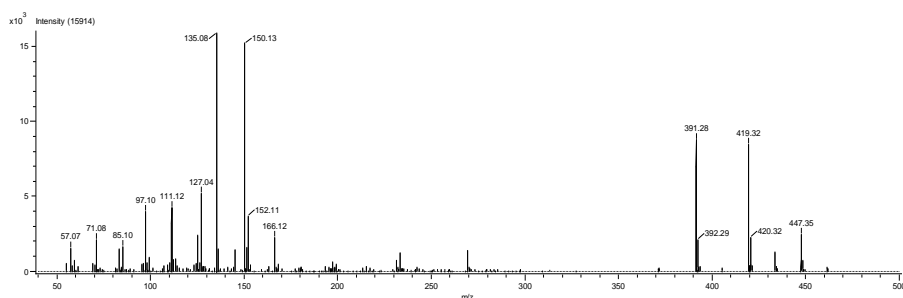
# Mushroom



MS File: F:\BIRMINGHAM LAB 7pj\04.01.09\mushroom.jsp  
 Search File: C:\Documents and Settings\Administrator\Desktop\DRUGS\_NEUTRAL\_MASSES.xls  
 Mass tolerance (mmu): 5 Threshold (%): 5  
 Date: 03-Apr-09 Time: 07:24 AM

Name	Neutral Comp.	Meas.	Calc.	Diff(u)	Abund.	
Isobutyl Nitrite	C4H9NO2	104.0716	104.0713	0.0005	9.7152	x
Nicotinic Acid	C6H5NO2	124.0384	124.0399	-0.0014	20.1042	x
Bufotenine	C12H16N2O	205.1338	205.1341	-0.0003	16.147671	H0800
5-MeO-AMT	C12H16N2O	205.1338	205.1341	-0.0003	16.1476	x
Psilocin	C12H16N2O	205.1338	205.1341	-0.0003	16.1476383	

# Methamphetamine Lab Liquid



MS File: F:\VALIDATION.7pj\03.10.09\09JV00871-1.jsp  
 Search File: C:\Documents and Settings\Administrator\Desktop\DRUGS\_NEUTRAL\_MASSES.xls  
 Mass tolerance (mmu): 5      Threshold (%): 20.  
 Date: 03-Apr-09      Time: 08:31 AM

Name	Neutral comp.	Meas.	Calc.	Diff(u)	Abund.	
Phentermine	C10H15N	150.1276	150.1283	-0.0006	100.0000	
Methamphetamine	C10H15N	150.1276	150.1283	-0.0006	100.0000	
Phenylpropanolamine	C9H13NO	152.1073	152.1075	-0.0002	28.3102	PPA01
Cathine (Norpseudoephedrine)	C9H13NO	152.1073	152.1075	-0.0002	28.3102	X

## Why do we want to use this new technology?

- The AccuTOF-DART provides reviewable data for screening of cases
- Samples that have been negative by traditional screening techniques have screened positive with the AccuTOF-DART
  - Compounds that do not have a traditional screening technique available can be easily analyzed with this instrumentation

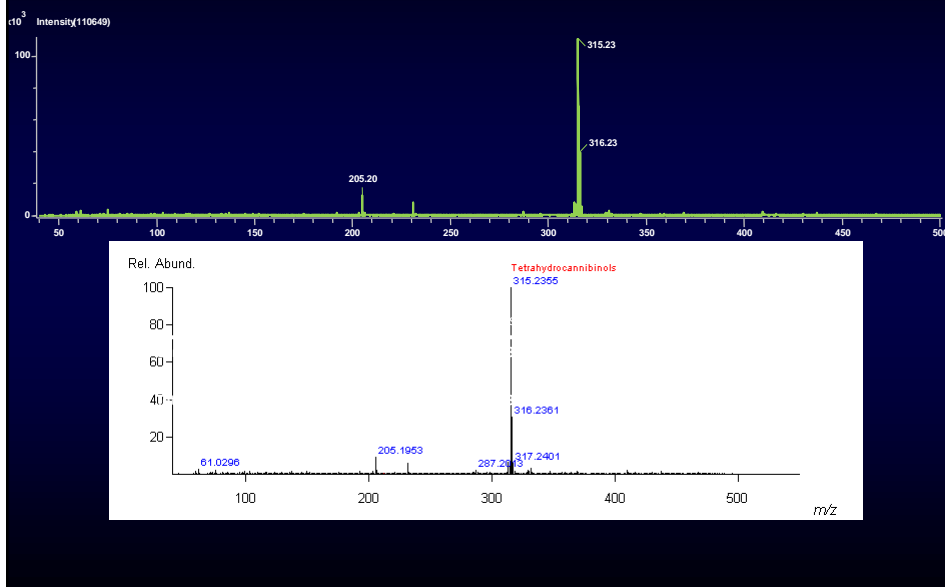
## 3200 QTRAP-DART MS

A DART coupled to a hybrid triple quadrupole/trap instrument produces molecular ions can be individually fragmented for identification of sample components.

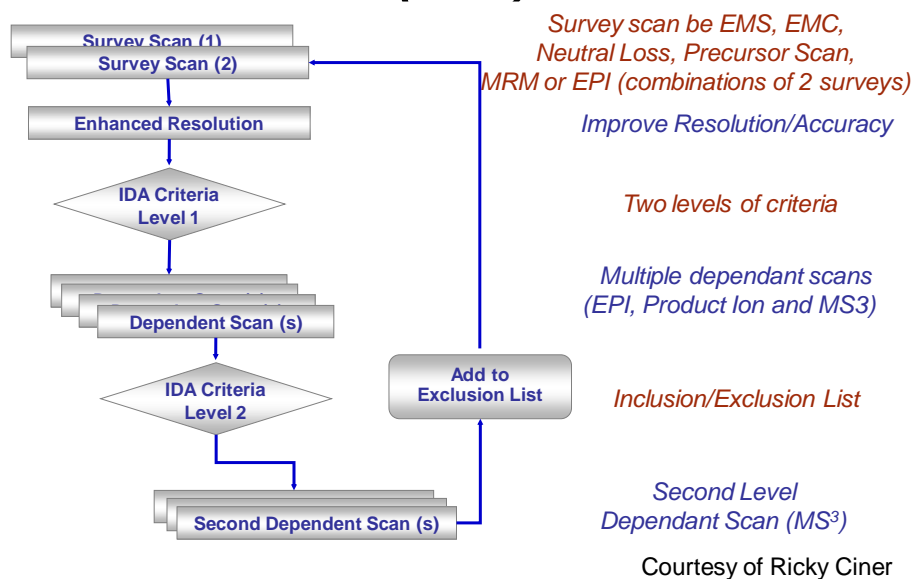


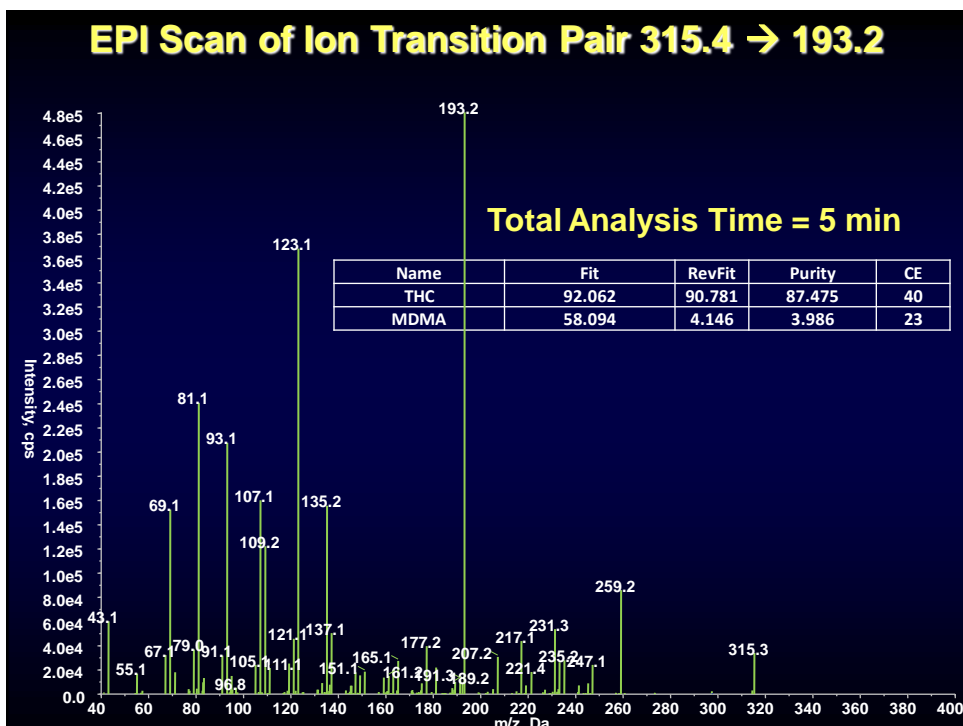
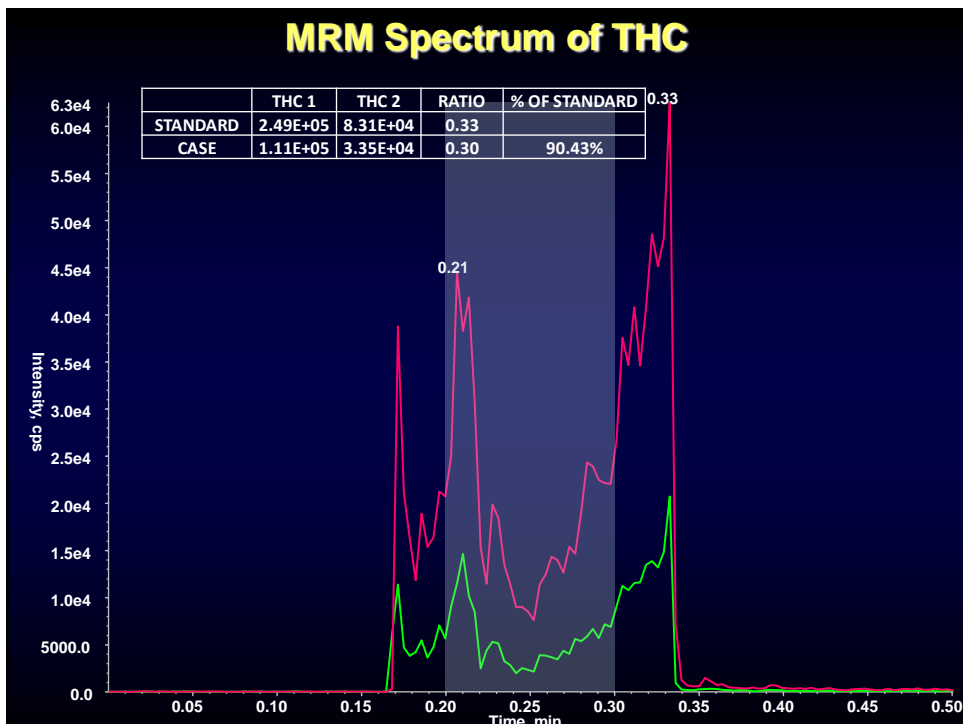


## Plant Material Analyzed with the AccuTOF-DART MS

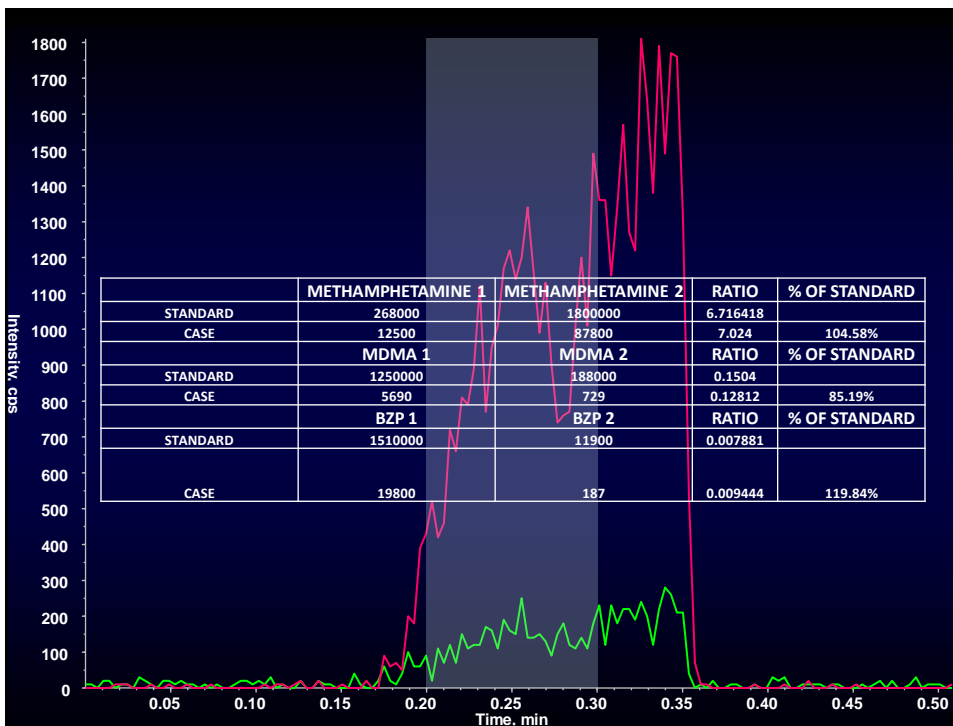
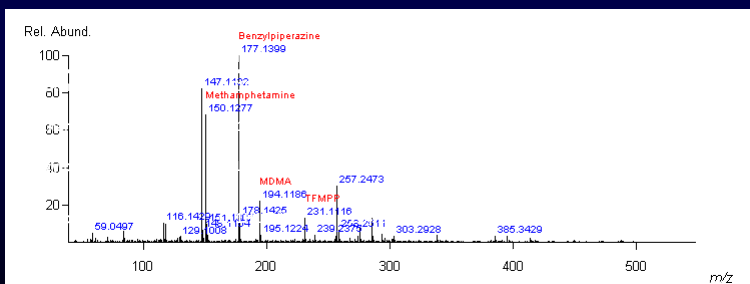
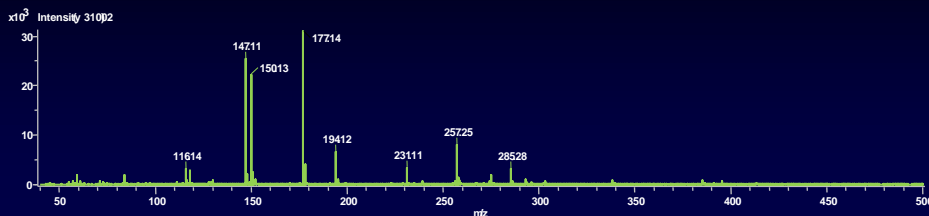


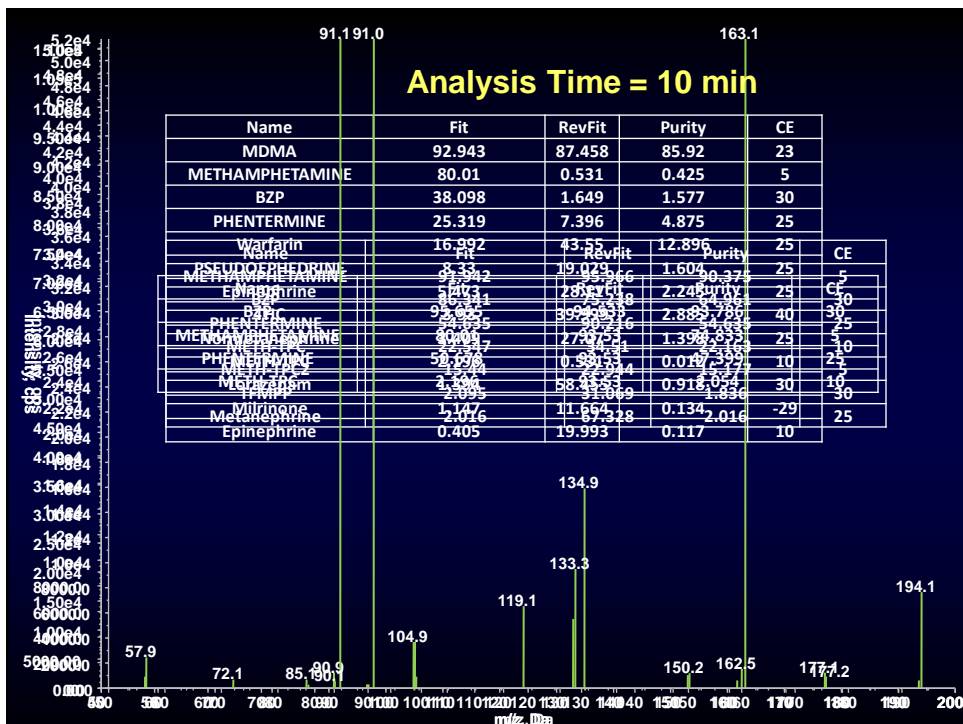
## Information Dependent Acquisition (IDA)





## Clandestine Tablet Analyzed with the AccuTOF-DART MS





### Clandestine Laboratory Samples Analyzed with the DART-QTRAP

	METHAMPHETAMINE 1	METHAMPHETAMINE 2	RATIO	% OF STANDAR
STANDARD	2.78E+05	7.88E+05	2.83	
White Powder	1.51E+04	3.97E+04	2.63	92.75%
Pen Tube	6.85E+04	1.88E+05	2.74	96.82%
Aluminum Foil	9.25E+03	2.31E+04	2.50	88.10%
Aluminum Foil	3.02E+04	7.99E+04	2.65	93.34%
Rubber Tubing	7.89E+04	2.13E+05	2.70	95.24%

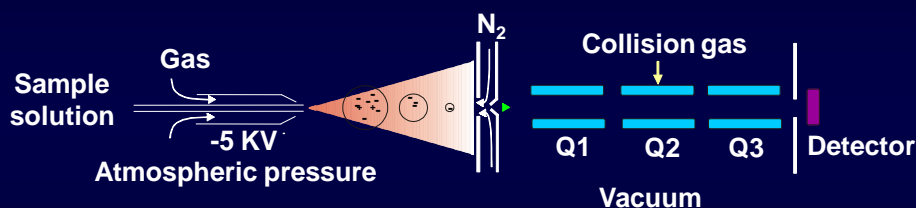
	PSEUDO 1	PSEUDO 2	RATIO	% OF STANDARD
STANDARD	5.75E+05	1.10E+05	0.19	
White Powder	5.17E+05	1.00E+05	0.19	101.11%
White Powder	4.08E+04	6.97E+03	0.17	89.30%
Pen Tube	5.75E+04	1.18E+04	0.21	107.27%
Blender	5.94E+05	1.14E+05	0.19	100.32%

Total Analysis Time for 7 Items = 1 hr  
Analyst Time for 7 Items = 2 days

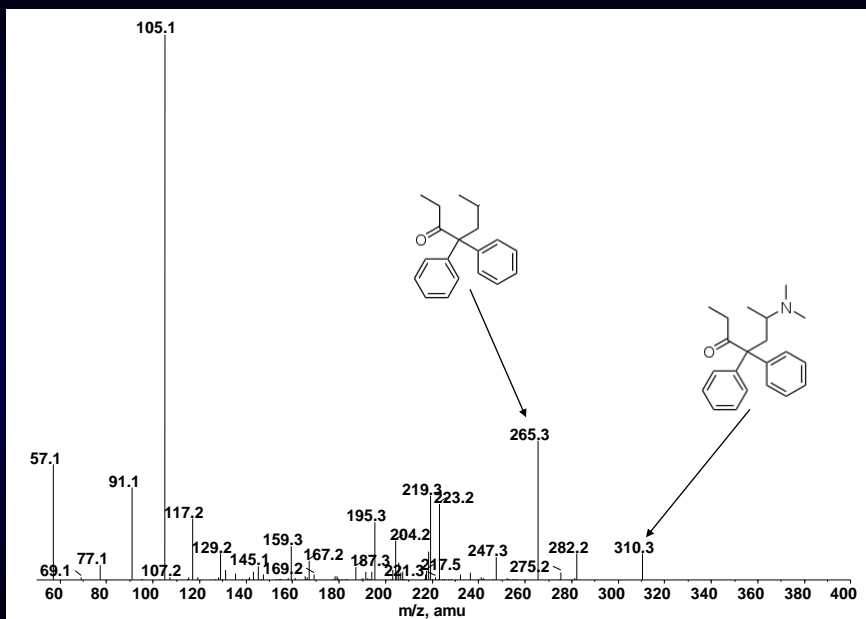
## Why do we want to use this new technology?

- Compound fragmentation is possible without extraction
- CID fragmentation allows retention of molecular ion in fragmentation spectrum
  - These can be searched against an in house library for identification
- MRM analysis gives ion ratios for a second level of compound identification in comparison to a standard
  - This can all be accomplished in a single analysis
- We are the only state forensic science lab with this technology

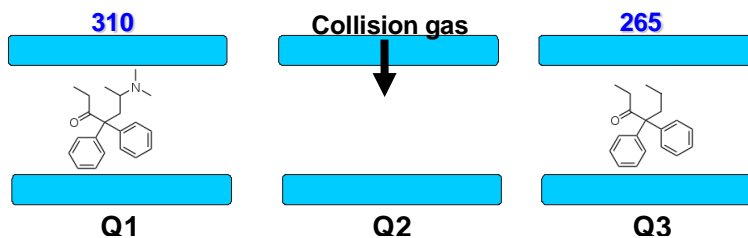
## LC-MRM-MS assay for Drug Detection and Quantitation



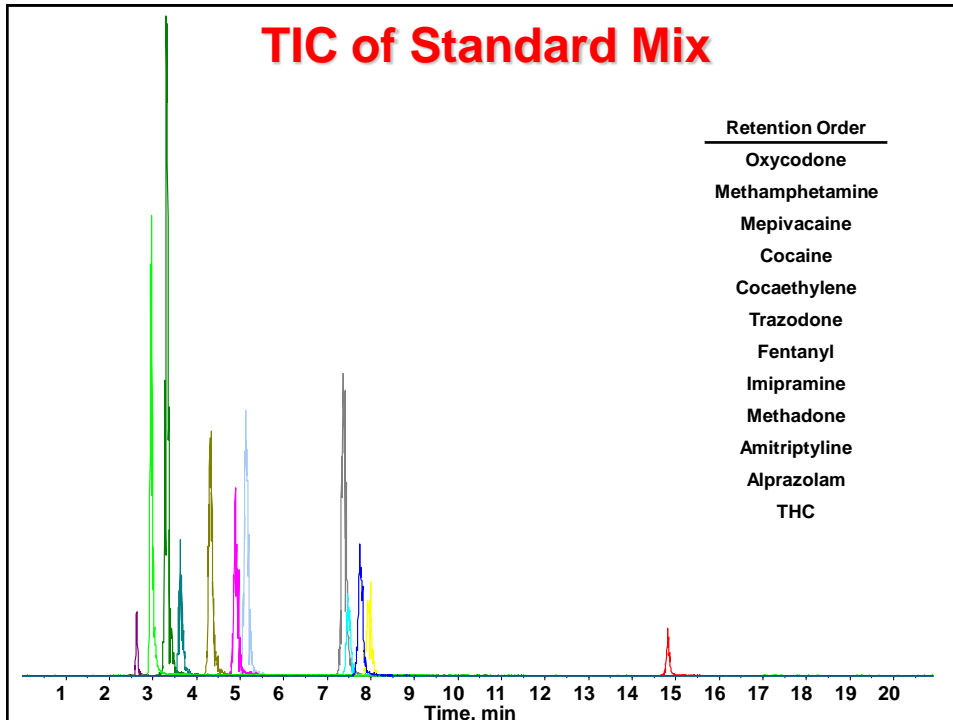
## LC-ESI-MS/MS Spectrum of Methadone



## LC-ESI-MRM-MS



Compound	Molecular Weight	Parent ion	Product ion	Dwell Time (msec)	Declustering Potential (DP)	Collision Energy (CE)	Retention Time	pKa
Alprazolam	308.0829	309.1	205	25	60	50	8.03	2.4
Amiriptryline	277.183	278.2	91	25	45	42	7.8	9.4
Cocaehtylene	317.37	318.2	196	25	40	39	4.32	--
Cocaine	303.1471	304.1	82	25	30	40	3.6	8.6
Fentanyl	336.2202	337.2	188	25	55	43	5.18	8.4
Imipramine	280.1939	281.2	86	25	35	32	7.41	9.5
Mepivacaine	246.1732	247.2	98	25	42	28	3.32	7.6
Methadone	309.2093	310.1	265	25	30	35	7.56	8.6
Methamphetamine	149.1204	150.1	91	25	34	27	2.96	8.6
Oxycodone	315.1471	316.1	241	25	50	40	2.6	8.5
THC	314.2246	315.1	193	25	37	34	14.91	10.6
Trazodone	371.1513	372.2	176	25	60	42	4.87	6.1



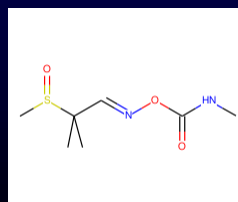
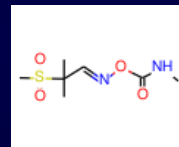
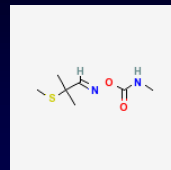
## New Methods with LC/MS/MS or LC-MRM/MS

**5** new methods have been brought online using LC/MS/MS analysis

- Aldicarb quantification and confirmation in blood and urine
- Cannabinoid quantification in whole blood
- Select benzodiazepines quantification in whole blood
- Zolpidem quantification in whole blood
- Fentanyl quantification in whole blood

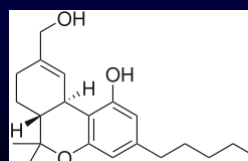
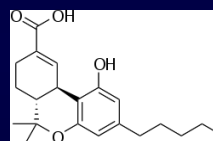
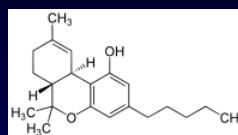
## Aldicarb and its Metabolites by LC-MS/MS

- Resulted in **3** new analytes that can be qualitatively identified and quantified in house
- The limit of quantification for all three compounds was 5.0 ng/mL
- Created **1** new section in the Toxicology SOP (TX-SOP-22)
- **2** scientists were trained on the extraction and technology



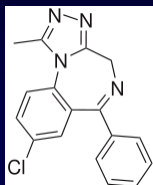
## Cannabinoids by LC-MRM/MS

- Resulted in **3** new analytes that can be quantified in house
- The limit of quantification for THC is 1.0 ng/mL, THC-COOH 2.0 ng/mL, and 11-OH-THC 0.5 ng/mL
- Created **1** new section in the Toxicology SOP (TX-SOP-23)
- **6** scientists were trained on the extraction and technology

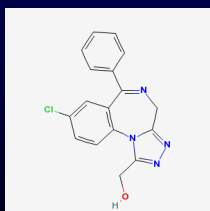
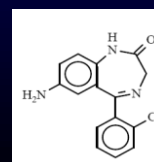
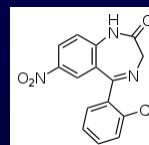
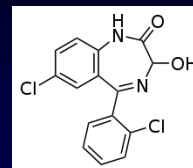




## Select Benzodiazepines by LC-MRM/MS

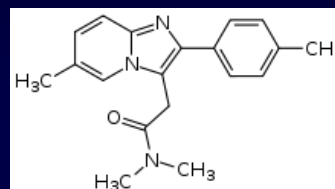


- Resulted in **5** new analytes that can be quantified in house and **1** that can be qualitatively identified
- The limit of quantification for alprazolam and  $\alpha$ -OH-alprazolam is 1.0 ng/mL, and clonazepam and lorazepam is 2.0 ng/mL
- Created **1** new section in the Toxicology SOP (TX-SOP-24)
- **3** scientists were trained on the extraction



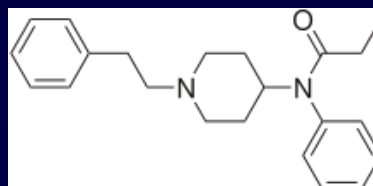
## Zolpidem by LC-MRM/MS

- Resulted in **1** new analyte that can be quantified in house
- The limit of quantification for the analyte was 50 ng/mL
- Created a new section in the Toxicology SOP (TX-SOP-24)
- **2** scientists were trained on the extraction



## Fentanyl by LC-MRM/MS

- Resulted in **1** new analyte that can be quantified in house
- The limit of quantification for the analyte was 0.1 ng/mL
- Added to an existing section in the Toxicology SOP (TX-SOP-24)
- **2** scientists were trained on the extraction



## LC-MRM/MS Summary

- **5** new methods brought online for either quantification or qualitative identification
- **14** new analytes can now be analyzed in house
- **3** additions to the Toxicology SOP to encompass the new methods
- **6** scientists trained on either extractions or the new technology
- **345+** total cases reported with these methods

## Why do we want to use this new technology?

- GC-MS is the traditional instrument for qualitative and quantitative analysis of drugs; however, there are limitations for its use
- LC-ESI-MS can also be used in the qualitative and quantitative analysis of drugs in toxicological specimens
  - Many of the analytes brought online were not analyzable using GC-MS
    - Sensitivity
    - Sample elution

## Summary

- Mass spectrometry is a powerful tool in a forensic science lab
- New instrumentation is expanding the capability of chemical analysis in the forensic lab to include improved detection as well as increased variety in sample types
- No one technique is robust enough for everything, therefore a combination of techniques are ideal for screening and confirmation of drug and toxicology samples

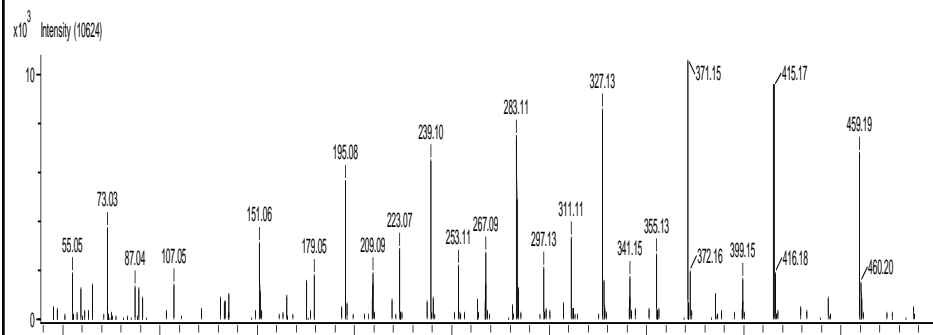
## Conclusion

Our goal is not to keep up with the forensic science community but to continue to lead the development and implementation of new methods and technology across the disciplines.

## Acknowledgements

UAB	ADFS
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## Polyethylene Glycol (PEG)



Every spectrum undergoes internal calibration with polyethylene glycol.

## Mushroom

