# Mass Spectrometry in Forensic Science

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## Overview

- Introduction to forensic sciences
- Uses of mass spectrometry in forensic sciences
- Typical instrumentation in forensic sciences
- Applications of new instrumentation

# 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

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

Firearms – Bullet pattern recognition and analysis of gun powder

Fire Debris -- Identification of ignitable liquids used in arsons

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

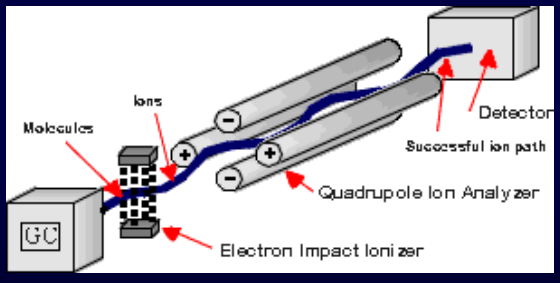
- Alabama
  - Frye standard: the court must decide if the questioned procedure, technique, and principles are "generally accepted" by a relevant community
  - Federal Rule 702: a witness qualified as an expert may testify in the form of an opinion
- Federal
  - Daubert:
    - Has it been tested?
    - 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

## Mass Spectrometry in Forensic Science

**A gas chromatograph with a mass spec detector is the final tool used in the analysis of drug chemistry and toxicology samples for identification and confirmation.**

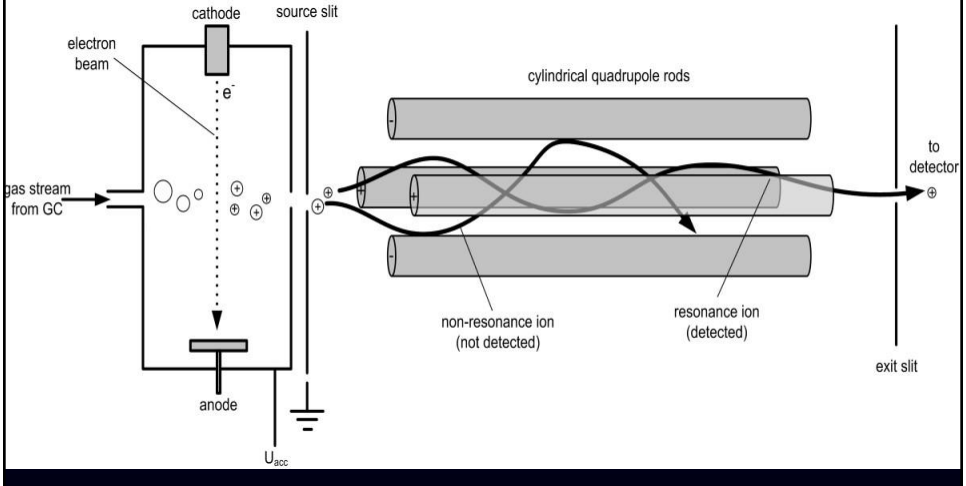
# Typical forms of Mass Spectrometry in Every Forensic Science Lab

**Gas Chromatography-Mass Spectrometry (GC-MS)**



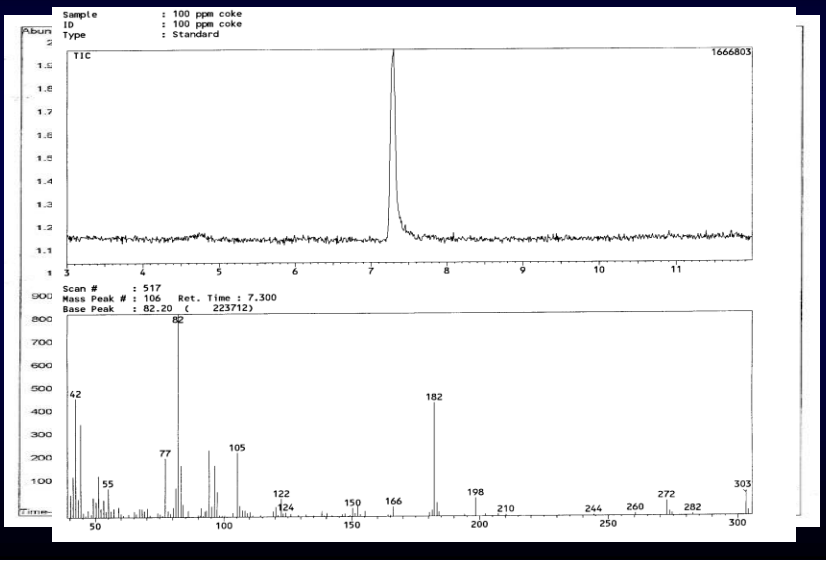

http://www.chem.arizona.edu/massspec/intro_html/intro.html

# Typical forms of Mass Spectrometry in Every Forensic Science Lab



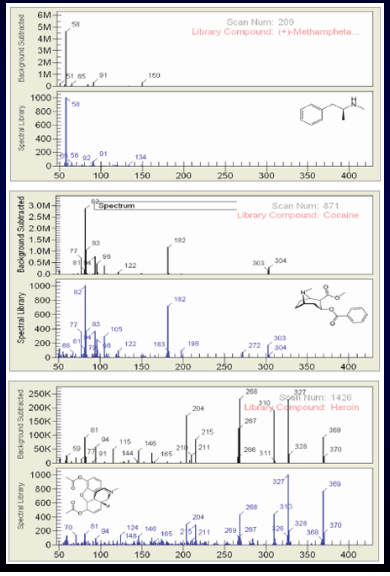

http://www.microbialcellfactories.com/content/figures/1475-2859-6-6-4-l.jpg

# Typical forms of Mass Spectrometry in Every Forensic Science Lab




# Typical forms of Mass Spectrometry in Every Forensic Science Lab

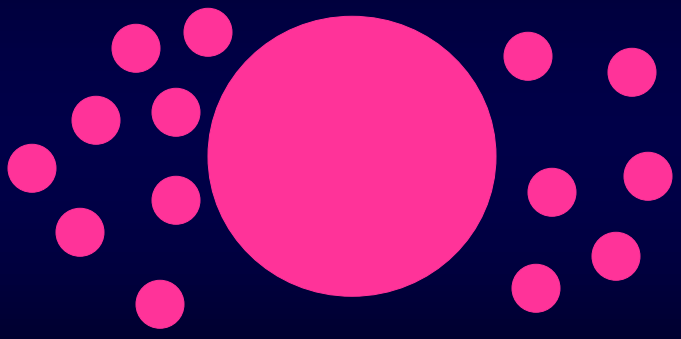
## Gas Chromatography-Mass Spectrometry (GC-MS) Spectrum




**Spectra are searched against a library of known compounds in an effort to identify every peak in the TIC**

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

# Problems Encountered with the GC/MS

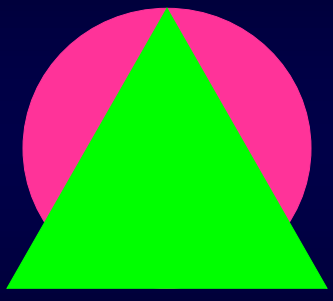


Lose the parent ion of the compound upon ionization in the instrument
Example: Methadone

# Problems Encountered with the GC/MS

Derivatize the compound for analysis with GC/MS which decreases detection of low level compounds
Example: THC

# Problems Encountered with the GC/MS



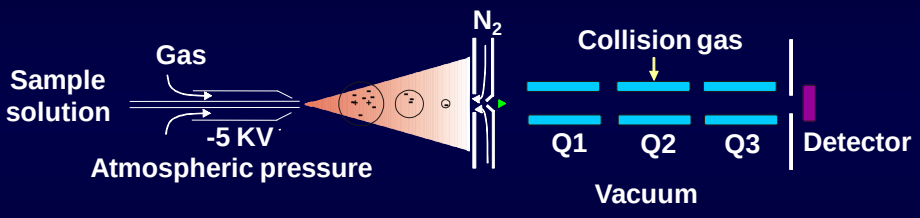
**Heat labile compound will be identified as a related compound, but not the actual compound**
**Example: Clorazepate to Nordiazepam**

# New Technology

- Four new instruments have been brought into the department in October 2008
  - AccuTOF-DART mass spectrometer
  - 3200 QTRAP mass spectrometer with LC
  - 3200 QTRAP mass spectrometer with DART
  - HS-GC-MSD

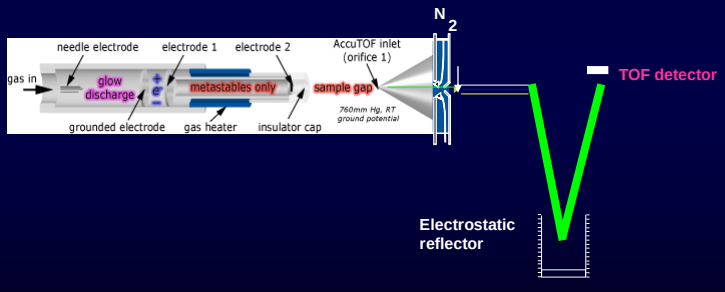

# Different forms of Mass Spectrometry

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




# Different forms of Mass Spectrometry

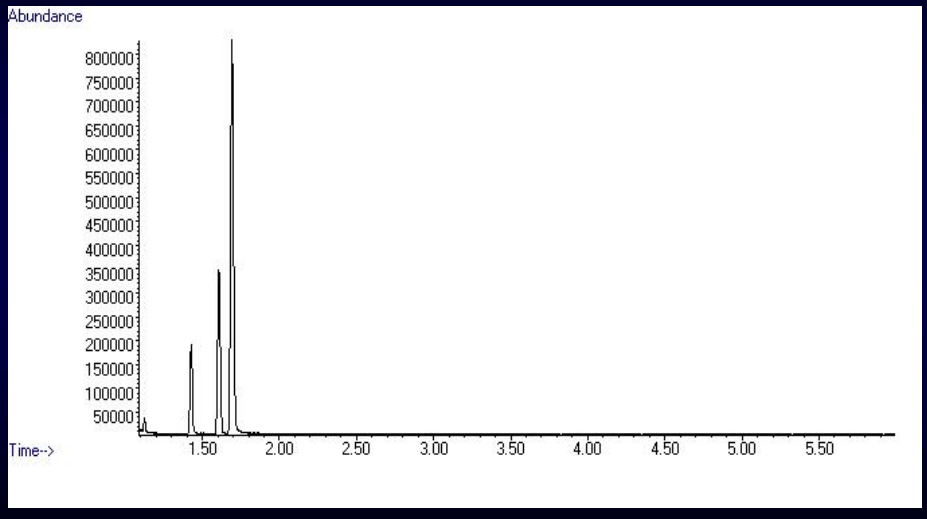
**Direct Analysis in Real Time with Time of Flight Mass Spectrometry**



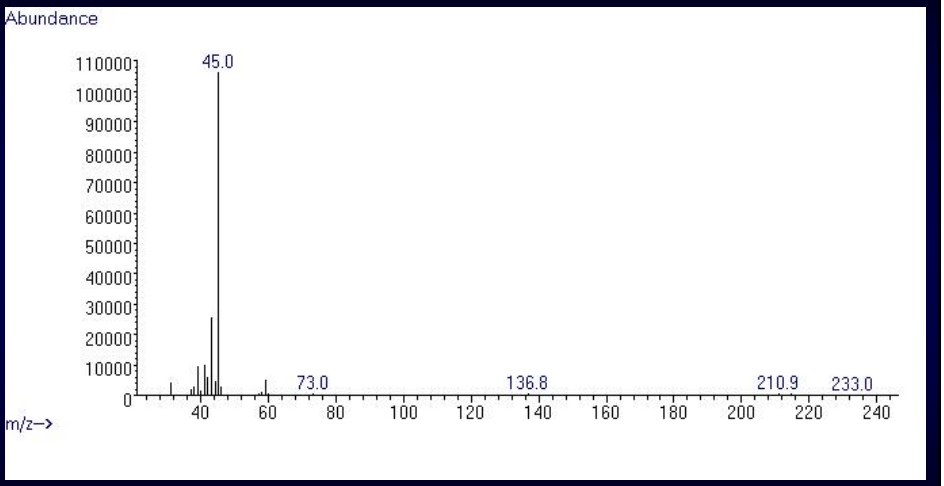
# HS-GC-MSD

- This instrument provides opportunity for qualitative and quantitative identification of volatile compounds

# TIC of Volatiles Mix

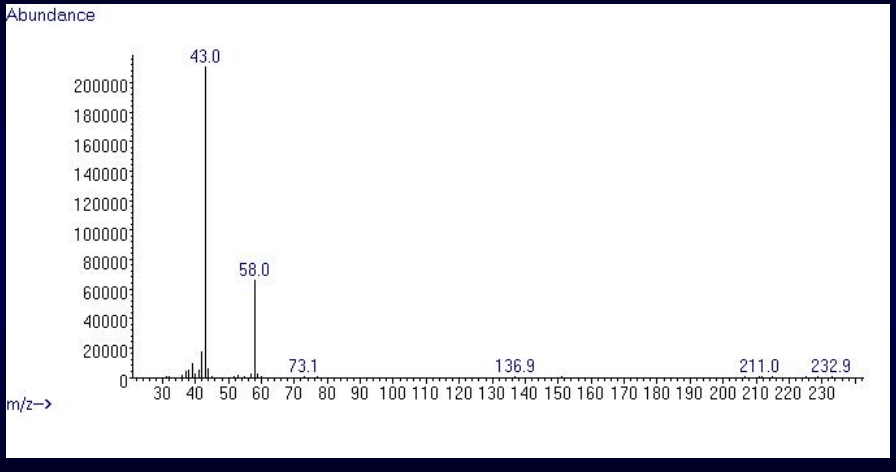


# Spectrum of Peak at 1.44 min



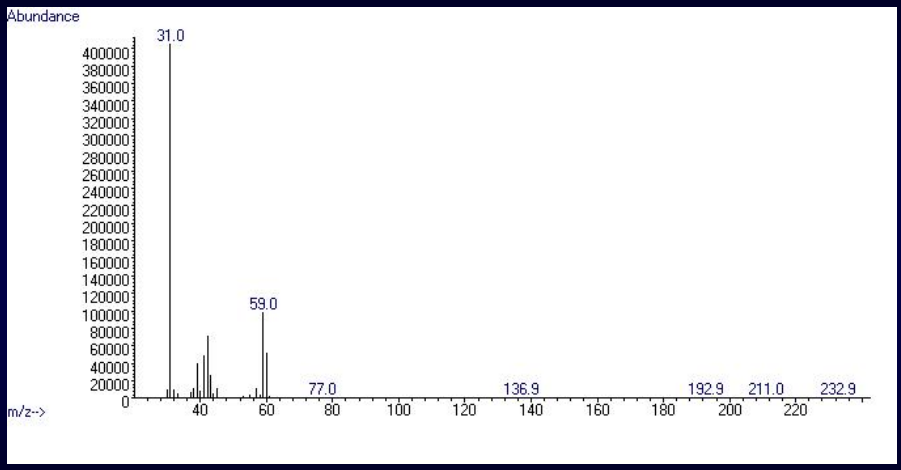

**Isopropanol**

# Spectrum of Peak at 1.61 min



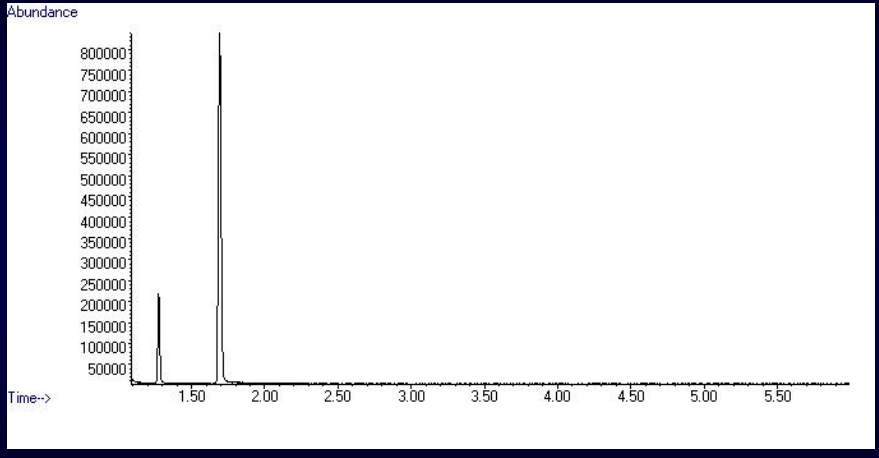

**Acetone**

# Spectrum of Peak at 1.70 min

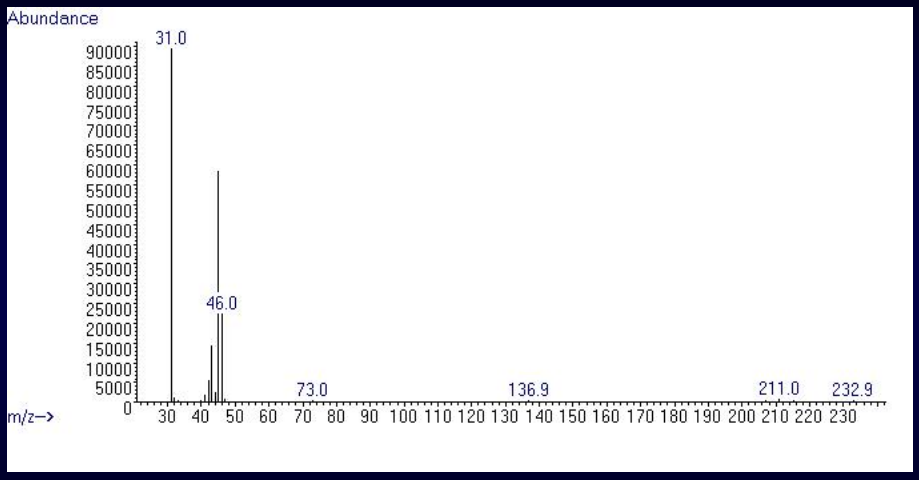



**1-propanol (IS)**

# TIC of Ethanol Standard

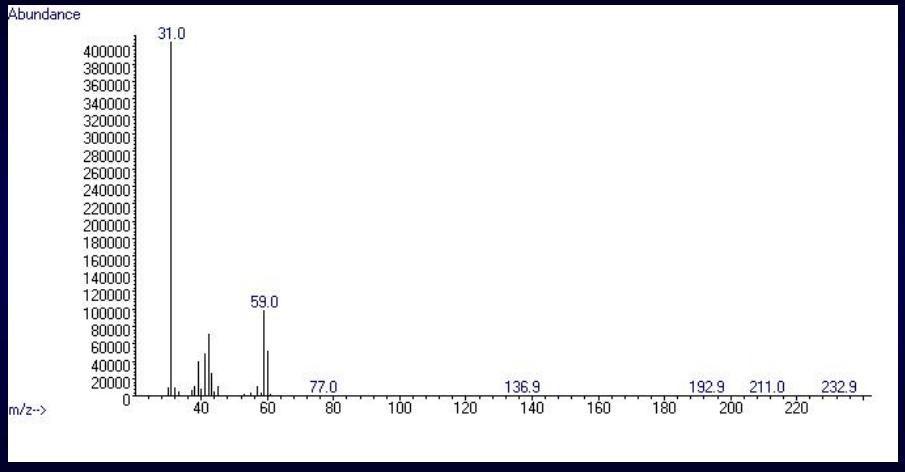


# Spectrum of Peak at 1.29 min




**Ethanol**

# Spectrum of Peak at 1.70 min




**1-propanol (IS)**

# Summary

- Method development is underway with the HS-GC-MSD
  - Good separation and spectra from the volatiles mix and ethanol standard
    - Ready to start validation
  - Developing method for commonly abused inhalants
  - Developing a screening for other volatile compounds
    - Example: GHB

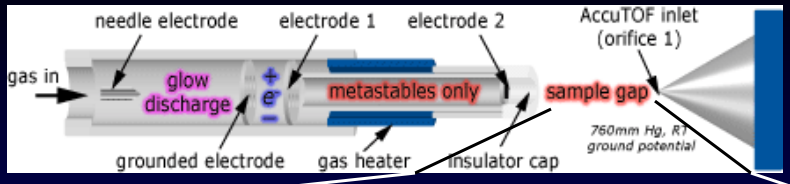
# AccuTOF-DART MS

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

# 3200 QTRAP-DART MS

- Coupled to a hybrid triple quadrupole/Trap instrument molecular ions can be individually fragmented for identification of sample components

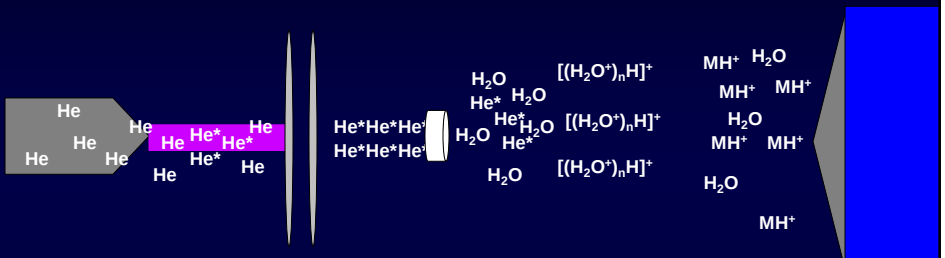
# DART Ionization




- 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

# DART Ionization




# Time of Flight Detector


$$t = (d/\sqrt{(2U)})((\sqrt{m/z}))$$

t = time

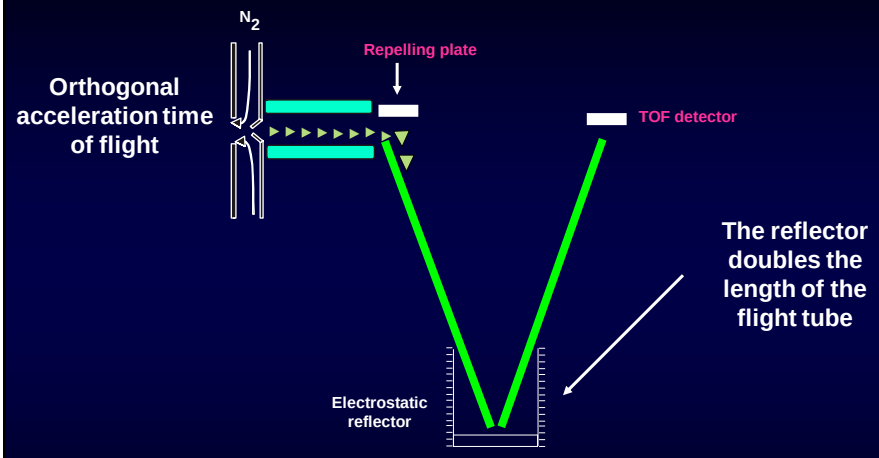
d = flight tube distance

U = accelerating voltage

m = mass

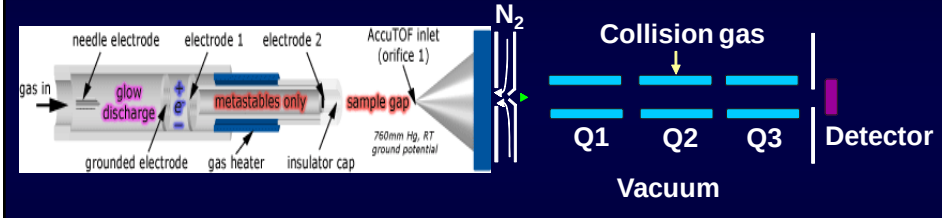
z = charge

# AccuTOF Mass Spectrometer

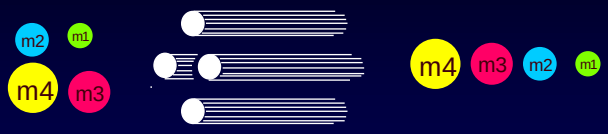



# Different forms of Mass Spectrometry

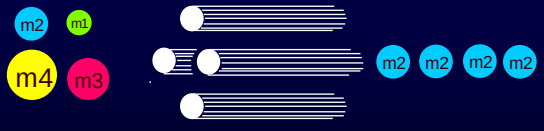
## DART Ionization Tandem Mass Spectrometry



## Quadrupoles have variable ion transmission modes

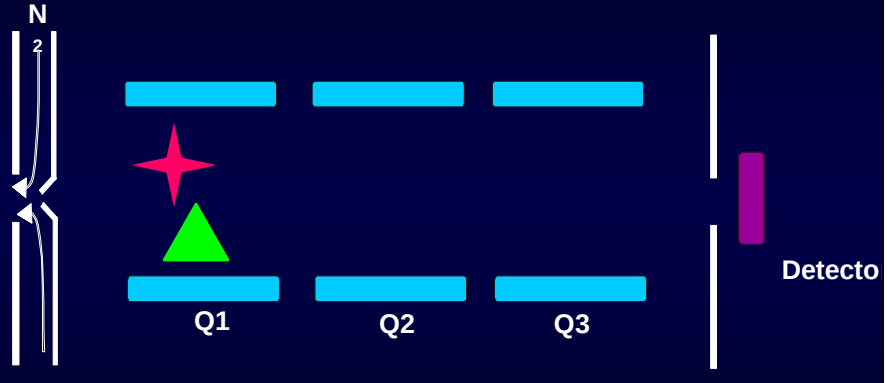


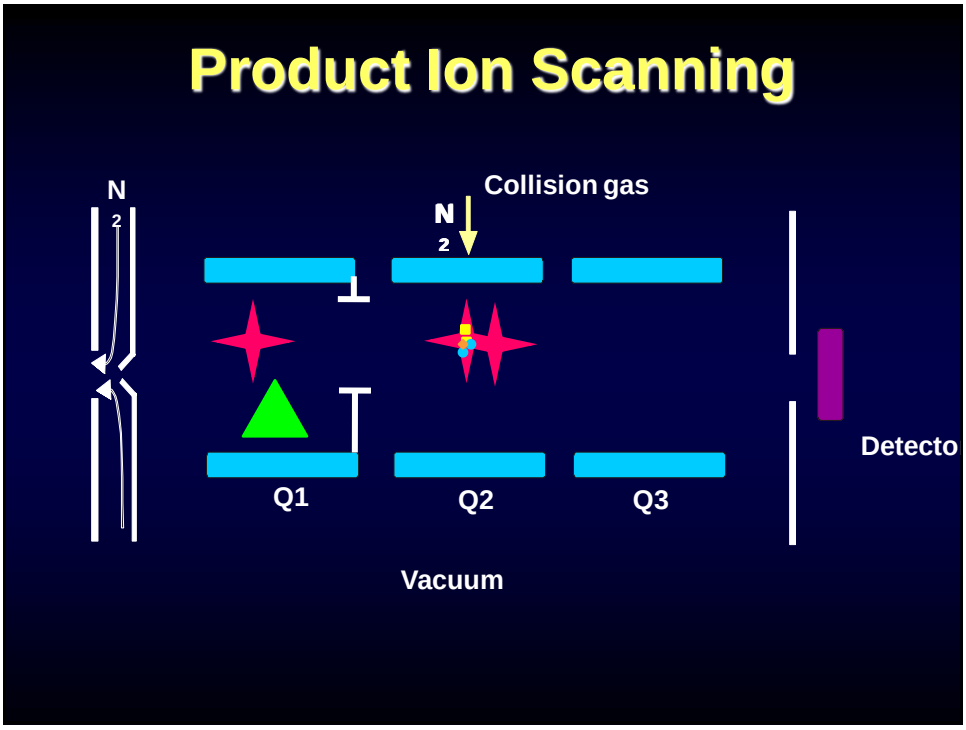

mass scanning mode

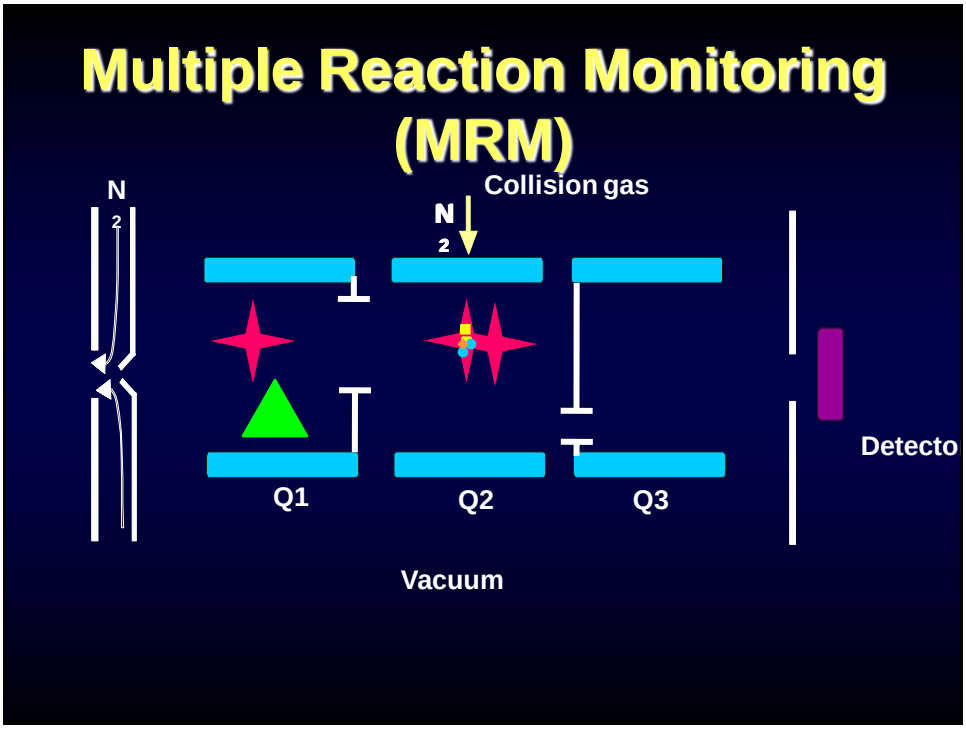



single mass transmission mode

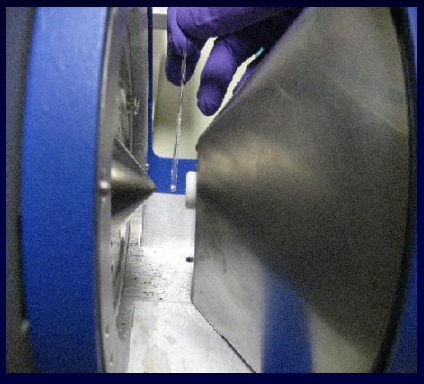
## Molecular Ion Scanning




Product Ion Scanning
N
2
Collision gas
N
2
Q1
Q2
Q3
Detecto
Vacuum


Multiple Reaction Monitoring
(MRM)
N
2
Collision gas
N
2
Q1
Q2
Q3
Detecto
Vacuum

# Sample Introduction with the AccuTOF-DART MS

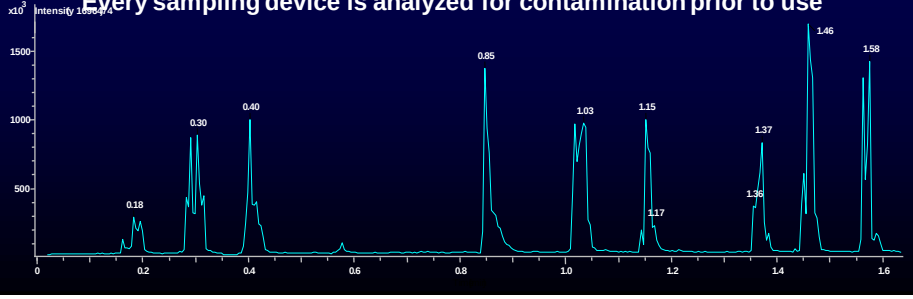


Liquid samples are introduced with a glass capillary tube closed at one end

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

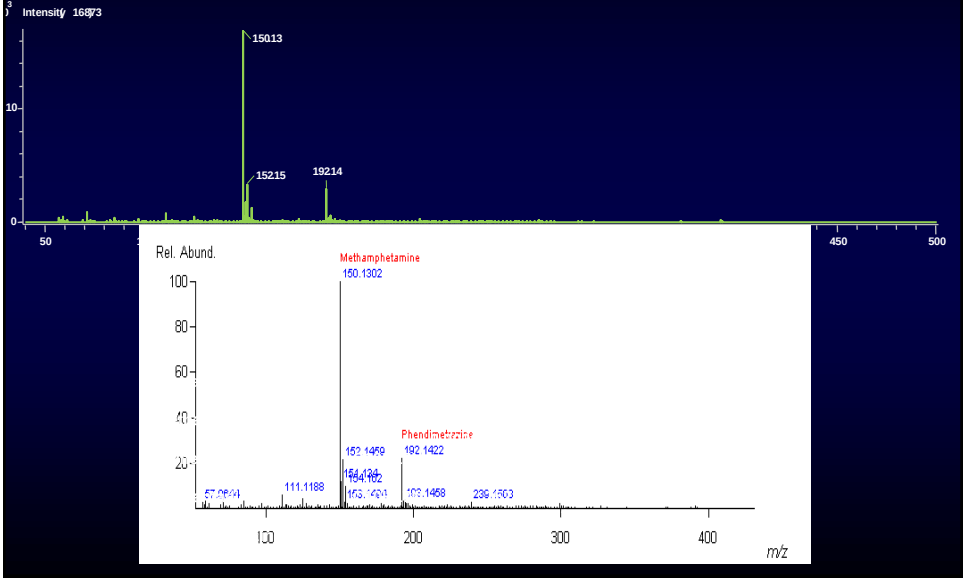


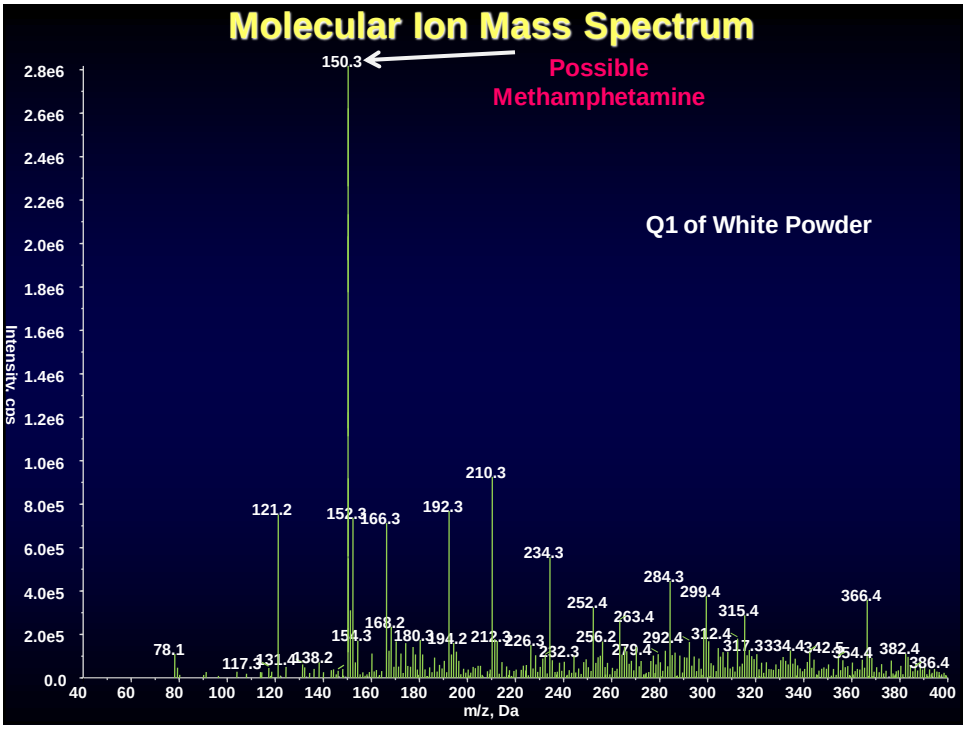
## Types of Samples Analyzed with the AccuTOF-DART MS

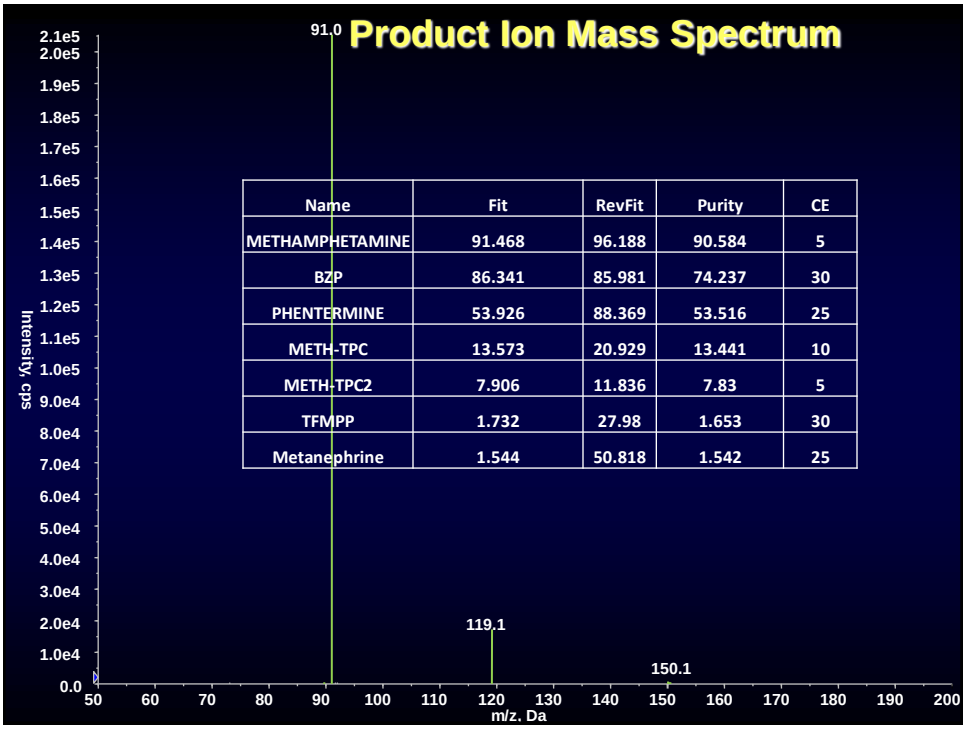
http://www.ecstasy2.com/img/ecstasy_pill_collage1.jpg



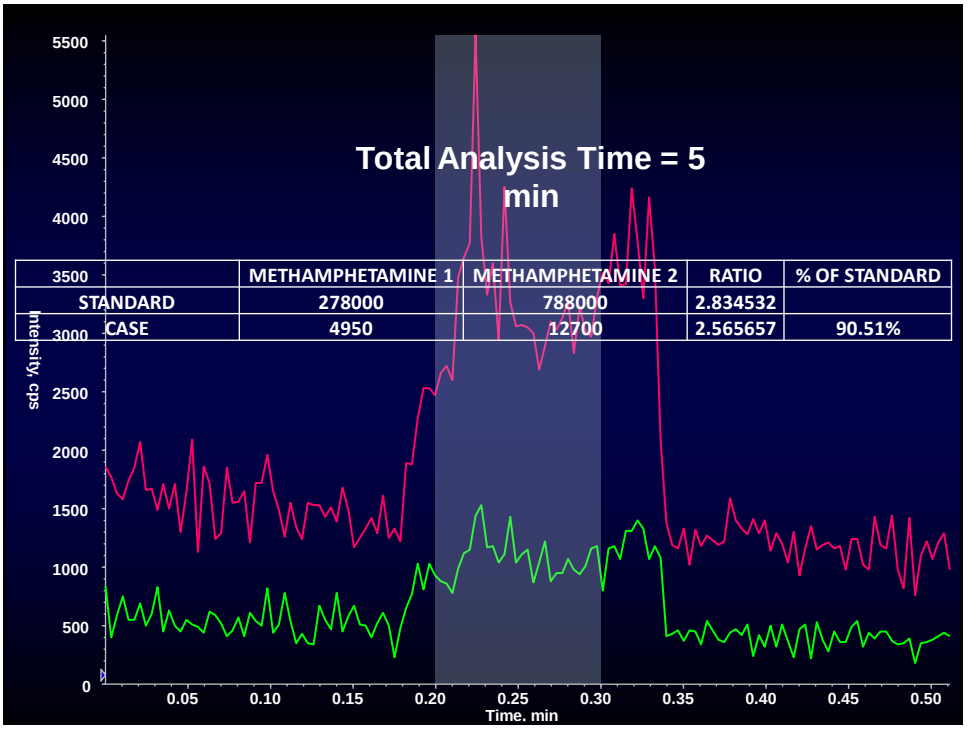
## White Powder Analyzed with the AccuTOF-DART MS





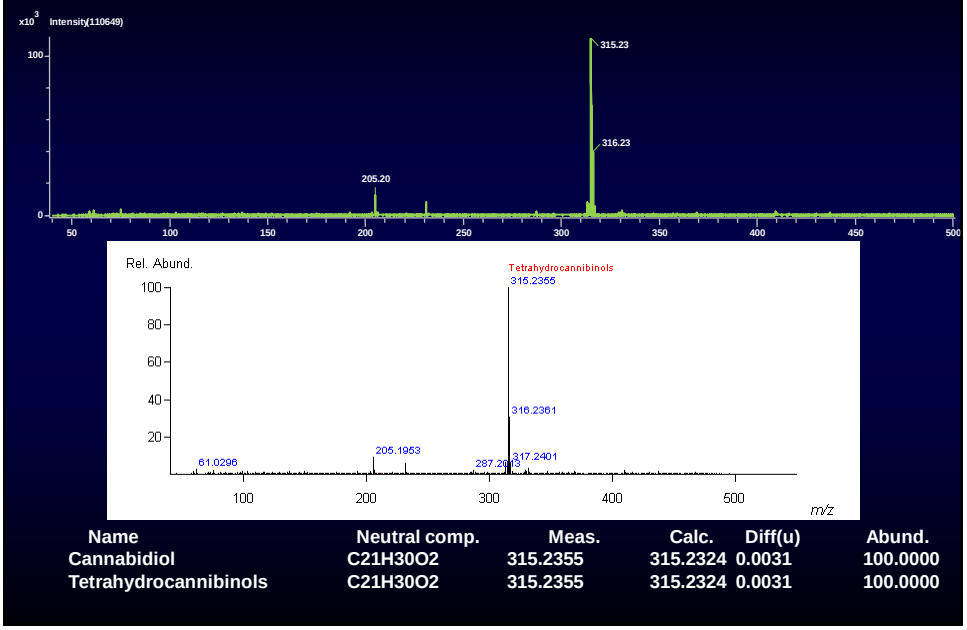




| Name | Fit | RevFit | Purity | CE |
|---|---|---|---|---|
| METHAMPHETAMINE | 91.468 | 96.188 | 90.584 | 5 |
| BZP | 86.341 | 85.981 | 74.237 | 30 |
| PHENTERMINE | 53.926 | 88.369 | 53.516 | 25 |
| METH-TPC | 13.573 | 20.929 | 13.441 | 10 |
| METH-TPC2 | 7.906 | 11.836 | 7.83 | 5 |
| TFMPP | 1.732 | 27.98 | 1.653 | 30 |
| Metanephrine | 1.544 | 50.818 | 1.542 | 25 |

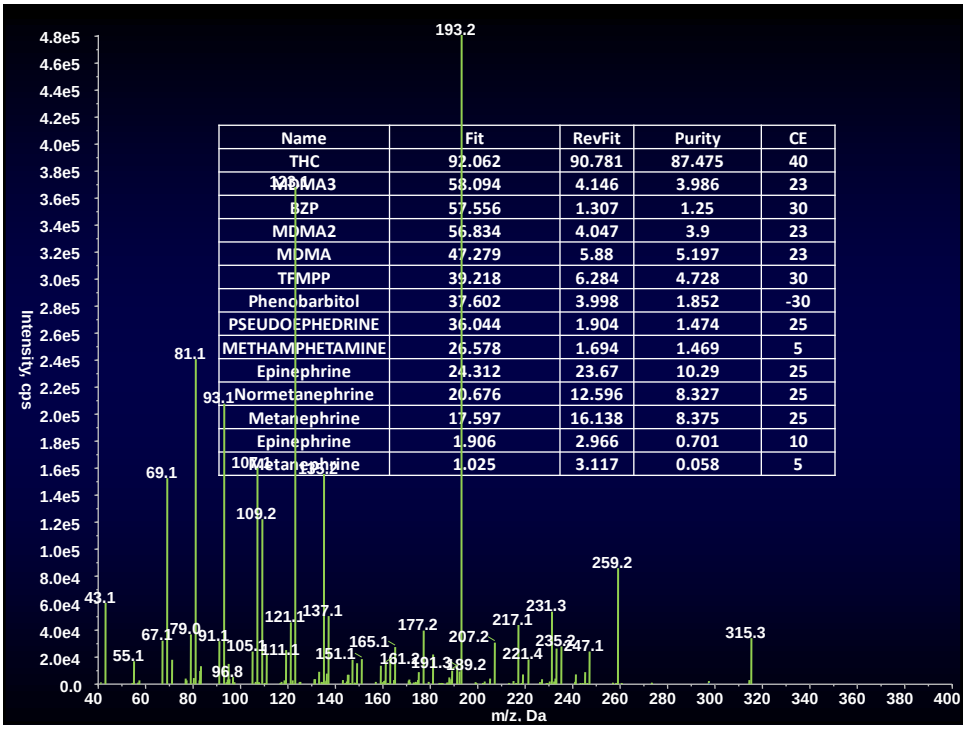



| | METHAMPHETAMINE 1 | METHAMPHETAMINE 2 | RATIO | % OF STANDARD |
|---|---|---|---|---|
| STANDARD | 278000 | 788000 | 2.834532 | |
| CASE | 4950 | 12700 | 2.565657 | 90.51% |

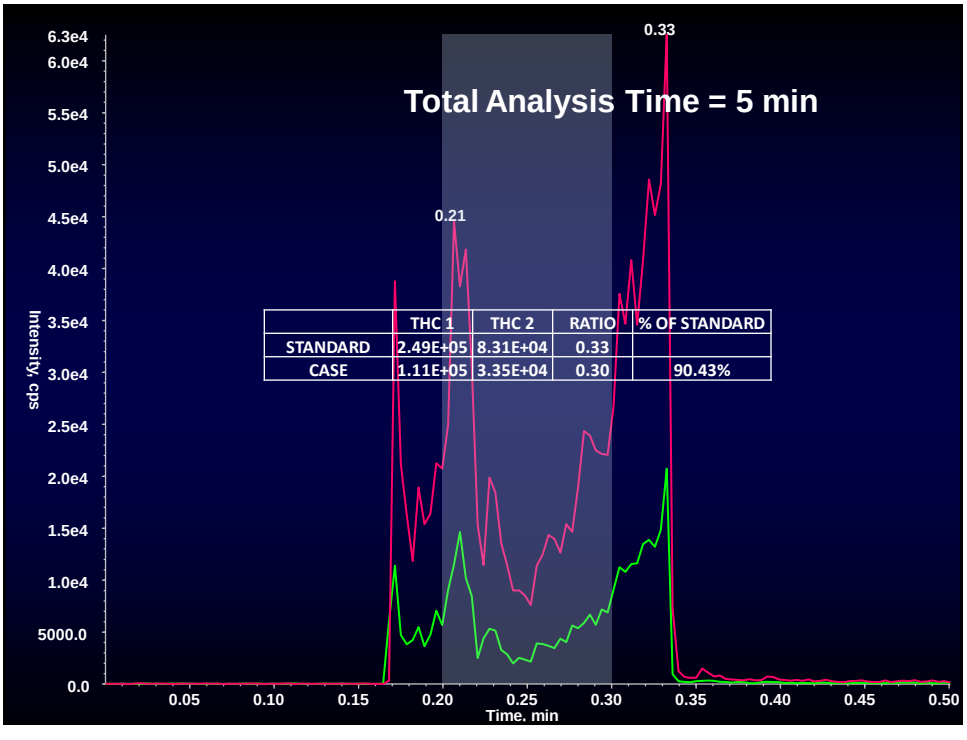
## Plant Material Analyzed with the AccuTOF-DART MS




| Name | Neutral comp. | Meas. | Calc. | Diff(u) | Abund. |
|---|---|---|---|---|---|
| Cannabidiol | C21H30O2 | 315.2355 | 315.2324 | 0.0031 | 100.0000 |
| Tetrahydrocannibinols | C21H30O2 | 315.2355 | 315.2324 | 0.0031 | 100.0000 |

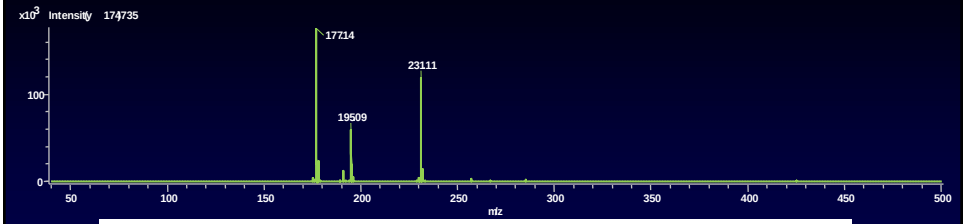


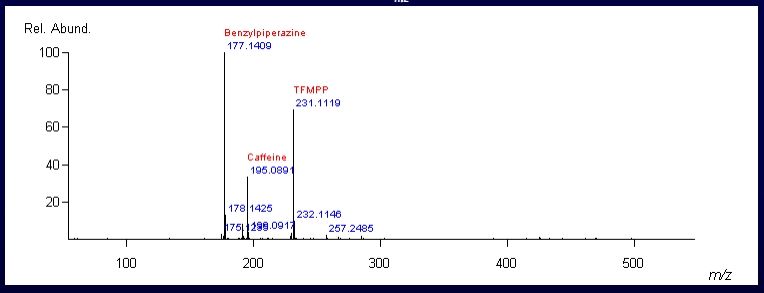

| Name | Fit | RevFit | Purity | CE |
|---|---|---|---|---|
| THC | 92.062 | 90.781 | 87.475 | 40 |
| MDMA3 | 58.094 | 4.146 | 3.986 | 23 |
| BZP | 57.556 | 1.307 | 1.25 | 30 |
| MDMA2 | 56.834 | 4.047 | 3.9 | 23 |
| MDMA | 47.279 | 5.88 | 5.197 | 23 |
| TFMPP | 39.218 | 6.284 | 4.728 | 30 |
| Phenobarbitol | 37.602 | 3.998 | 1.852 | -30 |
| PSEUDOEPHEDRINE | 36.044 | 1.904 | 1.474 | 25 |
| METHAMPHETAMINE | 26.578 | 1.694 | 1.469 | 5 |
| Epinephrine | 24.312 | 23.67 | 10.29 | 25 |
| Normetanephrine | 20.676 | 12.596 | 8.327 | 25 |
| Metanephrine | 17.597 | 16.138 | 8.375 | 25 |
| Epinephrine | 1.906 | 2.966 | 0.701 | 10 |
| Metanephrine | 1.025 | 3.117 | 0.058 | 5 |



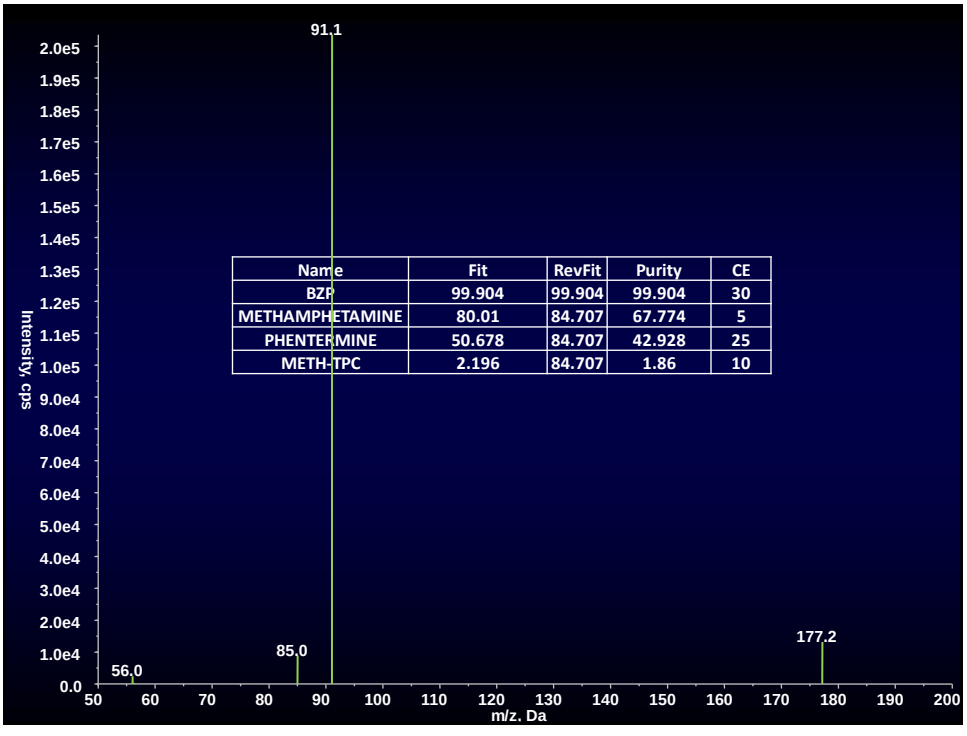

| | THC 1 | THC 2 | RATIO | % OF STANDARD |
|---|---|---|---|---|
| STANDARD | 2.49E+05 | 8.31E+04 | 0.33 | |
| CASE | 1.11E+05 | 3.35E+04 | 0.30 | 90.43% |

# Clandestine Tablet Analyzed with the AccuTOF-DART MS

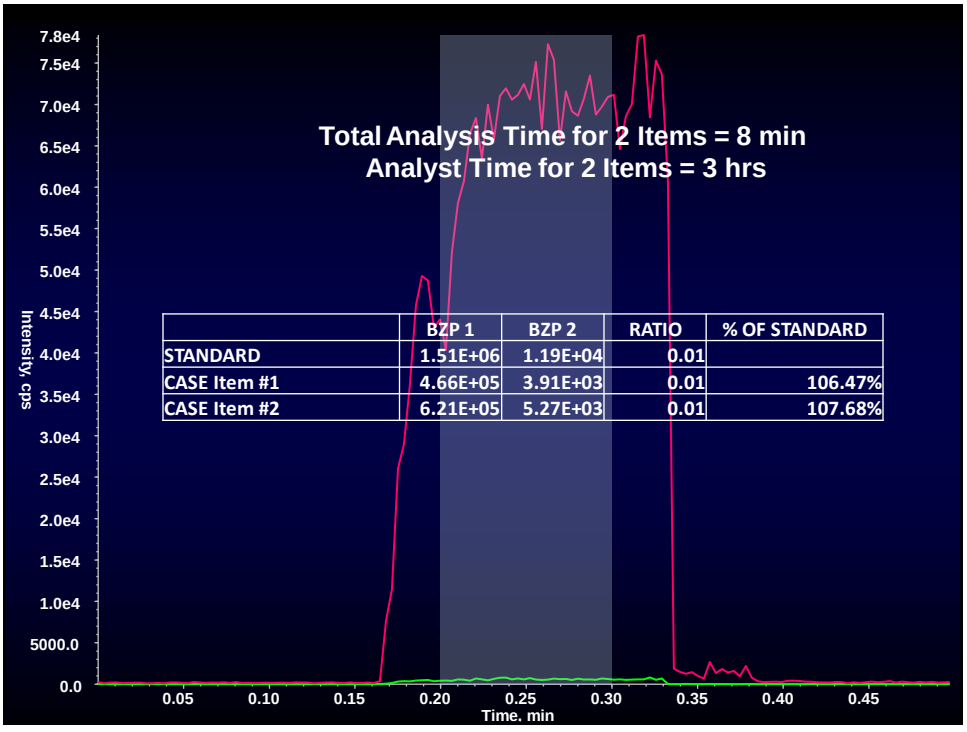



| Name | Neutral comp. | Meas. | Calc. | Diff(u) | Abund. |
|---|---|---|---|---|---|
| Benzylpiperazine | C11H16N2 | 177.1409 | 177.1392 | 0.0017 | 100.0000 |
| Caffeine | C8H10N4O2 | 195.0891 | 195.0882 | 0.0009 | 33.0937 |
| TFMPP | C11H13F3N2 | 231.1119 | 231.1109 | 0.0010 | 69.6525 |

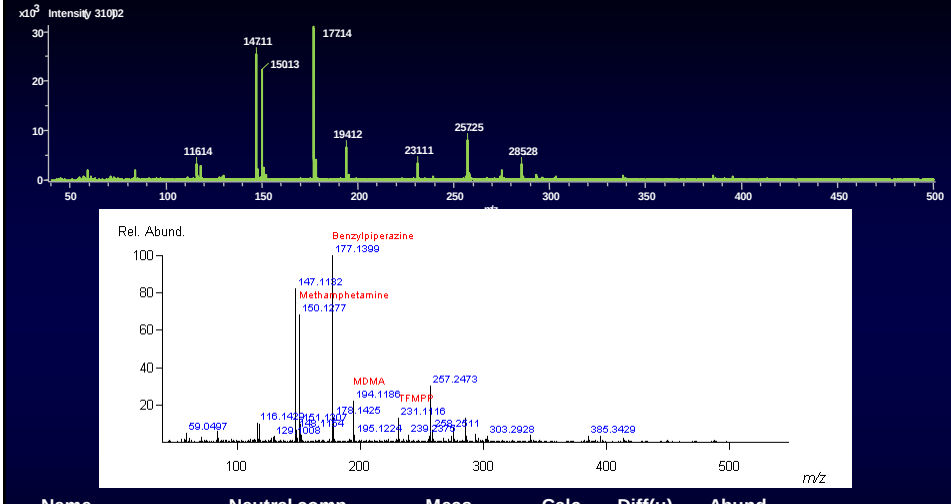



| Name | Fit | RevFit | Purity | CE |
|---|---|---|---|---|
| BZP | 99.904 | 99.904 | 99.904 | 30 |
| METHAMPHETAMINE | 80.01 | 84.707 | 67.774 | 5 |
| PHENTERMINE | 50.678 | 84.707 | 42.928 | 25 |
| METH-TPC | 2.196 | 84.707 | 1.86 | 10 |

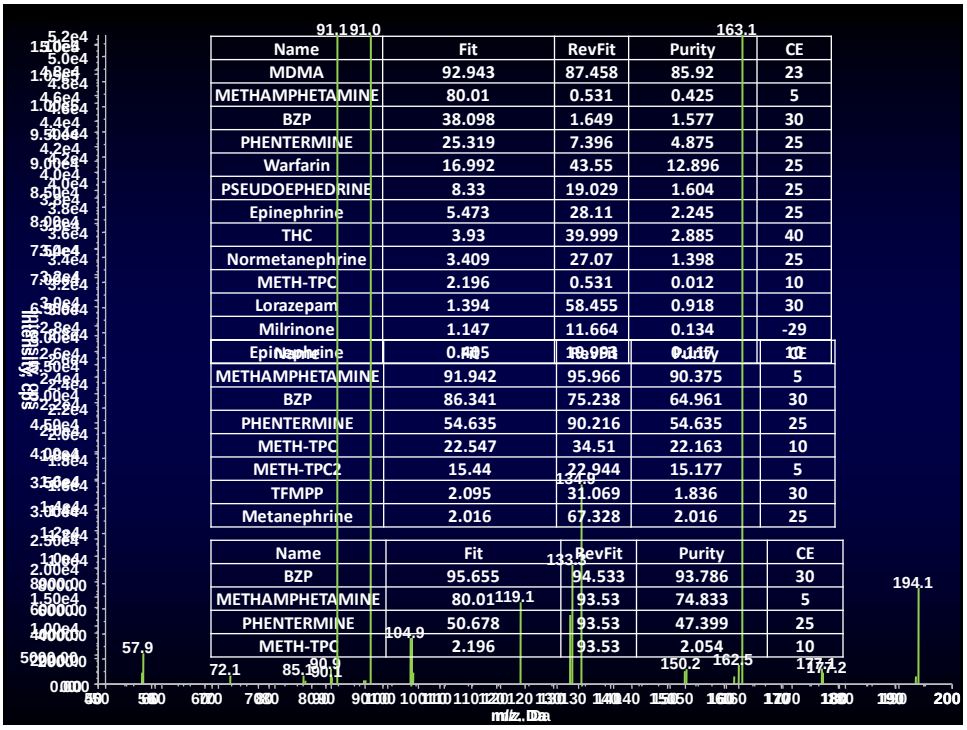



| | BZP 1 | BZP 2 | RATIO | % OF STANDARD |
|---|---|---|---|---|
| STANDARD | 1.51E+06 | 1.19E+04 | 0.01 | |
| CASE Item #1 | 4.66E+05 | 3.91E+03 | 0.01 | 106.47% |
| CASE Item #2 | 6.21E+05 | 5.27E+03 | 0.01 | 107.68% |

## Clandestine Tablet Analyzed with the AccuTOF-DART MS

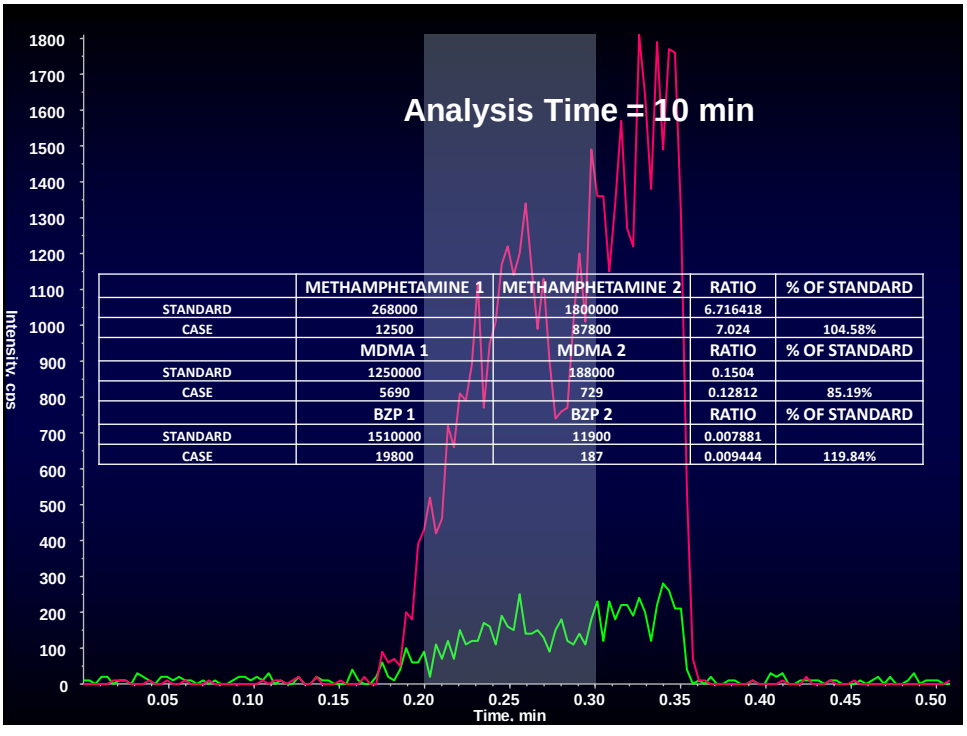



| Name | Neutral comp. | Meas. | Calc. | Diff(u) | Abund. |
|---|---|---|---|---|---|
| Phentermine | C10H15N | 150.1277 | 150.1283 | -0.0005 | 68.2766 |
| Methamphetamine | C10H15N | 150.1277 | 150.1283 | -0.0005 | 68.2766 |
| Benzylpiperazine | C11H16N2 | 177.1400 | 177.1392 | 0.0008 | 100.00 |
| Butamben | 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 |

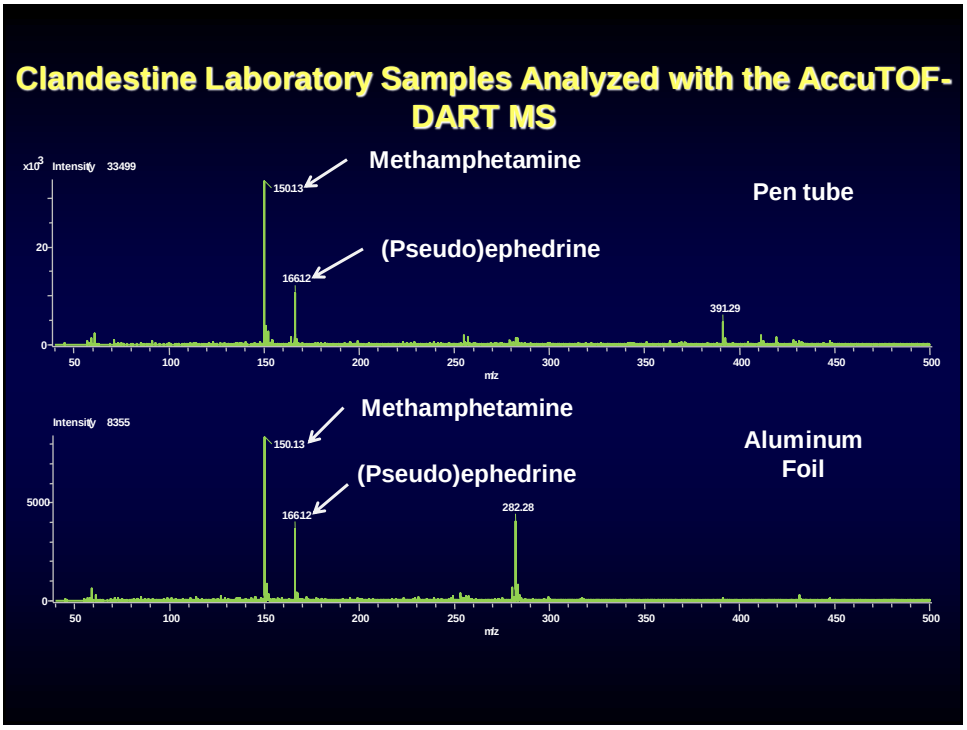


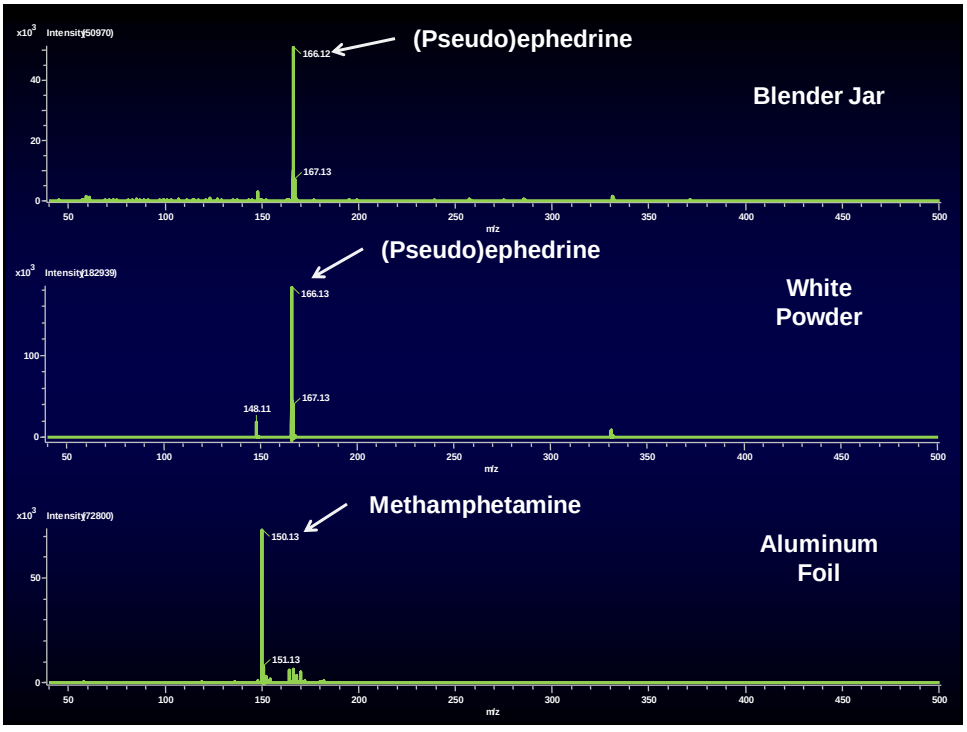

| Name | Fit | RevFit | Purity | CE |
|---|---|---|---|---|
| MDMA | 92.943 | 87.458 | 85.92 | 23 |
| METHAMPHETAMINE | 80.01 | 0.531 | 0.425 | 5 |
| BZP | 38.098 | 1.649 | 1.577 | 30 |
| PHENTERMINE | 25.319 | 7.396 | 4.875 | 25 |
| Warfarin | 16.992 | 43.55 | 12.896 | 25 |
| PSEUDOEPHEDRINE | 8.33 | 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 | 0.445 | 18.983 | 0.117 | 10 |
| 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 |

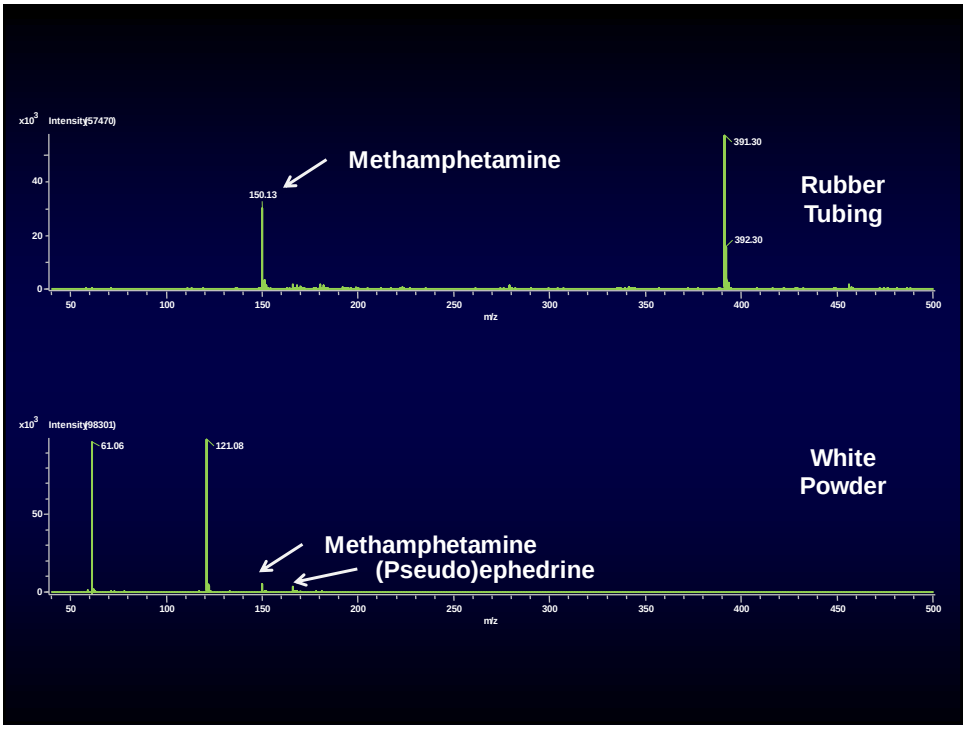
| Name | Fit | RevFit | Purity | CE |
|---|---|---|---|---|
| BZP | 95.655 | 94.533 | 93.786 | 30 |
| METHAMPHETAMINE | 80.01 | 93.53 | 74.833 | 5 |
| PHENTERMINE | 50.678 | 93.53 | 47.399 | 25 |
| METH-TPC | 2.196 | 93.53 | 2.054 | 10 |

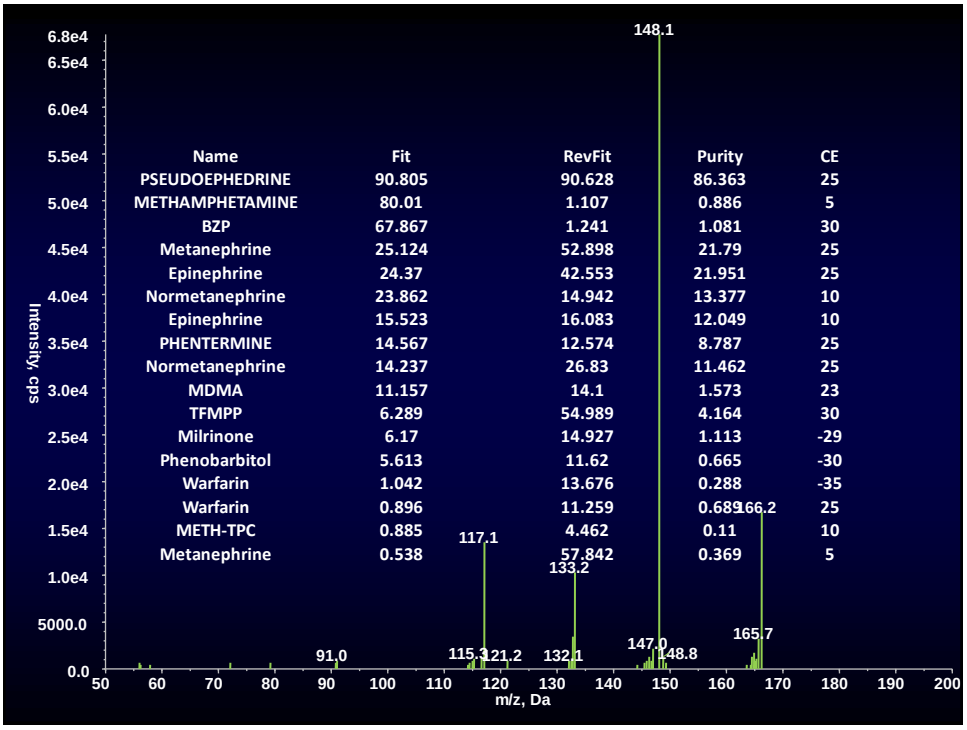



| | METHAMPHETAMINE 1 | METHAMPHETAMINE 2 | RATIO | % OF STANDARD |
|---|---|---|---|---|
| STANDARD | 268000 | 1800000 | 6.716418 | |
| CASE | 12500 | 87800 | 7.024 | 104.58% |
| | MDMA 1 | MDMA 2 | RATIO | % OF STANDARD |
| STANDARD | 1250000 | 188000 | 0.1504 | |
| CASE | 5690 | 729 | 0.12812 | 85.19%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


Clandestine Laboratory Samples Analyzed with the AccuTOF-DART MS
Methamphetamine
(Pseudo)ephedrine
Pen tube
Methamphetamine
(Pseudo)ephedrine
Aluminum Foil
m/z


(Pseudo)ephedrine
Blender Jar
(Pseudo)ephedrine
White Powder
Methamphetamine
Aluminum Foil
m/z


Methamphetamine
Rubber Tubing
White Powder
Methamphetamine
(Pseudo)ephedrine
m/z


Name Fit RevFit Purity CE
PSEUDOEPHEDRINE 90.805 90.628 86.363 25
METHAMPHETAMINE 80.01 1.107 0.886 5
BZP 67.867 1.241 1.081 30
Metanephrine 25.124 52.898 21.79 25
Epinephrine 24.37 42.553 21.951 25
Normetanephrine 23.862 14.942 13.377 10
Epinephrine 15.523 16.083 12.049 10
PHENTERMINE 14.567 12.574 8.787 25
Normetanephrine 14.237 26.83 11.462 25
MDMA 11.157 14.1 1.573 23
TFMPP 6.289 54.989 4.164 30
Milrinone 6.17 14.927 1.113 -29
Phenobarbitol 5.613 11.62 0.665 -30
Warfarin 1.042 13.676 0.288 -35
Warfarin 0.896 11.259 0.689 25
METH-TPC 0.885 4.462 0.11 10
Metanephrine 0.538 57.842 0.369 5
Intensity, cps
m/z, Da

## Clandestine Laboratory Samples Analyzed with the DART-QTRAP

| | METHAMPHETAMINE 1 | METHAMPHETAMINE 2 | RATIO | % OF STANDARD |
|---|---|---|---|---|
| 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 | |
|---|---|---|---|---|
| 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**

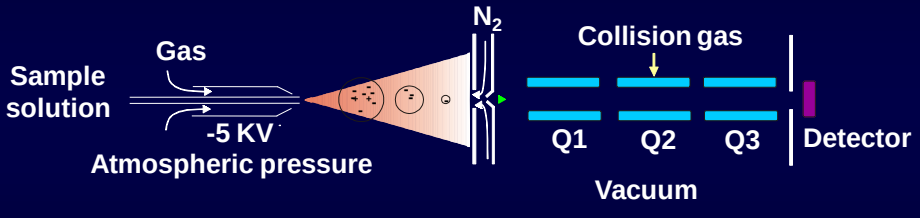
## Real Time Sample Analysis with the AccuTOF-DART MS

- Efficient screening instrument
  - Soft ionization keeps the molecular ion intact
    - Mass accuracy allows matches within 5 mmu of the theoretical mass of a compound
  - No extraction is required for sample analysis
    - Raw samples the preferred sample
  - High-throughput
    - Typical analysis time for a sample is 1-2 min

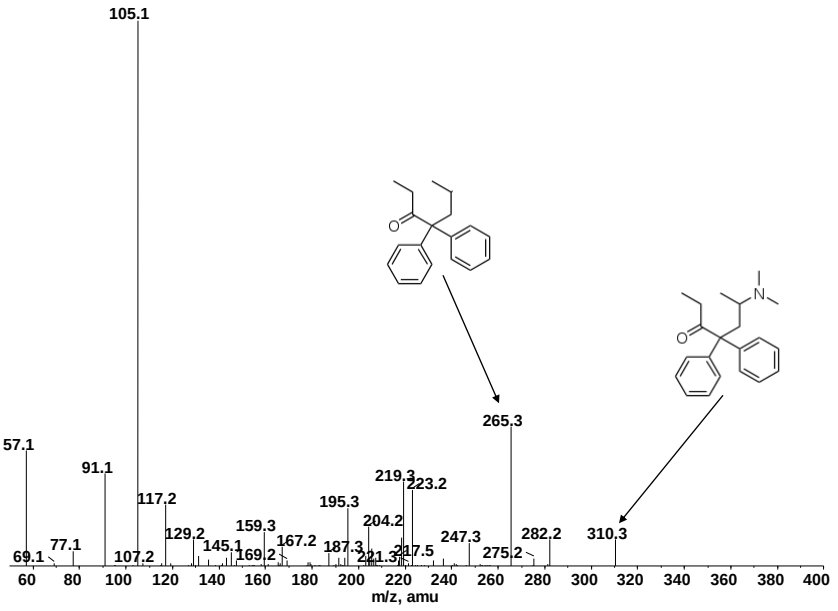
## Real Time Sample Analysis with the DART-QTRAP MS

- 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
- Complex mixtures do not present a problem for analysis
  - The instrument has the ability to isolate a single compound for fragmentation

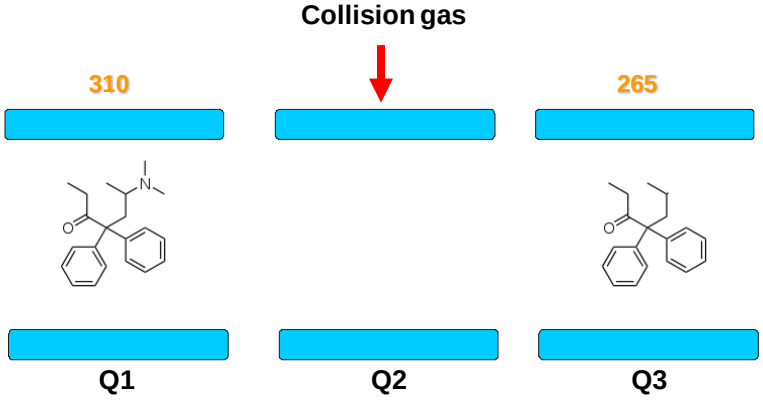
## LC-MRM-MS assay for Drug Detection and Quantitation



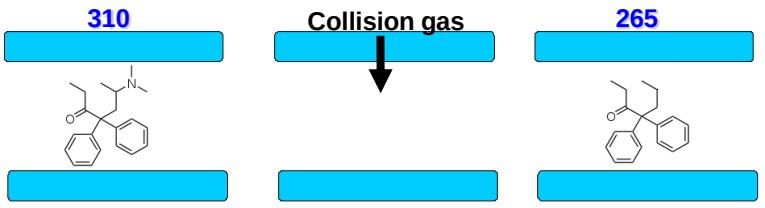
## LC-ESI-MS/MS Spectrum of Methadone




## LC-MRM-MS analysis for Drug Quantitation

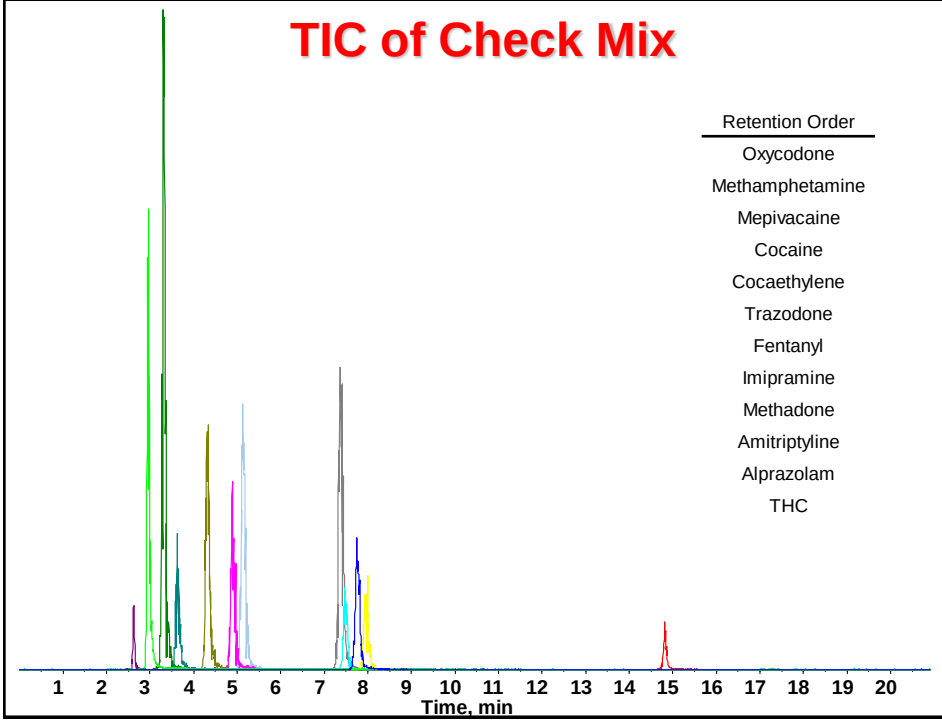


# LC-ESI-MRM-MS

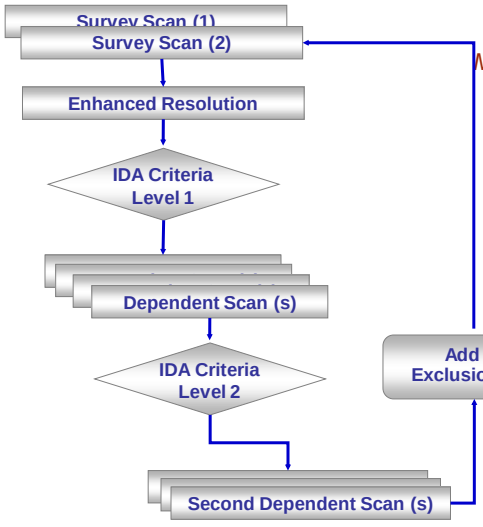



| Compound | Molecular Weight | Parent ion | Product ion | Dwell Time (msec) | Declustering Potential (DP) | Collision Energy (CE) | Retention Time | pKa |
|---|---|---|---|---|---|---|---|---|
| Alprazolam | 308.0829 | 309.1 | 205 | 25 | 60 | 50 | 8.03 | 2.4 |
| Amitriptyline | 277.183 | 278.2 | 91 | 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 Check Mix



# Information Dependent Acquisition (IDA)




*Survey scan be EMS, EMC, Neutral Loss, Precursor Scan, MRM or EPI (combinations of 2 surveys)*

*Improve Resolution/Accuracy*

*Two levels of criteria*

*Multiple dependant scans (EPI, Product Ion and MS3)*

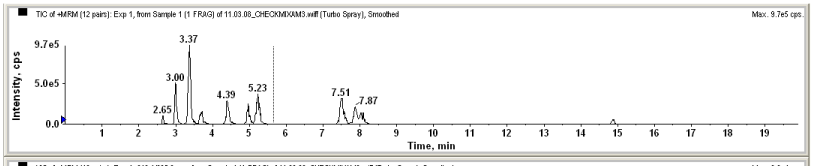
*Inclusion/Exclusion List*

*Second Level Dependant Scan ($MS^3$)*

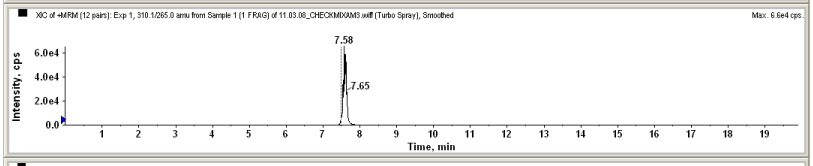
Courtesy of Ricky Ciner

# IDA Analysis of Check Mix

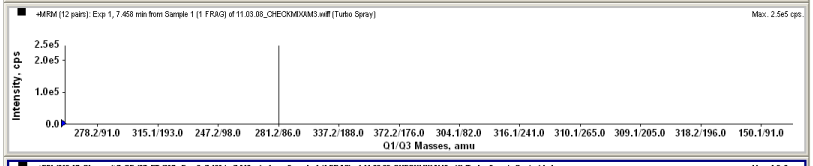
**TIC**



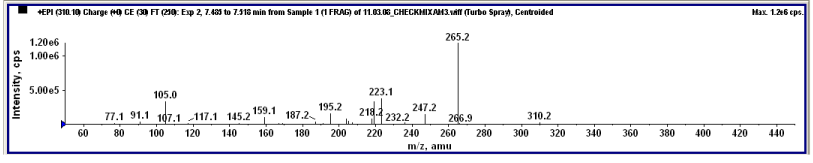

**XIC**



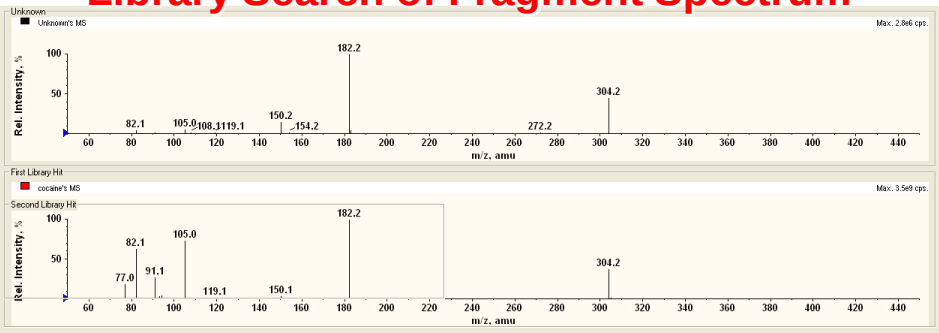

**MRM**




**EPI**



# Library Search of Fragment Spectrum




| | Name | Formula | Molecular weight (amu) | Fit | RevFit | Purity | CE |
|---|---|---|---|---|---|---|---|
| 1 | cocaine | C17H21NO4 | 303.1470 | 76.392 | 75.193 | 74.384 | 20.000 |
| 2 | norpropoxyphene (3200) | C21H27NO2 | 325.2041 | 56.738 | 0.392 | 0.222 | 20.000 |
| 3 | phenytoin (3200) | C15H12N2O2 | 252.0898 | 45.442 | 45.056 | 22.367 | 25.000 |
| 4 | atomoxetine | C17H21NO | 255.1623 | 44.991 | 43.158 | 21.116 | 20.000 |
| 5 | methadone | C21H27NO | 309.2092 | 33.970 | 2.092 | 1.360 | 20.000 |
| 6 | diltiazem | C22H26N2O4S | 414.1613 | 32.815 | 6.350 | 3.150 | 20.000 |
| 7 | gabapentin (3200) | C9H17NO2 | 171.1259 | 32.126 | 1.195 | 0.730 | 20.000 |
| 8 | scopolamine | C17H21NO4 | 303.1470 | 28.540 | 36.795 | 10.921 | 20.000 |
| 9 | cocaethylene | C18H23NO4 | 317.1627 | 27.416 | 6.327 | 3.437 | 20.000 |
| 10 | MDMA | C11H15NO2 | 193.1102 | 26.564 | 0.828 | 0.615 | 20.000 |
| 11 | phencyclidine | C17H25N | 243.1987 | 20.907 | 0.410 | 0.323 | 20.000 |

# Summary

- LC-ESI-MS can be used in the qualitative and quantitative analysis of drugs in toxicological specimens
  - The instrumentation is advantageous in that chemicals do not have to be derivatized
  - The soft ionization aids detection of the parent ion of the compound

# Overall Summary

- Mass spectrometry is a powerful tool in a forensic science lab
- New instrumentation is expanding the sample analysis possibilities beyond current limitations
- No one technique is robust enough for everything, therefore a combination of techniques is ideal for screening and confirmation of drug and toxicology samples

# Acknowledgements


| UAB | ADFS |
| --- | --- |
| • Dr. Stephen Barnes<br>• Marion Kirk<br>• Ray Moore<br>• Dr. Matthew Renfrow<br>• Landon Wilson | • Dr. Dale Carpenter<br>• Andrea Headrick<br>• Dr. Jack Kalin<br>• Gary Wallace |