Analysis of Synthetic Drugs Using Electron and Chemical Ionization High Resolution Time-of-Flight Mass Spectrometry

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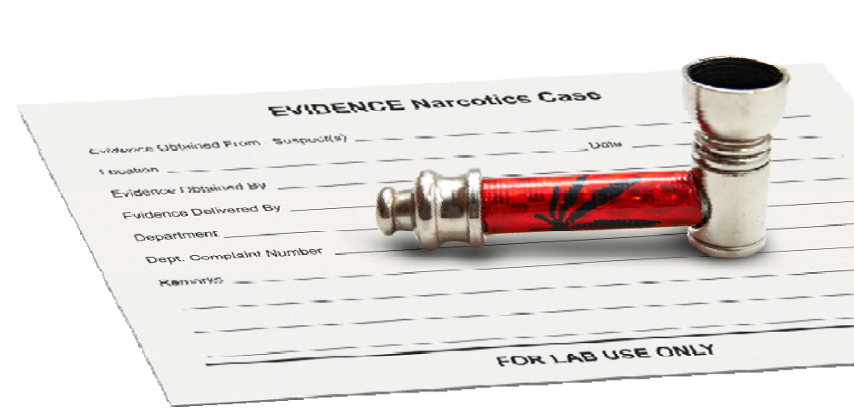


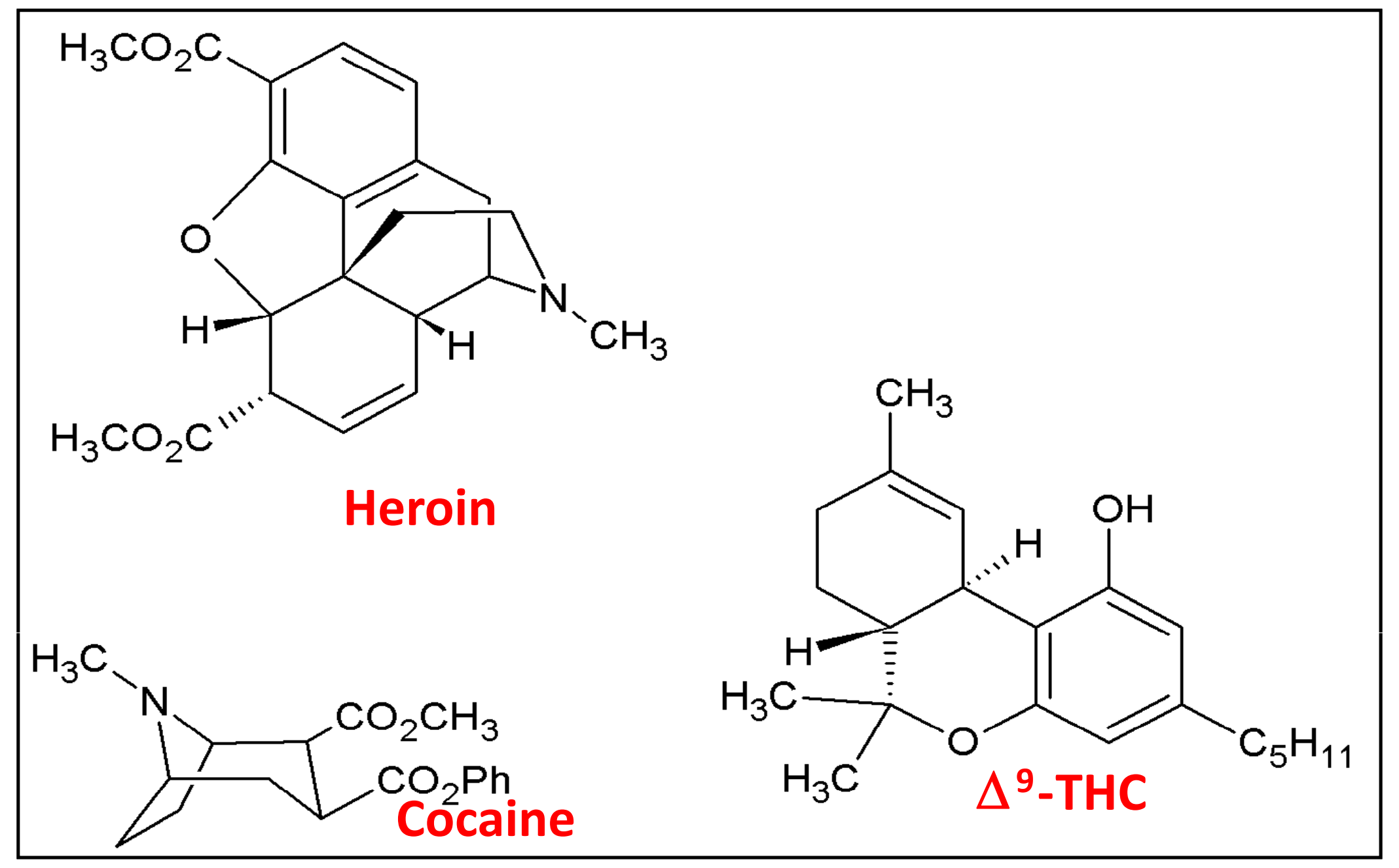
Introduction

The development of unregulated recreational drugs has been increasing at an alarming rate since the mid-2000s. Relatively simple organic transformations produce novel and licit psychotics that can elude detection by standard analytical methods (e.g., GC-MS). This is a major problem for law enforcement agencies, forensic laboratories, and the medical community since the legal status, physiological effects, and long-term toxicity of these synthetic drugs are unclear. Detection and characterization of these substances through routine forensic analyses is challenging due to their novelty and lack of appropriate instrumental methods. Matters are complicated further by the wide range of active ingredients and variety of botanical blends in samples which can be difficult to handle instrumentally.

High performance time-of-flight mass spectrometry (TOFMS) is a practical choice for the analysis of these substances:

- Data acquisition is comprehensive and robust
- Single acquisition, high-resolution accurate mass data can be probed multiple times for traditional and emerging drugs
- High resolving power minimizes background interferences
- Excellent mass accuracy values facilitate confident elemental formula determination for compound characterization
- Complete and high-quality spectral data can be searched against databases

Traditional



Synthetic

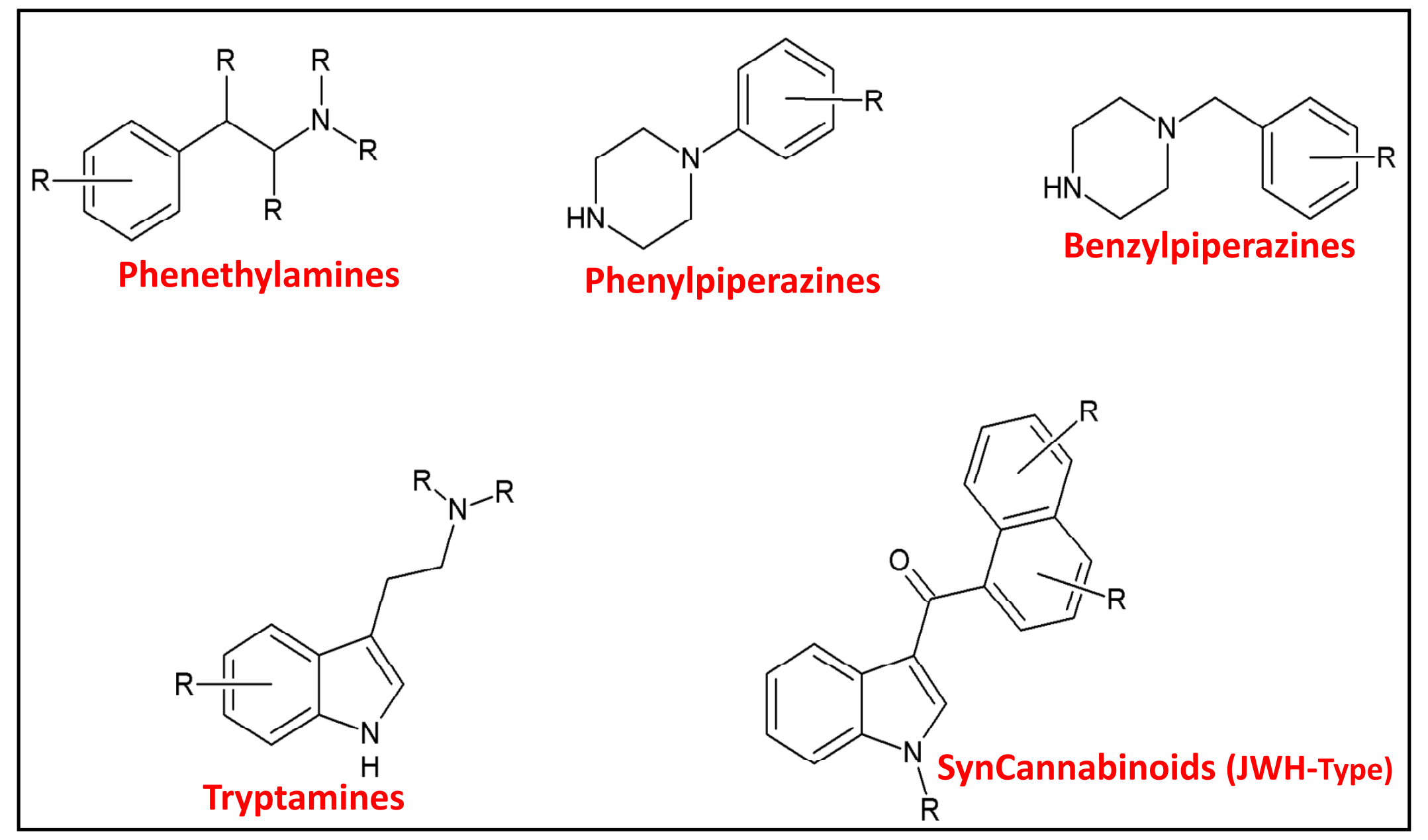
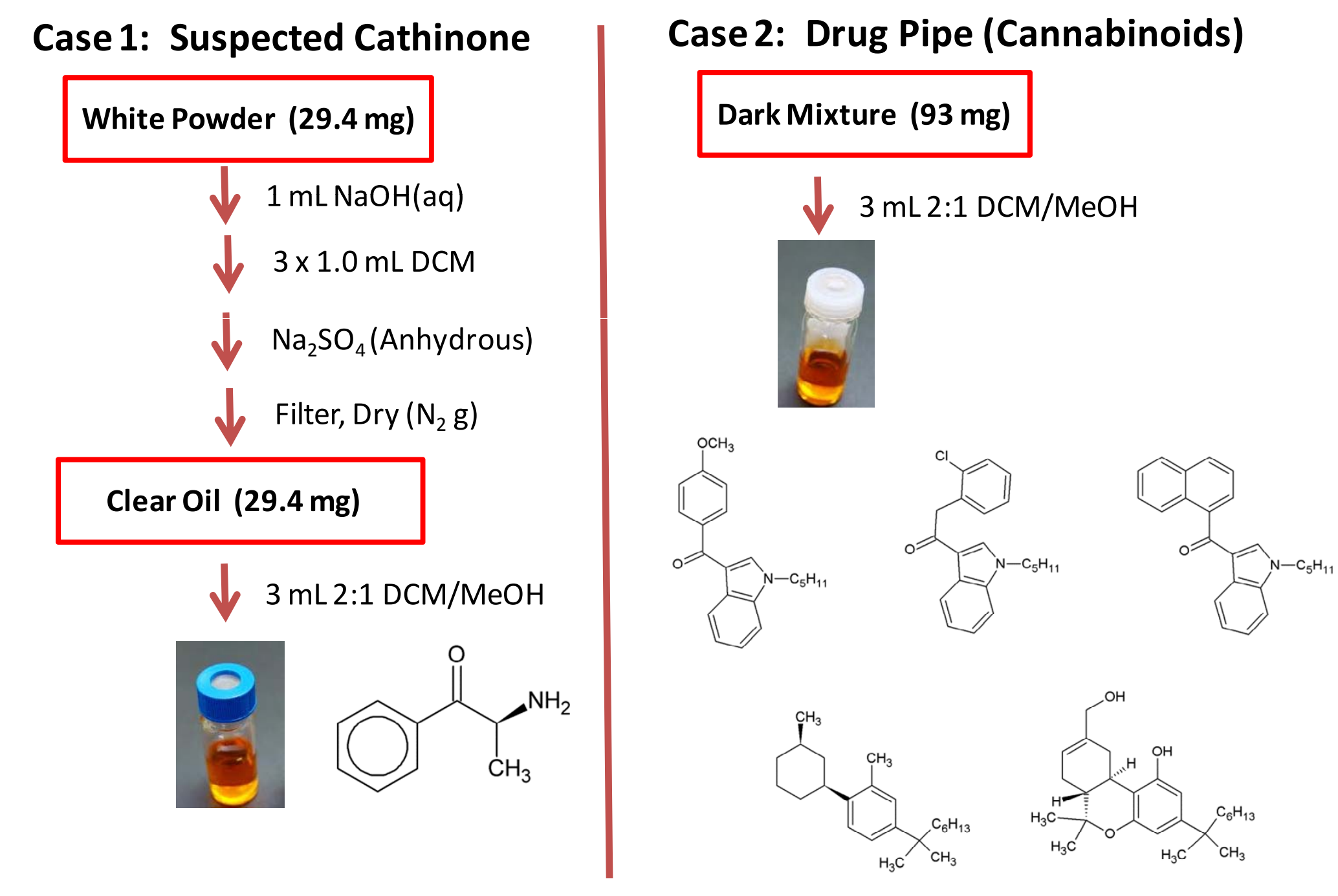


Figure 1: Traditional and Synthetic Drug Examples

In this study, two samples (Cases 1 and 2) obtained from a collaborating forensics laboratory were analyzed and found to contain traditional and new psychotropic substances. The combination of electron impact and chemical ionization high resolution TOFMS was vital for unambiguous identification of new drugs in these mixtures.

Experimental

Sample Preparation



Case 1: Suspected Cathinone

White Powder (29.4 mg)
1 mL NaOH(aq)
3 x 1.0 mL DCM
Na_2SO_4 (Anhydrous)
Filter, Dry (N_2 g)
Clear Oil (29.4 mg)
3 mL 2:1 DCM/MeOH

Case 2: Drug Pipe (Cannabinoids)

Dark Mixture (93 mg)
3 mL 2:1 DCM/MeOH

Figure 2. Sample Preparation Procedures

Instrument Parameters

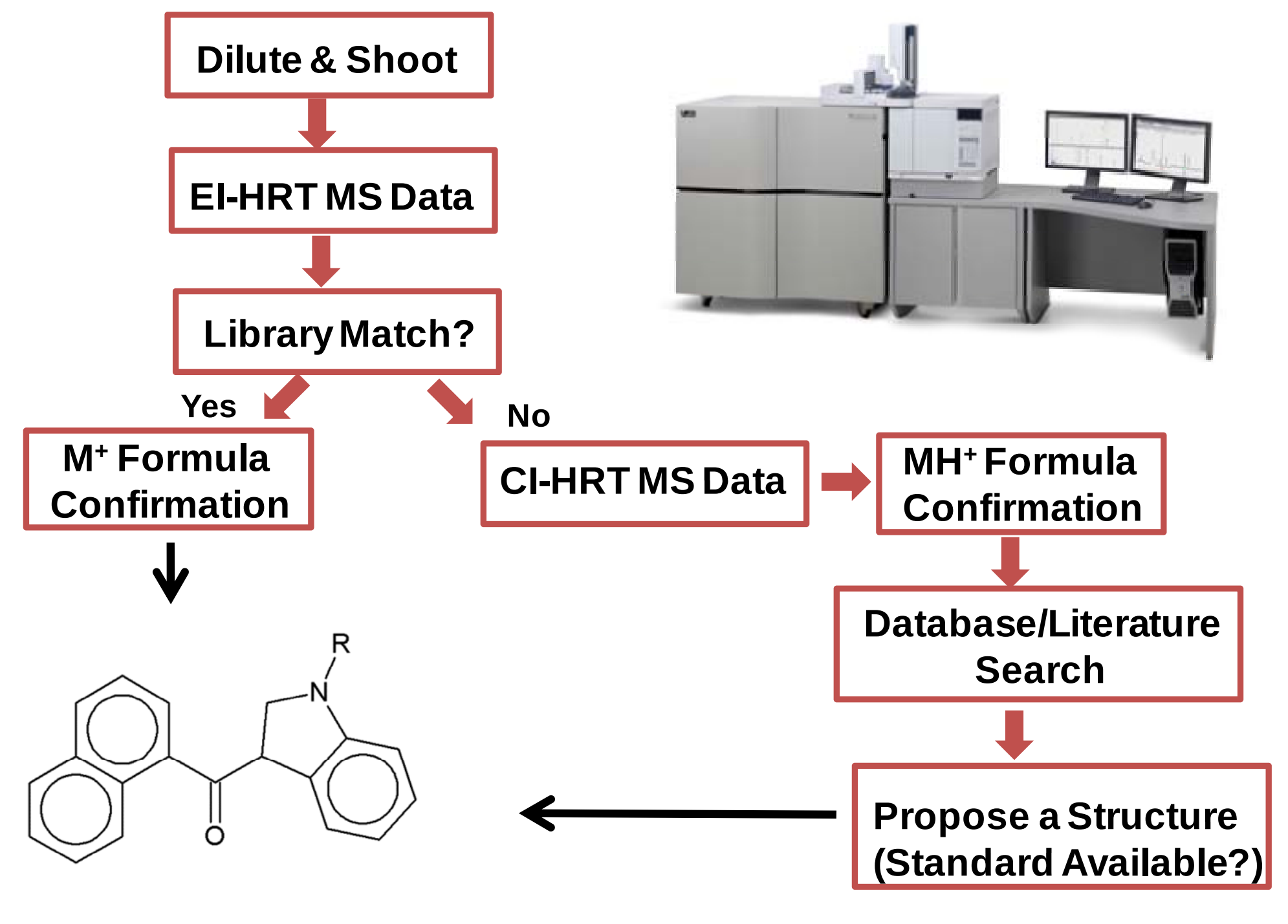
GC Parameters

GC:	Agilent 7890 and 7693 Auto Sampler
Column Type:	Restek R*xi*-5Sil MS (30 m, 0.25 mm ID, 0.25 mm *df*)
Injection:	0.5 mL, Split 150:1 (CI 100:1); Inlet Temp. 250°C
Oven:	60°C (2 min) to 300°C at 20°C/min (1 min)
Carrier Gas:	He, Constant Flow (1.00 mL/min)

MS Parameters

Spectrometer:	LECO Pegasus® GC-HRT
Ion Sources:	LECO EI, CI
Folded Flight Path:	HR (R = 25,000 FWHM)
Spectral Acquisition:	6 spectra/second
Mass Range (m/z):	30–650 (CI 60–650)
Calibration:	PFTBA (Internal)
CI Reagent Gas:	5% Ammonia in Methane

Analysis Workflow

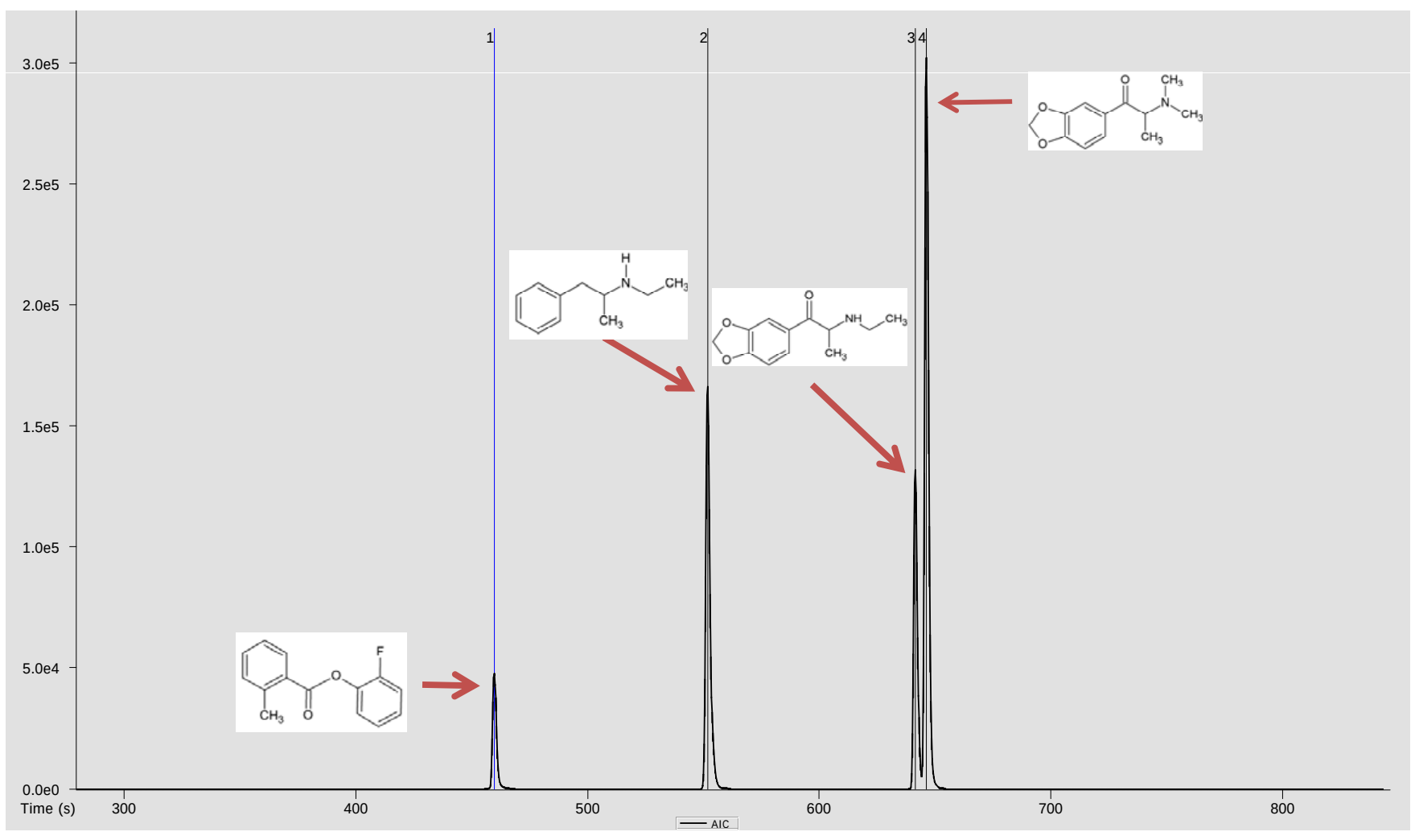


Dilute & Shoot
EI-HRT MS Data
Library Match?
Yes
M⁺ Formula Confirmation
No
CI-HRT MS Data
MH⁺ Formula Confirmation
Database/Literature Search
Propose a Structure (Standard Available?)

Figure 3: Drug Analysis Workflow; EI and CI-HRT Analysis

Results (Case 1: White Powder)

Analysis of the sample produced the analytical ion chromatogram shown in Figure 4. The four major components of the mixture identified through library searches were 2-Fluorophenyl 2-methylbenzoate, *N*-Ethylamphetamine, Ethylone and Dimethylone (Figure 4). Library matches for these compounds were very good (799 to 935/1000); however, quick inspection of the CI-HRT data demonstrated that the number one hit for peak #2, *N*-ethylamphetamine ($C_{11}H_{17}N$), was incorrect (Figure 5). A formula search on the analyte's protonated molecular ion at m/z = 192.13825 resulted in the formula $C_{12}H_{18}NO$ (-0.21 ppm). An internet search using the formula $C_{12}H_{17}NO$, led to Cayman Chemical and Scientific Working Group Sites and 4-methylethcathinone as a potential candidate for this compound.



Peak #	Name	Formula	R.T. (s)	Area	LM (1000)
1	2-Methylbenzoic acid, 2-fluorophenyl ester	C14H11FO2	459.7	265777	935
2	N-Ethylamphetamine	C11H17N	551.8	1081942	809
3	Ethylone	C12H15NO3	641.4	731528	799
4	Dimethylone	C12H15NO3	646.2	1882302	802

Figure 4: AIC - Major Components, Sample 1

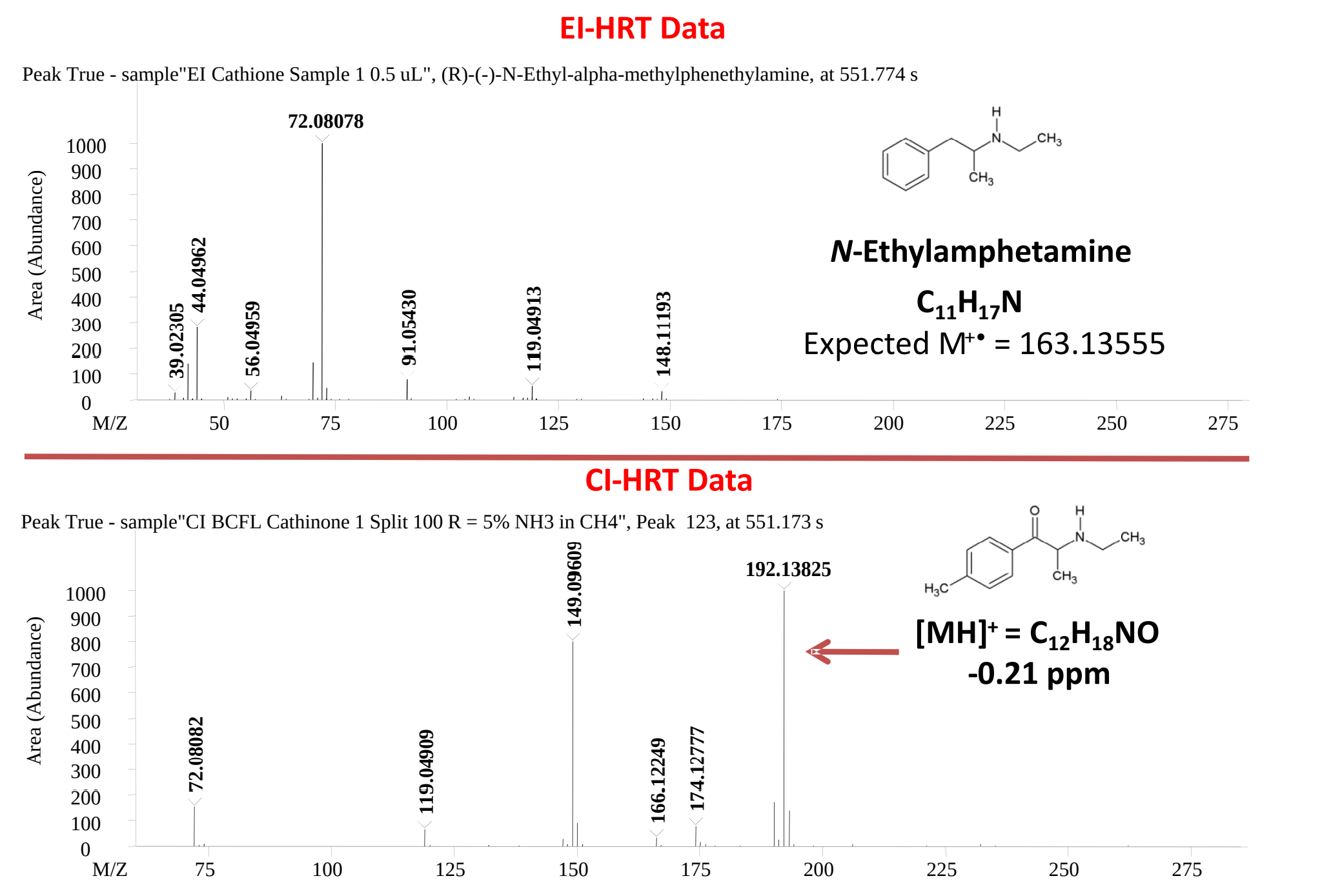


Figure 5: EI (top) and CI-HRT Data (bottom) for Analyte 2, Sample 1

EI and CI-HRT data for ethylone and dimethylone is shown in Figs. 6 and 7. Protonated molecular ions ($C_{12}H_{15}NO_3$) for these structural isomers were found at m/z = 222.11249 (0.08 ppm) and m/z = 222.11247 (-0.015 ppm) respectively.

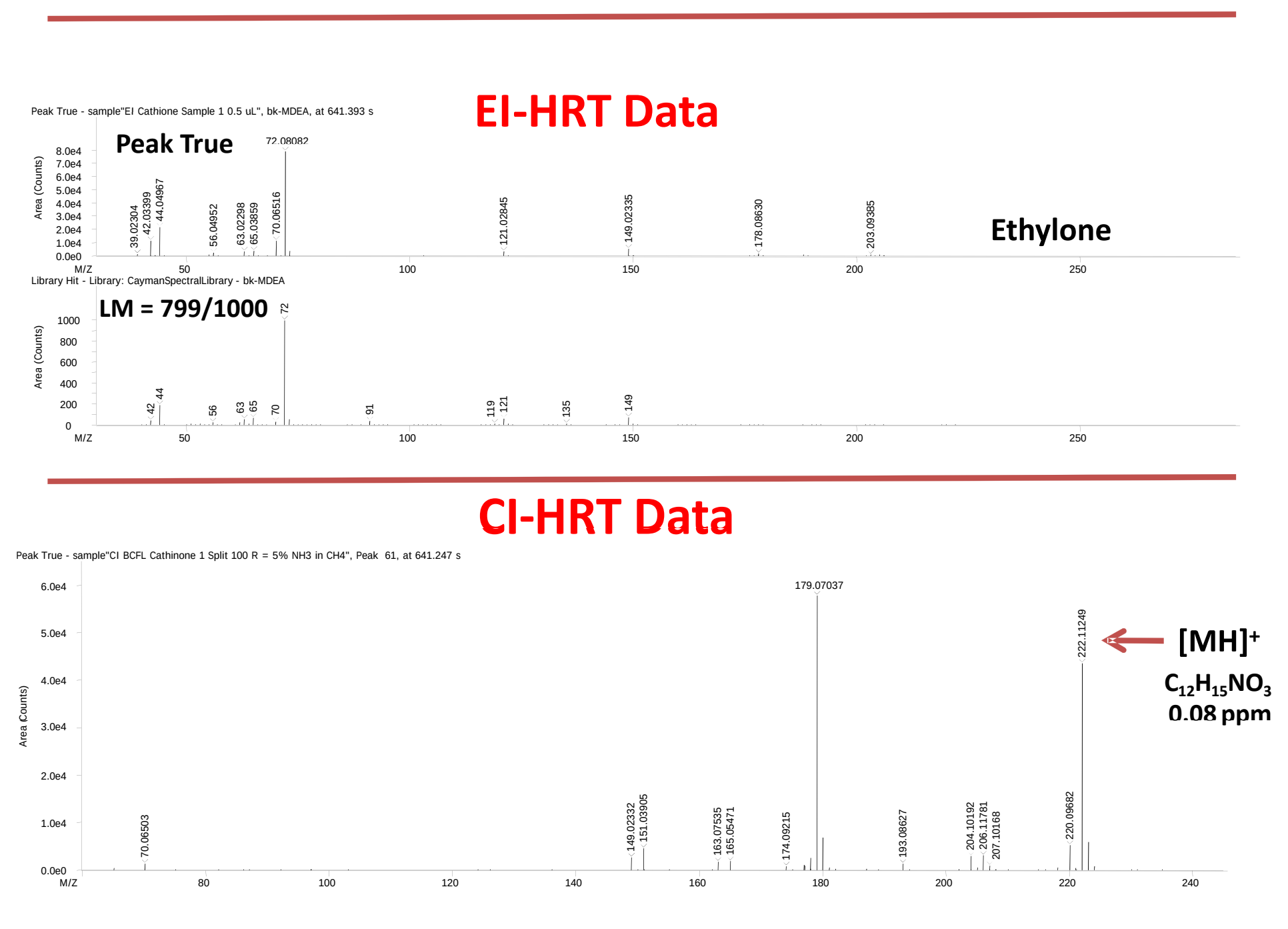


Figure 6: EI (top) and CI-HRT Data (bottom) for Ethylone, Sample 1

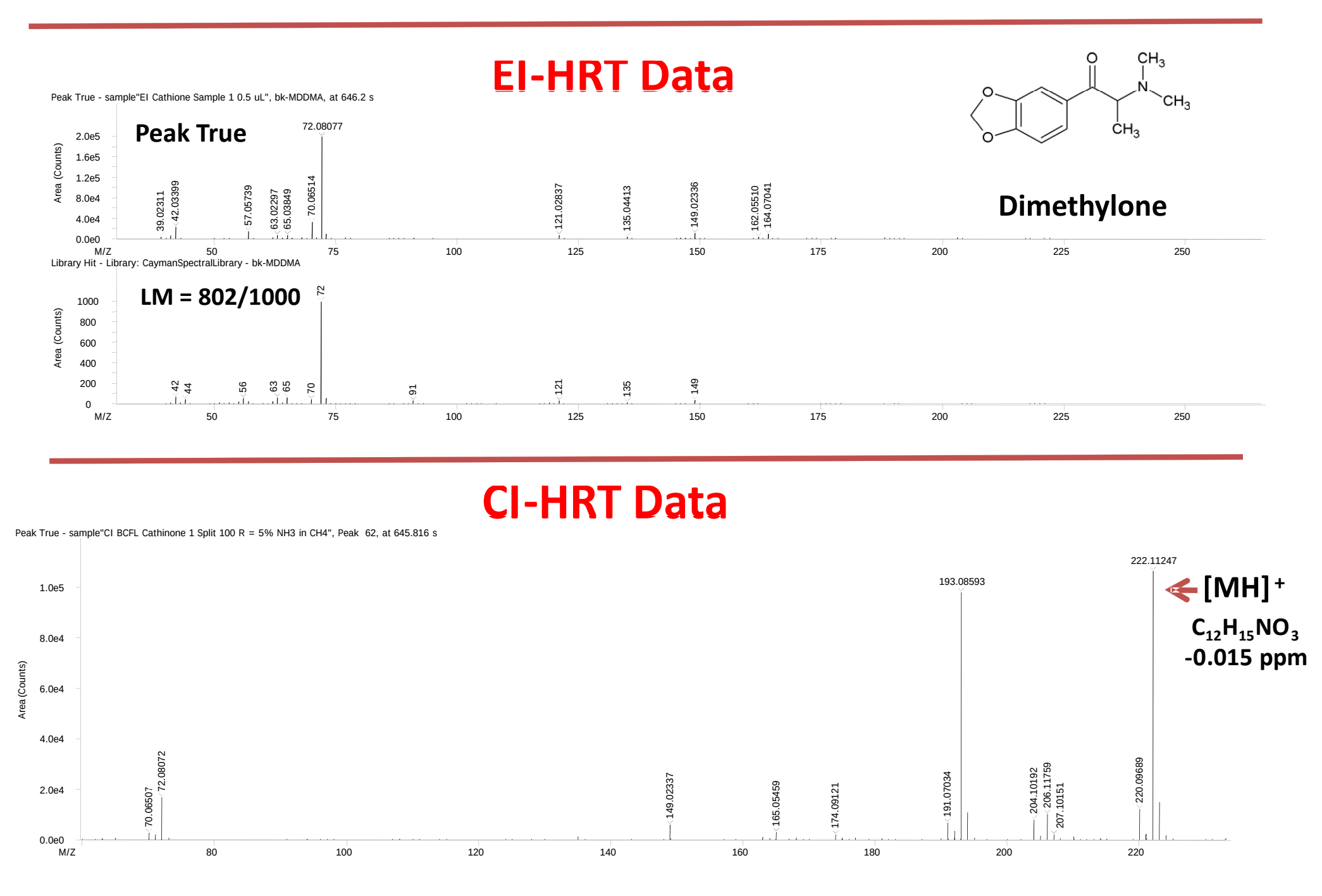


Figure 7: EI (top) and CI-HRT Data (bottom) for Dimethylone, Sample 1

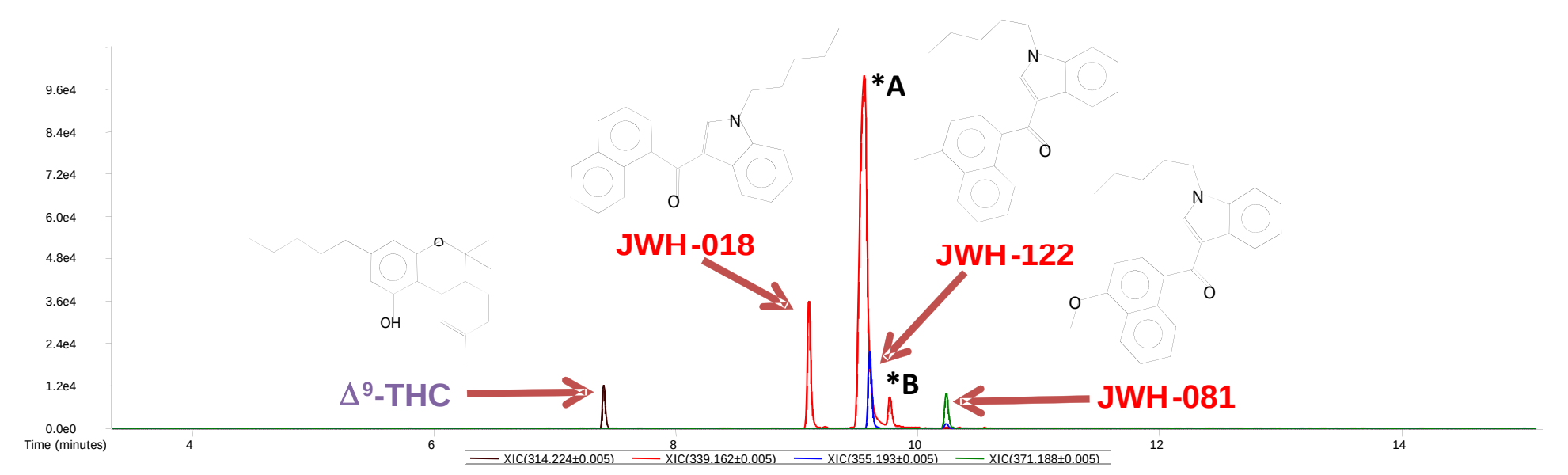
Results (Case 2: Drug Pipe)

Sample 2 was a heterogeneous, dark, solid material that was scraped from the inside of a confiscated drug pipe. A representative set of the over 130 compounds in the sample are listed in Table 1. These include nitriles, phenols, sterols and various heterocyclic compounds. An extracted ion chromatogram XIC (Figure 8) of the mixture shows the major component A, which was not present in any of the commercial libraries used during data processing. The XIC also displays an additional unknown (*B), as well as various drugs detected in the sample.

Table 1: Compounds In Sample 2

Name	Formula	R.T. (s)	Area	Mass Accuracy (ppm)
Phenol, 3-methyl- (CAS)	C7H8O	205.149	282908	-0.87
1H-Imidazole, 2-methyl- (CAS)	C4H6N2	206.376	263641	0.44
Phenol, 2-methoxy- (CAS)	C7H8O2	208.767	45543	0.26
4(1H)-Pyridinone (CAS)	C5H5NO	213.948	397571	-0.33
Phenol, 3-ethyl- (CAS)	C8H10O	228.263	162252	0.13
Naphthalene	C10H8	234.075	51896	0.29
Methylphloroglucinol	C7H8O3	236.362	83989	0.41
Catechol	C6H6O2	237.614	185354	-0.64
4-vinylphenol	C8H8O	240.441	512207	0.24
Benzenepropanenitrile	C9H9N	243.873	42046	-0.50
Cinnamonitrile	C9H7N	247.059	44083	0.21
1,4-Benzenediol	C6H6O2	253.334	516706	-0.47
1H-Indole	C8H7N	257.923	508162	-0.18
4 - vinyl - guaiacol	C9H10O2	261.488	80190	-0.09
5 - ethyl - pyrogallol	C8H10O3	268.299	181932	0.35
1H-Indole, 3-methyl-	C9H9N	277.262	251291	-0.46
ATRANOL	C8H8O3	278.703	38855	-0.34
4-methyl-6(2-methylpropenyl)-2H-pyran-2-one	C10H12O2	288.459	50850	-0.94
1-cyano-naphthalene	C11H7N	298.871	58725	-0.21
2,3,5-Trimethoxytoluene	C10H14O3	301.771	39244	1.90
(3aS,5a-trans)-dodecahydropyrrolo[1,2-a]quinoxaline	C11H20N2	308.857	58336	-0.51
octylphenol	C14H22O	317.535	1871006	-0.85
(+,-)-3-(2'-METHYLBUTYL)INDOLE	C13H17N	318.341	67100	-0.94
9H-Carbazole	C12H9N	357.229	65287	-0.47
octylphenol-ethoxylate	C16H26O2	357.776	5107445	-0.38
9H-Pyrido[3,4-b]indole, 1-methyl-	C12H10N2	376.271	51137	0.26
9H-Pyrido[3,4-b]indole	C11H8N2	377.278	132740	-0.52
Pentalene, 1,2,3,3a,4,6a-hexahydro-	C8H12	395.052	17052	-0.87
Ethanol, 2-[2-[4-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]-	C18H30O3	398.148	2909944	-0.18
9-OCTADECENAMIDE	C18H35NO	423.04	4358976	0.87
Oleanitrile	C18H33N	423.416	150791	0.35
octylphenol-triethoxylate	C20H34O4	433.317	1111259	-0.33
Ethanol, 2-[2-[2-[2-[p-(1,1,3,3-tetramethylbutyl)phenoxy]ethoxy]ethoxy]ethoxy]-	C22H38O5	465.887	322409	-0.23
9H-Carbazole, 9-phenyl-	C18H13N	473.068	51386	-1.13
1H-Quinolin-2-one, 3,4-diphenyl-	C21H15NO	498.817	179143	-1.28
Tocopherol	C28H48O2	510.43	82866	-1.13
Vitamin E	C29H50O2	523.46	312844	0.21
Stigmastan-3,5-diene	C29H48	525.666	49994	-0.47
Sitosterol	C29H50O	568.389	64162	0.02
2-isopropyl-3,4-diphenyl-1-isoquinolone	C24H21NO	572.918	3961353	0.17
3-benzoyl-7-methyl-2-(2-methylphenylamino)indole	C23H20N2O	586.043	161486	-1.45

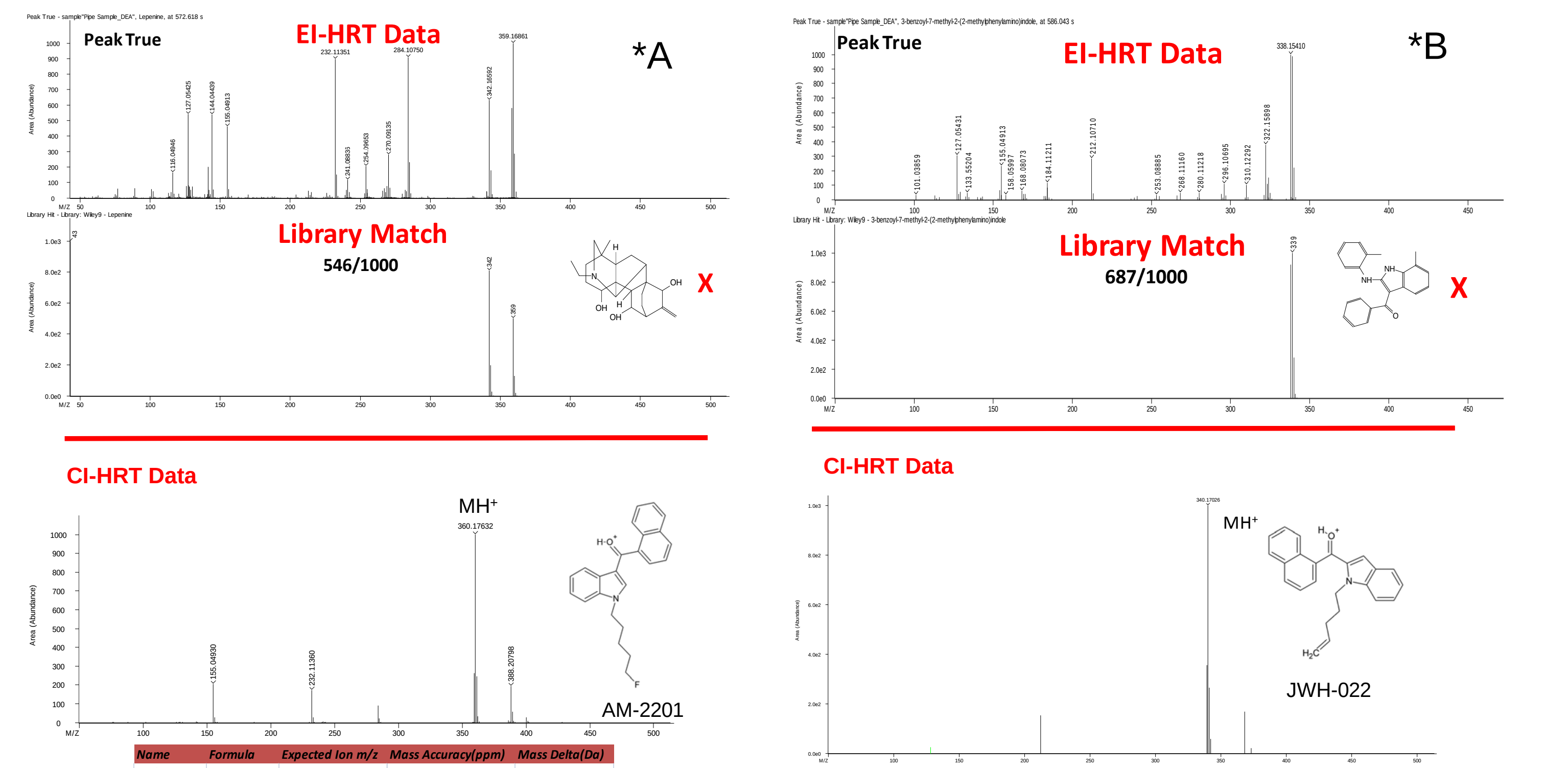
Ave. = 0.53 ppm



Name	Similarity	Formula	Expected Ion m/z	Mass Accuracy	Mass Delta
Delta 9-THC	864	C21H30O2	314.22403	-0.12	-0.00004
JWH-018	731	C24H23NO	341.17742	-1.39	-0.00047
JWH-122 (1-pentyl-3-(4-methyl-1-napthoyl)indole)	779	C25H25NO	355.19307	-0.65	-0.00023
JWH-081 (1-pentyl-3-(4-methoxy-1-napthoyl)indole)	863	C25H25NO2	371.18798	-0.88	-0.00033

Figure 8: XIC of Sample 2

CI-HRT data was critical for the identification of unknowns A and B (Figure 9). EI-HRT Library hits for these unknowns were poor. Confirmation of unidentified compound formulas was accomplished using CI-HRT data. Internet and database searches of the data sets led to AM-2201 and JWH-022 as probable hits, which were then confirmed through the analysis of standard spectra.



Name	Formula	Expected Ion m/z	Mass Accuracy(ppm)	Mass Delta(Da)
Unknown A	C24H23FNO	360.17582	1.38	0.00050

Name	Formula	Expected Ion m/z	Mass Accuracy (ppm)	Mass Delta (Da)
Unknown B	C24H22NO	340.16959	1.95	0.00066

Figure 9: EI (top) and CI-HRT Data (bottom) for Unknowns A and B

Summary

A combination of EI and CI-HRT resulted in confident identification of traditional and emerging synthetic drugs. The GC-HRT's high resolution, quality spectral data, and excellent mass accuracy values are very useful for the identification of novel compounds in complex matrices. The accurate mass data provided formulas that were searched against forensic databases for identification of unknowns in drug samples.