Mass Spectrometry in Forensic Science

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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

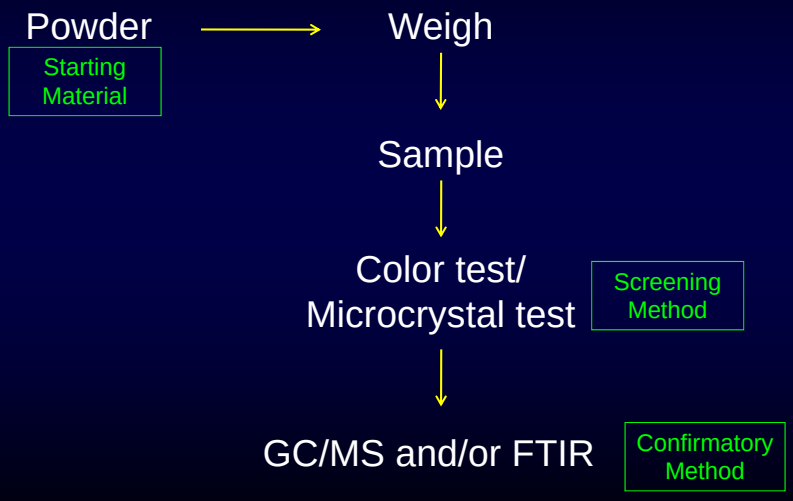
Alabama

- 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

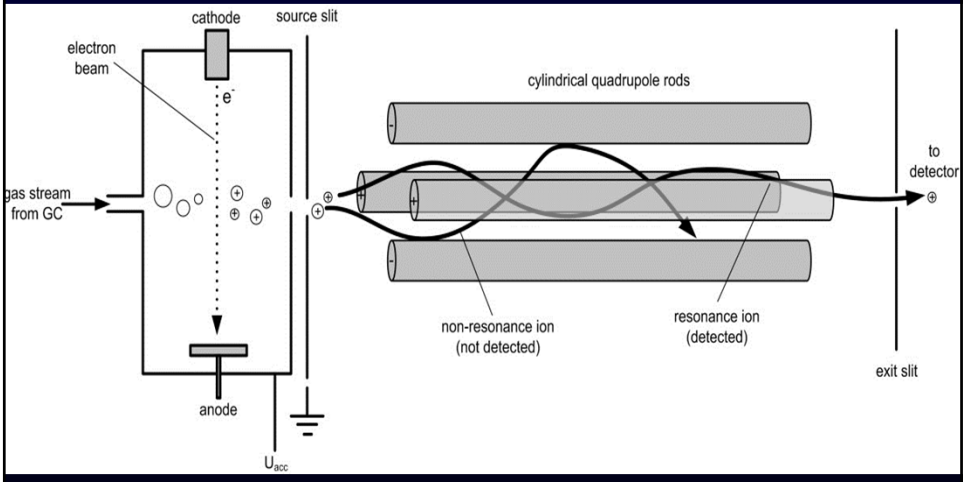


Mass Spectrometry in Forensic Science

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.

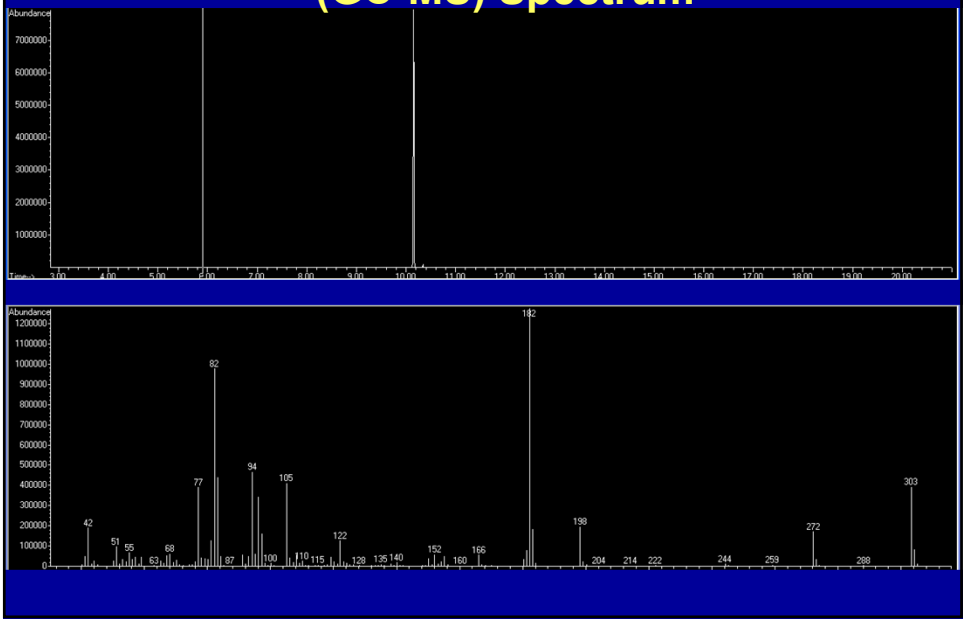
The Hoover Laboratory has 40 analysts, 12 of which work in either chemistry or toxicology. There are 9 GC-MS in the lab.

Typical forms of Mass Spectrometry in Most Forensic Science Laboratories

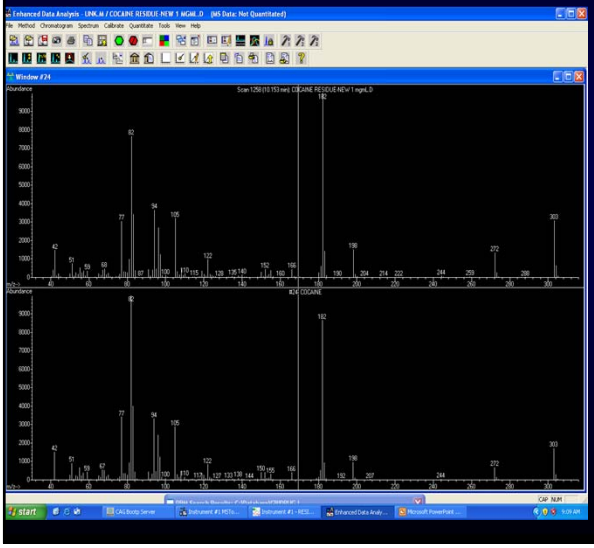


http://www.microbialcellfactories.com/content/figures/1475-2859-6-6-4-l.jpg

Gas Chromatography-Mass Spectrometry (GC-MS) Spectrum



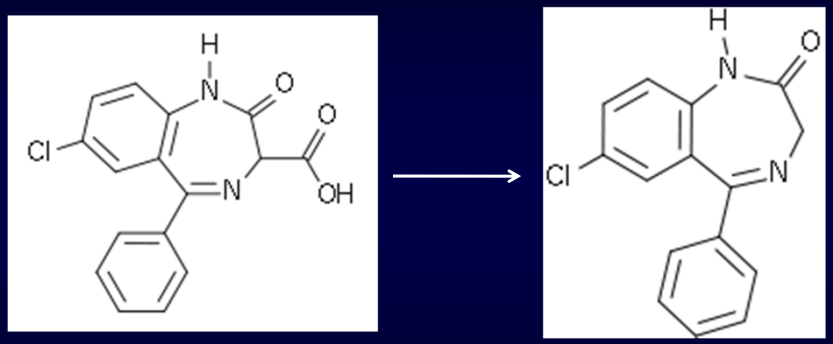
Gas Chromatography-Mass Spectrometry (GC-MS) Spectrum



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



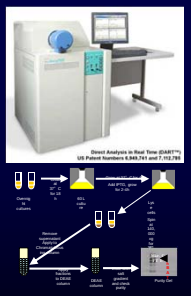
Clorazepate Nordiazepam

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

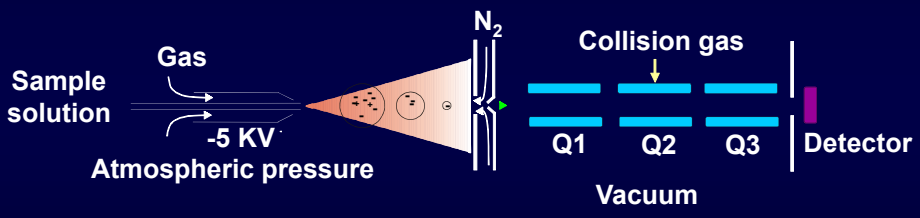
New Technology at the Alabama Department of Forensic Science (ADFS)

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

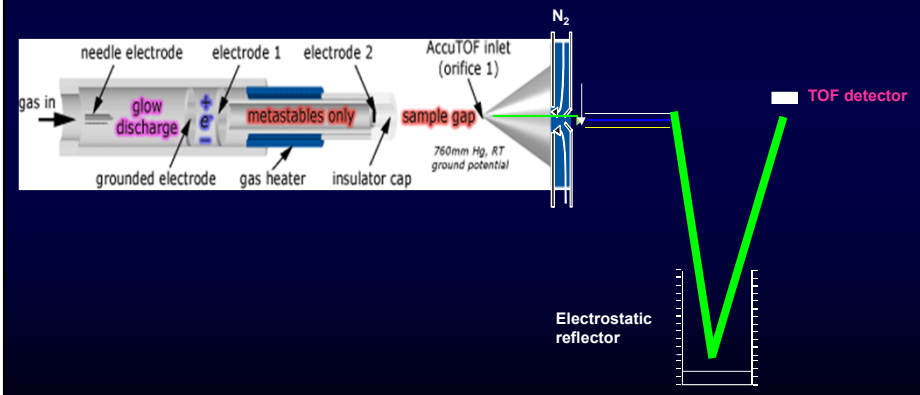
Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC-ESI-MS)



Courtesy of S. Barnes

Different forms of Mass Spectrometry

Direct Analysis in Real Time with Time of Flight Mass Spectrometry

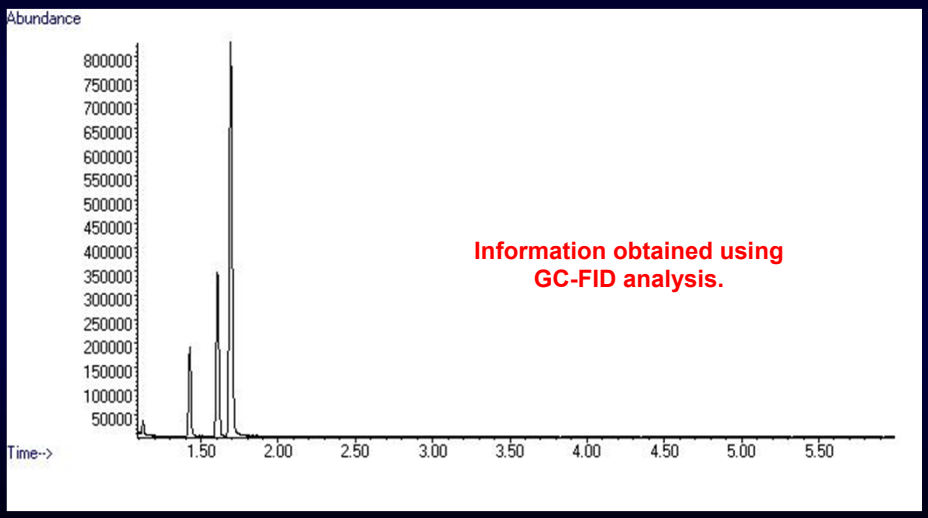


Courtesy of S. Barnes

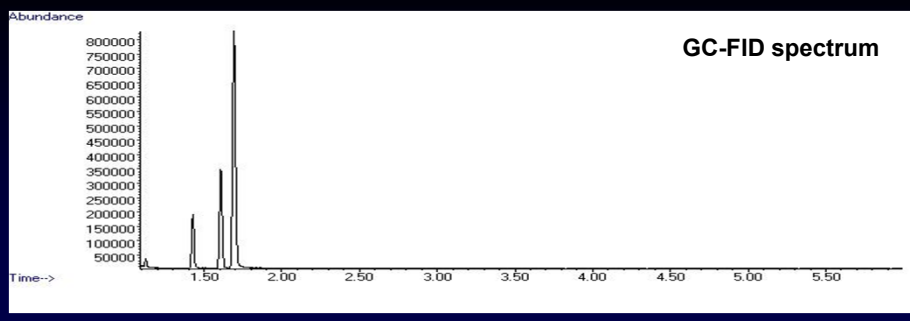
HeadSpace-GC-MSD

- 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

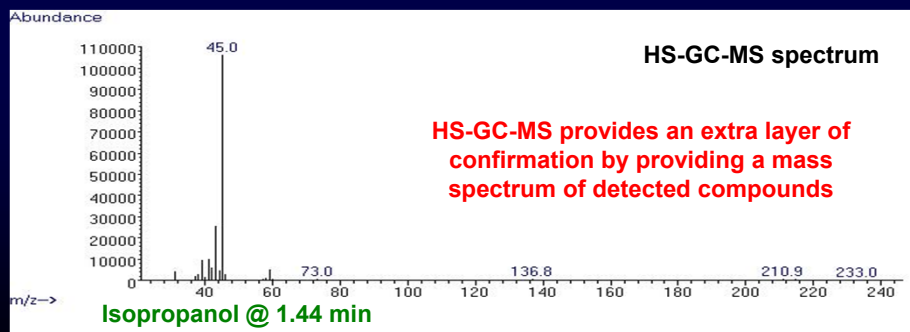
TIC of Volatiles Mix



Information obtained using GC-FID analysis.



GC-FID spectrum



HS-GC-MS spectrum

HS-GC-MS provides an extra layer of confirmation by providing a mass spectrum of detected compounds

Isopropanol @ 1.44 min

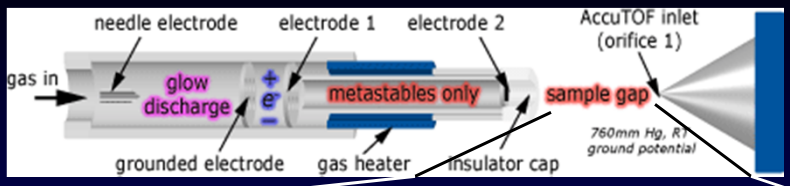
Why do we want to use this new technology?

- 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

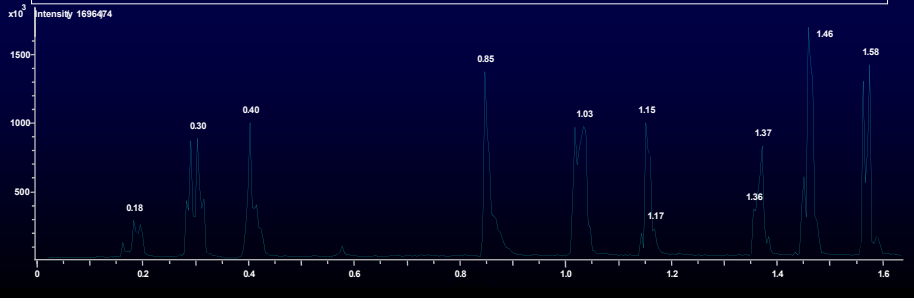
Sample Introduction with the AccuTOF-DART MS

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

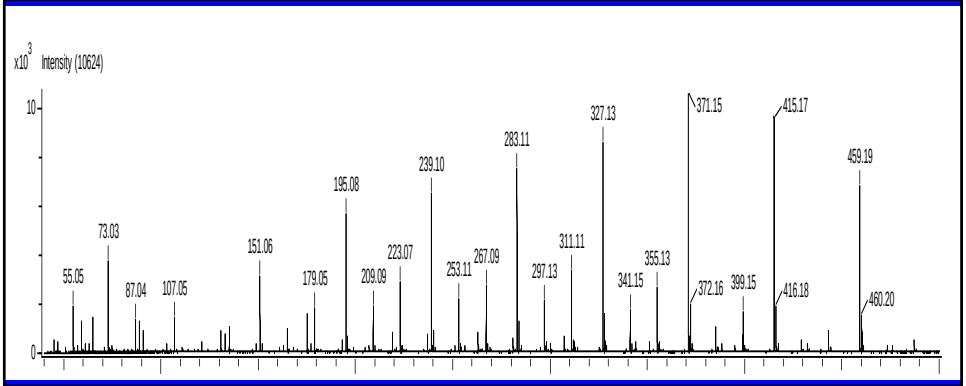
Solid samples are introduced into the stream with tweezers

Sample Introduction with the AccuTOF-DART MS

Every sampling device is analyzed for contamination prior to use.

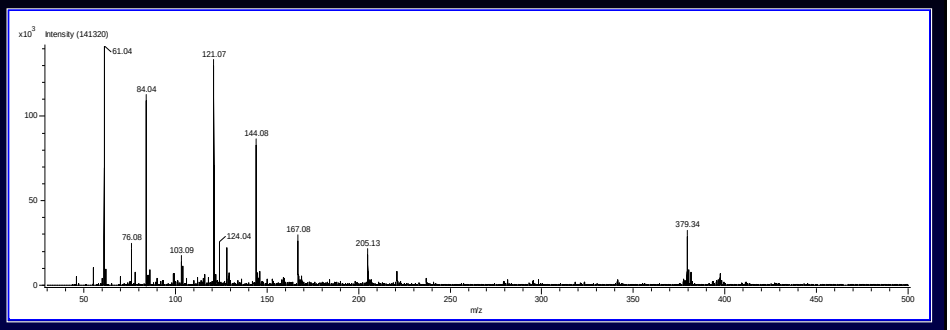


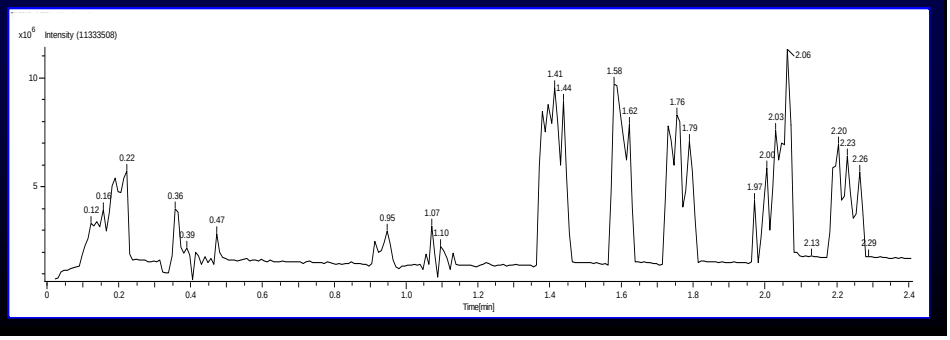
Polyethylene Glycol (PEG)

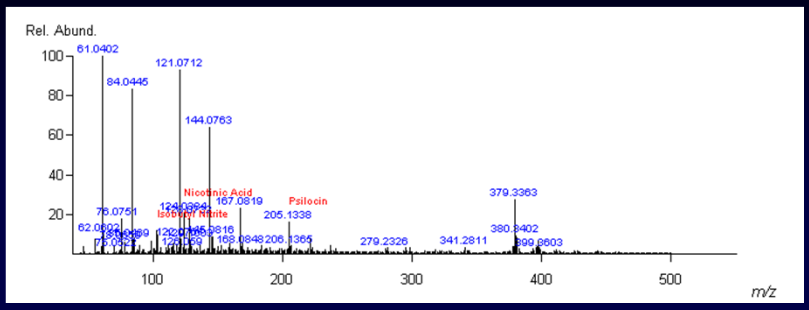


Every spectrum undergoes internal calibration with polyethylene glycol.

Mushroom



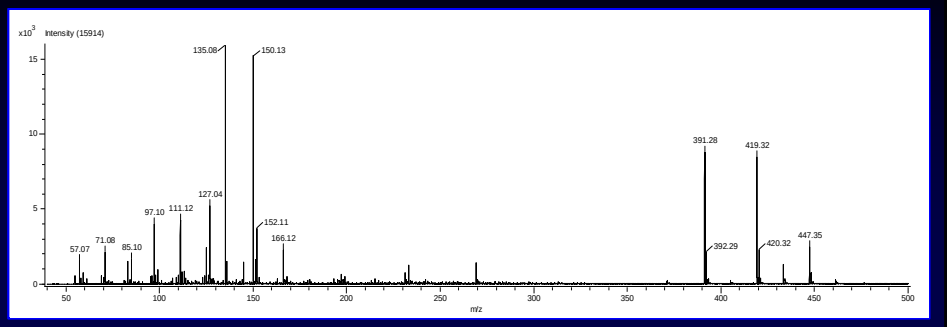


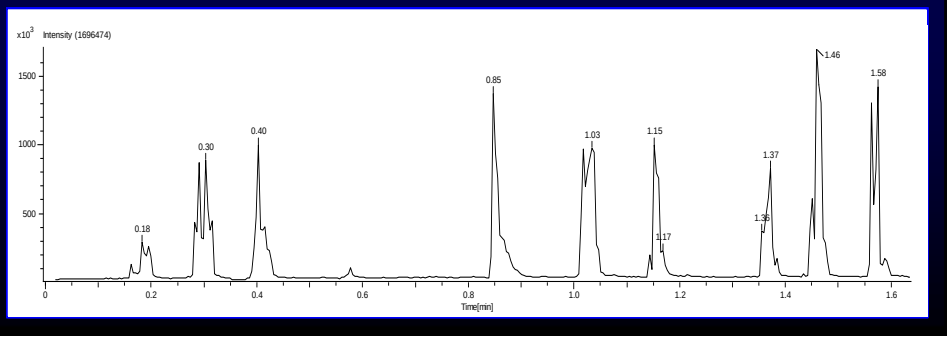


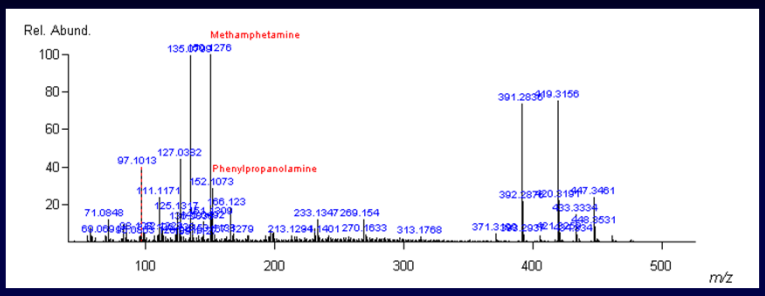
MS File: F:\BIRMINGHAM LAB.7pj\04.01.09\mushroom.jsp
Search File: C:\Documents and Settings\Administrator\Desktop\DRUGS_NEUTRAL_MASSES.xls
Mass tolerance (mmu): 5 Threshold (%): 5
Date: 03-Apr-09 Time: 07:24 AM

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

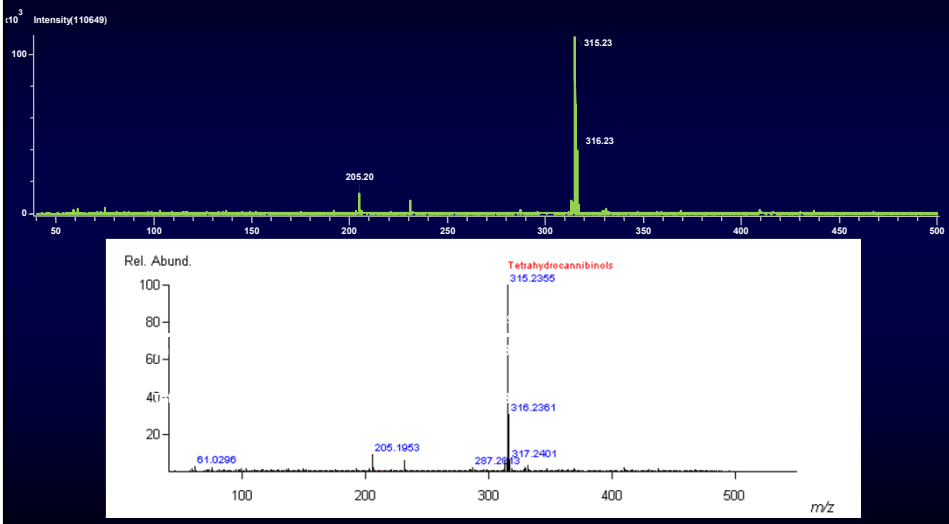
Why do we want to use this new technology?

- 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

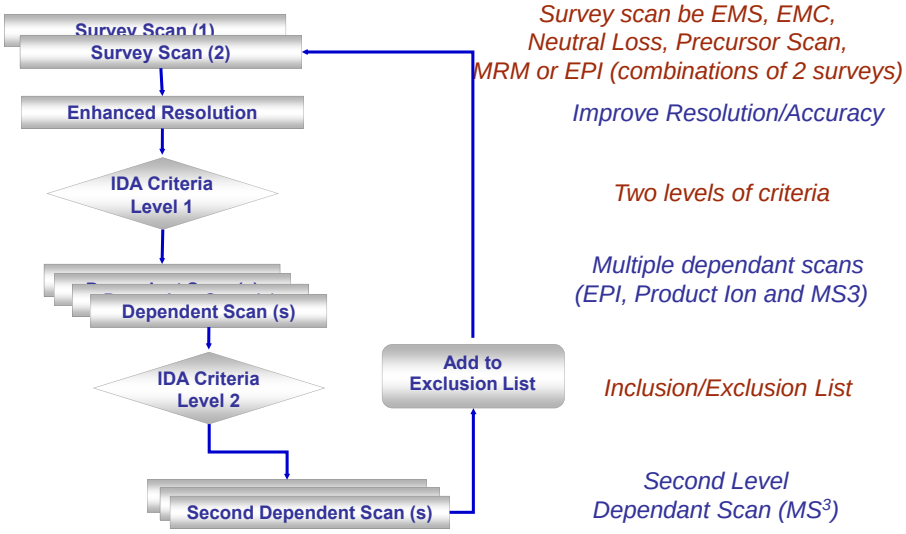
3200 QTRAP-DART MS

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

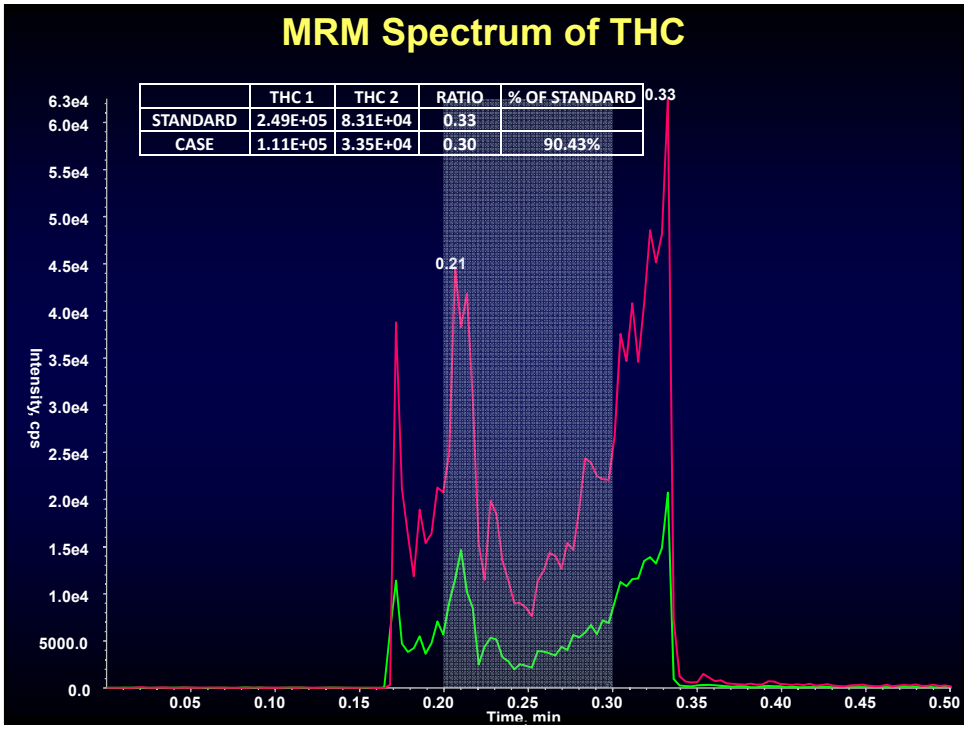
Plant Material Analyzed with the AccuTOF-DART MS

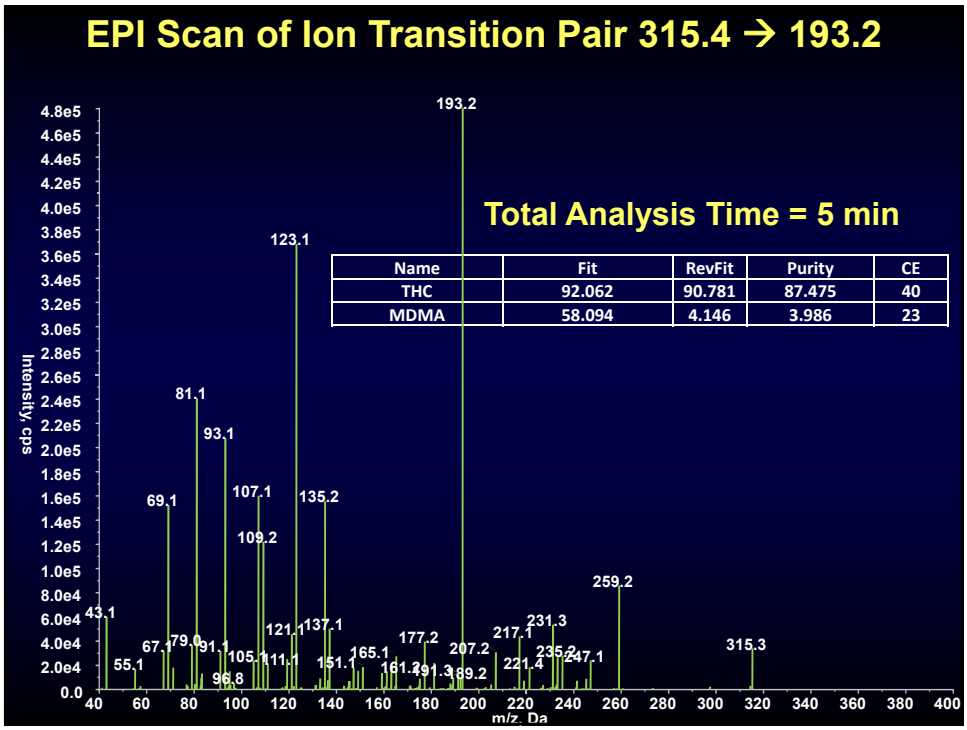


*I*nformation *D*ependent *A*cquisition *(IDA)*

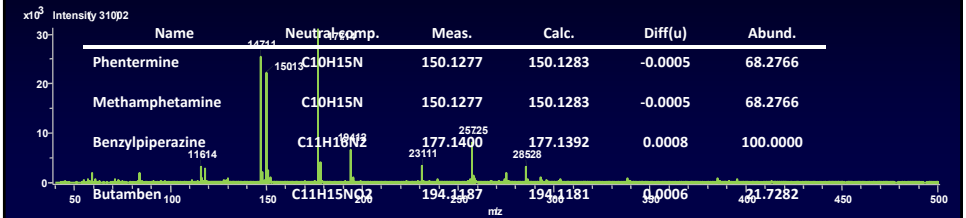


Courtesy of Ricky Ciner

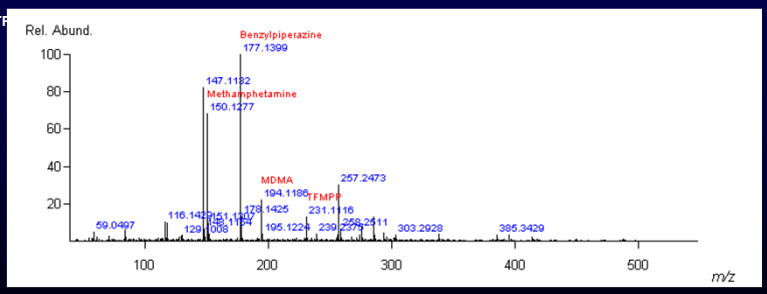

MRM Spectrum of THC
THC 1
THC 2
RATIO
% OF STANDARD
STANDARD
2.49E+05
8.31E+04
0.33
CASE
1.11E+05
3.35E+04
0.30
90.43%
0.33
0.21
Intensity, cps
Time, min


EPI Scan of Ion Transition Pair 315.4 → 193.2
Total Analysis Time = 5 min
Name
Fit
RevFit
Purity
CE
THC
92.062
90.781
87.475
40
MDMA
58.094
4.146
3.986
23
193.2
123.1
81.1
93.1
69.1
107.1
135.2
109.2
259.2
315.3
Intensity, cps
m/z, Da

Clandestine Tablet Analyzed with the AccuTOF-DART MS



Name	Neutral comp.	Meas.	Calc.	Diff(u)	Abund.
Phentermine	C10H15N	150.1277	150.1283	-0.0005	68.2766
Methamphetamine	C10H15N	150.1277	150.1283	-0.0005	68.2766
Benzylpiperazine	C11H16N2	177.1400	177.1392	0.0008	100.0000
Butamben	C11H15NO2	194.1187	194.1181	0.0006	21.7282
MDMA	C11H15NO2	194.1187	194.1181	0.0006	21.7282
TF					



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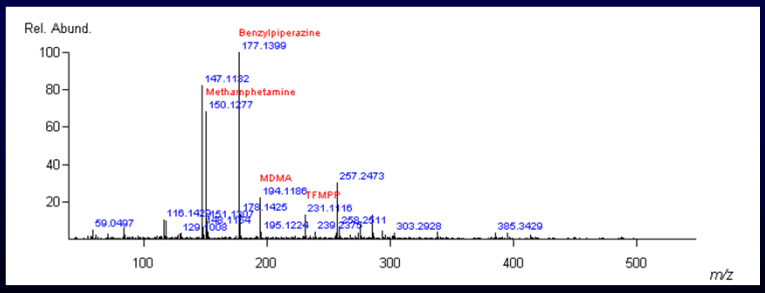
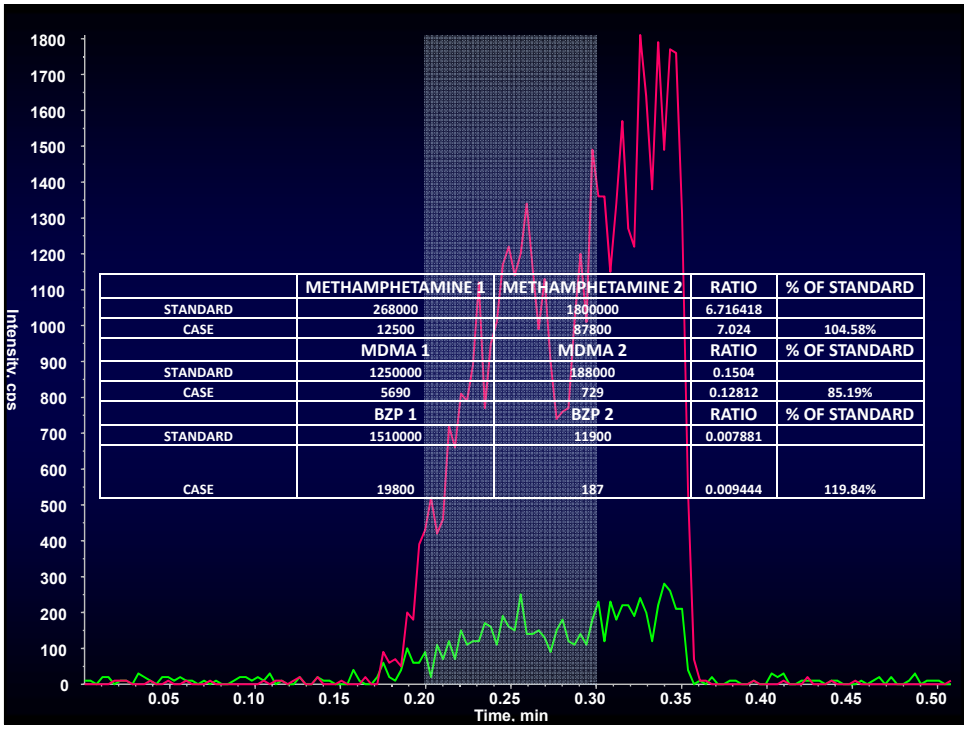
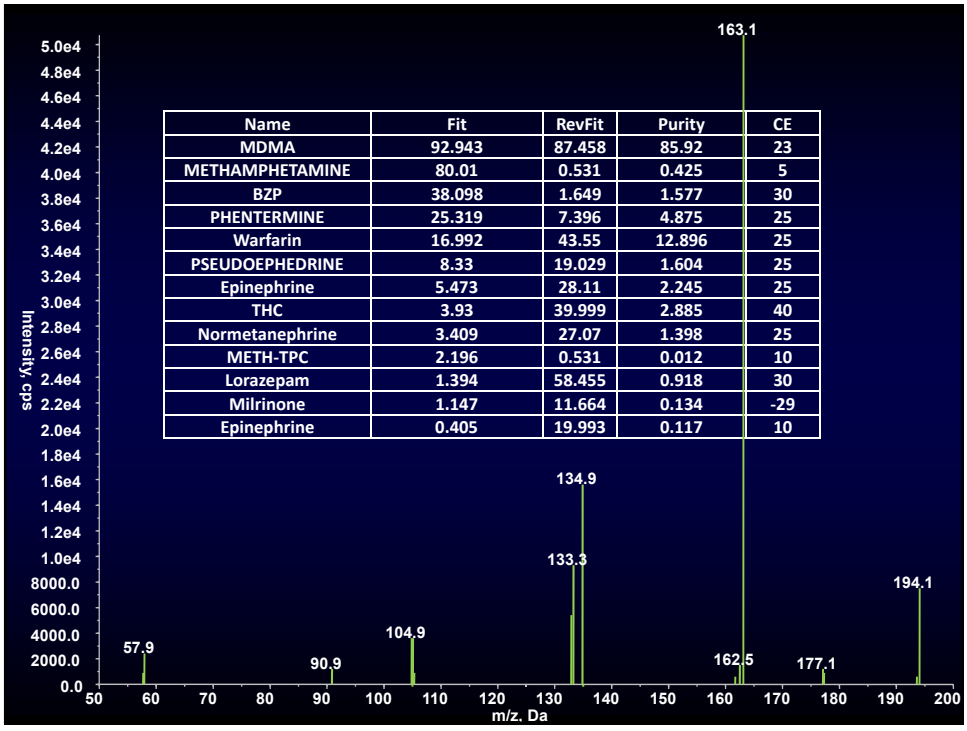
Butamben	C11H15NO2	194.1187	194.1181	0.0006	21.7282
MDMA	C11H15NO2	194.1187	194.1181	0.0006	21.7282
TFMPP	C11H13F3N2	231.1117	231.1109	0.0008	12.8296

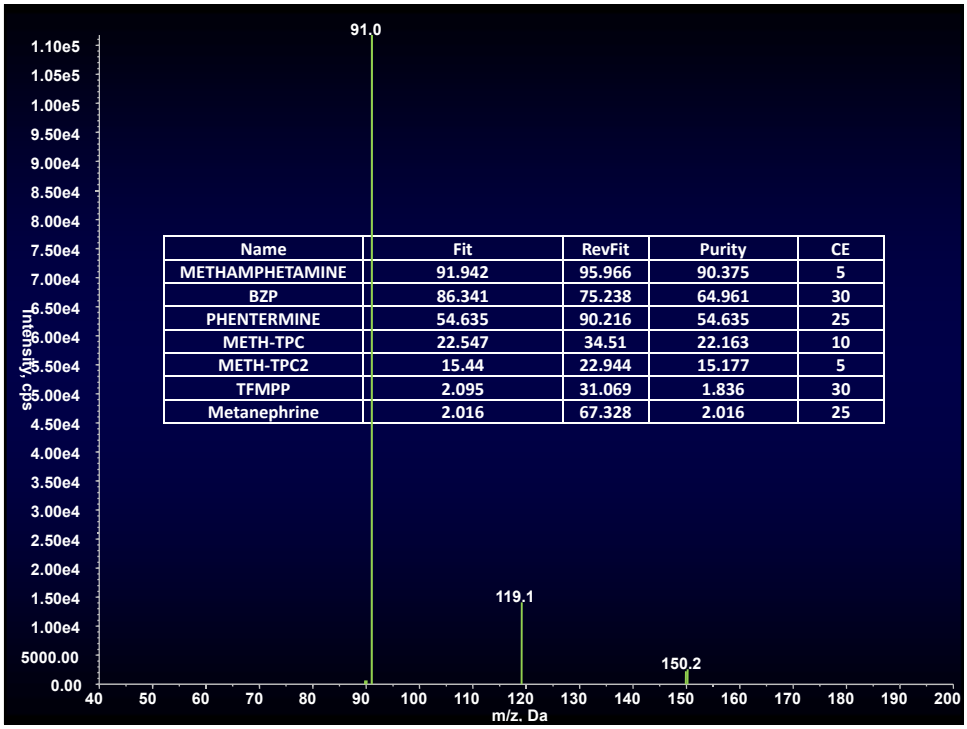




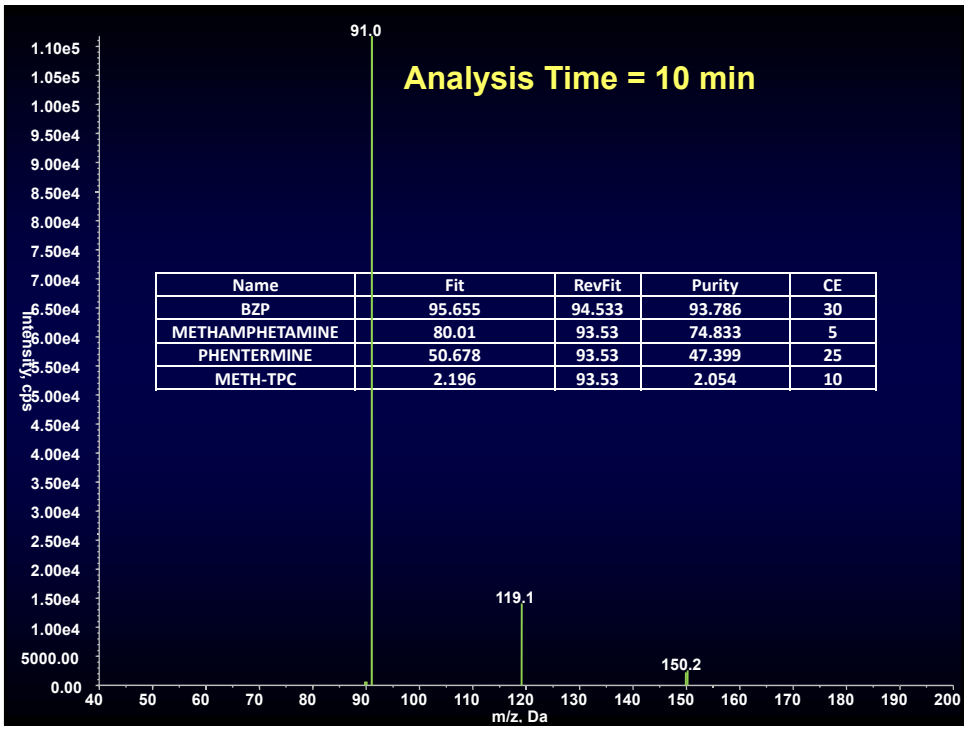
	METHAMPHETAMINE 1	METHAMPHETAMINE 2	RATIO	% OF STANDARD
STANDARD	268000	1800000	6.716418	
CASE	12500	87800	7.024	104.58%
	MDMA 1	MDMA 2	RATIO	% OF STANDARD
STANDARD	1250000	188000	0.1504	
CASE	5690	729	0.12812	85.19%
	BZP 1	BZP 2	RATIO	% OF STANDARD
STANDARD	1510000	11900	0.007881	
CASE	19800	187	0.009444	119.84%



Name	Fit	RevFit	Purity	CE
MDMA	92.943	87.458	85.92	23
METHAMPHETAMINE	80.01	0.531	0.425	5
BZP	38.098	1.649	1.577	30
PHENTERMINE	25.319	7.396	4.875	25
Warfarin	16.992	43.55	12.896	25
PSEUDOEPHEDRINE	8.33	19.029	1.604	25
Epinephrine	5.473	28.11	2.245	25
THC	3.93	39.999	2.885	40
Normetanephrine	3.409	27.07	1.398	25
METH-TPC	2.196	0.531	0.012	10
Lorazepam	1.394	58.455	0.918	30
Milrinone	1.147	11.664	0.134	-29
Epinephrine	0.405	19.993	0.117	10



Name	Fit	RevFit	Purity	CE
METHAMPHETAMINE	91.942	95.966	90.375	5
BZP	86.341	75.238	64.961	30
PHENTERMINE	54.635	90.216	54.635	25
METH-TPC	22.547	34.51	22.163	10
METH-TPC2	15.44	22.944	15.177	5
TFMPP	2.095	31.069	1.836	30
Metanephrine	2.016	67.328	2.016	25



Name	Fit	RevFit	Purity	CE
BZP	95.655	94.533	93.786	30
METHAMPHETAMINE	80.01	93.53	74.833	5
PHENTERMINE	50.678	93.53	47.399	25
METH-TPC	2.196	93.53	2.054	10

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%

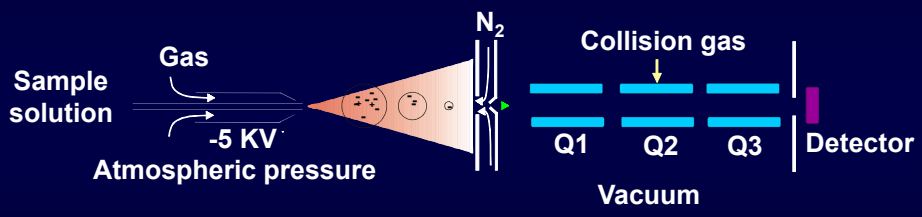
	PSEUDO 1	PSEUDO 2	RATIO	% OF STANDARD
STANDARD	5.75E+05	1.10E+05	0.19	
White Powder	5.17E+05	1.00E+05	0.19	101.11%
White Powder	4.08E+04	6.97E+03	0.17	89.30%
Pen Tube	5.75E+04	1.18E+04	0.21	107.27%
Blender	5.94E+05	1.14E+05	0.19	100.32%

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

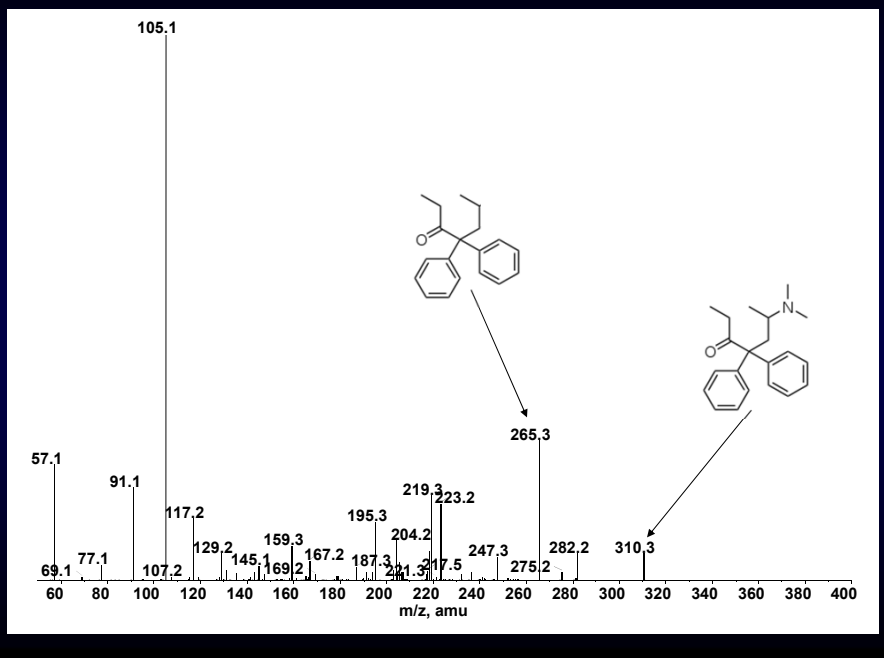
Why do we want to use this new technology?

- 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

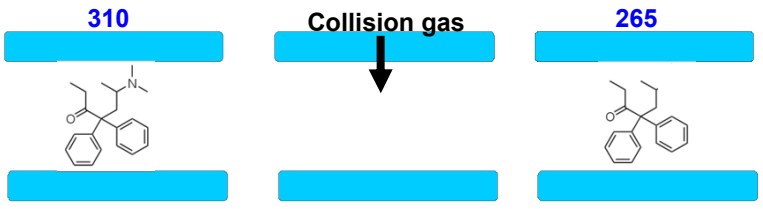
LC-MRM-MS assay for Drug Detection and Quantitation



LC-ESI-MS/MS Spectrum of Methadone

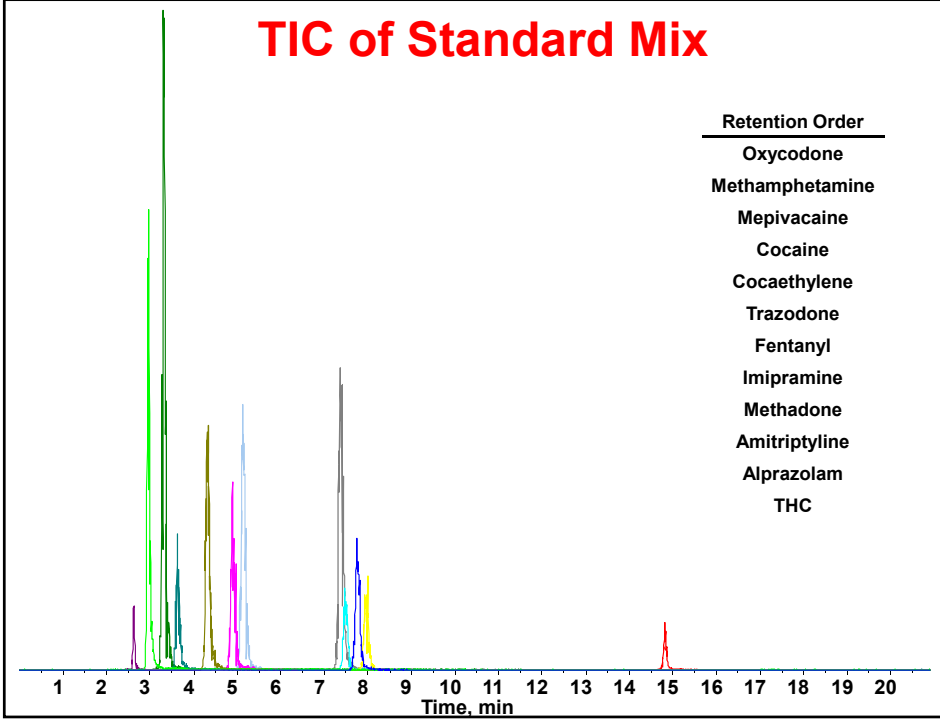


LC-ESI-MRM-MS



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
Amitriptyline	277.183	278.2	91	25	45	42	7.8	9.4
Cocaethylene	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

TIC of Standard Mix



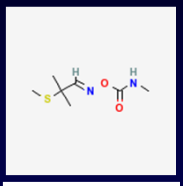
New Methods with LC/MS/MS or LC-MRM/MS

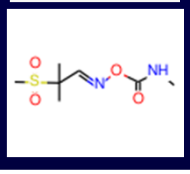
5 new methods have been brought online using LC/MS/MS analysis

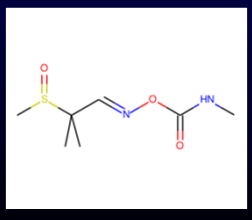
- Aldicarb quantification and confirmation in blood and urine
- Cannabinoid quantification in whole blood
- Select benzodiazepines quantification in whole blood
- Zolpidem quantification in whole blood
- Fentanyl quantification in whole blood

Cannabinoids by LC-MS/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**

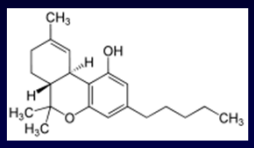
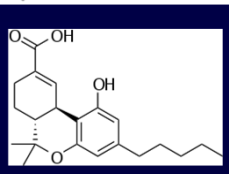
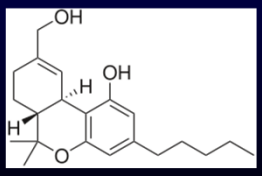




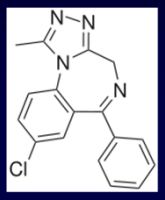
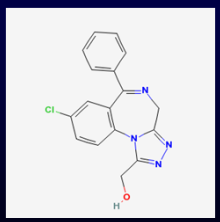


Cannabinoids by LC-MRM/MS

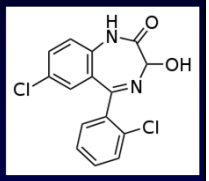
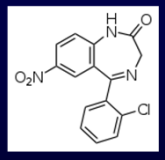
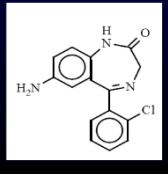
- Resulted in 3 new analytes that can be quantified in house
- The limit of quantification for THC is 1.0 ng/mL, THC-COOH 2.0 ng/mL, and 11-OH-THC 0.5 ng/mL
- Created 1 new section in the Toxicology SOP (TX-SOP-23)
- 6 scientists were trained on the extraction and technology

Select Benzodiazepines by LC-MRM/MS

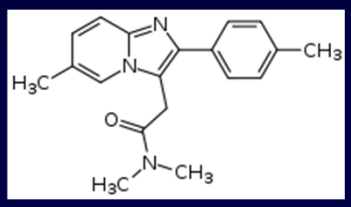



- Resulted in 5 new analytes that can be quantified in house and 1 that can be qualitatively identified
- The limit of quantification for alprazolam and α-OH-alprazolam is 1.0 ng/mL, and clonazepam and lorazepam is 2.0 ng/mL
- Created 1 new section in the Toxicology SOP (TX-SOP-24)
- 3 scientists were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

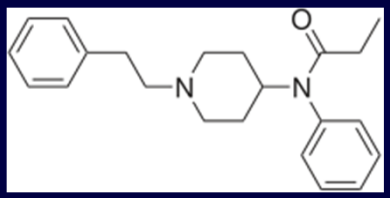
Zolpidem by LC-MRM/MS

- 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

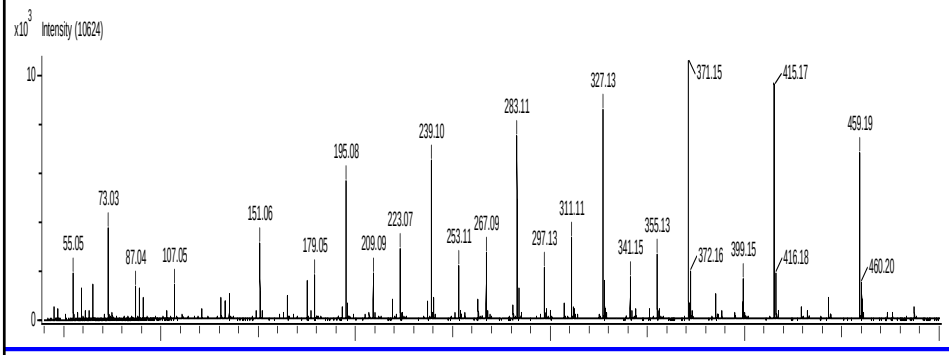
Conclusion

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

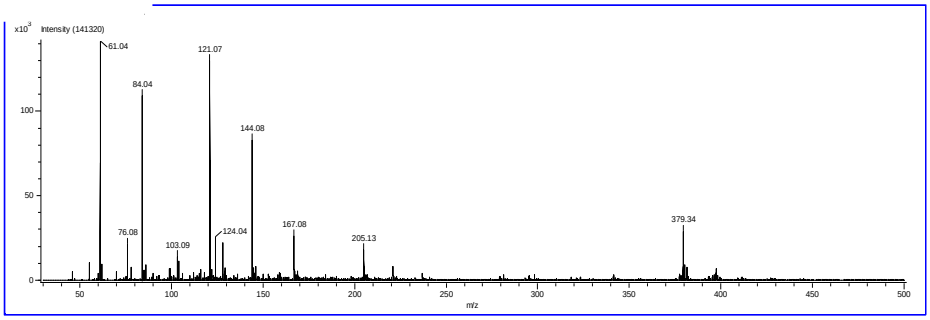
UAB	ADFS
• Stephen Barnes, PhD • Marion Kirk • Ray Moore • Matthew Renfrow, PhD • Landon Wilson • Tracy D'Alessandro, PhD	• Mike Sparks • Dale Carpenter, PhD • Rod Kennette • Andrea Headrick • Jack Kalin, PhD

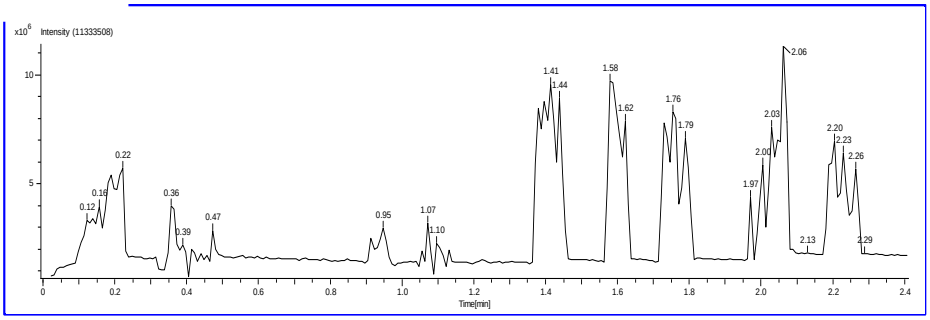
Polyethylene Glycol (PEG)

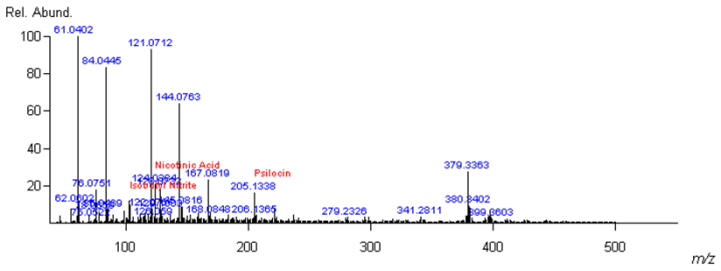


Every spectrum undergoes internal calibration with polyethylene glycol.

Mushroom



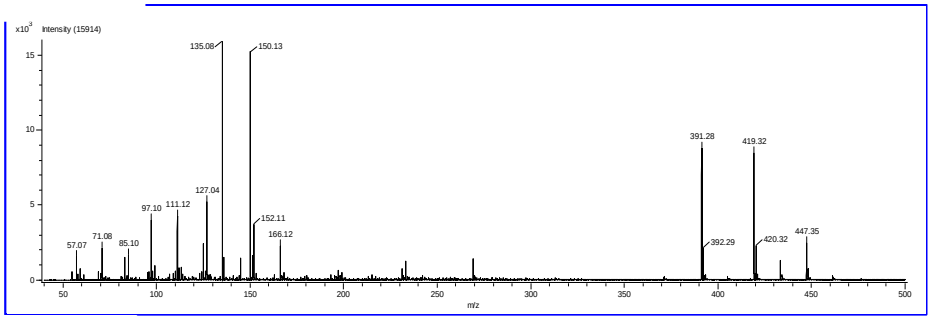


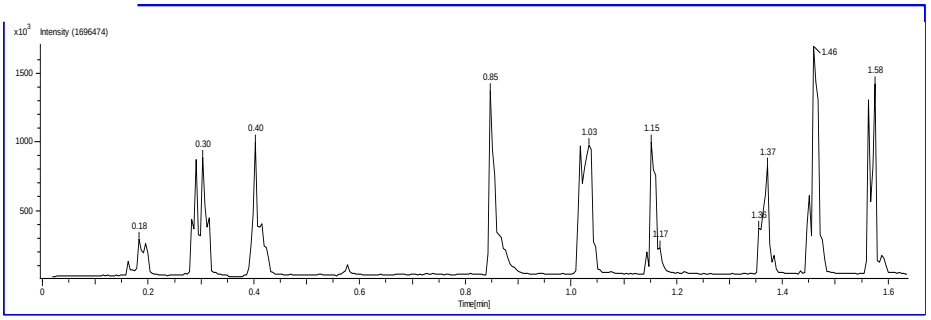


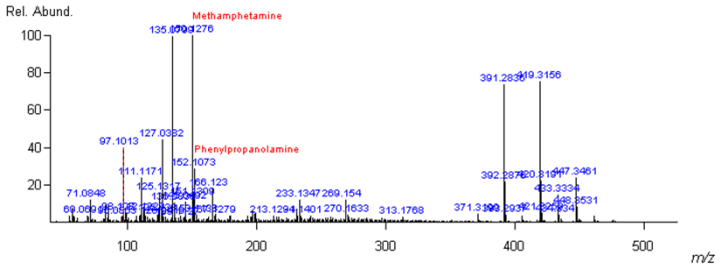
MS File: F:\BIRMINGHAM LAB.7pj\04.01.09\mushroom.jsp
Search File: C:\Documents and Settings\Administrator\Desktop\DRUGS_NEUTRAL_MASSES.xls
Mass tolerance (mmu): 5Threshold (%): 5
Date: 03-Apr-09 Time: 07:24 AM

Name	Neutral comp.	Meas.	Calc.	Diff(u)	Abund.	
Isobutyl Nitrite	C4H9NO2	104.0716	104.0712	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	16.1476	71H0800
5-MeO-AMT	C12H16N2O	205.1338	205.1341	-0.0003	16.1476	X
Psilocin	C12H16N2O	205.1338	205.1341	-0.0003	16.1476	383

Meth Lab Liquid







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Search File: C:\Documents and Settings\Administrator\Desktop\DRUGS_NEUTRAL_MASSES.xls
Mass tolerance (mmu): 5Threshold (%): 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	
PHE07							
Methamphetamine	C10H15N	150.1276	150.1283	-0.0006	100.0000	MAA28	
Phenylpropanolamine	C9H13NO	152.1073	152.1075	-0.0002	28.3102	PPA01	
Cathine (Norpseudoephedrine)		C9H13NO	152.107	152.1075	-0.0002	28.3102	X

LC-ESI-MS/MS Spectrum of Methadone