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

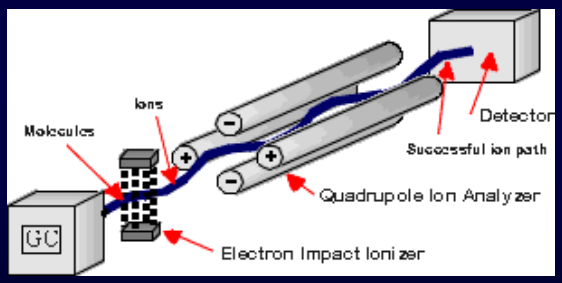
- Alabama
  - Frye standard: the court must decide if the questioned procedure, technique, and principles are "generally accepted" by a relevant community
  - Federal Rule 702: a witness qualified as an expert may testify in the form of an opinion
- Federal
  - Daubert:
    - Has it been tested?
   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

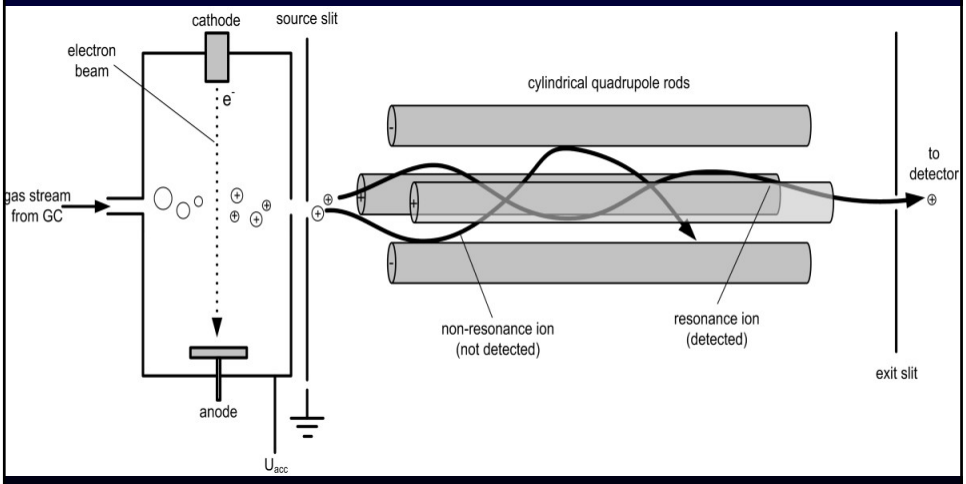
Typical forms of Mass Spectrometry in Every Forensic Science Lab

Gas Chromatography-Mass Spectrometry (GC-MS)



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

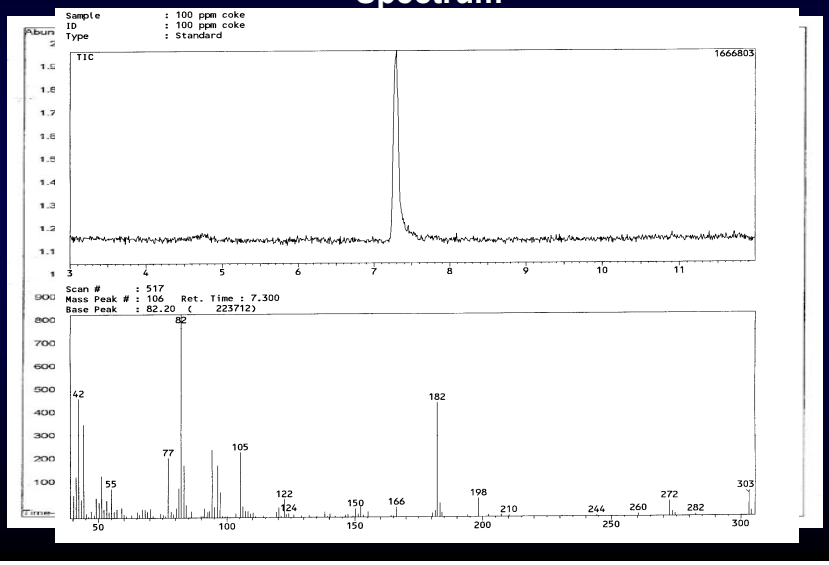
Typical forms of Mass Spectrometry in Every Forensic Science Lab



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

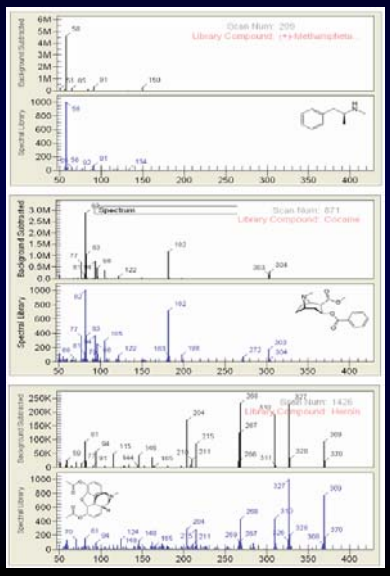
Typical forms of Mass Spectrometry in Every Forensic Science Lab

Gas Chromatography-Mass Spectrometry (GC-MS) Spectrum



Typical forms of Mass Spectrometry in Every Forensic Science Lab

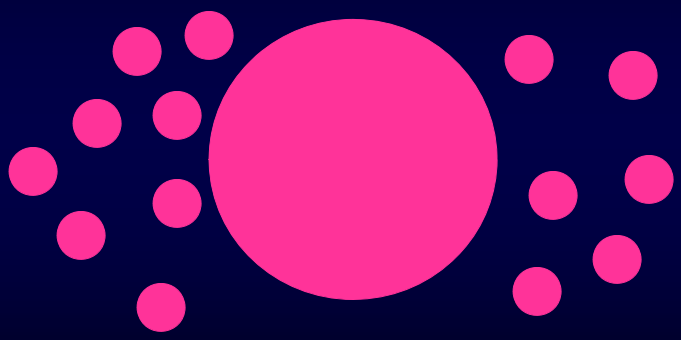
Gas Chromatography-Mass Spectrometry (GC-MS) Spectrum



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

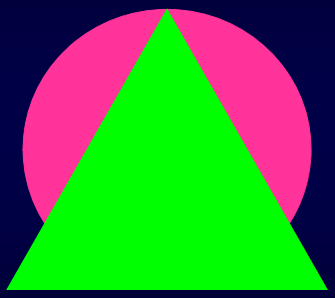


Lose the parent ion of the compound upon ionization in the instrument
Example: Methadone

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



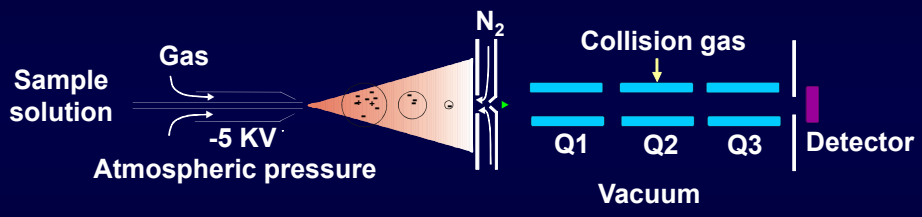
Heat labile compound will be identified as a related compound, but not the actual compound
Example: Clorazepate to Nordiazepam

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

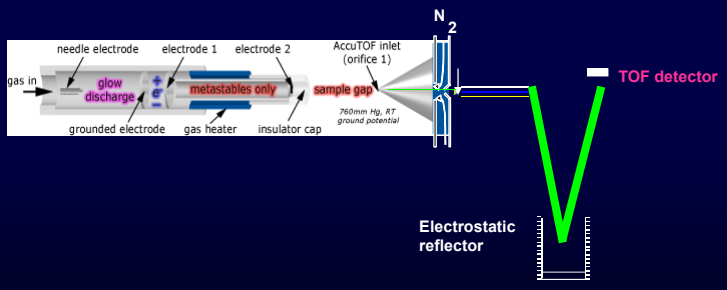
Different forms of Mass Spectrometry

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



Different forms of Mass Spectrometry

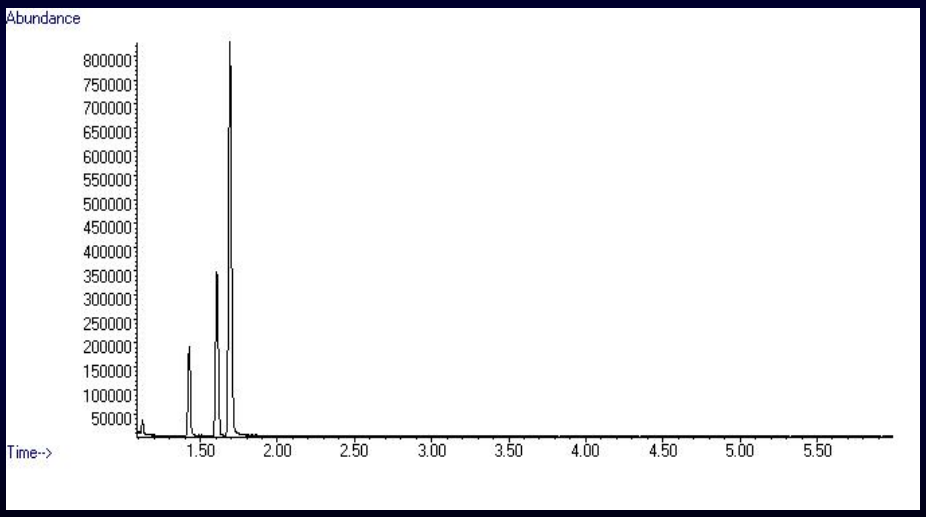
Direct Analysis in Real Time with Time of Flight Mass Spectrometry



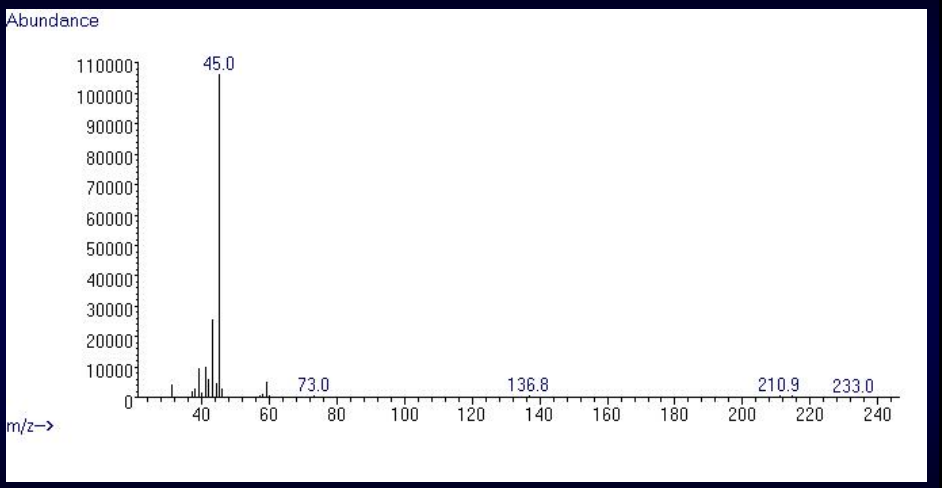
HS-GC-MSD

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

TIC of Volatiles Mix

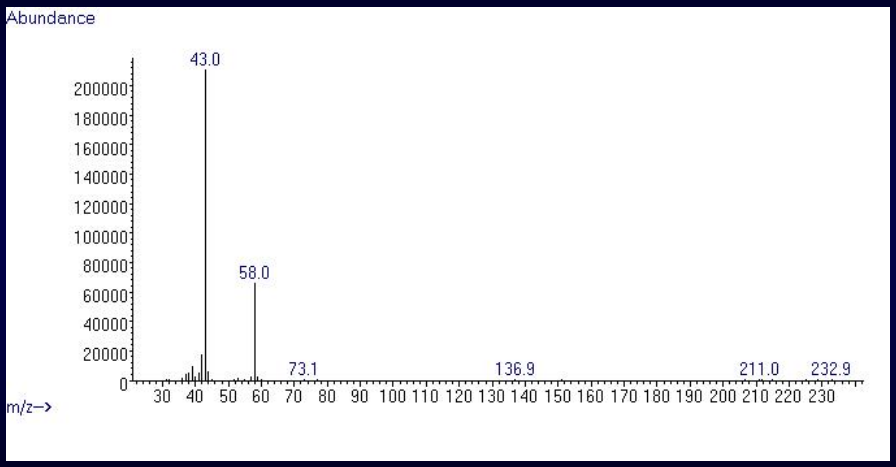


Spectrum of Peak at 1.44 min



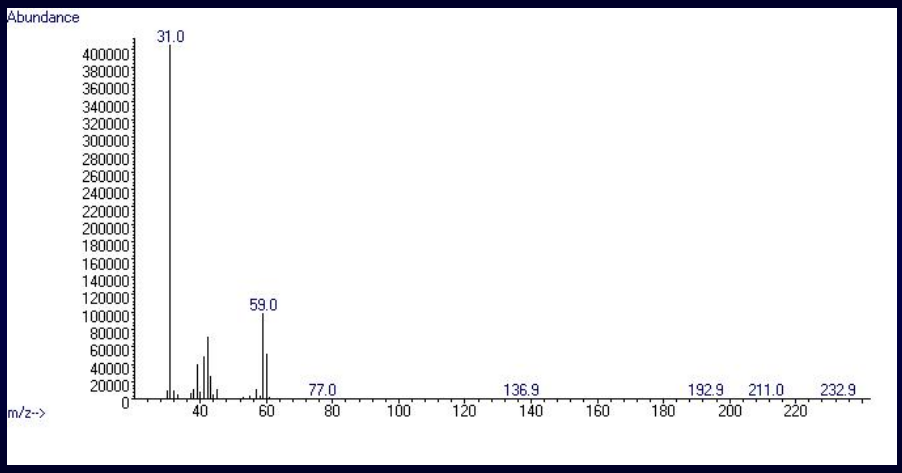
Isopropanol

Spectrum of Peak at 1.61 min



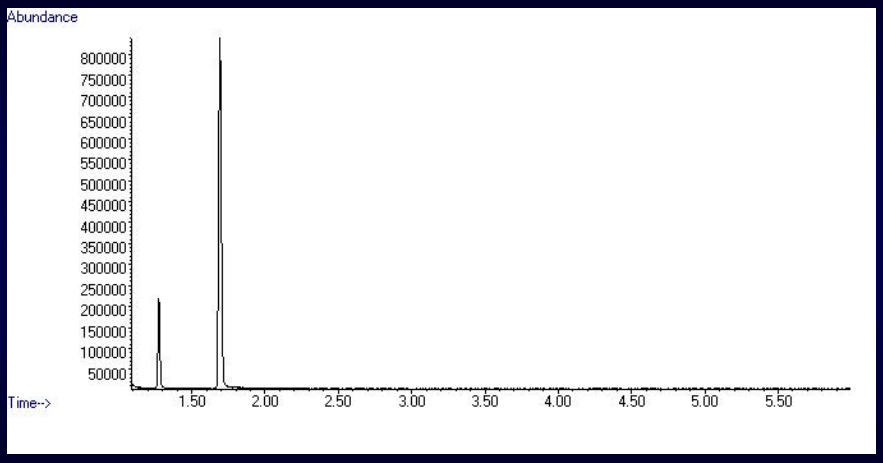
Acetone

Spectrum of Peak at 1.70 min

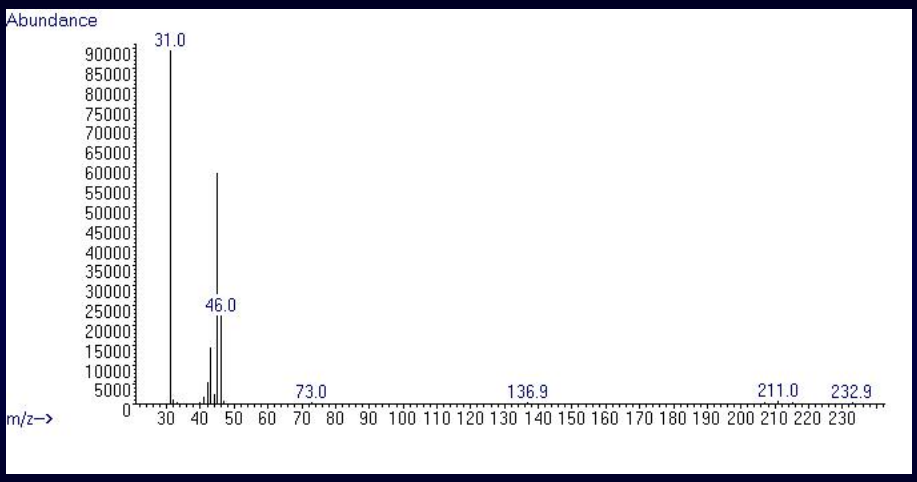


1-propanol (IS)

TIC of Ethanol Standard

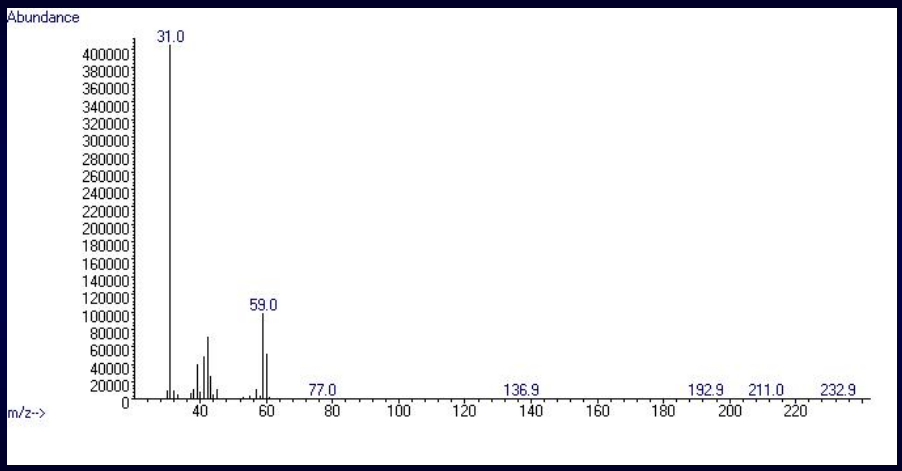


Spectrum of Peak at 1.29 min



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

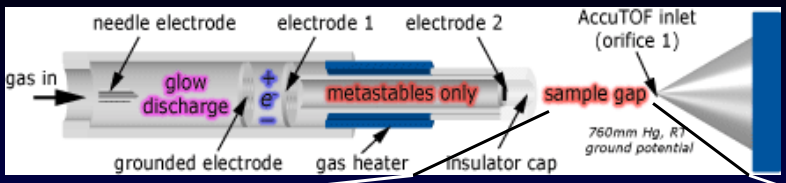
AccuTOF-DART MS

- 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

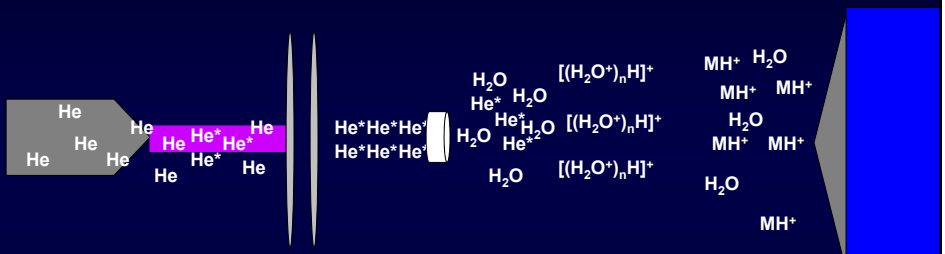
DART Ionization



- 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

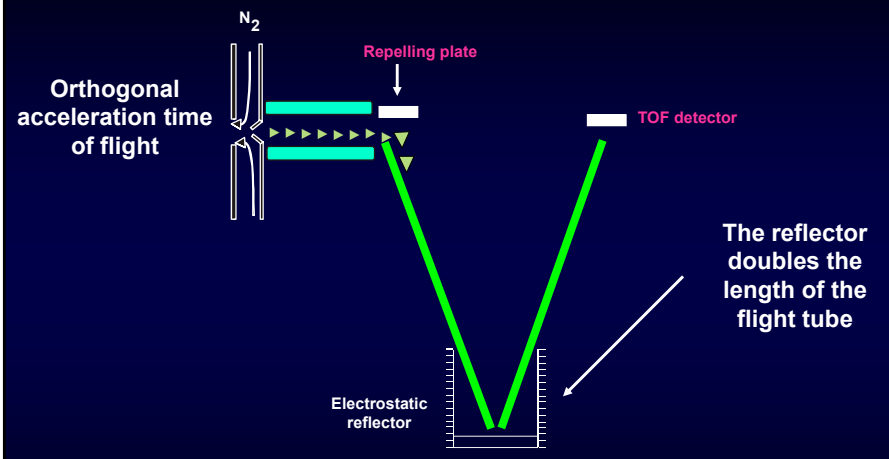
d = flight tube distance

U = accelerating voltage

m = mass

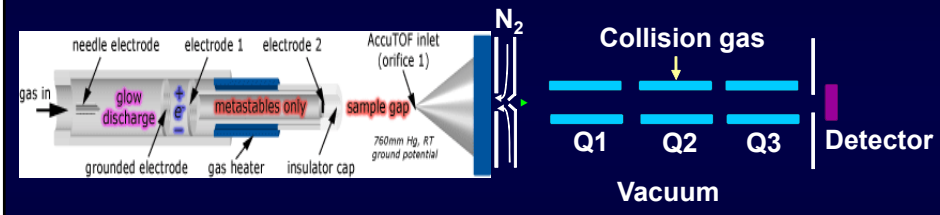
z = charge

AccuTOF Mass Spectrometer

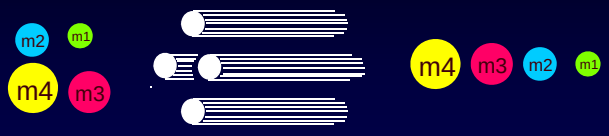


Different forms of Mass Spectrometry

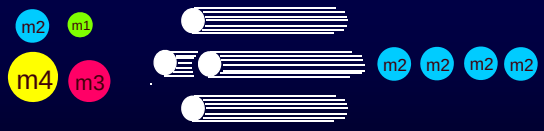
DART Ionization Tandem Mass Spectrometry



Quadrupoles have variable ion transmission modes

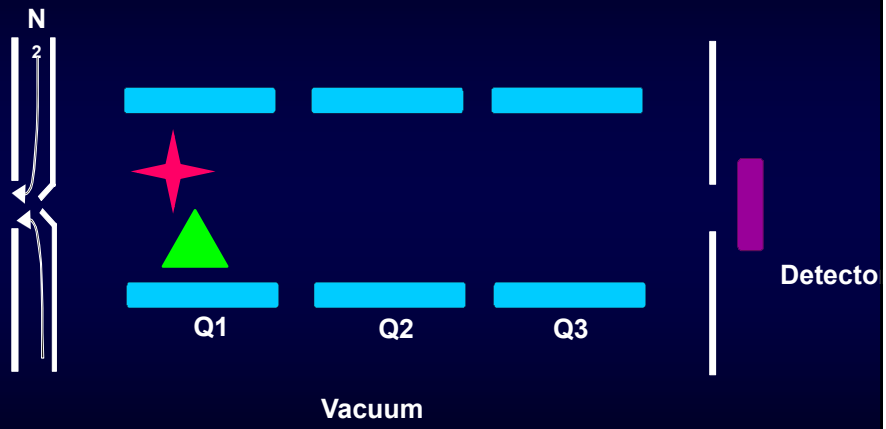


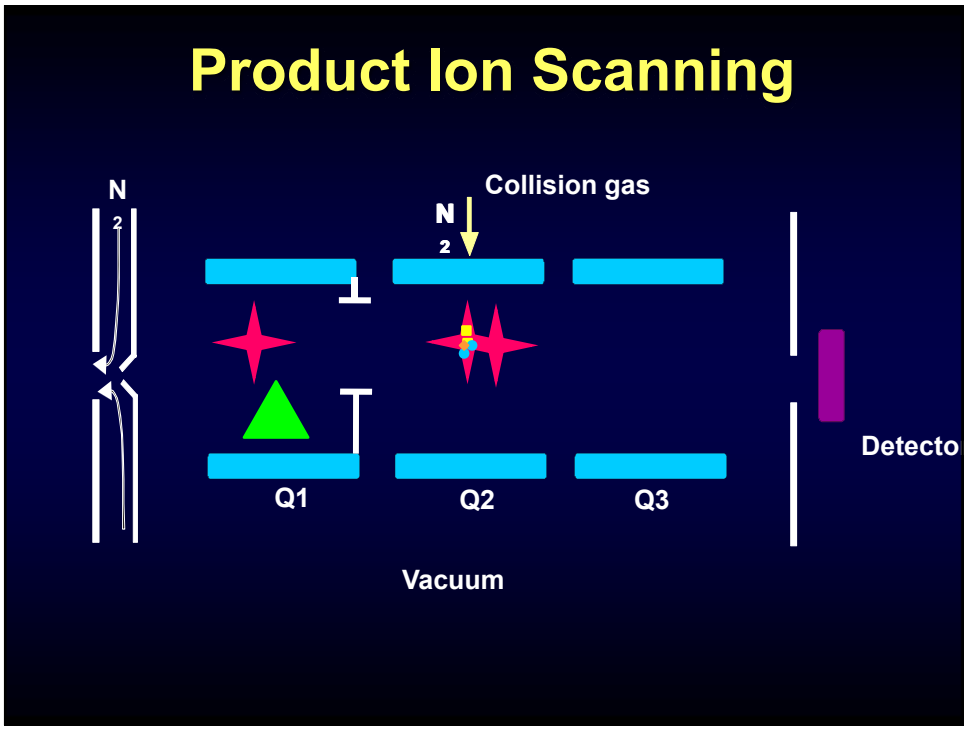
mass scanning mode

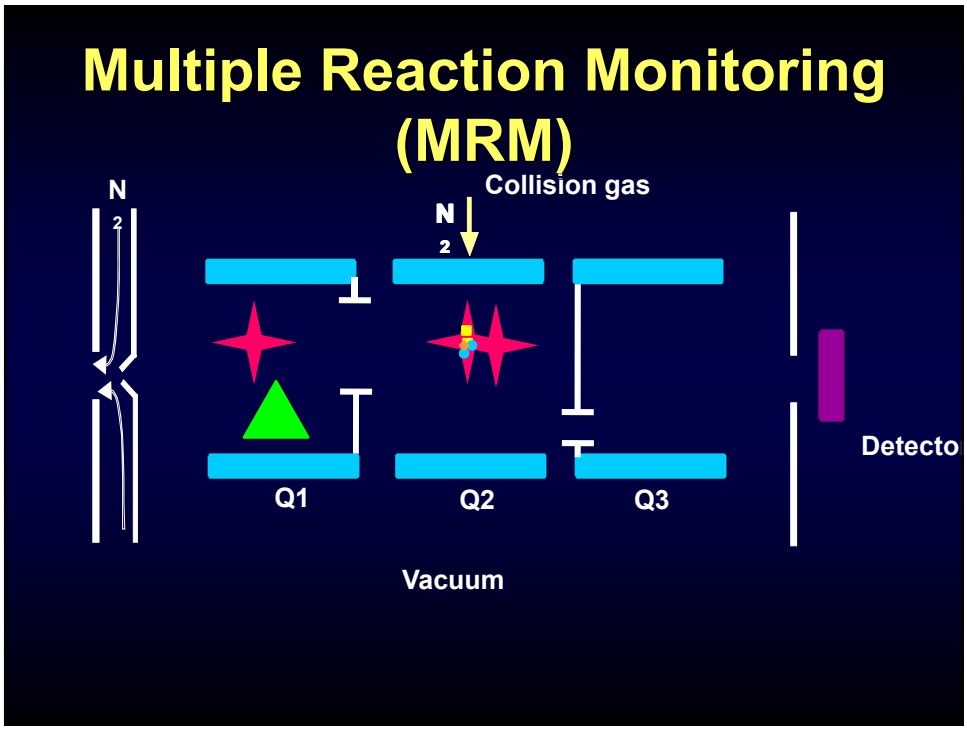


single mass transmission mode

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

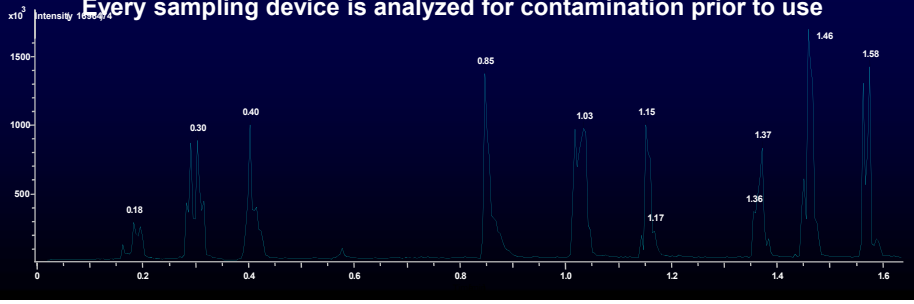
Sample Introduction with the AccuTOF-DART MS

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

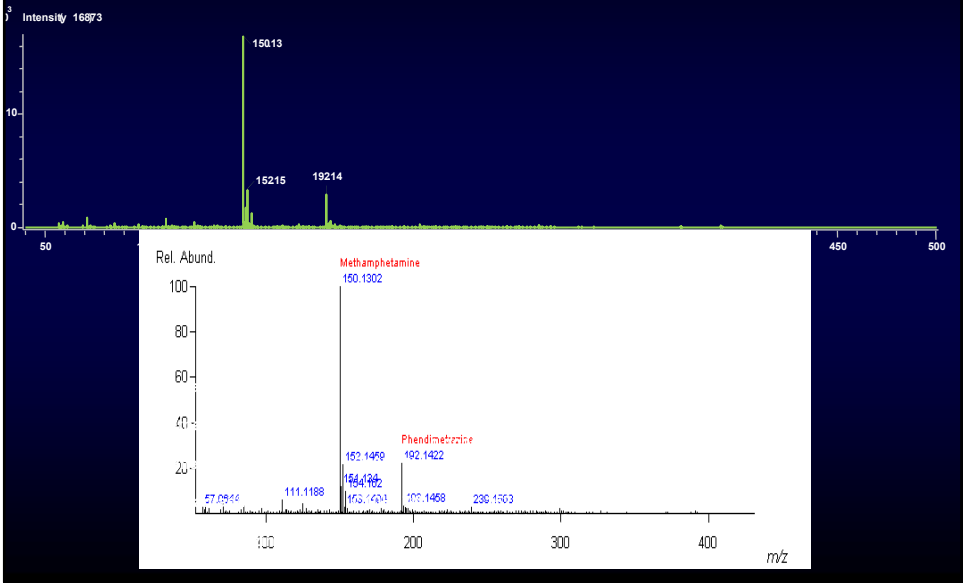
Every sampling device is analyzed for contamination prior to use

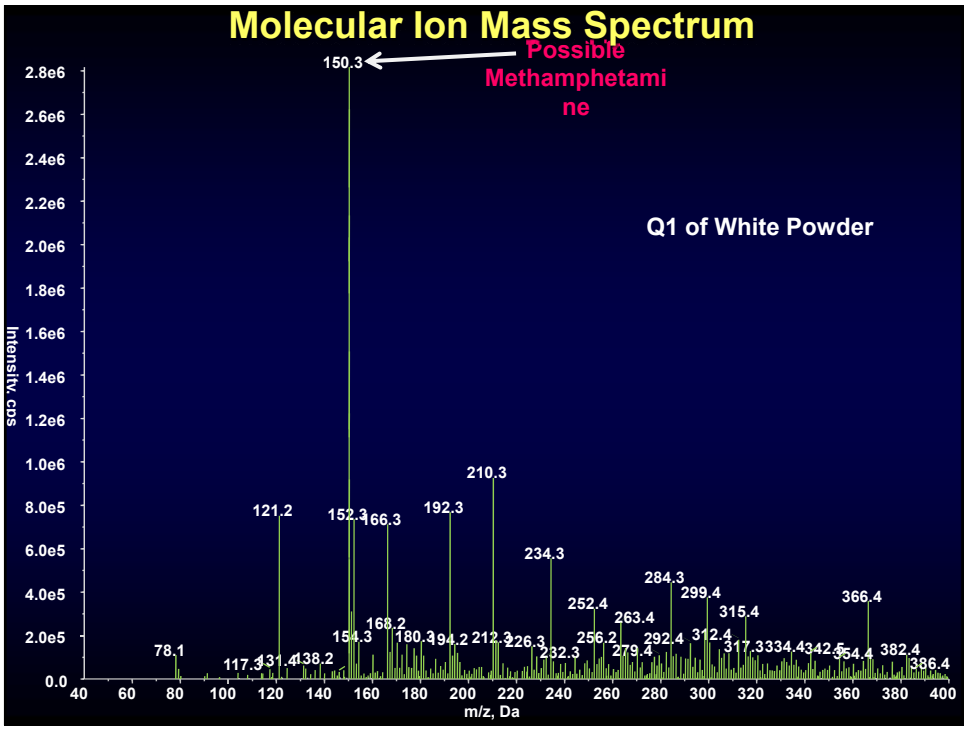


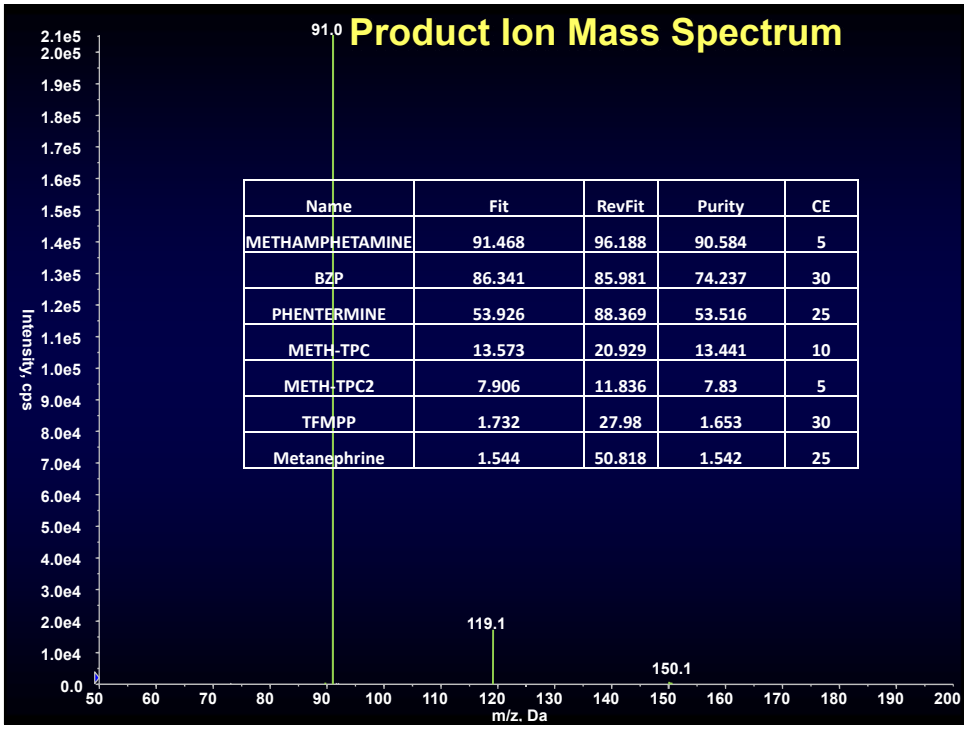
Types of Samples Analyzed with the AccuTOF-DART MS

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

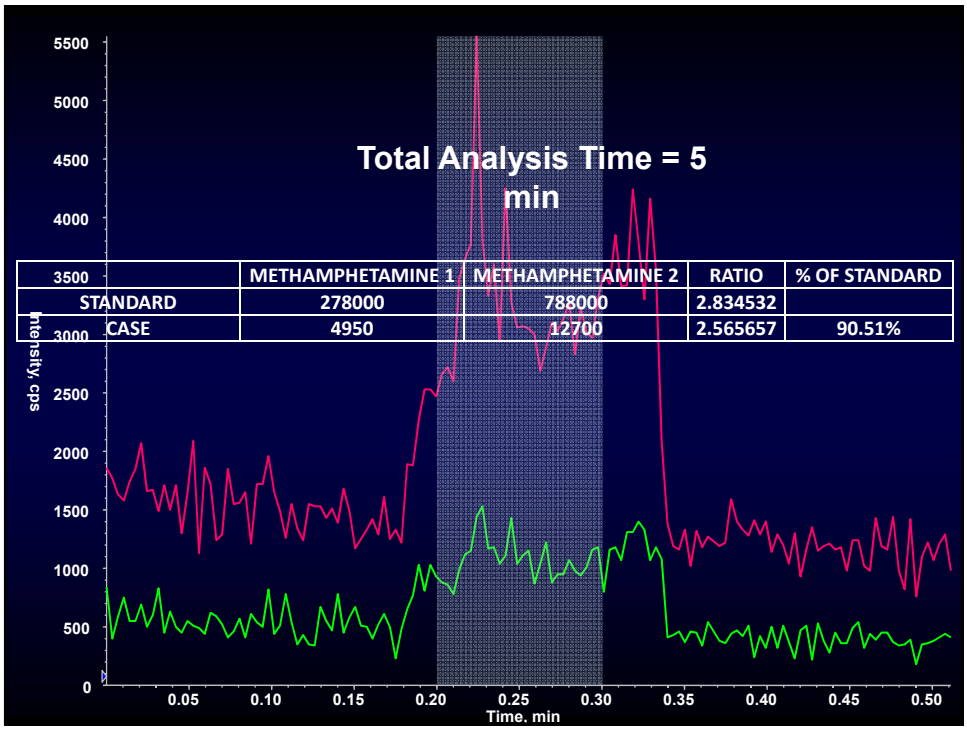
White Powder Analyzed with the AccuTOF-DART MS



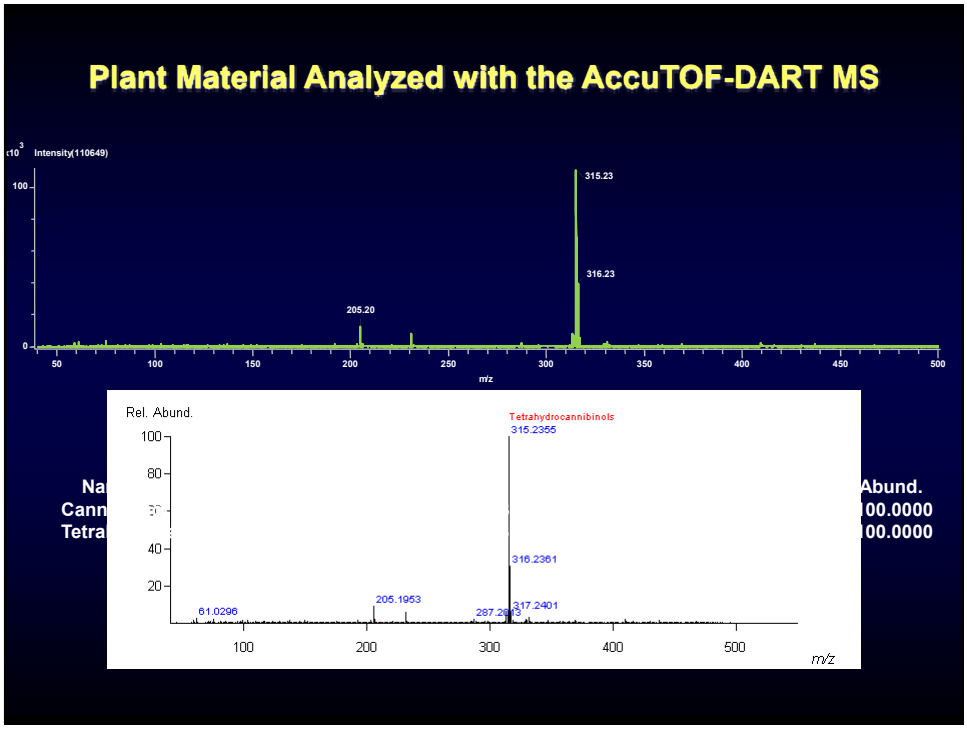


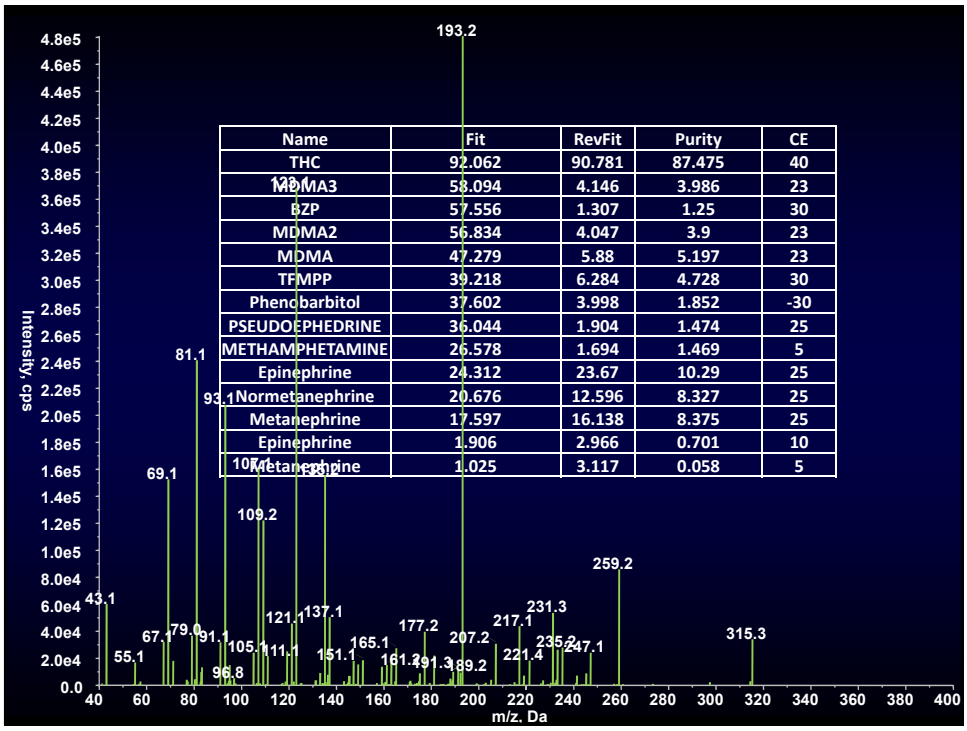


Name	Fit	RevFit	Purity	CE
METHAMPHETAMINE	91.468	96.188	90.584	5
BZP	86.341	85.981	74.237	30
PHENTERMINE	53.926	88.369	53.516	25
METH-TPC	13.573	20.929	13.441	10
METH-TPC2	7.906	11.836	7.83	5
TFMPP	1.732	27.98	1.653	30
Metanephrine	1.544	50.818	1.542	25

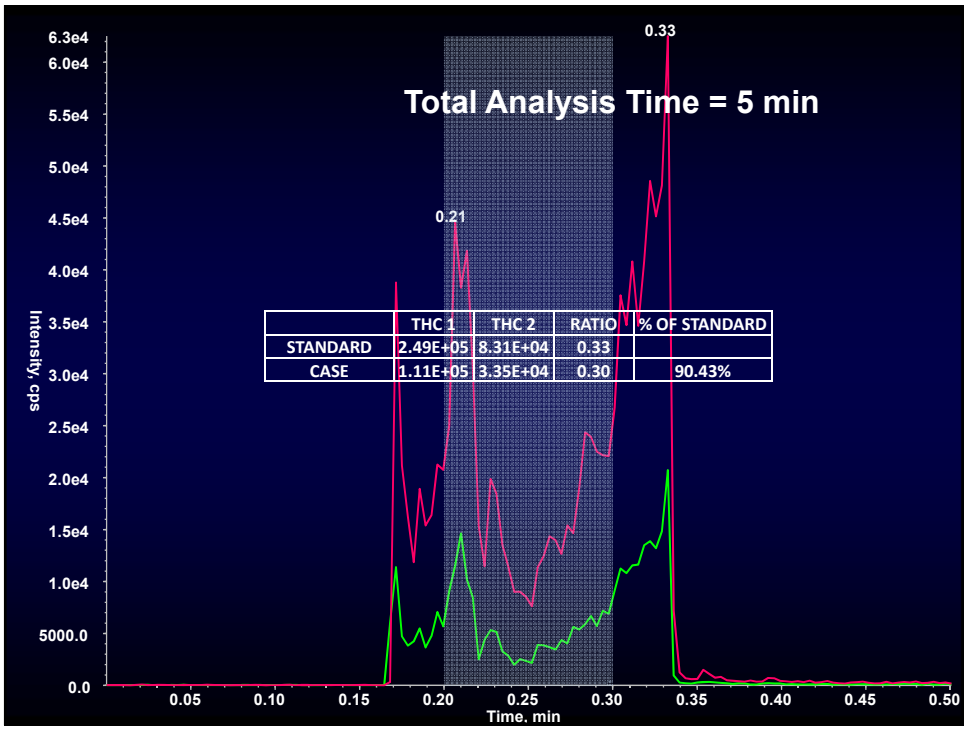


	METHAMPHETAMINE 1	METHAMPHETAMINE 2	RATIO	% OF STANDARD
STANDARD	278000	788000	2.834532	
CASE	4950	12700	2.565657	90.51%

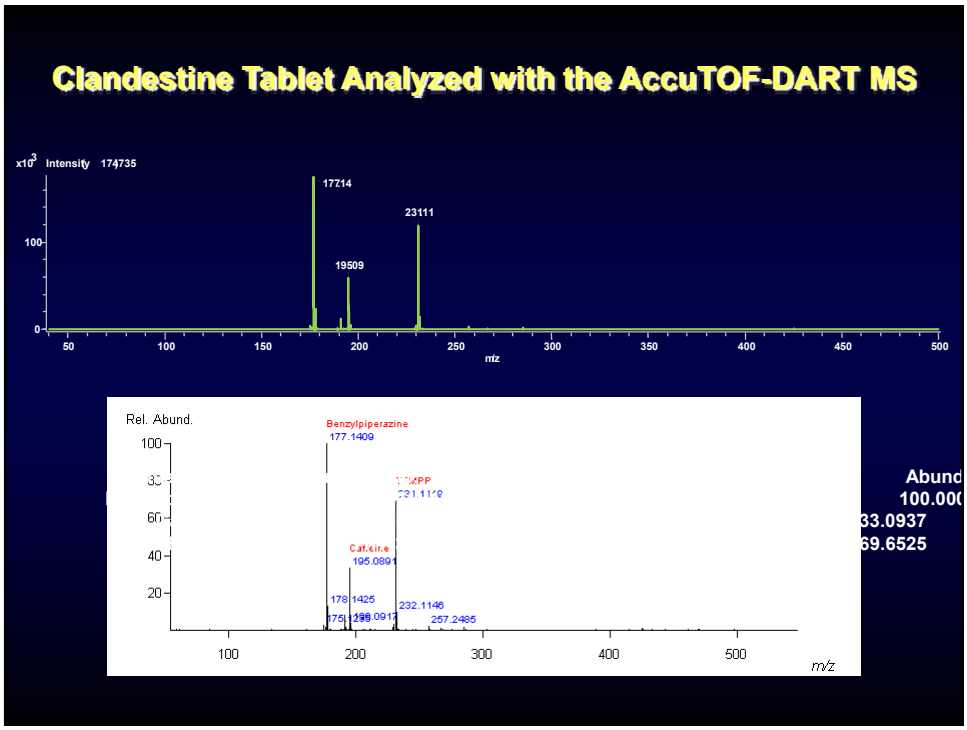


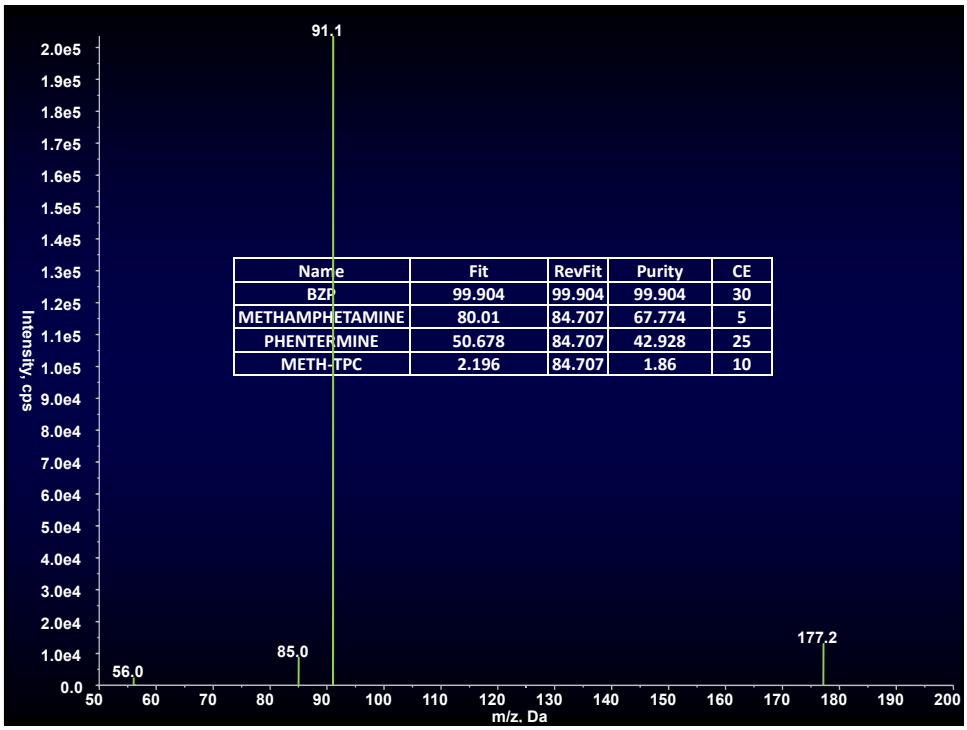


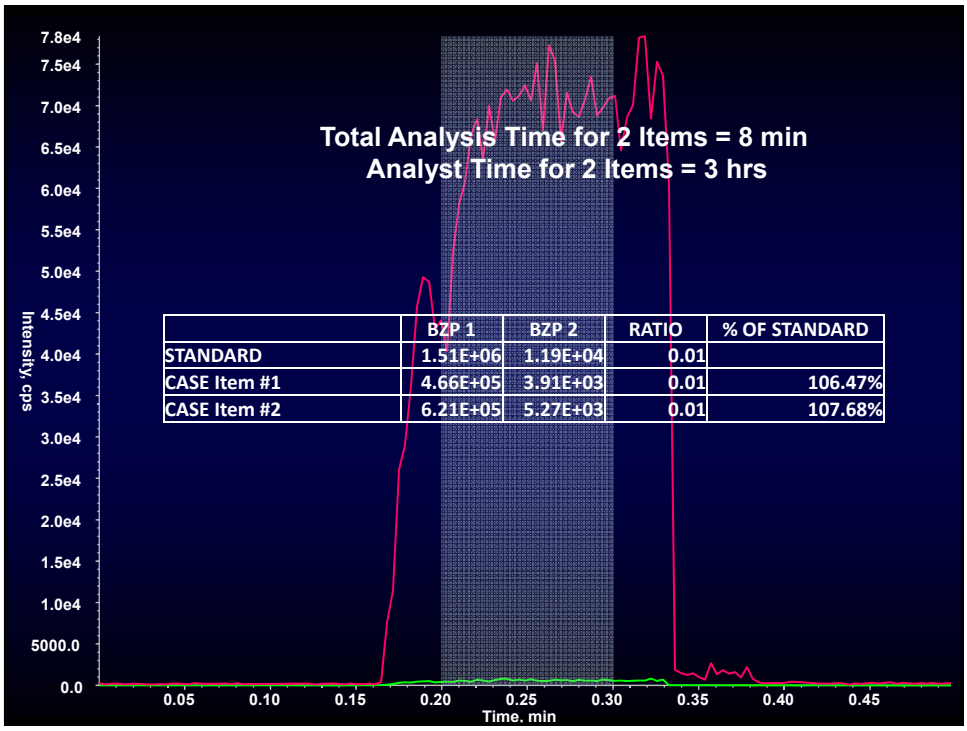
Name	Fit	RevFit	Purity	CE
THC	92.062	90.781	87.475	40
MDMA3	58.094	4.146	3.986	23
BZP	57.556	1.307	1.25	30
MDMA2	56.834	4.047	3.9	23
MDMA	47.279	5.88	5.197	23
TFMPP	39.218	6.284	4.728	30
Phenobarbitol	37.602	3.998	1.852	-30
PSEUDOEPHEDRINE	36.044	1.904	1.474	25
METHAMPHETAMINE	26.578	1.694	1.469	5
Epinephrine	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



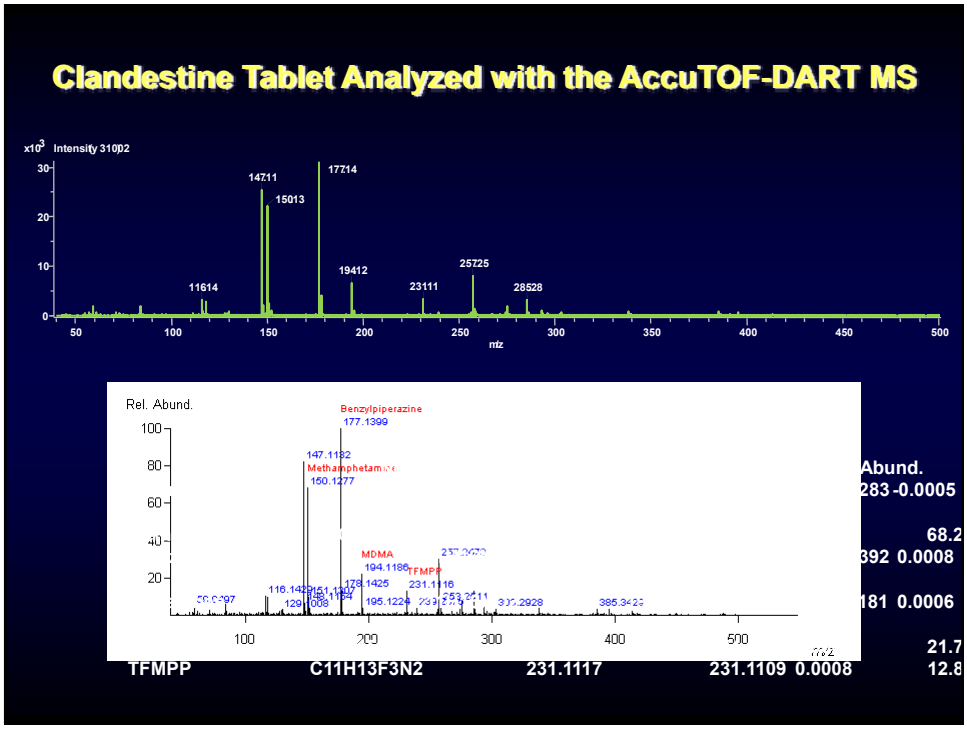
	THC 1	THC 2	RATIO	% OF STANDARD
STANDARD	2.49E+05	8.31E+04	0.33	
CASE	1.11E+05	3.35E+04	0.30	90.43%

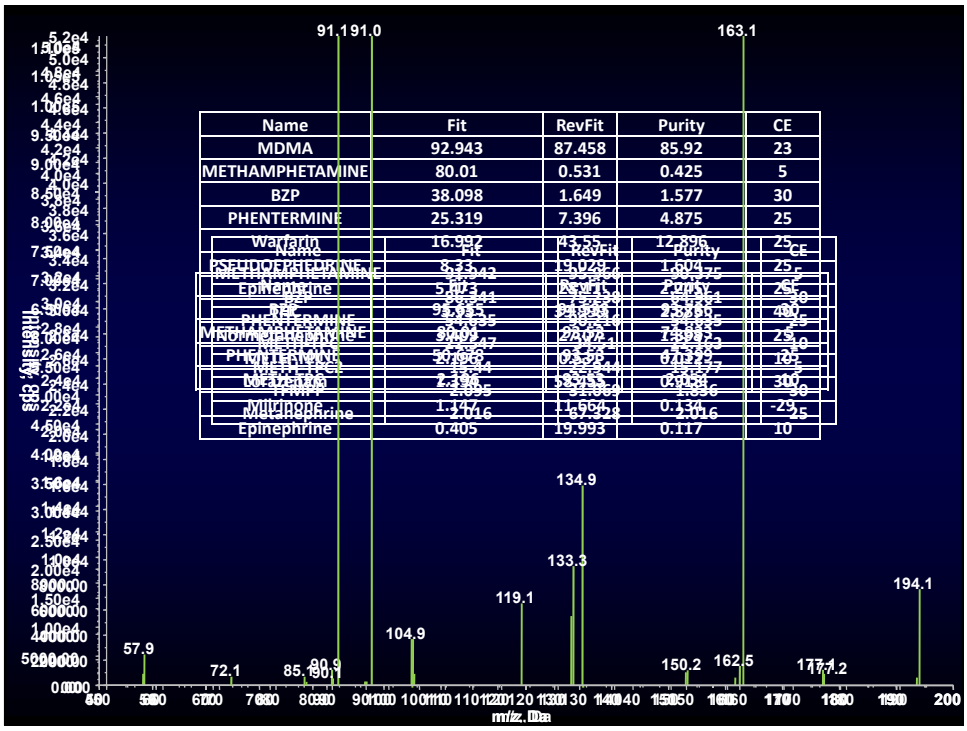

Clandestine Tablet Analyzed with the AccuTOF-DART MS
x10^3 Intensity 174735
17214
23111
19509
mz
Rel. Abund.
Benzylpiperazine
177.1409
Caf.kir.e
195.0891
178.1425
232.1146
257.2485
m/z
Abund
100.000
33.0937
69.6525


91.1
Intensity, cps
m/z, Da
56.0
85.0
177.2
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

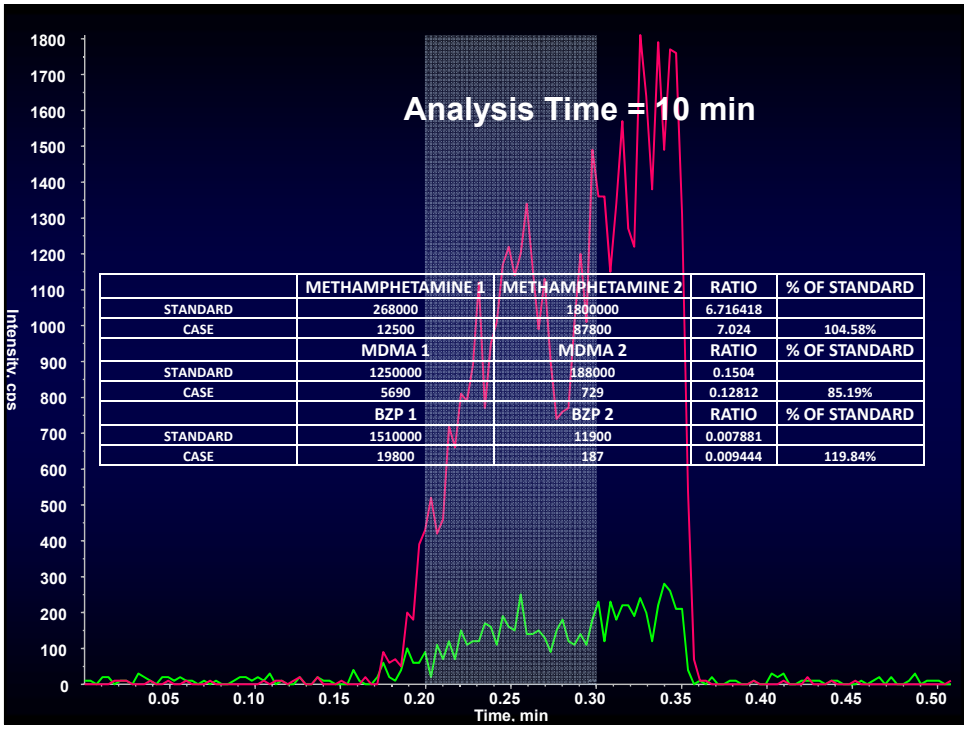


	BZP 1	BZP 2	RATIO	% OF STANDARD
STANDARD	1.51E+06	1.19E+04	0.01	
CASE Item #1	4.66E+05	3.91E+03	0.01	106.47%
CASE Item #2	6.21E+05	5.27E+03	0.01	107.68%

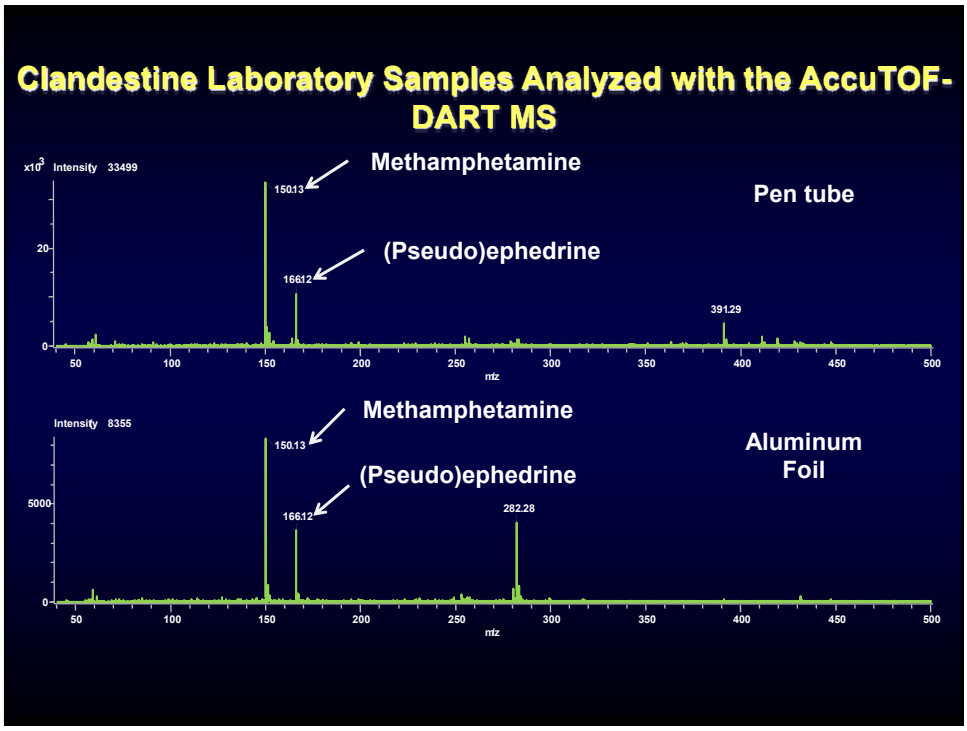


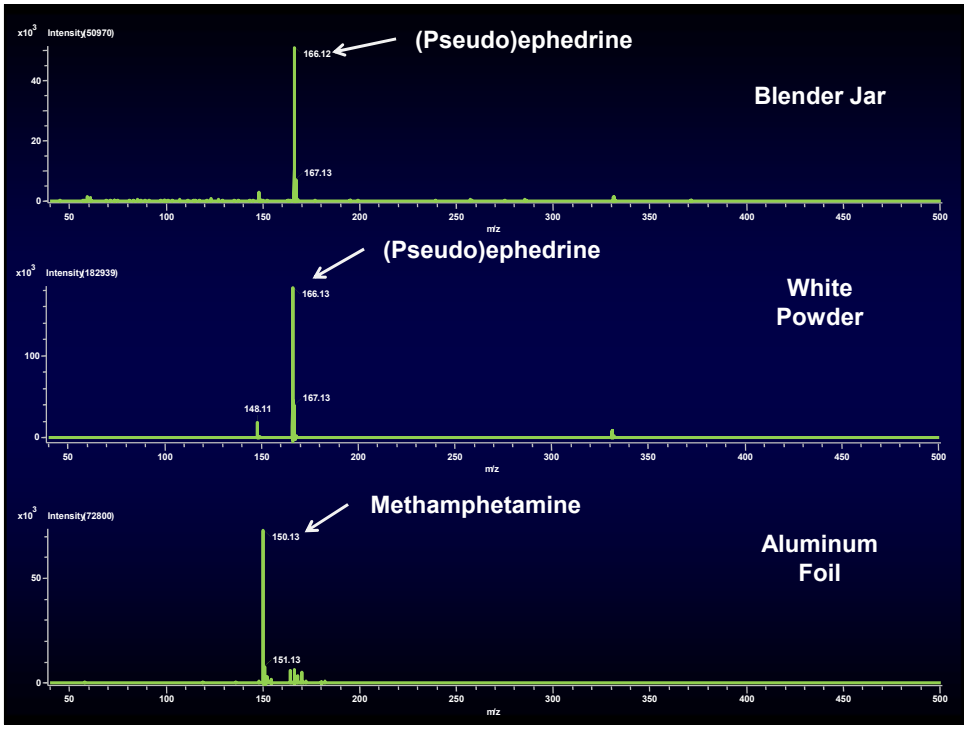


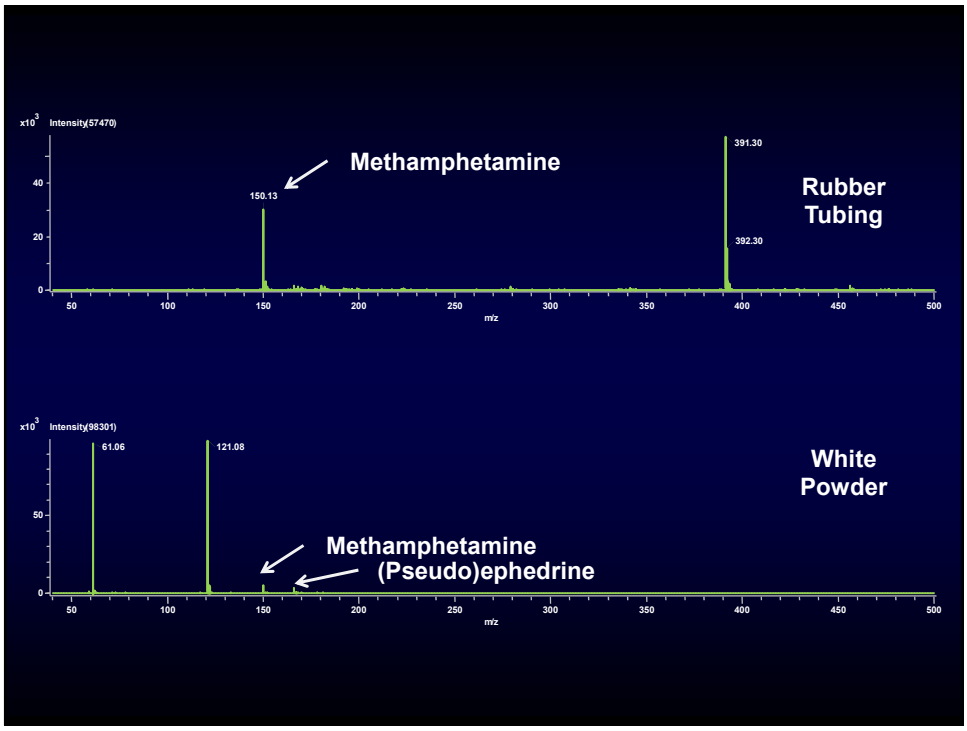
Name	Fit	RevFit	Purity	CE
MDMA	92.943	87.458	85.92	23
METHAMPHETAMINE	80.01	0.531	0.425	5
BZP	38.098	1.649	1.577	30
PHENTERMINE	25.319	7.396	4.875	25
Warfarin	16.997	43.55	12.896	25
Epinephrine	0.405	19.993	0.117	10

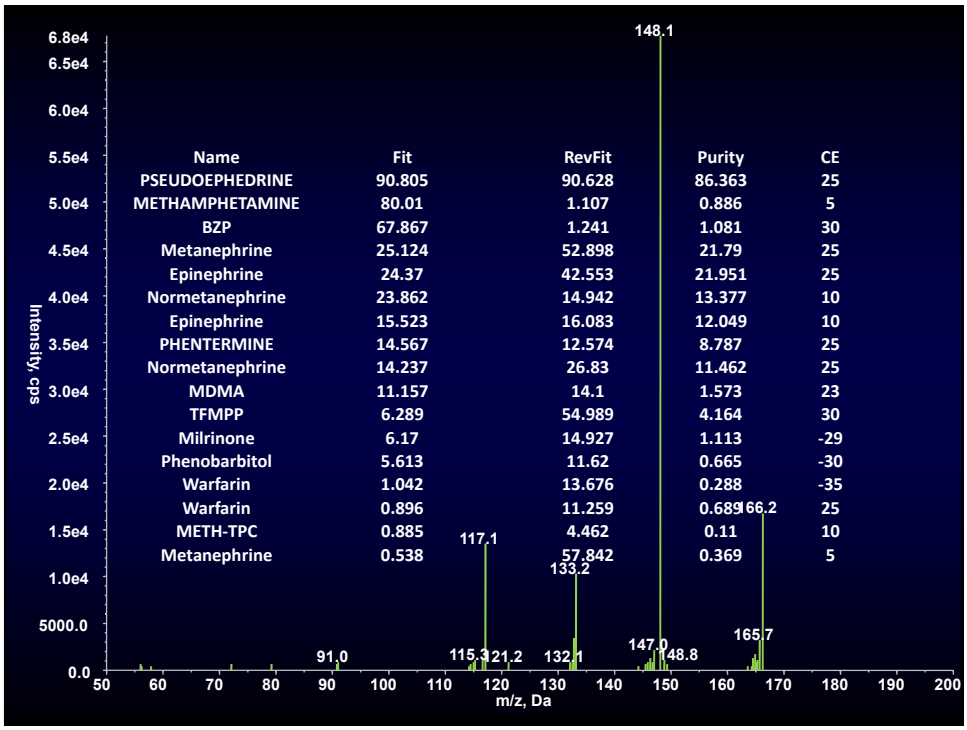


	METHAMPHETAMINE 1	METHAMPHETAMINE 2	RATIO	% OF STANDARD
STANDARD	268000	1800000	6.716418	
CASE	12500	87800	7.024	104.58%
	MDMA 1	MDMA 2	RATIO	% OF STANDARD
STANDARD	1250000	188000	0.1504	
CASE	5690	729	0.12812	85.19%
	BZP 1	BZP 2	RATIO	% OF STANDARD
STANDARD	1510000	11900	0.007881	
CASE	19800	187	0.009444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


Clandestine Laboratory Samples Analyzed with the AccuTOF-DART MS
x10^3 Intensity 33499
Methamphetamine
150.13
Pen tube
(Pseudo)ephedrine
166.12
391.29
m/z
Intensity 8355
Methamphetamine
150.13
Aluminum Foil
(Pseudo)ephedrine
166.12
282.28
m/z


x10^3 Intensity(50970)
(Pseudo)ephedrine
166.12
Blender Jar
167.13
m/z
x10^3 Intensity(182939)
(Pseudo)ephedrine
166.13
White Powder
148.11
167.13
m/z
x10^3 Intensity(72800)
Methamphetamine
150.13
Aluminum Foil
151.13
m/z


x10^3 Intensity(57470)
Methamphetamine
150.13
391.30
392.30
Rubber Tubing
m/z
x10^3 Intensity(98301)
61.06
121.08
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
148.1
166.2
117.1
133.2
165.7
147.0
91.0
115.3
121.2
132.1
148.8
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

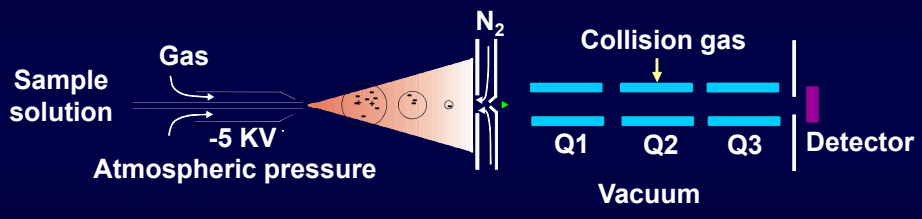
Real Time Sample Analysis with the AccuTOF-DART MS

- 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

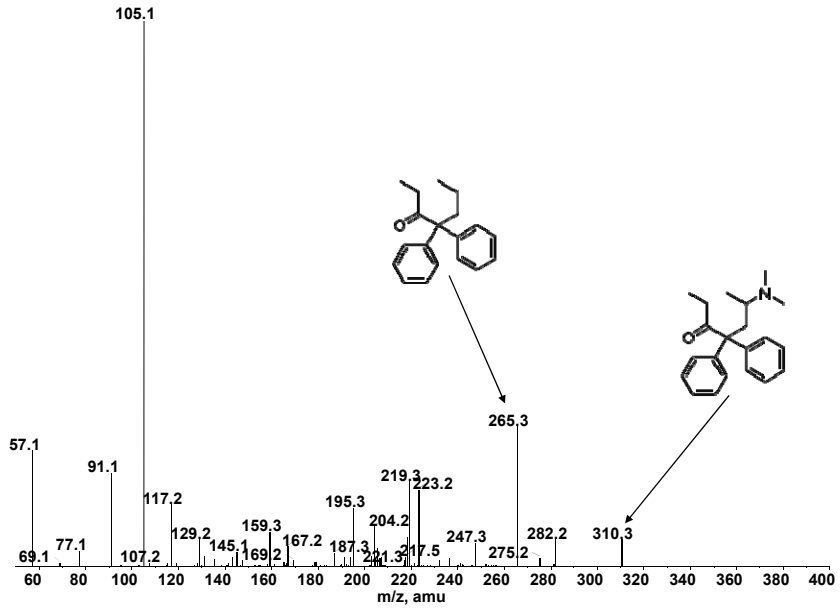
Real Time Sample Analysis with the DART-QTRAP MS

- 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

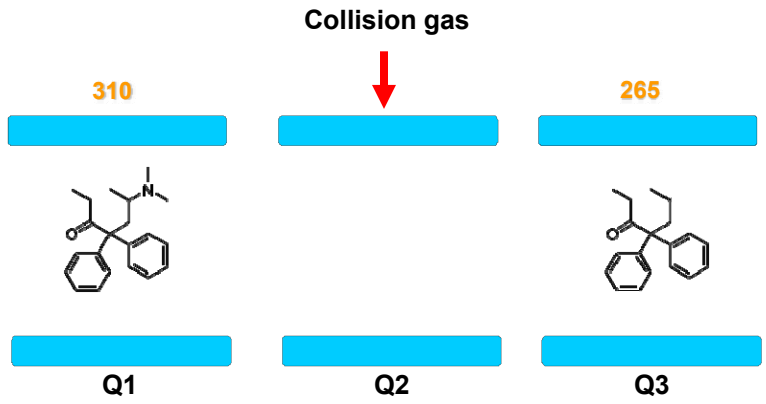
LC-MRM-MS assay for Drug Detection and Quantitation



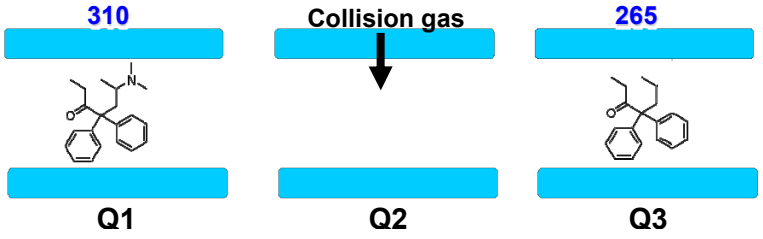
LC-ESI-MS/MS Spectrum of Methadone



LC-MRM-MS analysis for Drug Quantitation

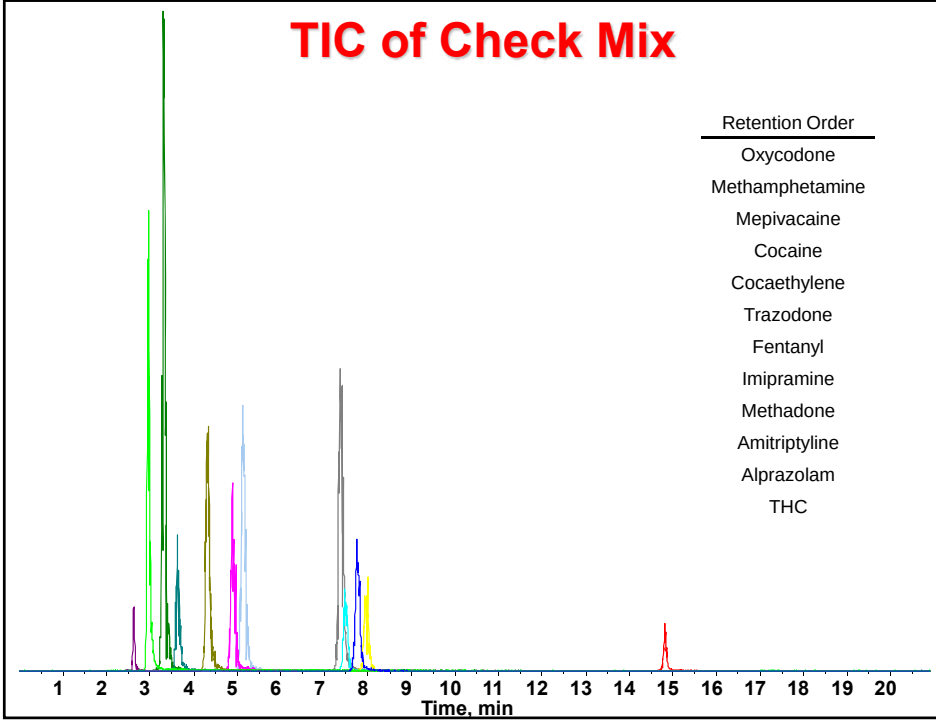


LC-ESI-MRM-MS

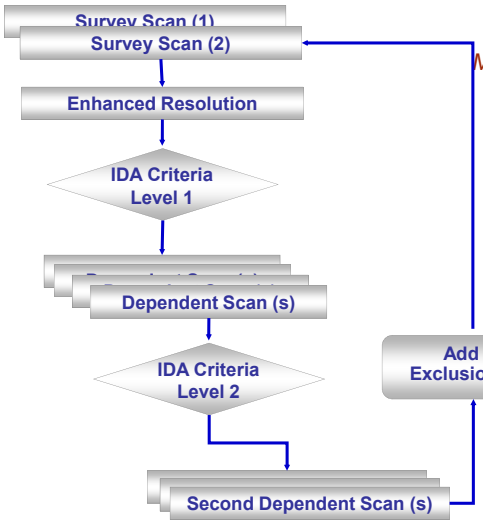


Compound	Molecular Weight	Parent ion	Product ion	Dwell Time (msec)	Declustering Potential (DP)	Collision Energy (CE)	Retention Time	pKa
Alprazolam	308.0829	309.1	205	25	60	50	8.03	2.4
Amitriptyline	277.183	278.2	91	25	45	42	7.8	9.4
Cocaethylene	317.37	318.2	196	25	40	39	4.32	--
Cocaine	303.1471	304.1	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



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

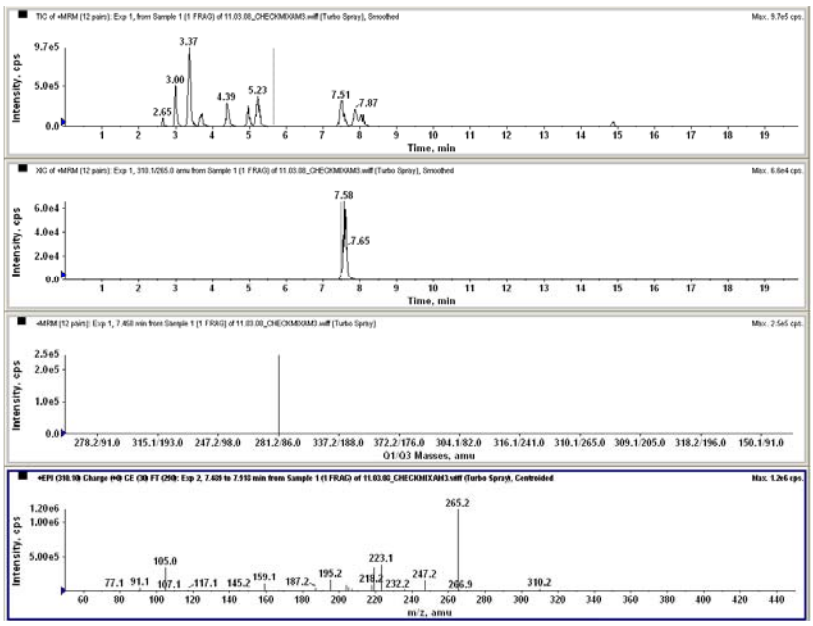
IDA Analysis of Check Mix

TIC

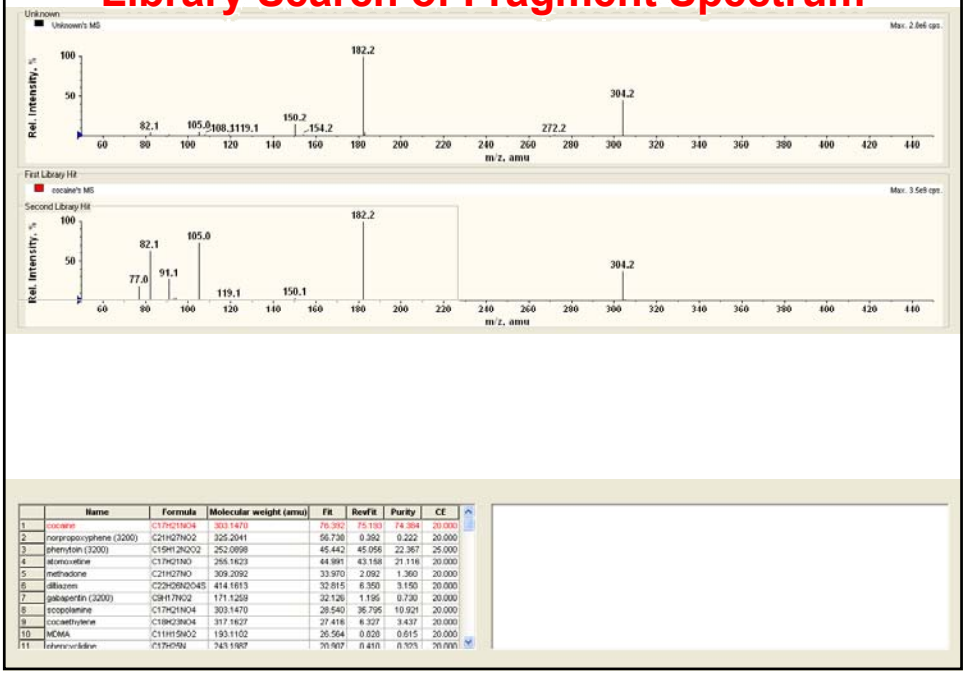
XIC

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	44.891	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	29.540	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	ephenylpridine	C17H19N	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

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