A Workflow for Comprehensive Analysis of Forensic Samples Using Electron and Chemical Ionization High Resolution Time-of-Flight Mass Spectrometry

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Introduction

Background

Controlled substances such as marijuana, cocaine, heroin and methamphetamines are commonly seized by law enforcement officials and routinely analyzed by forensic laboratories. More recently, synthetic drugs such as bath salts and cannabinoids, as well as naturally occurring "herbal remedies" and hallucinogenic mushrooms, have gained popularity as chemical stimulants and opiate substitutes. This study focuses on the comprehensive profiling of complex botanicals obtained from drug seizures.

Botanicals

Bath Salts

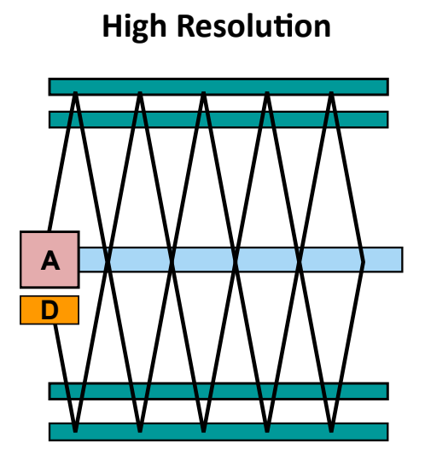
Magic Mushrooms

Analytical Challenges

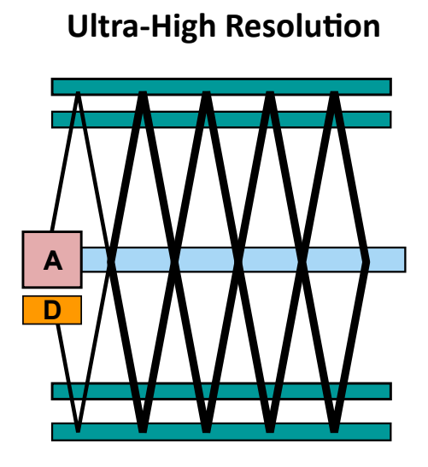
- Chemical Diversity of Compounds
- Novelty of Synthetic Compounds
- Concentration Range of Sample Constituents
- Complexity of Botanical Matrices
- Inappropriate Sample Preparation Methods
- Unsuitable Instrumental Analysis Protocol
- Absence of Library Spectra for Unknowns
- Lack of Standards

The Solution

- ***Tailored Sample Preparation***
- ***GC-HR TOFMS – Pegasus® GC-HRT:***
  - High Quality Spectral Data
    - Comprehensive
    - Search Against Well-Established Databases (e.g., NIST, Wiley)
  - Excellent Mass Accuracy Values (<1 ppm) = Robust Formulas for Fragment, Molecular and Adduct Ions
  - High Resolution Deconvolution™ (HRD™)
  - High Resolving Power (up to 50,000)

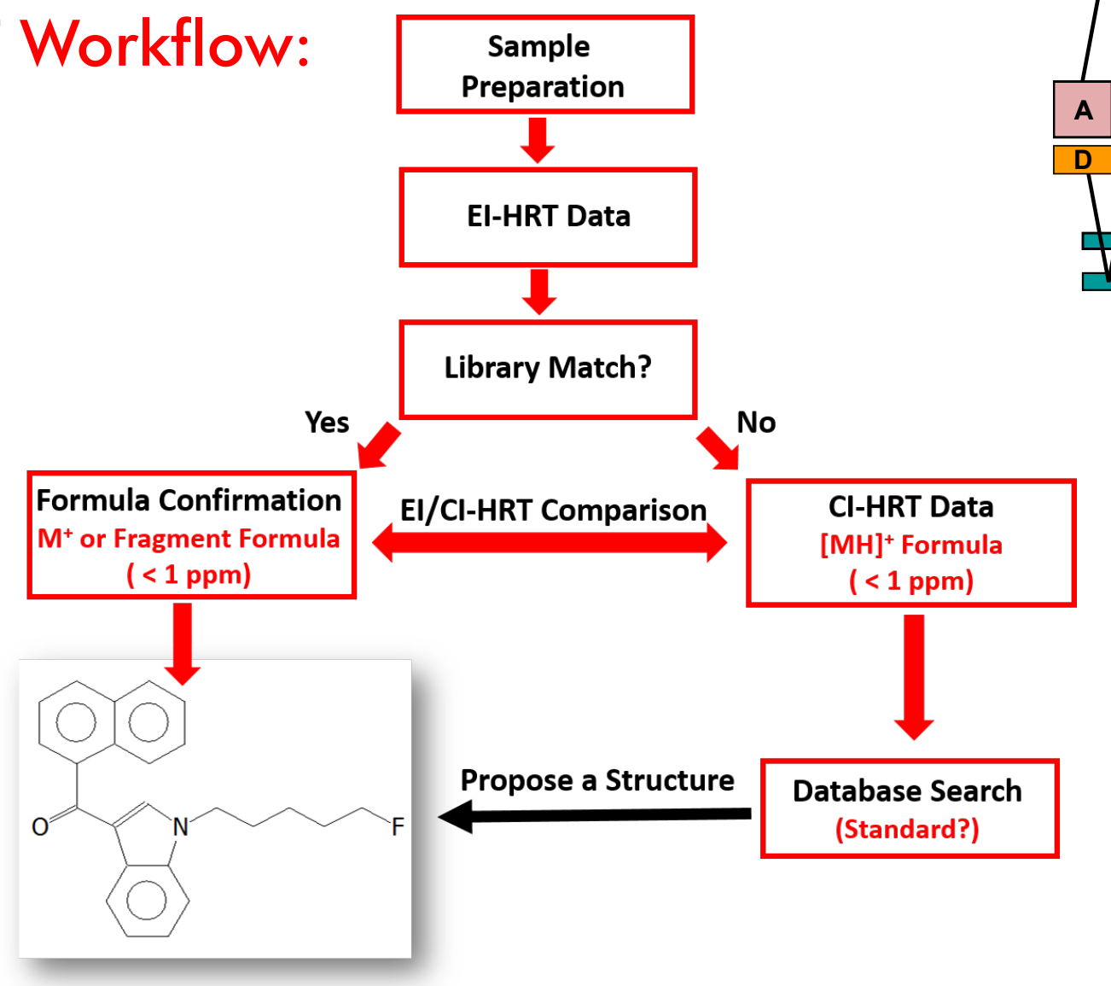


L = 20m
R = 25,000



L = 40m
R = 50,000

EI/CI-HRT Workflow:



Experimental

Sample Preparation Methods

Botanical samples were obtained from a forensic laboratory after they had been analyzed and pertinent cases were completed. General analysis for volatile materials was accomplished through head-space solid phase microextraction (HS-SPME). Solvent extraction followed by derivatization was utilized for comprehensive profiling of samples.

HS-SPME (Volatiles)

Placed 0.05 g of crushed sample (mortar & pestle) in 10 mL headspace vial. Added 0.5 g NaCl and 1 mL of either deionized water or 10% NaOH (aq). Extraction was carried out using a 50/30 mm DVB/CAR/PDMS Stableflex, 24 Ga SPME fiber. The procedure included fiber conditioning (1 hr at 270°C), incubation (3 min at 95°C), extraction (15 min at 95°C, 250 rpm interval agitation (5s on, 2s off)), followed by desorption in the GC-injector (5 min at 250°C).

Solvent Extraction/Derivatization (Profiling)

Samples (0.025 g) were mixed with methanol (2 mL) and sonicated for 25 minutes. The heterogeneous mixture was filtered and dried with N_2 gas. The samples were then treated with MEOX (25 µL, 60°C, 1 hr), MSTFA (50 µL, 60°C, 1 hr) and transferred to 2 mL GC vials for analysis.

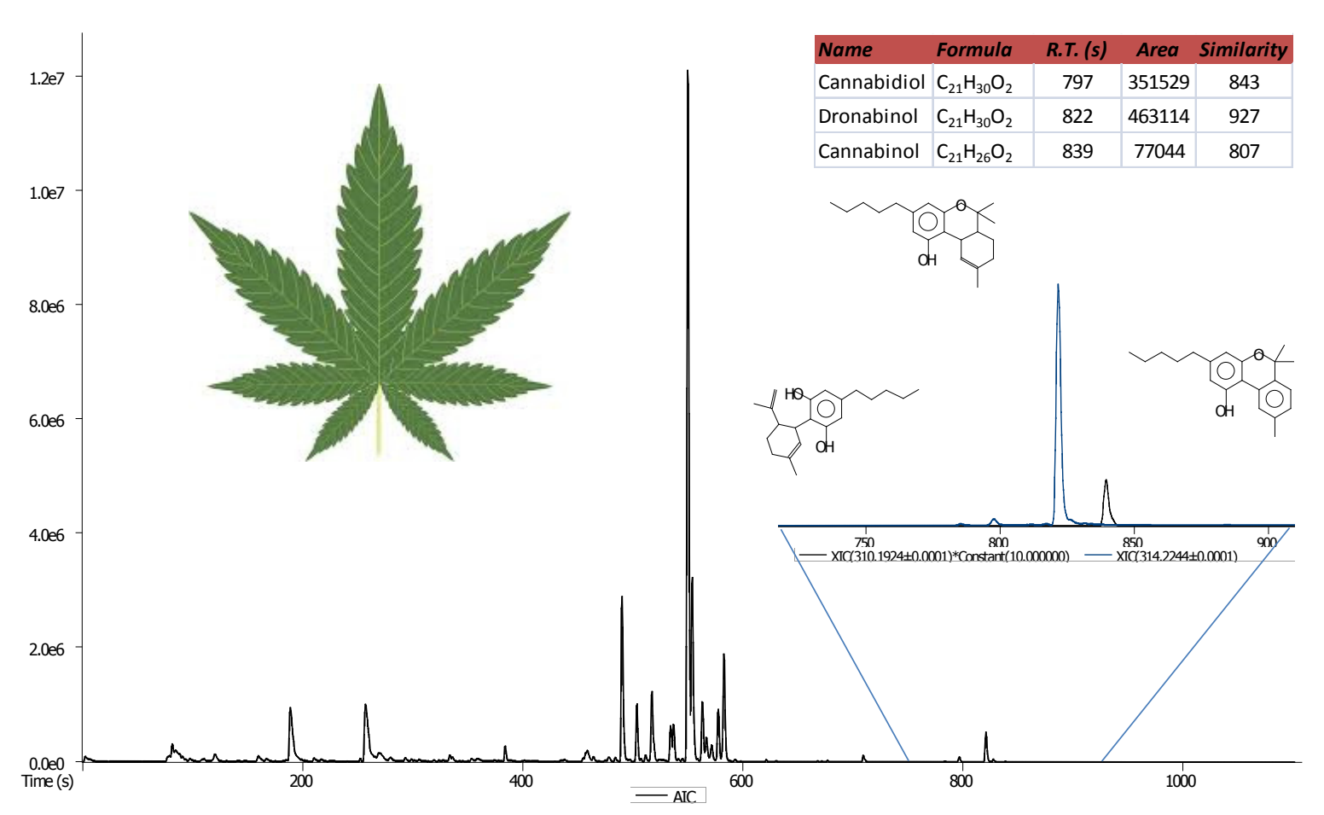
Instrument Parameters

GC	Agilent 7890 with Gerstel MPS Auto Sampler
Column	Restek Rxi-5 MS (30 m x 0.25 mm x 0.25 µm)
Carrier Gas, Flow	He, 1.0 mL/min Constant Flow
Injection	1 µL, Split 5:1, SPME
Inlet Temperature	270°C
Temp. Program	70°C (2 min) to 280°C at 20°C/min (3 min)

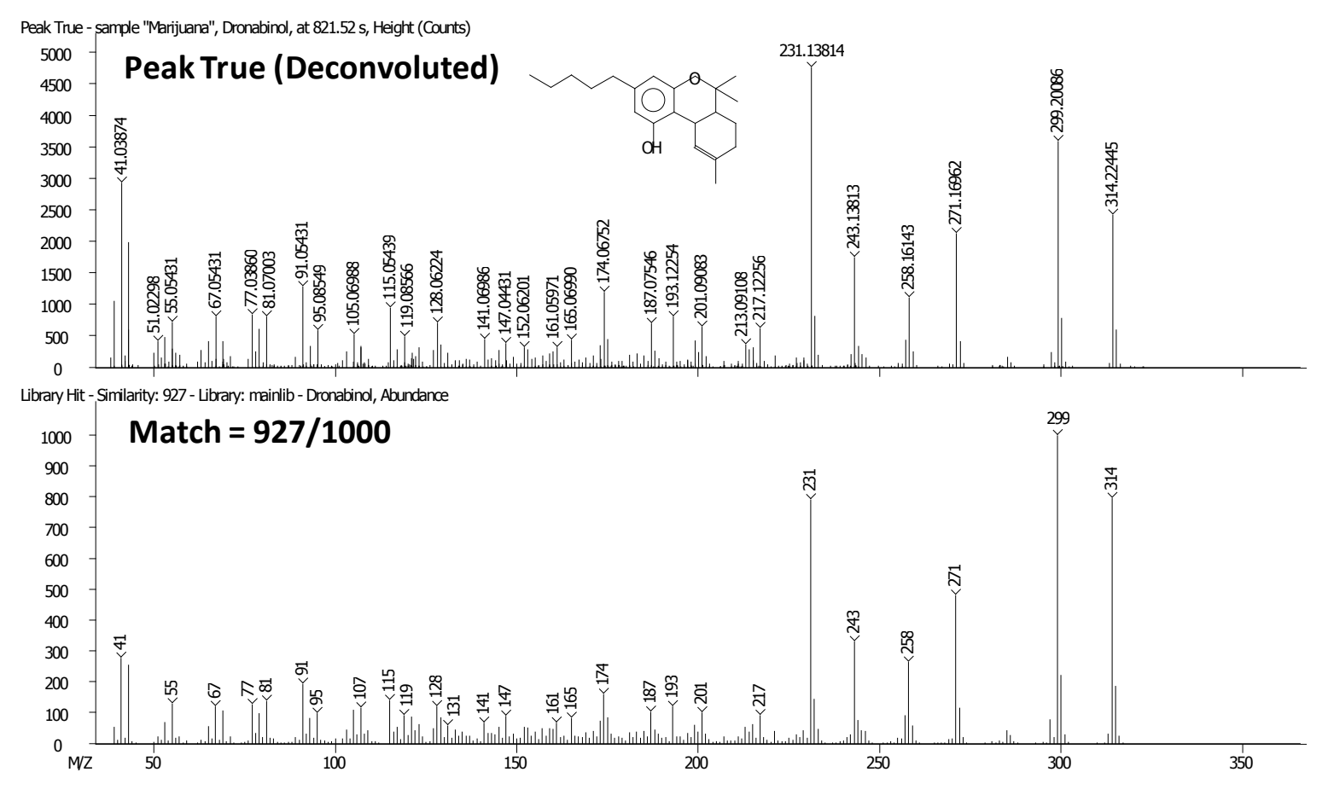
MS	LECO *Pegasus* GC-HRT
Transfer Line Temp.	300°C
Ion Source Temp.	EI 250°C; CI 200°C
Ionization	EI (70 eV); CI (140 eV)
Mass Range	EI 35 to 510; CI 60 to 510
Acquisition Rate	6 spectra/second
Calibration (Internal)	PFTBA
Reagent Gas	5% Ammonia in Methane

HS-SPME Results

A) Marijuana (High Quality Spectral Data)

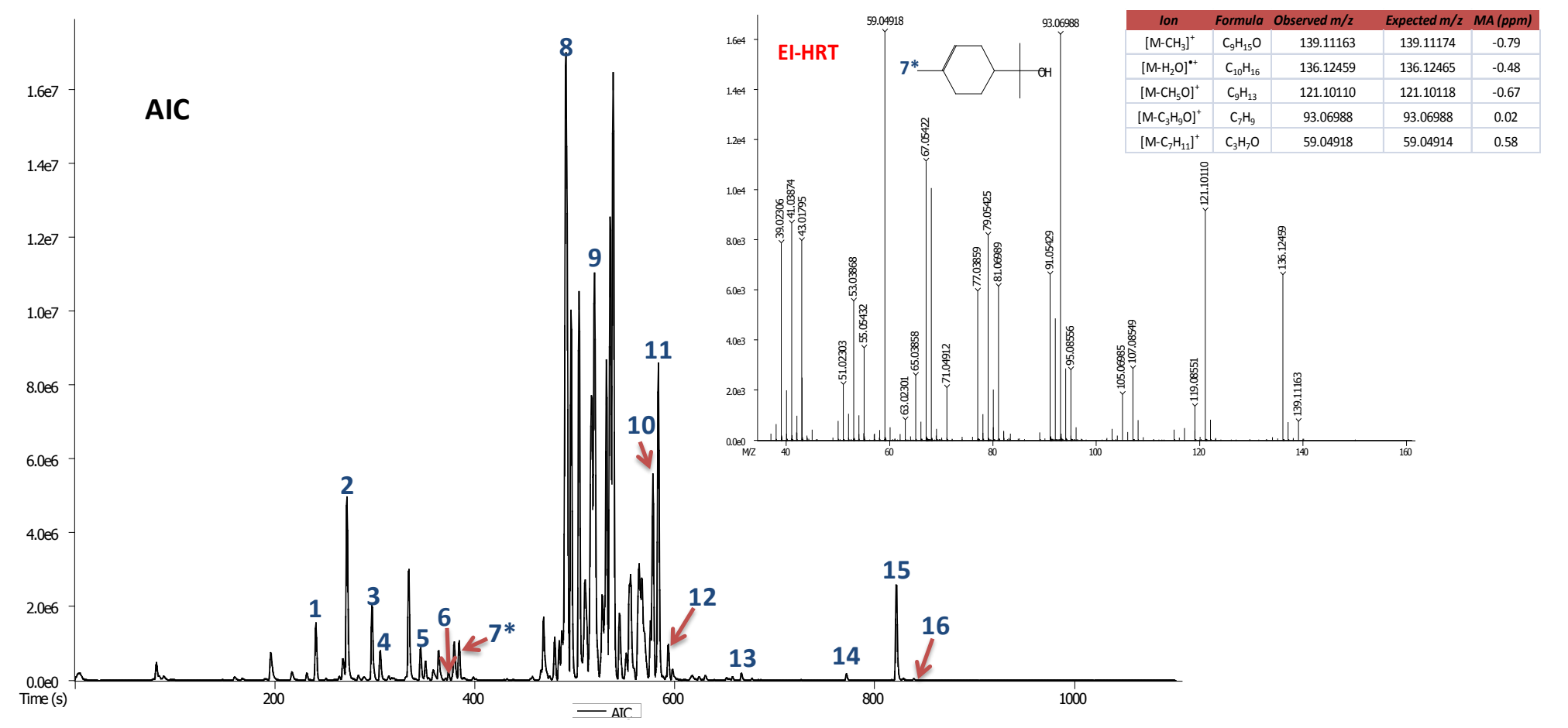


Name	Formula	R.T. (s)	Area	Similarity
Cannabidiol	$C_{21}H_{30}O_2$	797	351529	843
Dronabinol	$C_{21}H_{30}O_2$	822	463114	927
Cannabinol	$C_{21}H_{26}O_2$	839	77044	807



B) Compressed Hash (Excellent Mass Accuracy)

Peak #	Name	Formula	R.T. (s)	Observed m/z	Expected m/z	MA(ppm)
1	α-Pinene	$C_{10}H_{16}$	241	136.12465	136.12465	-0.04
2	β-Myrcene	$C_{10}H_{16}$	272	136.12454	136.12465	-0.85
3	D-Limonene	$C_{10}H_{16}$	297	136.12454	136.12465	-0.79
4	(Z)-Ocimene	$C_{10}H_{16}$	305	136.12462	136.12465	-0.23
5	D-Fenchyl alcohol	$C_{10}H_{18}O$	346	154.13513	154.13522	-0.57
6	Isoborneol	$C_{10}H_{18}O$	374	154.13536	154.13522	0.95
7*	α-Terpineol	$C_{10}H_{18}O$	384	🟩	🟩	🟩
8	Caryophyllene	$C_{15}H_{24}$	491	204.18717	204.18725	-0.42
9	α-selinene	$C_{15}H_{24}$	520	204.18734	204.18725	0.44
10	α-Guaiene	$C_{15}H_{24}$	578	204.18722	204.18725	-0.14
11	Cadalene	$C_{15}H_{18}$	584	198.14058	198.1403	1.38
12	Selina-3,7(11)-diene	$C_{15}H_{24}$	594	204.18723	204.18725	-0.09
13	Cembrene	$C_{20}H_{32}$	667	272.25	272.24985	0.54
14	Tetrahydrocannabivarin	$C_{19}H_{26}O_2$	772	286.19311	286.19273	1.31
15	Dronabinol	$C_{21}H_{30}O_2$	822	314.22423	314.22403	0.64
16	Cannabinol	$C_{21}H_{26}O_2$	839	310.19246	310.19273	-0.88

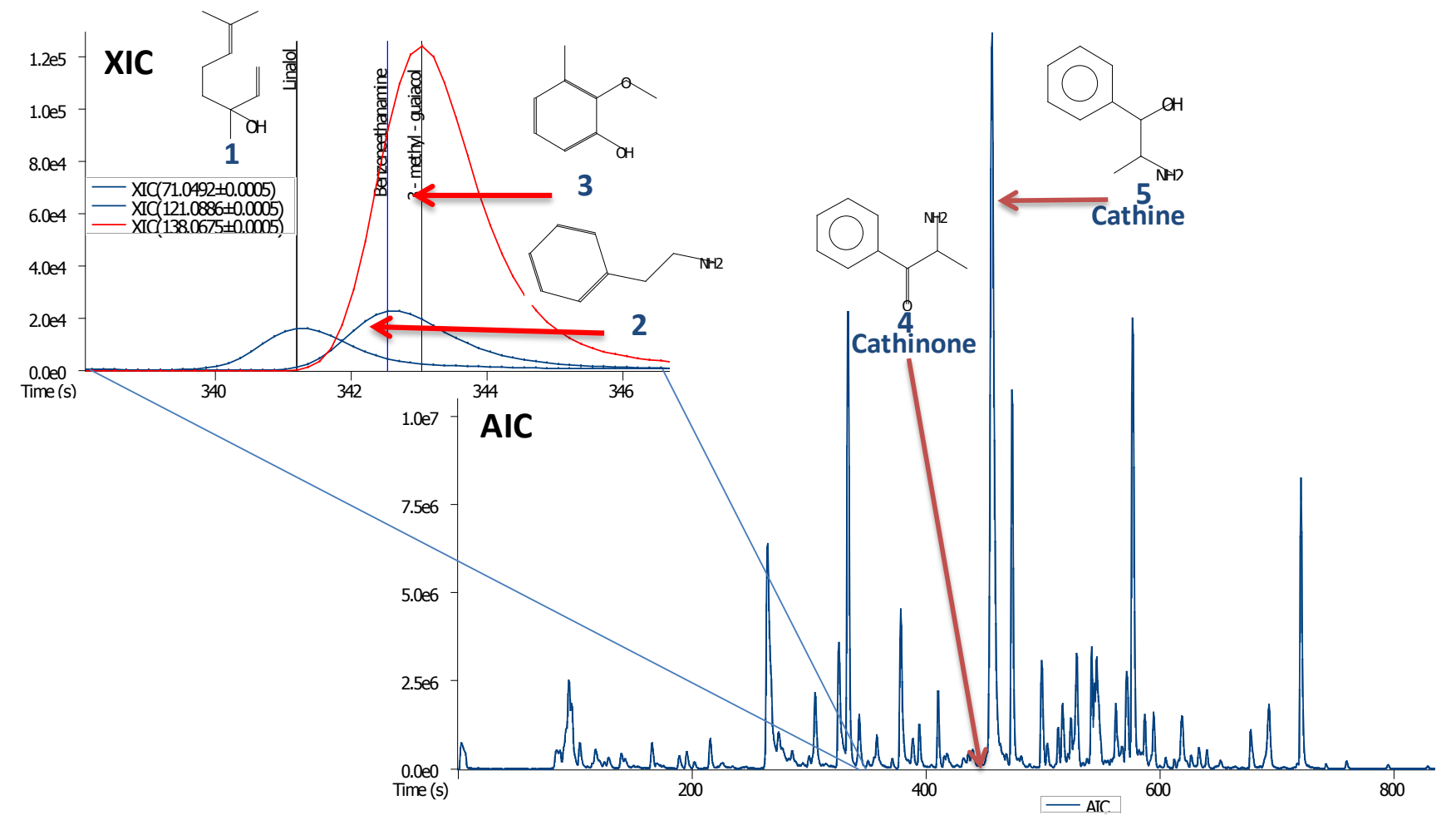


Ion	Formula	Observed m/z	Expected m/z	MA (ppm)
$[M-CH_3]^+$	$C_9H_{15}O$	139.11163	139.11174	-0.79
$[M-H_2O]^{\bullet+}$	$C_{10}H_{16}$	136.12459	136.12465	-0.48
$[M-CH_5O]^+$	C_9H_{13}	121.10110	121.10118	-0.67
$[M-C_3H_9O]^+$	C_7H_9	93.06988	93.06988	0.02
$[M-C_7H_{11}]^+$	C_3H_7O	59.04918	59.04914	0.58

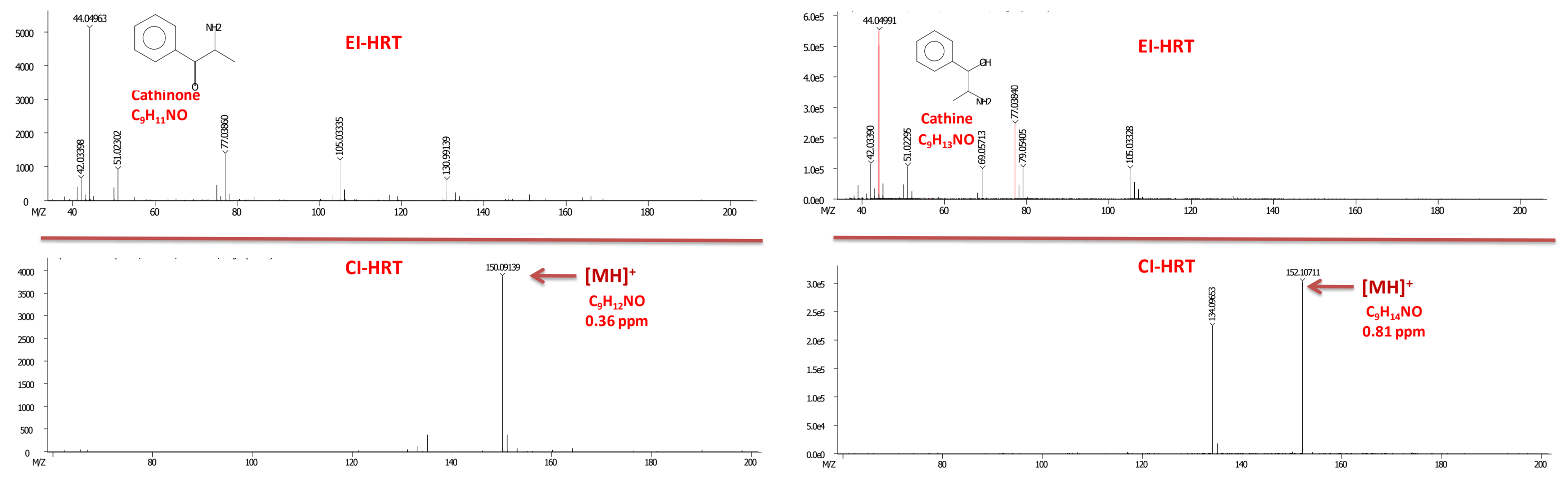
C) Khat *(High Resolution Deconvolution)*

Catha edulis

- Slow growing shrub (warm climates)
- Chewed (fresh) or consumed as tea (dry)
- Wide variety of components: Amines, terpenes sugar alcohols, amino acids, etc.
- Active compounds: Cathinone and Cathine



Peak #	Name	Formula	R.T. (s)	Area	Species	Observed m/z	Expected m/z	MA (ppm)
1	Linalol	$C_{10}H_{18}O$	341	275092	$[M-C_6H_{11}]^+$	71.04915	71.04914	0.13
2	Benzeneethanamine	$C_8H_{11}N$	343	1819808	$M^{\bullet+}$	121.08860	121.08860	0.02
3	3 - methylguaiacol	$C_8H_{10}O_2$	343	3454017	$M^{\bullet+}$	138.06753	138.06753	0.02
4	Cathinone	C9H11NO	448	397722	🟩	🟩	🟩	🟩
5	Cathine	C9H13NO	456	207231670	🟩	🟩	🟩	🟩

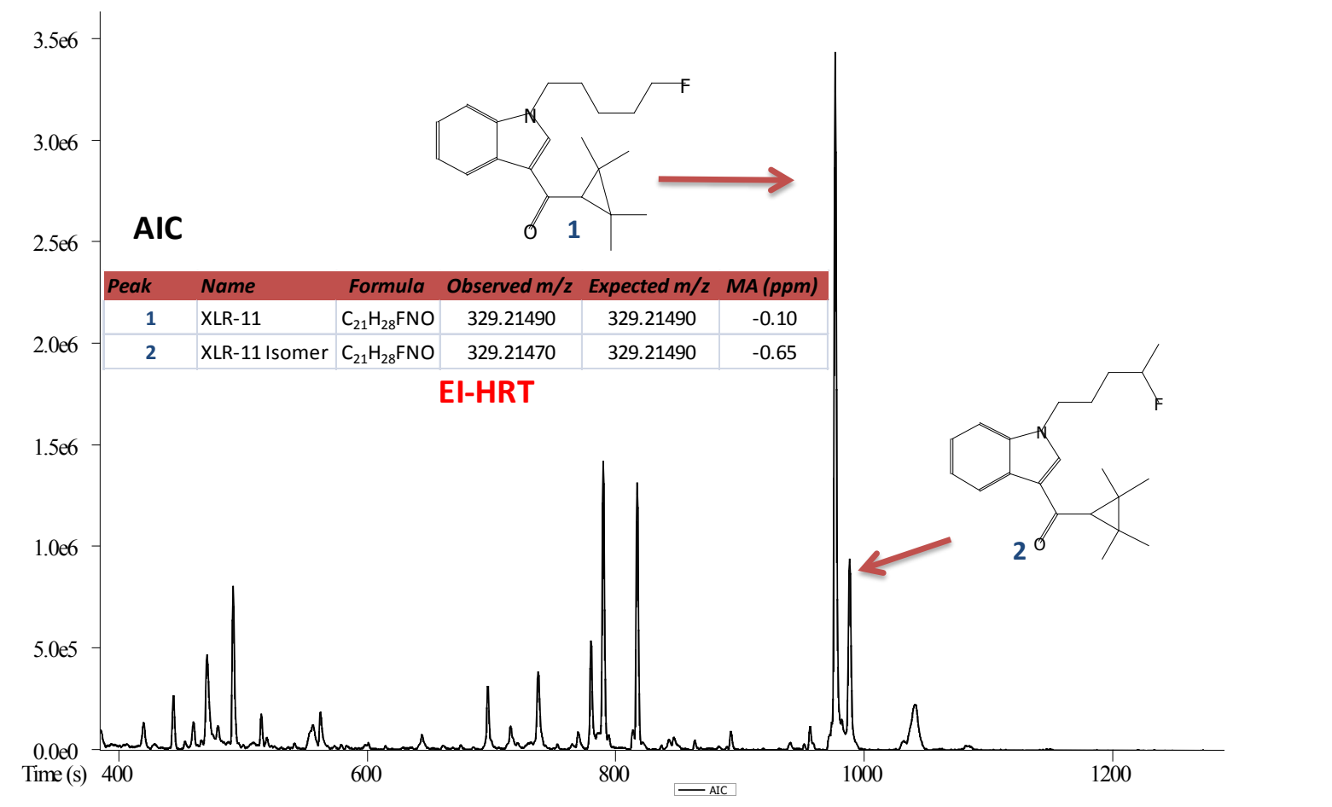


Liquid Extraction/Derivatization Results

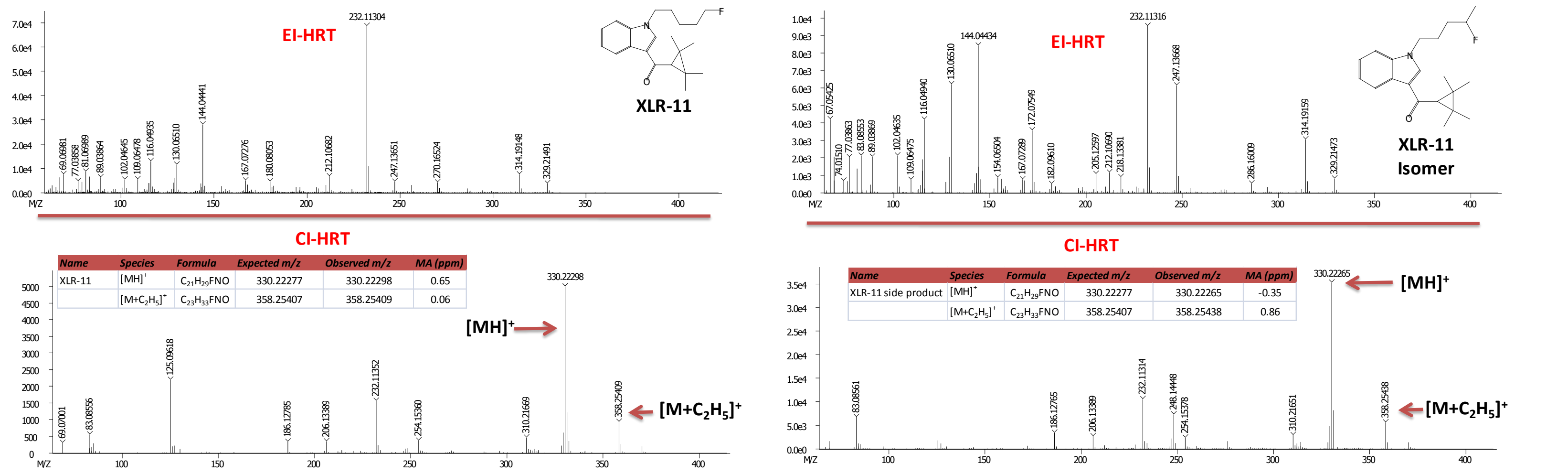
D) Pokeweed (Discovery & Confirmation)

Phytolacca americana

- Perennial herbaceous plant
- Native to North America
- Has been used as medicine and food
- Can be dried & then laced with synthetic drugs



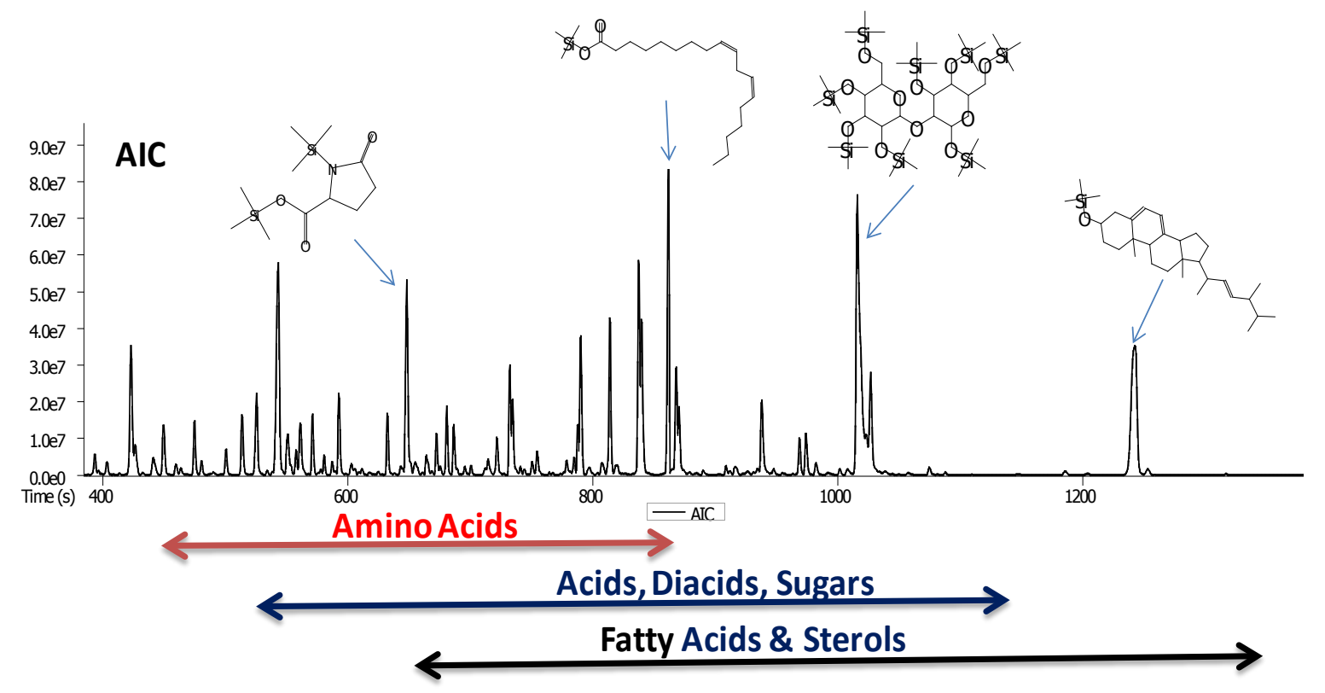
Peak	Name	Formula	Observed m/z	Expected m/z	MA (ppm)
1	XLR-11	$C_{21}H_{28}FNO$	329.21490	329.21490	-0.10
2	XLR-11 Isomer	$C_{21}H_{28}FNO$	329.21470	329.21490	-0.65



Name	Species	Formula	Expected m/z	Observed m/z	MA (ppm)
XLR-11	$[MH]^+$	$C_{21}H_{29}FNO$	330.22277	330.22298	0.65
	$[M+C_2H_5]^+$	$C_{23}H_{33}FNO$	358.25407	358.25409	0.06

Name	Species	Formula	Expected m/z	Observed m/z	MA (ppm)
XLR-11 side product	$[MH]^+$	$C_{21}H_{29}FNO$	330.22277	330.22265	-0.35
	$[M+C_2H_5]^+$	$C_{23}H_{33}FNO$	358.25407	358.25438	0.86

E) Magic Mushrooms (Comprehensive Profiling)



Amino Acids

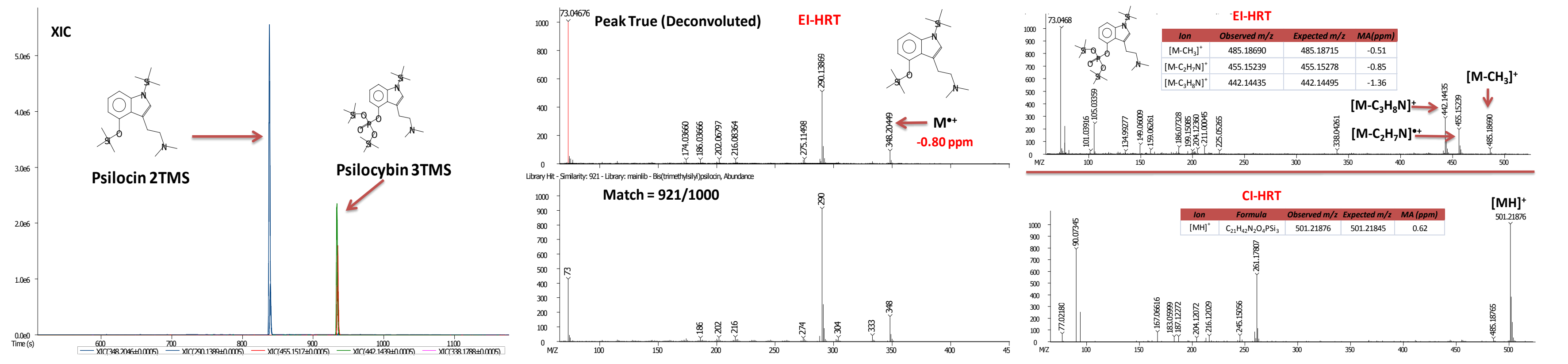
Name	R.T. (s)	Area	Similarity
Alanine 2TMS	449	43996321	815
Valine 2TMS	514	48268049	896
Leucine 2TMS	541	18578254	847
Isoleucine 2TMS	551	19852690	916
Serine 3TMS	581	14573232	880
Threonine 3TMS	593	55414717	925
Aspartic acid 3TMS	645	3185848	736
Proline 2TMS	648	1.8E+08	927
Glutamic acid 3TMS	681	31922702	915
Phenylalanine 2TMS	687	33922597	914
Glutamine 4TMS	722	26862656	872
Glutamine 3TMS	735	45836513	882
Tyrosine 3TMS	788	39598092	892
Tryptophan 3TMS	870	20651921	889

Acids, Diacids, Sugars

Name	R.T. (s)	Area	Similarity
Benzoic acid TMS	528	1248494	736
Succinic acid 2TMS	558	16781610	746
Glyceric acid 3TMS	568	1657183	732
Malic acid 3TMS	632	32993298	863
α-Hydroxyglutaric acid 3TMS	664	19774037	796
Citric acid 4TMS	750	10831424	727
Glucitol 6TMS	790	97770051	881
Pantothenic acid 3TMS	804	1706270	707
Myo-Inositol 6TMS	838	1.08E+08	920
Galactose MEOX 5TMS	874	7974144	747
Dehydroabietic acid TMS	915	571487	737
Mannobiose 8TMS	1016	2.43E+08	776
Octacosanol TMS	1148	1341165	850

Fatty Acids & Sterols

Name	R.T. (s)	Area	Similarity
Dodecanoic acid TMS	690	1452344	758
Tetradecanoic acid TMS	755	14043258	832
palmitoleic acid TMS	807	7474583	823
Hexadecanoic acid TMS	814	90087936	867
Heptadecanoic acid TMS	841	3011456	766
Octadecadienoic acid TMS	862	1.1E+08	863
Octadecanoic acid TMS	868	45409877	872
Eicosanoic acid TMS	917	1378267	719
1-Monopalmitin 2TMS	955	1583360	802
Docosanoic acid TMS	966	1118307	764
2-Monolinoleoylglycerol 2TMS	1002	3786880	780
Ergosterol TMS	1243	53461688	908
Stigmasterol TMS	1253	3338790	692
Sitosterol TMS	1317	1037436	754



Ion	Observed m/z	Expected m/z	MA(ppm)
$[M-CH_3]^+$	485.18690	485.18715	-0.51
$[M-C_2H_7N]^+$	455.15239	455.15278	-0.85
$[M-C_3H_8N]^+$	442.14435	442.14495	-1.36

Ion	Formula	Observed m/z	Expected m/z	MA (ppm)
$[MH]^+$	$C_{21}H_{42}N_2O_4PSi_3$	501.21876	501.21845	0.62

Summary

- EI/CI workflow facilitates confident compound identification
- Proper sample preparation is critical for the analysis of complex samples
- GC-HRT provides high quality, accurate mass data for:
  - Spectral similarity searches (NIST, Wiley, etc.)
  - Formula determination (fragment, molecular, and adduct ions)