Comprehensive and Confident Identification of Narcotics, Steroids and Pharmaceuticals in Urine

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Introduction

Monitoring of patients in hospitals and clinics has traditionally relied on targeted methods of analysis. These screening methods are not comprehensive and result in an incomplete picture of a patient's activities. Gas chromatography high resolution time-of-flight mass spectrometry (GC-HRT) provides a fast and convenient method for analysis of urine samples. The objective of this study was to develop a comprehensive, fast and accurate method for compound characterization and effective molecular profiling of urine samples.

Why Urine?

- Used since ancient times for medical diagnosis (Urology → Urinalysis)
- In ancient times considered "a divine fluid and window to the body"

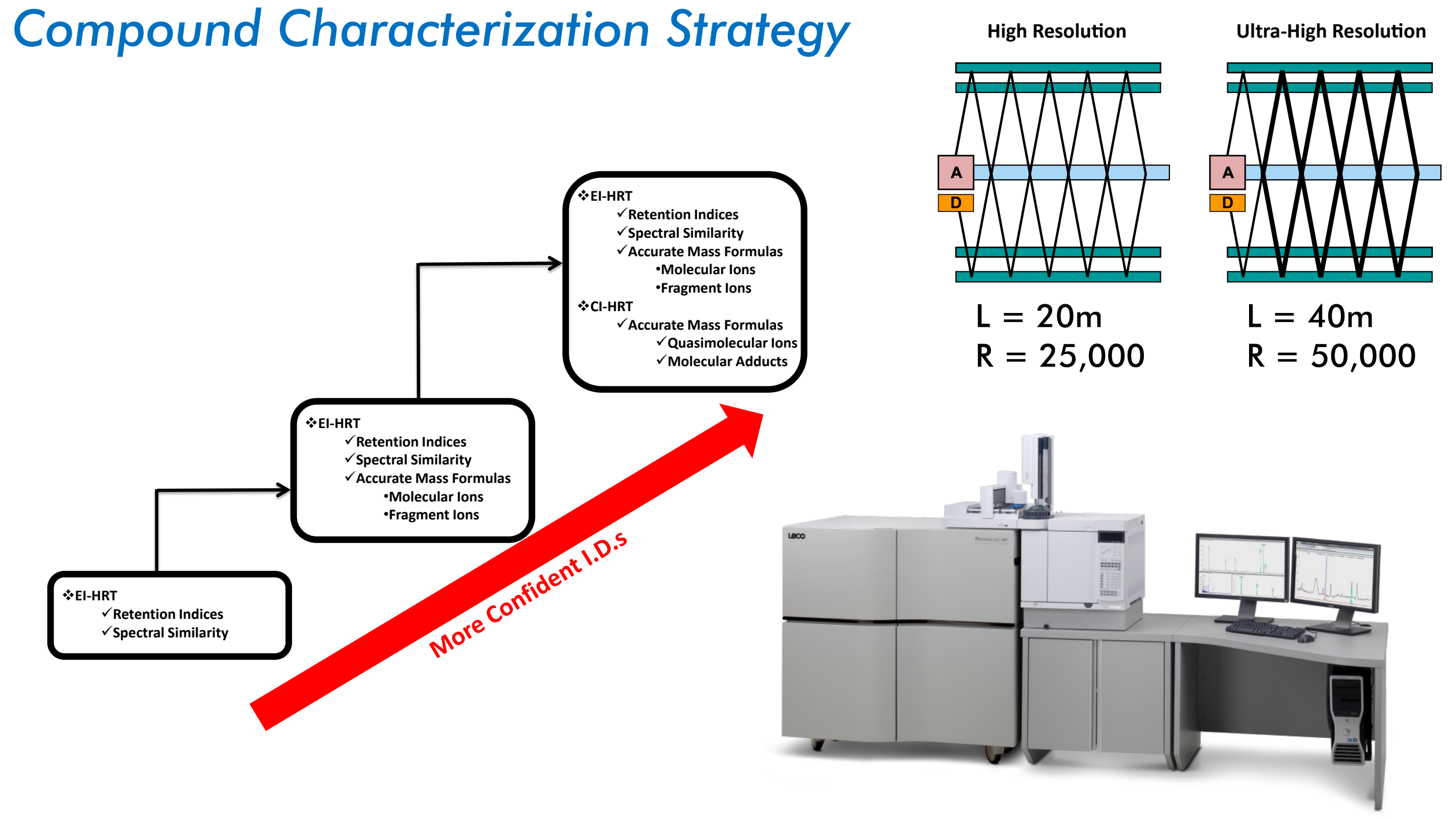
Fig. 1: A Physician Examining Urine through a Matula (Left). A Urine Color Chart (Right).

- Urine-based measurements exhibit advantages over other biological fluids:
  - Samples are easily collected in large quantities
  - Contain relatively high concentrations of pharmaceuticals, illicit drugs, and steroids
  - Compounds are typically detectable over extended periods of time

Instrument: LECO 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 & Adduct Ions
- High Resolution Deconvolution™ (HRD™)
- High Resolving Power (up to 50,000)

Compound Characterization Strategy



- ❖EI-HRT
  - ✓Retention Indices
  - ✓Spectral Similarity
- ❖EI-HRT
  - ✓Retention Indices
  - ✓Spectral Similarity
  - ✓Accurate Mass Formulas
    - •Molecular Ions
    - •Fragment Ions
- ❖EI-HRT
  - ✓Retention Indices
  - ✓Spectral Similarity
  - ✓Accurate Mass Formulas
    - •Molecular Ions
    - •Fragment Ions
- ❖CI-HRT
  - ✓Accurate Mass Formulas
    - ✓Quasimolecular Ions
    - ✓Molecular Adducts

Fig. 2: The Utilization of EI- and CI-HRT Technology for Confident Compound Characterization.

Experimental

Samples

- Obtained from a collaborating European hospital
- 52 patient monitoring samples
- Sample preparation

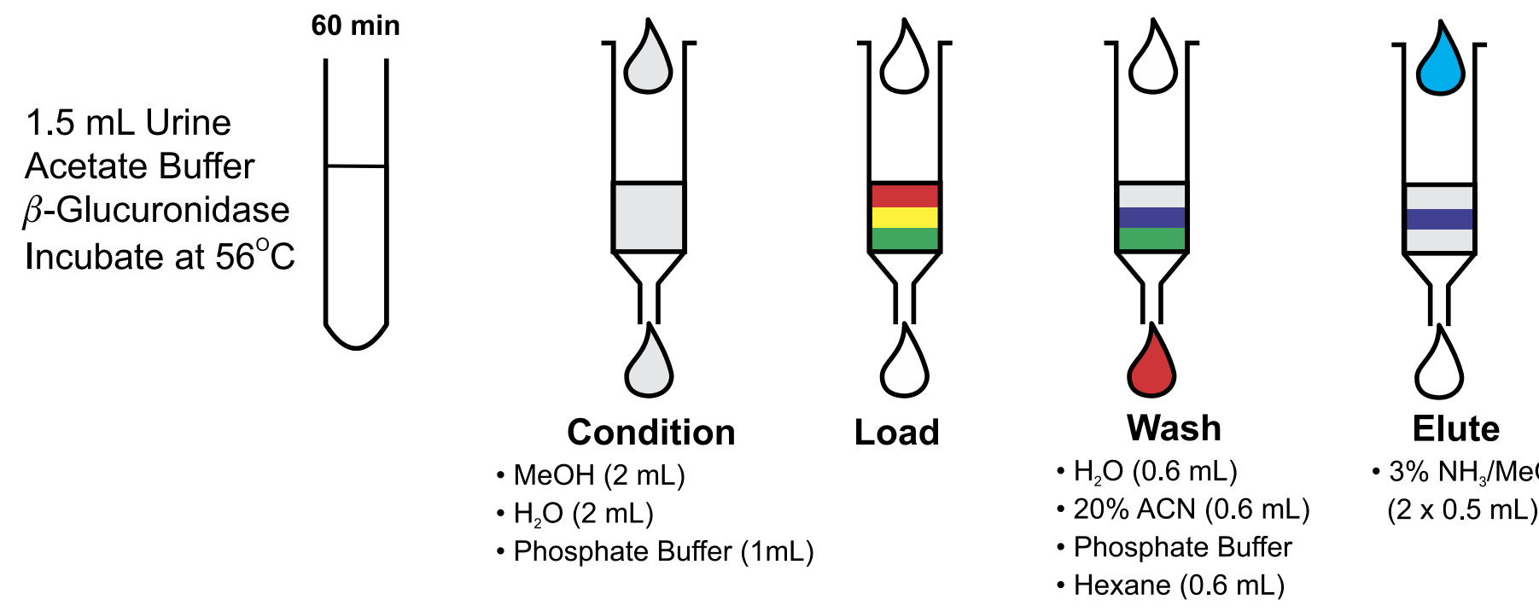


Fig. 3: SPE Urine Sample Preparation.

Acquisition Parameters

GC	**Agilent 7890B**
Column	Agilent J&W *VF-DA* (12m x 0.20 mm x 0.33μm)
Carrier Gas, Flow	He, 1.0 mL/min Constant Flow
Injection/Volume	Splitless/2 μL
Injector Temp.	280 °C
Temp. Program	70 °C (1 min) to 320OC at 25 °C/min (5 min)
MS	**LECO Pegasus® GC-HRT**
Transfer Line Temp.	300 °C
Ion Source Temp.	250 °C (CI 200 °C)
Ionization	EI (70 eV); CI (140 eV)
Mass Range	45 – 520 (CI 60 – 800, Reagent Gas = 5% NH_3 in CH_4)
Acquisition Rate	10 sps
Mass Calibration	PFTBA (Internal)

Results: Illustrative Urine Samples:

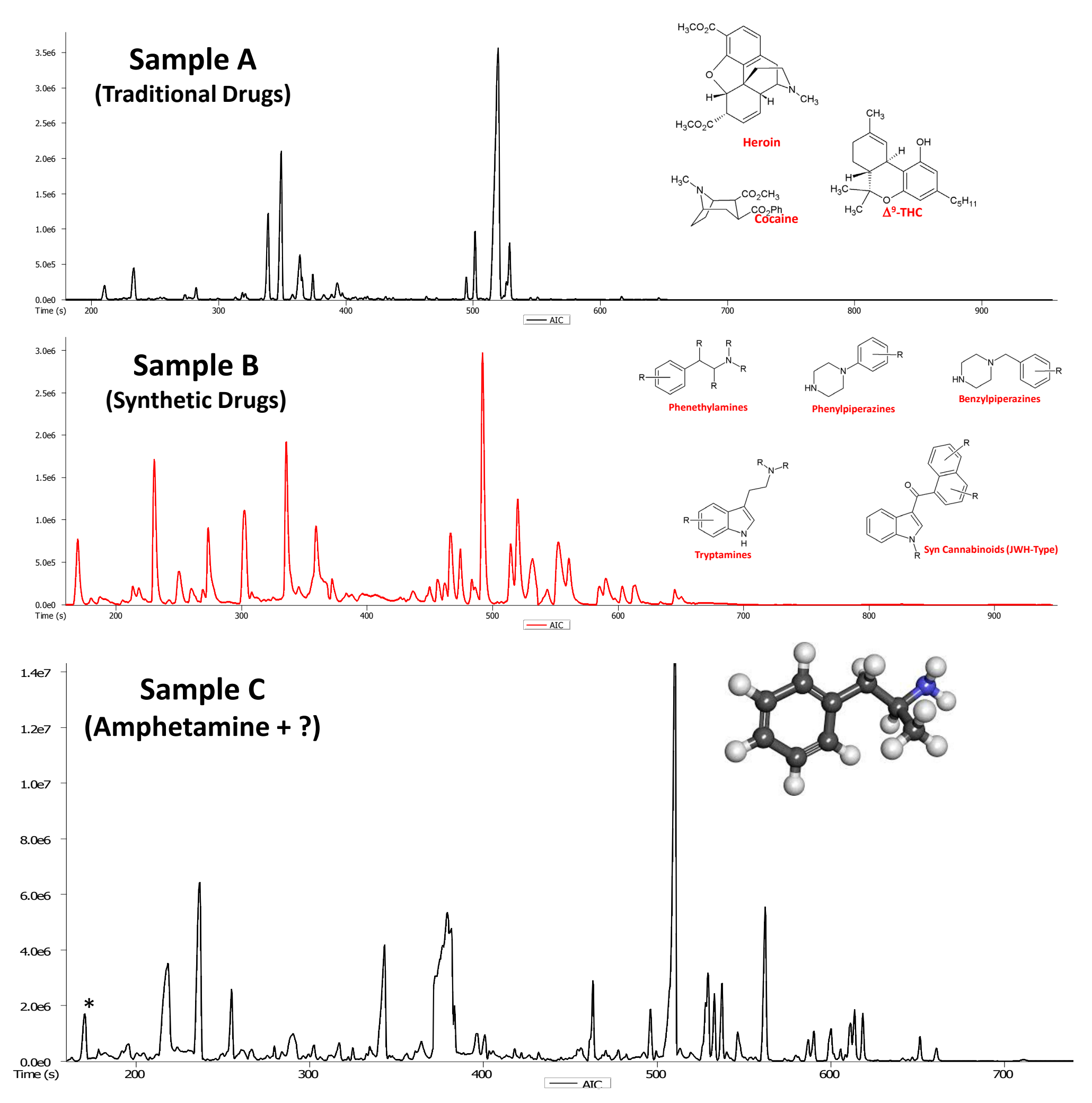


Fig. 4: Illustrative Urine Samples A, B, C.

Sample A (Traditional Drugs)

Representative Compounds

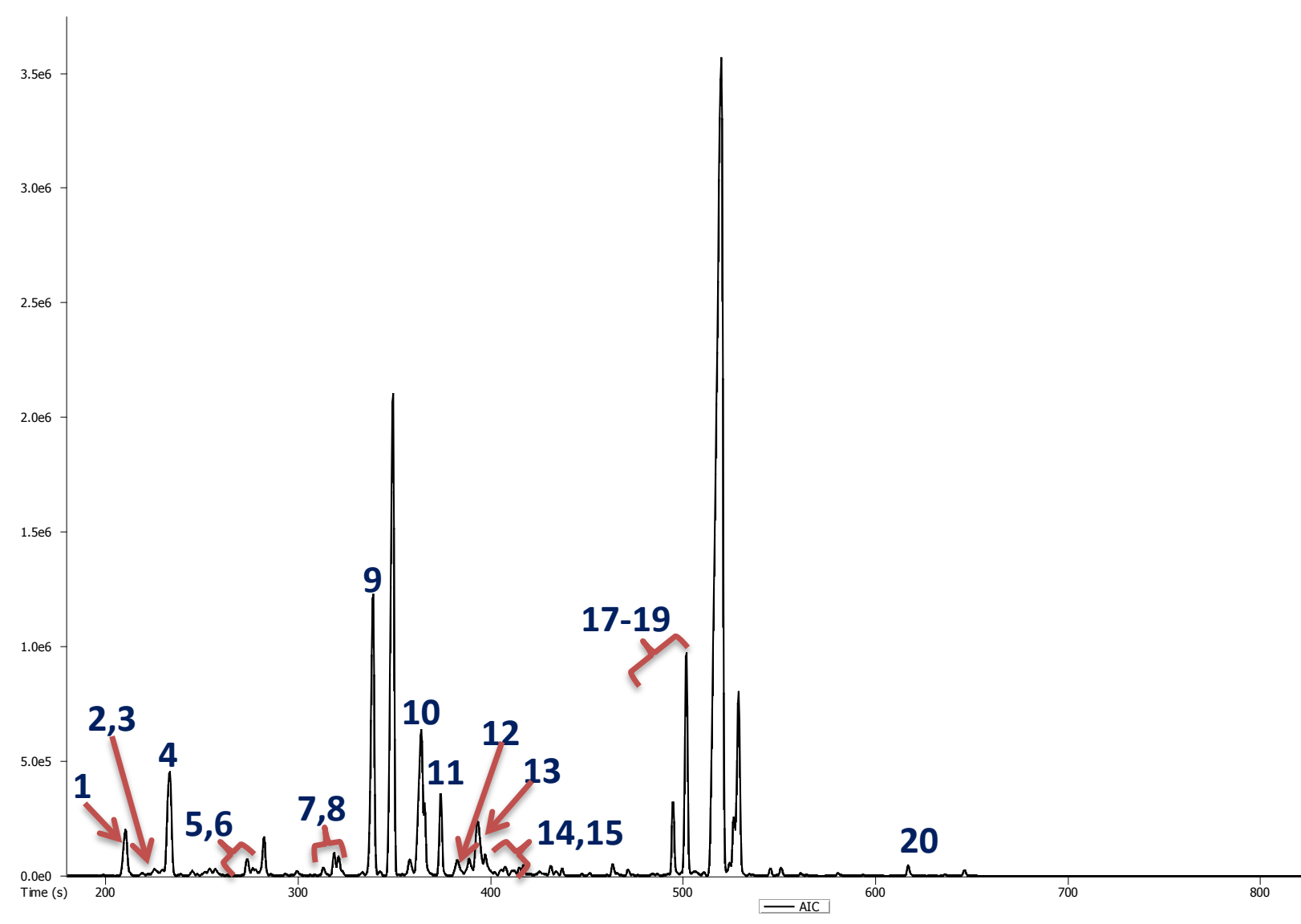
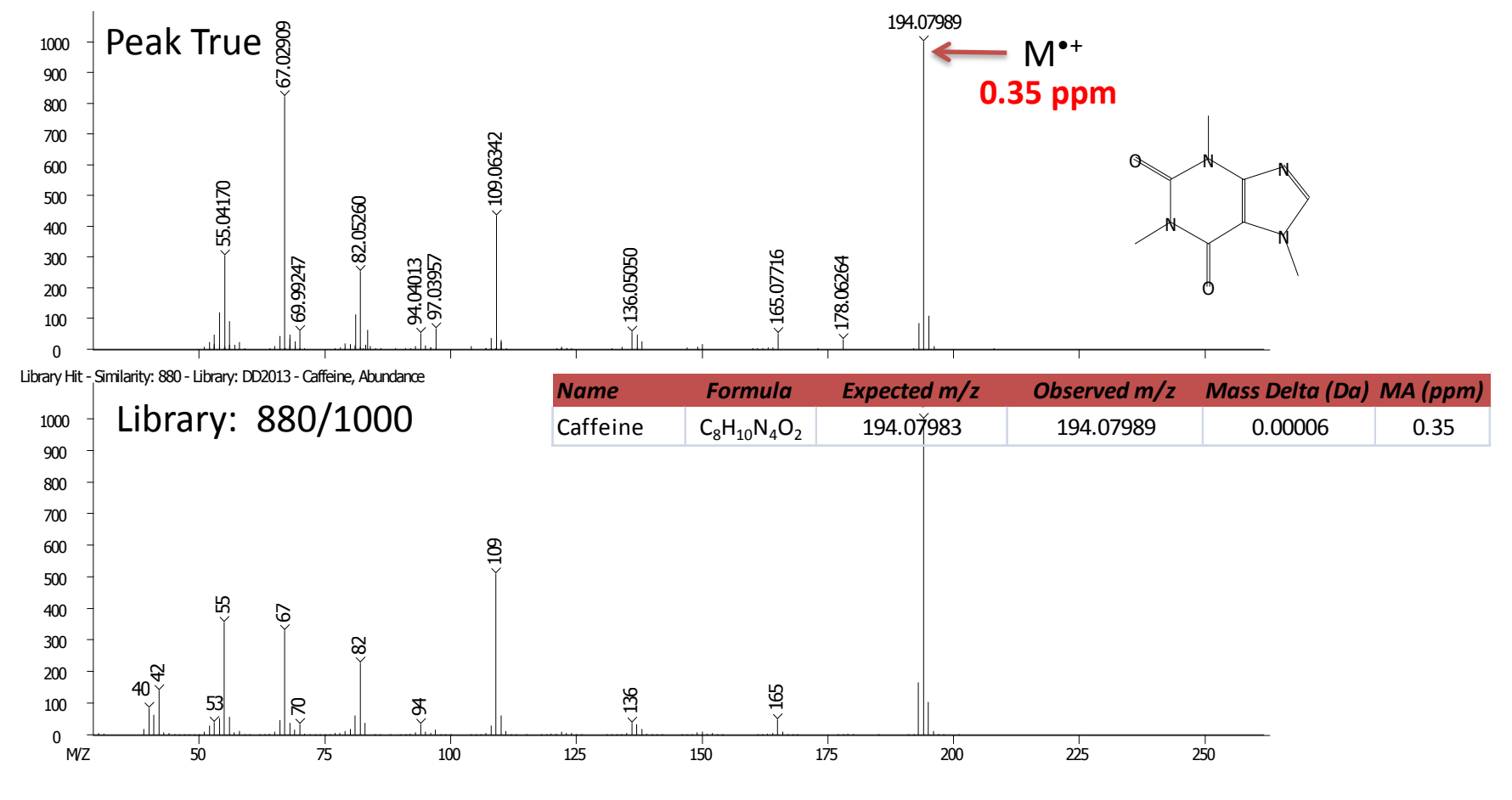


Fig. 5: Analytical Ion Chromatogram (AIC) – Sample A.

Peak #	Name	Formula	R.T. (s)	Area	Similarity	Mass Delta (mDa)	MA (ppm)
1	Creatinine ME	$C_5H_9N_3O$	210	1326229	800	-0.05	-0.43
2	o-Ethynylaniline	C_8H_7N	219	167436	893	0.07	0.63
3	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	223	65764	805	0.11	0.73
4	Nicotine	$C_{10}H_{14}N_2$	234	3249121	898	-0.21	-1.29
5	Hordenin	$C_{10}H_{15}NO$	274	949734	775	-0.14	-0.84
6	Methylecgonine	$C_{10}H_{17}NO_3$	276	104640	835	-0.22	-1.1
7	4-(3-Pyridyl-tetrahydrofuran-2-one	$C_9H_9NO_2$	313	93817	849	-0.11	-0.68
8	6-Methoxycoumaran-7-ol-3-one	$C_9H_8O_4$	321	161718	727	0.02	0.12
9	Cotinine	$C_{10}H_{12}N_2O$	339	5866842	870	-0.07	-0.38
10	3-Hydroxycotinine	$C_{10}H_{12}N_2O_2$	364	2087294	899	0.05	0.24
*11	**Caffeine**	$C_8H_{10}N_4O_2$	374	1179748	880	0.07	0.35
12	Theobromine	$C_7H_8N_4O_2$	383	261387	743	0.12	0.65
13	N-[4-Hydroxy-3-(methylthio)phenyl]-acetamide	$C_9H_{11}NO_2S$	393	1382915	888	0.17	0.89
14	Proline anhydride	$C_{10}H_{14}N_2O_2$	397	441603	872	0.04	0.2
15	4-Methyl-5H-carboline	$C_{12}H_{10}N_2$	406	109887	757	-0.01	-0.06
16	Acridine, 9-methyl-	$C_{14}H_{11}N$	408	253214	860	0.2	1.06
17	1-Methyl-6-(phenylethynyl)-1H-indole	$C_{17}H_{13}N$	488	6799	808	-0.16	-0.71
18	5H-benzo[f]pyrido[4,3-b]indole	$C_{15}H_{10}N_2$	490	50619	839	-0.03	-0.15
19	Phenylalanylproline lactam	$C_{14}H_{16}N_2O_2$	492	53239	835	0.04	0.15
*20	**Cholesterol**	$C_{27}H_{46}O$	617	38109	723	0.27	0.71

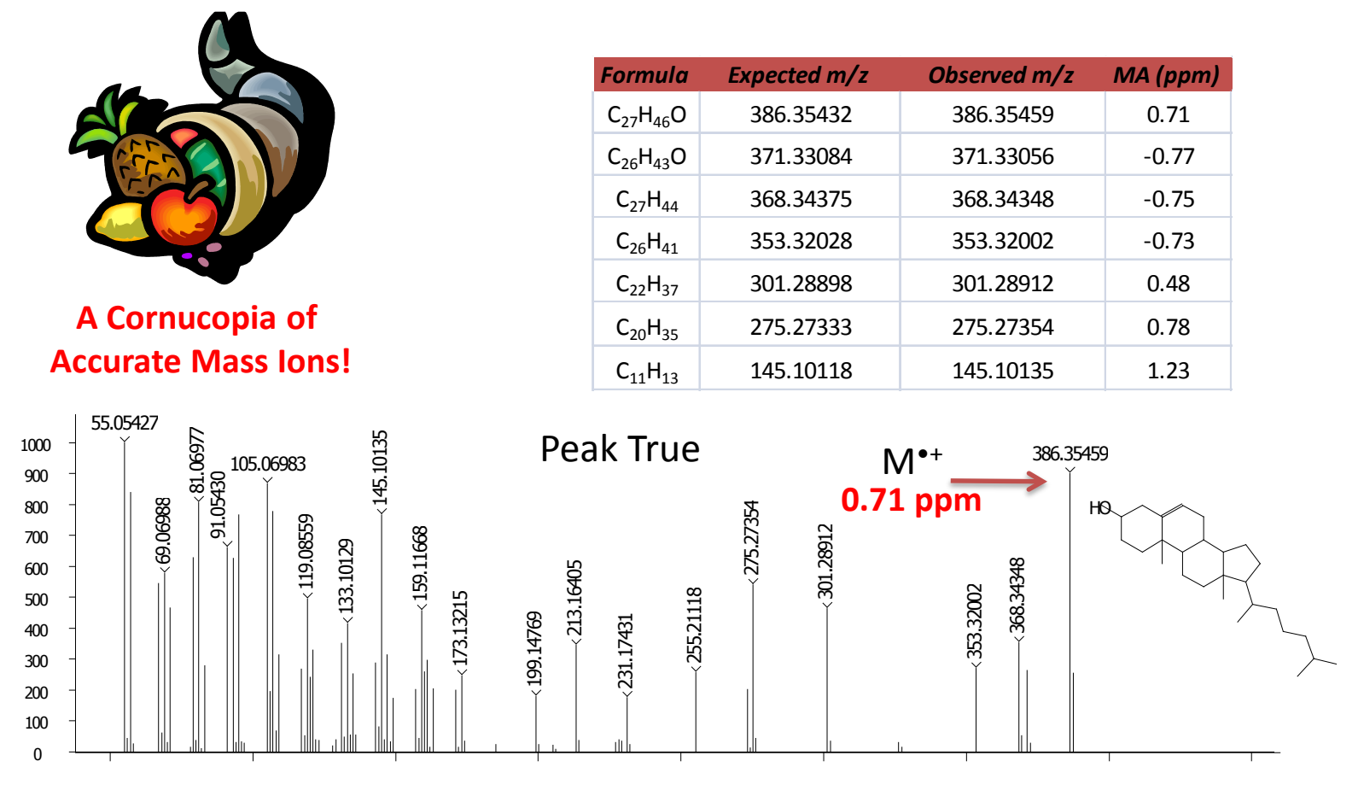
Ave.|ppm| = 0.57

Table 1: Compounds in Sample A.



Name	Formula	Expected m/z	Observed m/z	Mass Delta (Da)	MA (ppm)
Caffeine	$C_8H_{10}N_4O_2$	194.07983	194.07989	0.00006	0.35

Fig. 6: Peak True, Deconvoluted (Top) and Library Mass Spectra (Bottom) for Caffeine in Sample A.

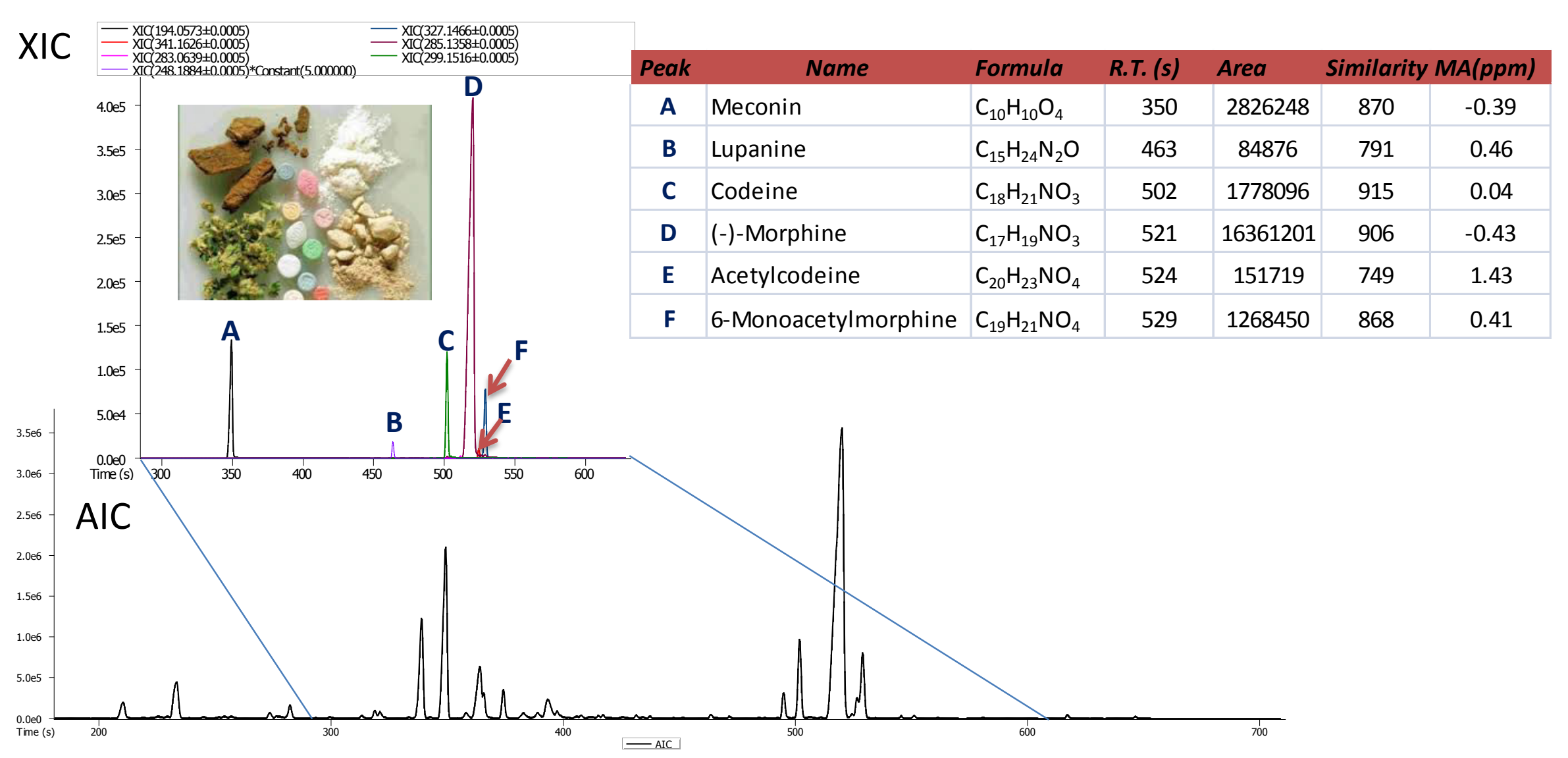


A Cornucopia of Accurate Mass Ions!

Formula	Expected m/z	Observed m/z	MA (ppm)
$C_{27}H_{46}O$	386.35432	386.35459	0.71
$C_{26}H_{43}O$	371.33084	371.33056	-0.77
$C_{27}H_{44}$	368.34375	368.34348	-0.75
$C_{26}H_{41}$	353.32028	353.32002	-0.73
$C_{22}H_{37}$	301.28898	301.28912	0.48
$C_{20}H_{35}$	275.27333	275.27354	0.78
$C_{11}H_{13}$	145.10118	145.10135	1.23

Fig. 7: Peak True Mass Spectrum and Table of Accurate Mass Ions for Cholesterol in Sample A.

Traditional Drugs



Peak	Name	Formula	R.T. (s)	Area	Similarity	MA(ppm)
A	Meconin	$C_{10}H_{10}O_4$	350	2826248	870	-0.39
B	Lupanine	$C_{15}H_{24}N_2O$	463	84876	791	0.46
C	Codeine	$C_{18}H_{21}NO_3$	502	1778096	915	0.04
D	(-)-Morphine	$C_{17}H_{19}NO_3$	521	16361201	906	-0.43
E	Acetylcodeine	$C_{20}H_{23}NO_4$	524	151719	749	1.43
F	6-Monoacetylmorphine	$C_{19}H_{21}NO_4$	529	1268450	868	0.41

Fig. 8: Extracted Ion Chromatogram (XIC) Displaying Drugs (Top) and AIC (Bottom) for Sample A.

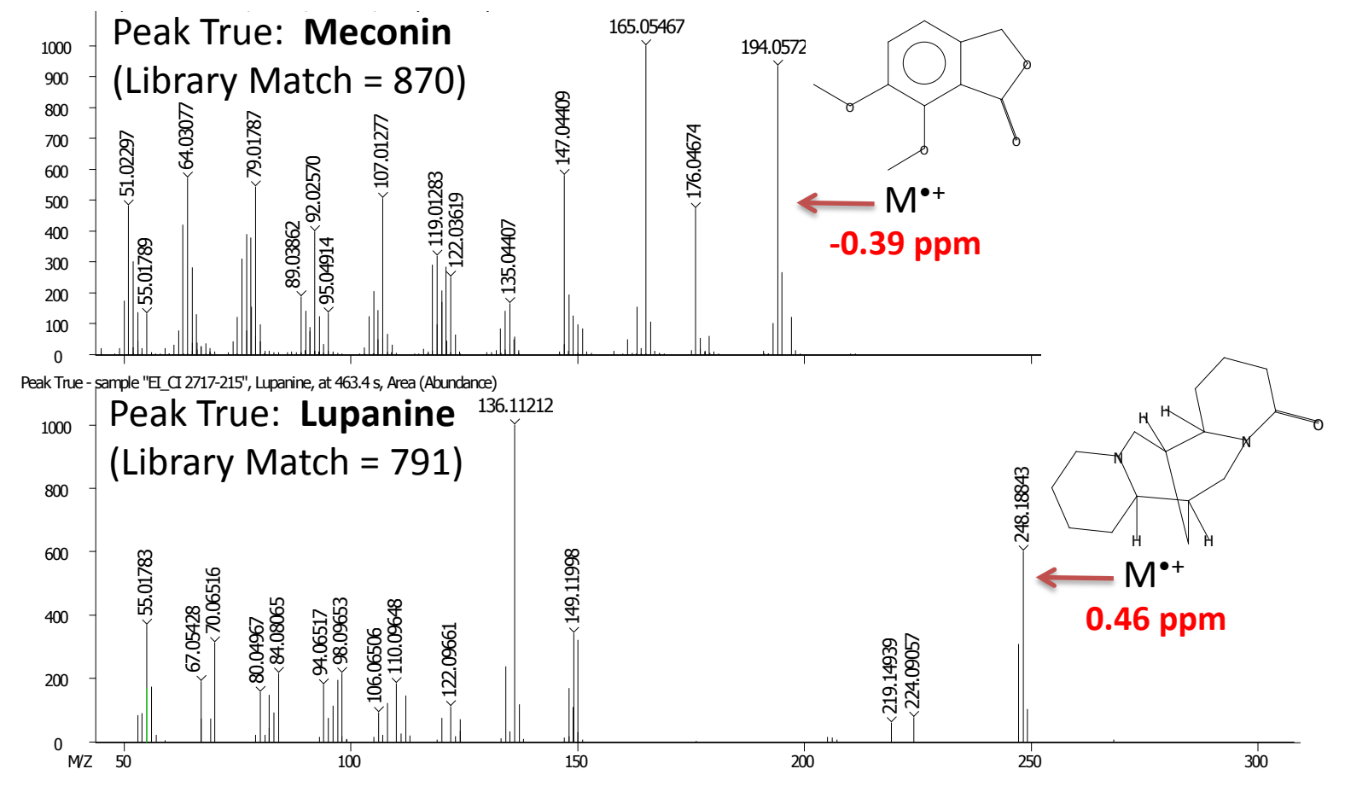


Fig. 9: Peak True Mass Spectra for Meconin (Top) and Lupanine (Bottom) in Sample A.

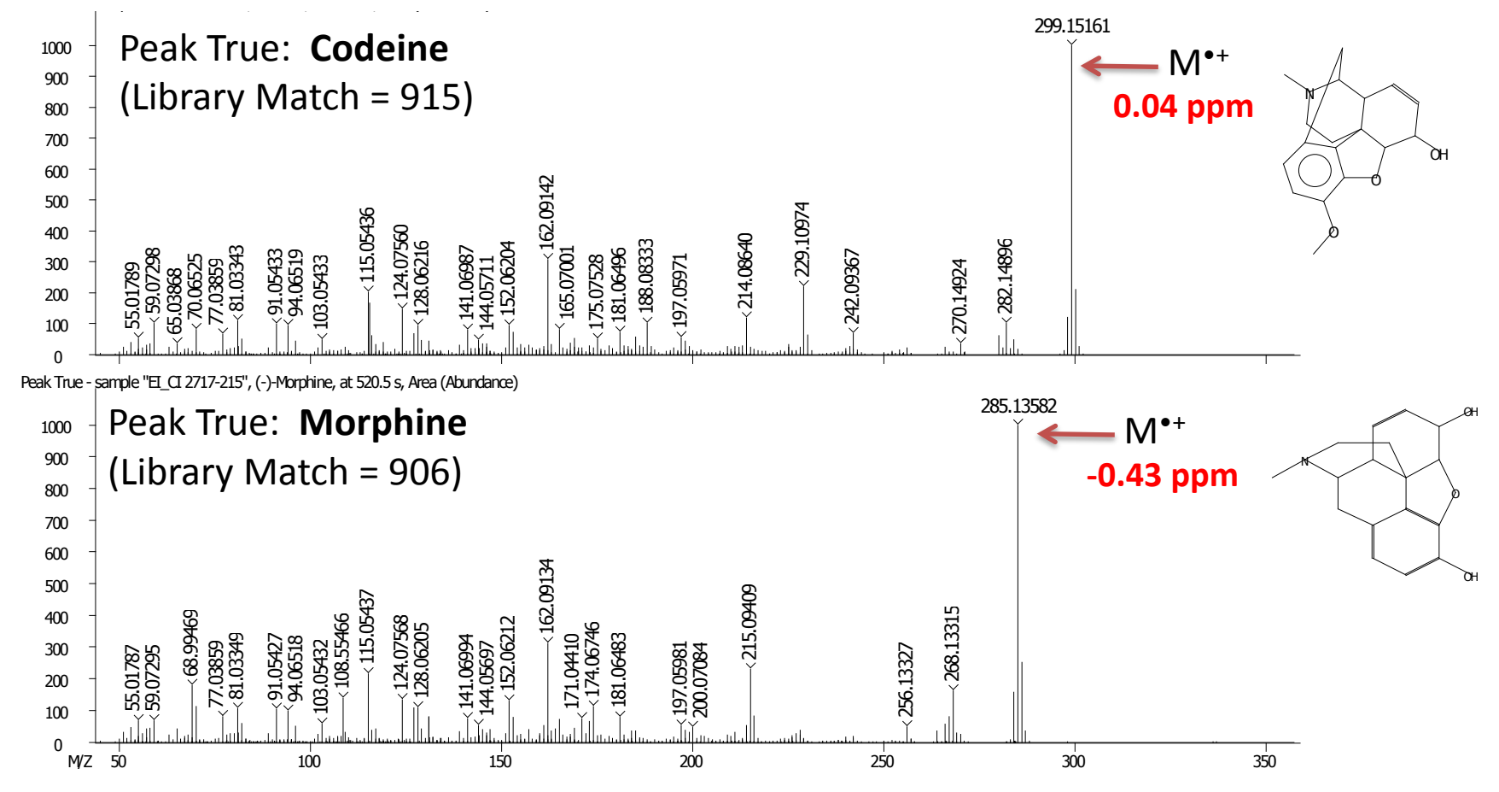
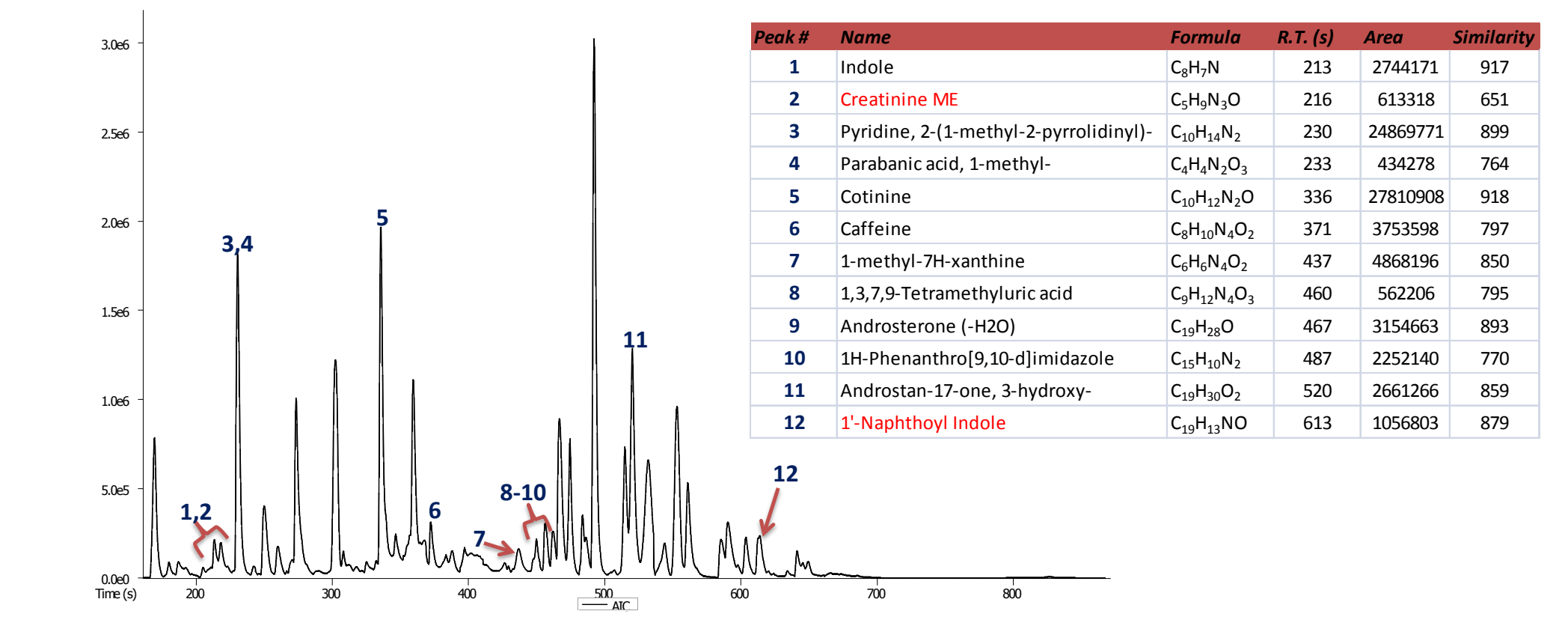


Fig. 10: Peak True Mass Spectra for Codeine (Top) and Morphine (Bottom) in Sample A.

Sample B (Synthetic Drugs)

Representative Compounds



Peak #	Name	Formula	R.T. (s)	Area	Similarity
1	Indole	C_8H_7N	213	2744171	917
2	Creatinine ME	$C_5H_9N_3O$	216	613318	651
3	Pyridine, 2-(1-methyl-2-pyrrolidinyl)-	$C_{10}H_{14}N_2$	230	24869771	899
4	Parabanic acid, 1-methyl-	$C_4H_4N_2O_3$	233	434278	764
5	Cotinine	$C_{10}H_{12}N_2O$	336	27810908	918
6	Caffeine	$C_8H_{10}N_4O_2$	371	3753598	797
7	1-methyl-7H-xanthine	$C_6H_6N_4O_2$	437	4868196	850
8	1,3,7,9-Tetramethyluric acid	$C_9H_{12}N_4O_3$	460	562206	795
9	Androsterone (-H2O)	$C_{19}H_{28}O$	467	3154663	893
10	1H-Phenanthro[9,10-d]imidazole	$C_{15}H_{10}N_2$	487	2252140	770
11	Androstan-17-one, 3-hydroxy-	$C_{19}H_{30}O_2$	520	2661266	859
12	1'-Naphthoyl Indole	$C_{19}H_{13}NO$	613	1056803	879

Fig. 11: AIC and Table Listing Compounds in Sample B.

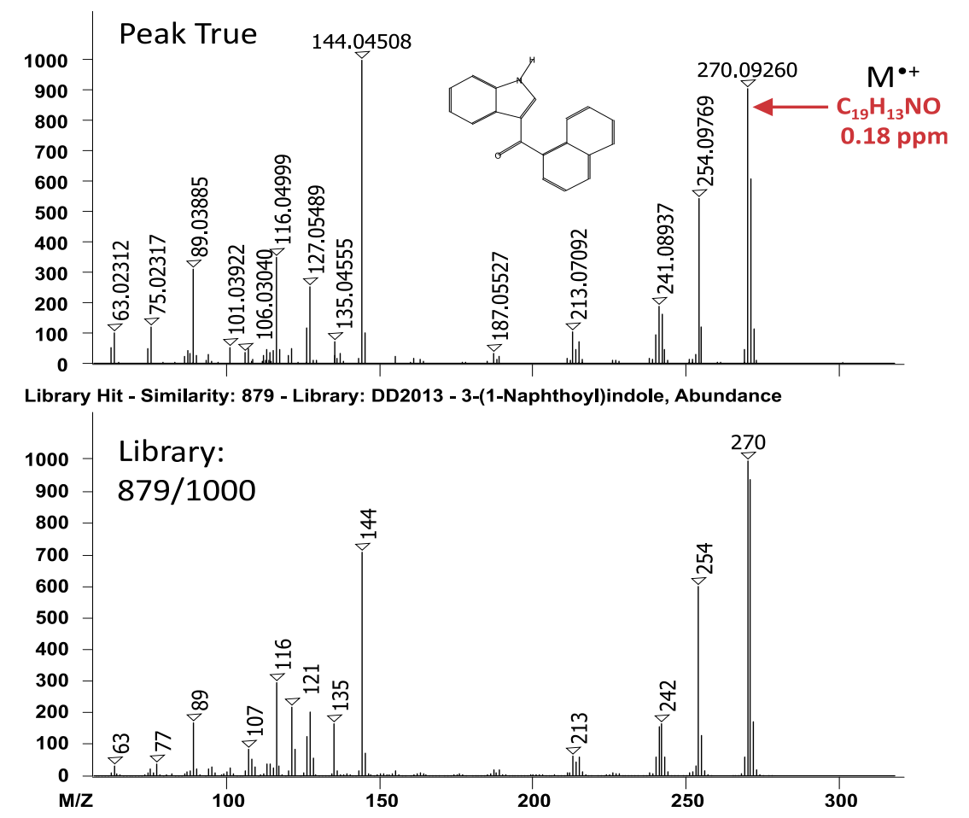
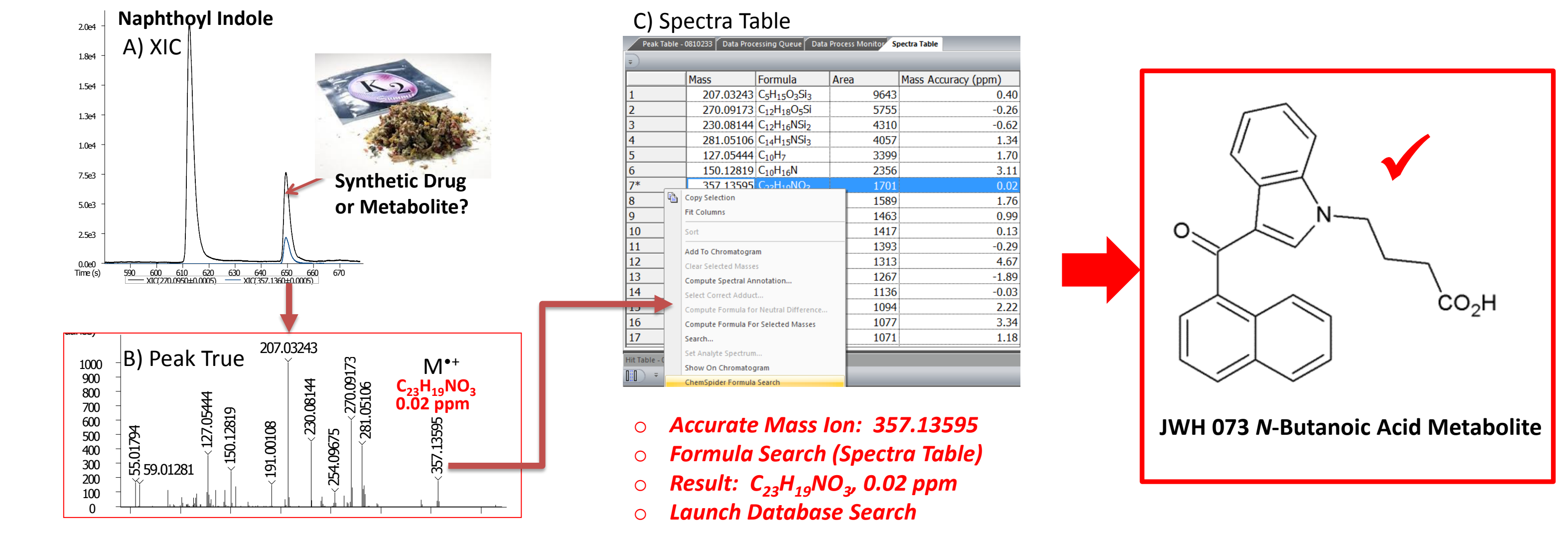


Fig. 12: Peak True (Top) and Library Mass Spectra (Bottom) for 3-(1-Naphthoyl)indole in Sample B.

Synthetic Drugs



	Mass	Formula	Area	Mass Accuracy (ppm)
1	207.03243	$C_5H_{15}O_3Si_3$	9643	0.40
2	270.09173	$C_{12}H_{18}O_5Si$	5755	-0.26
3	230.08144	$C_{12}H_{16}NSi_2$	4310	-0.62
4	281.05106	$C_{14}H_{15}NSi_3$	4057	1.34
5	127.05444	$C_{10}H_7$	3399	1.70
6	150.12819	$C_{10}H_{16}N$	2356	3.11
7*	357.13595	$C_{23}H_{19}NO_3$	1701	0.02
8			1589	1.76
9			1463	0.99
10			1417	0.13
11			1393	-0.29
12			1313	4.67
13			1267	-1.89
14			1136	-0.03
15			1094	2.22
16			1077	3.34
17			1071	1.18

- ○ *Accurate Mass Ion: 357.13595*
- ○ *Formula Search (Spectra Table)*
- ○ *Result: $C_{23}H_{19}NO_3$, 0.02 ppm*
- ○ *Launch Database Search*

Fig. 13: An XIC showing an unknown synthetic drug or metabolite not present in the NIST or Wiley libraries (A). Peak True mass spectrum for the unknown (B). The ChromaTOF-HRT® Spectra Table (C) showing the formula $C_{23}H_{19}NO_3$ for m/z = 357.13595 (mass accuracy = 0.02 ppm) and the initiation of a spectral database search resulting in the potential hit: 4-(3-(1-naphthoyl)1H-indol-1-yl, a JWH 073 N-butanoic acid metabolite.

Sample C (Amphetamine + Pharmaceuticals + Metabolites)

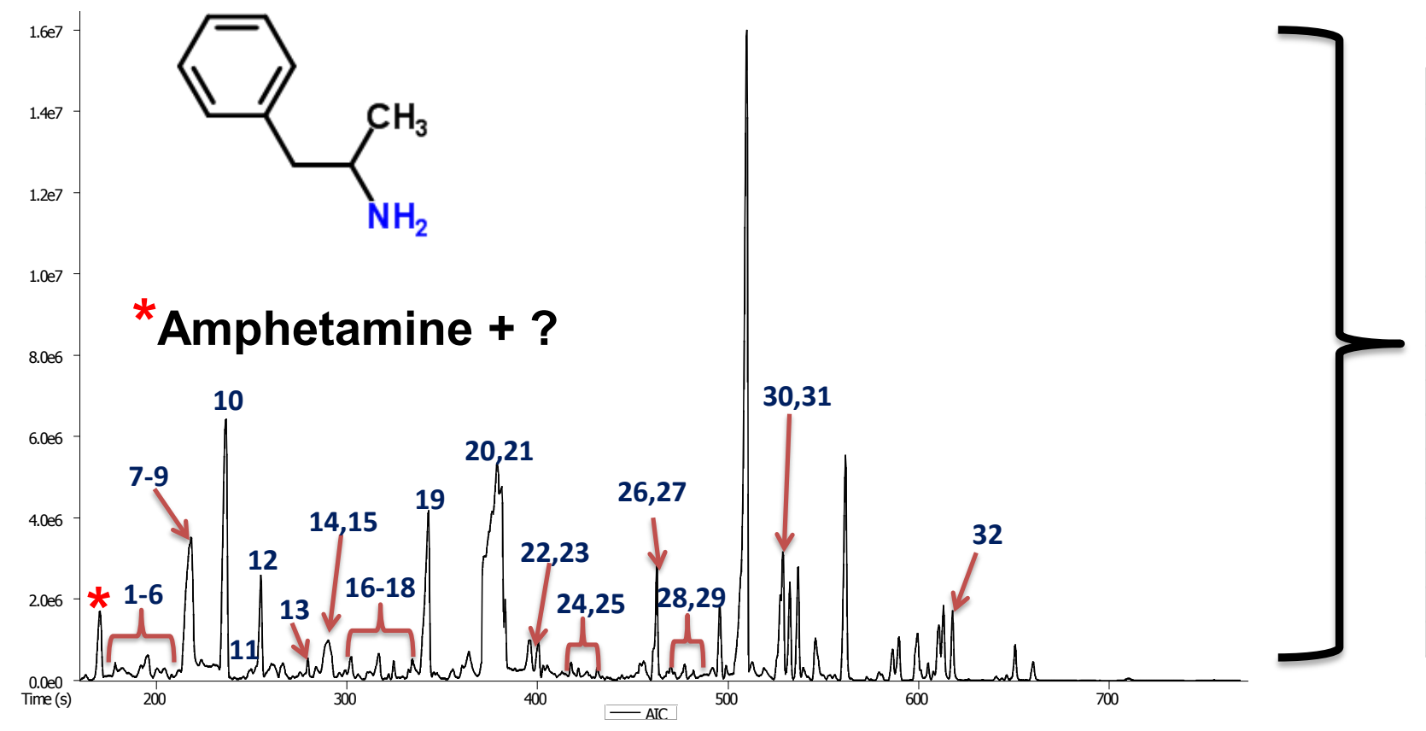


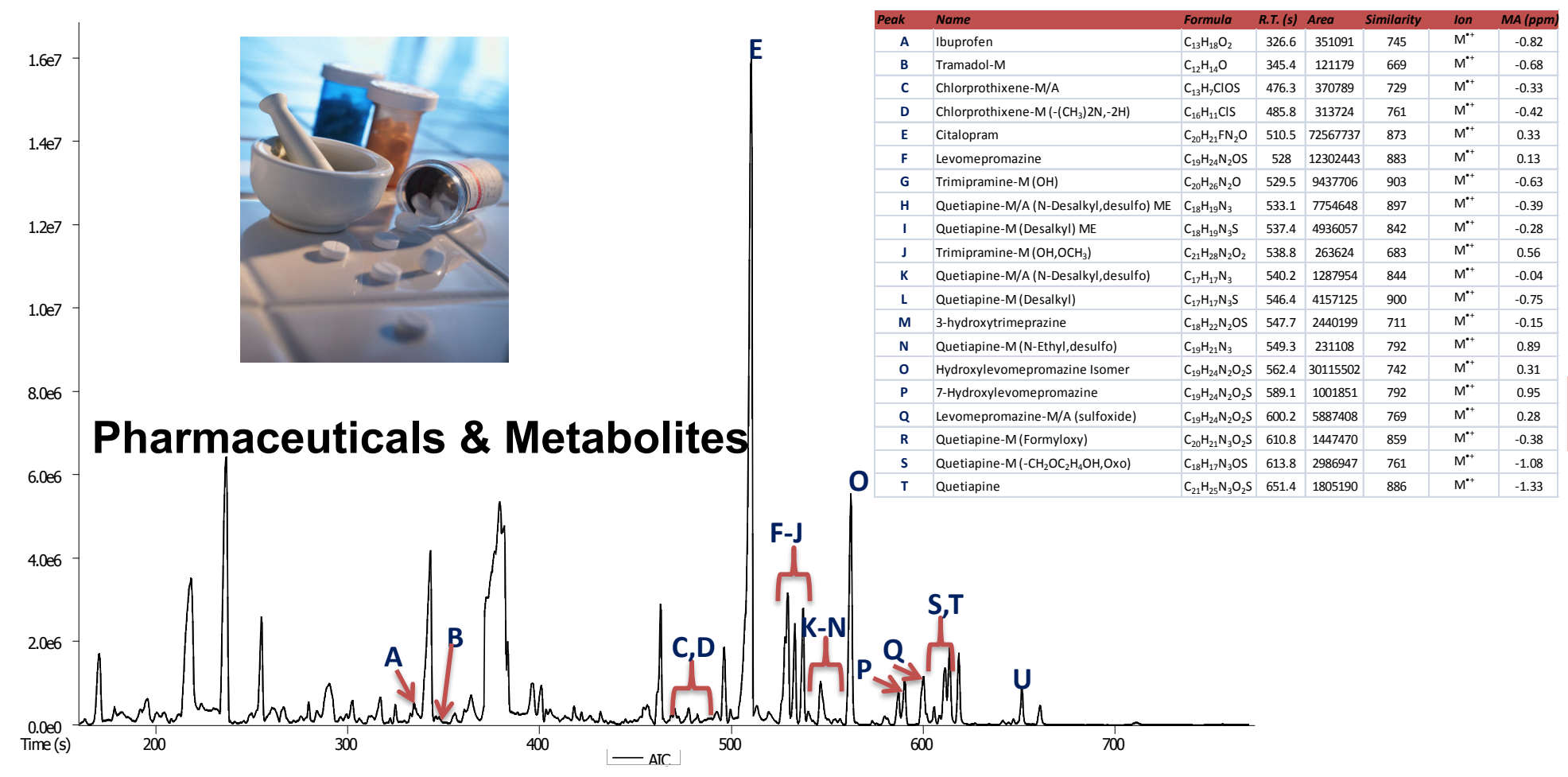
Fig. 14: AIC Displaying Representative Compounds in Sample C.

Peak	Name	Formula	R.T. (s)	Area	Similarity	MA(ppm)
*	Amphetamine	$C_9H_{13}N$	170	9913299	841	-0.14
1	3-Pyridinol	C_5H_5NO	183	6185856	812	-0.09
2	Dihydrothymine	$C_5H_8N_2O_2$	187	621464	746	0.73
3	N,2-Dimethylimidazole	$C_5H_8N_2$	188	178643	813	-0.47
4	Ethanol, 2-phenoxy-	$C_8H_{10}O_2$	194	4463665	809	-0.68
5	Octanoic acid, 2-methyl-	$C_9H_{18}O_2$	204	3662983	726	-0.29
6	Dihydrobenzofuran	C_8H_8O	208	399549	737	0.00
7	Creatinine ME	$C_5H_9N_3O$	218	36375929	773	-0.92
8	o-Ethynylaniline	C_8H_7N	221	3330816	792	-0.19
9	5-methylhydantoin	$C_4H_6N_2O_2$	232	24651088	793	-0.46
10	Nicotin	$C_{10}H_{14}N_2$	237	43628594	889	-1.04
11	1H-Indole, 3-methyl-	C_9H_9N	249	1481839	808	-1.11
12	2-(Methylmercapto)benzonitrile	C_8H_7NS	255	5421426	868	-0.74
13	Creatinine, 1-acetyl-	$C_6H_9N_3O_2$	276	1017562	767	-0.13
14	Methyl 4-hydroxyphenylacetate	$C_9H_{10}O_3$	288	1417270	738	-0.68
15	2,3,5-Trimethyl-6-ethylpyrazine	$C_9H_{14}N_2$	292	207732	734	-0.91
16	4 - vinyl - syringol	$C_{10}H_{12}O_3$	298	219900	764	0.44

Peak	Name	Formula	R.T. (s)	Area	Similarity	MA(ppm)
17	Hexahydropyrrolizin-3-one	$C_7H_{11}NO$	302	2541535	730	-0.10
18	4-(3-Pyridyl-tetrahydrofuran-2-one	$C_9H_9NO_2$	317	1453261	862	-0.95
19	Cotinine	$C_{10}H_{12}N_2O$	343	23921315	865	-0.91
20	methyl 1H-indole-3-acetate	$C_{11}H_{11}NO_2$	371	1792478	792	-0.85
21	Hydroxycotinine	$C_{10}H_{12}N_2O_2$	382	63335156	795	-0.57
22	Theobromine	$C_7H_8N_4O_2$	397	5447784	790	-0.31
23	Proline anhydride	$C_{10}H_{14}N_2O_2$	401	5113606	854	-0.52
24	Acridine, 9-methyl-	$C_{14}H_{11}N$	410	519170	707	-0.47
25	Imidazo[2,1-a]isoquinoline	$C_{11}H_8N_2$	419	1196366	741	-0.13
26	Hydroxyandrostene	$C_{19}H_{30}O$	461	1102975	824	-0.81
27	Trimepramine	$C_{20}H_{26}N_2$	463	8095369	783	-0.47
28	Androsta-5,16-dien-3-ol	$C_{19}H_{28}O$	478	541953	813	0.22
29	Cyclo (Phe-Pro)	$C_{14}H_{16}N_2O_2$	492	1442590	747	-1.17
30	Androsterone	$C_{19}H_{30}O_2$	526	252420	834	0.06
31	Androstenediol	$C_{19}H_{30}O_2$	529	798453	736	0.06
32	Cholesterol	$C_{27}H_{46}O$	619	799390	909	-0.35

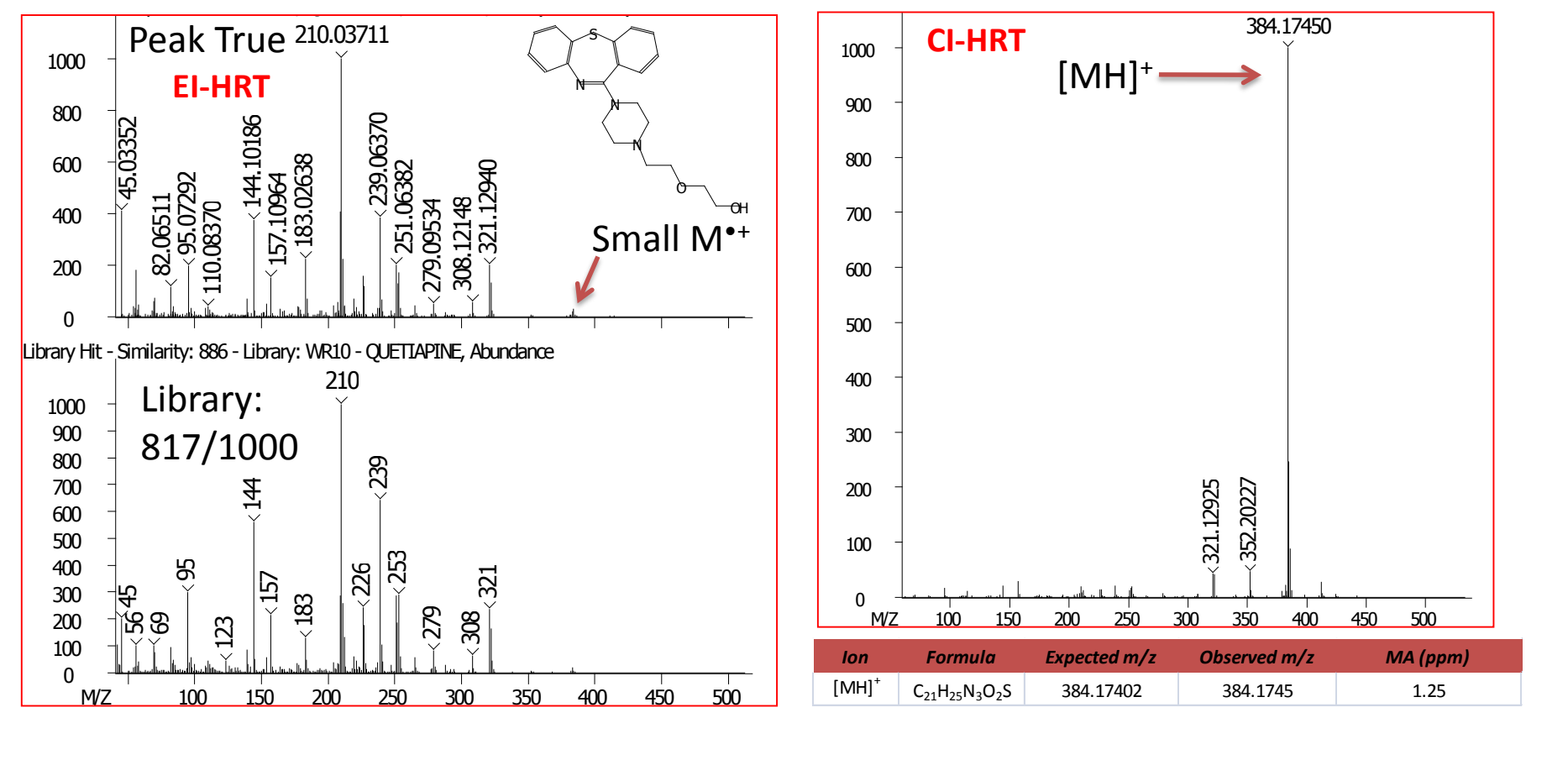
Ave.|ppm| = 0.51

Table 2: Representative Compounds in Sample C.



Peak	Name	Formula	R.T. (s)	Area	Similarity	Ion	MA (ppm)
A	Ibuprofen	$C_{13}H_{18}O_2$	326.6	351091	745	$M^{\bullet+}$	-0.82
B	Tramadol-M	$C_{12}H_{14}O$	345.4	121179	669	$M^{\bullet+}$	-0.68
C	Chlorprothixene-M/A	$C_{13}H_7ClOS$	476.3	370789	729	$M^{\bullet+}$	-0.33
D	Chlorprothixene-M (-(CH₃)2N,-2H)	$C_{16}H_{11}ClS$	485.8	313724	761	$M^{\bullet+}$	-0.42
E	Citalopram	$C_{20}H_{21}FN_2O$	510.5	72567737	873	$M^{\bullet+}$	0.33
F	Levomepromazine	$C_{19}H_{24}N_2OS$	528	12302443	883	$M^{\bullet+}$	0.13
G	Trimipramine-M (OH)	$C_{20}H_{26}N_2O$	529.5	9437706	903	$M^{\bullet+}$	-0.63
H	Quetiapine-M/A (N-Desalkyl,desulfo) ME	$C_{18}H_{19}N_3$	533.1	7754648	897	$M^{\bullet+}$	-0.39
I	Quetiapine-M (Desalkyl) ME	$C_{18}H_{19}N_3S$	537.4	4936057	842	$M^{\bullet+}$	-0.28
J	Trimipramine-M (OH,OCH₃)	$C_{21}H_{28}N_2O_2$	538.8	263624	683	$M^{\bullet+}$	0.56
K	Quetiapine-M/A (N-Desalkyl,desulfo)	$C_{17}H_{17}N_3$	540.2	1287954	844	$M^{\bullet+}$	-0.04
L	Quetiapine-M (Desalkyl)	$C_{17}H_{17}N_3S$	546.4	4157125	900	$M^{\bullet+}$	-0.75
M	3-hydroxytrimeprazine	$C_{18}H_{22}N_2OS$	547.7	2440199	711	$M^{\bullet+}$	-0.15
N	Quetiapine-M (N-Ethyl,desulfo)	$C_{19}H_{21}N_3$	549.3	231108	792	$M^{\bullet+}$	0.89
O	Hydroxylevomepromazine Isomer	$C_{19}H_{24}N_2O_2S$	562.4	30115502	742	$M^{\bullet+}$	0.31
P	7-Hydroxylevomepromazine	$C_{19}H_{24}N_2O_2S$	589.1	1001851	792	$M^{\bullet+}$	0.95
Q	Levomepromazine-M/A (sulfoxide)	$C_{19}H_{24}N_2O_2S$	600.2	5887408	769	$M^{\bullet+}$	0.28
R	Quetiapine-M (Formyloxy)	$C_{20}H_{21}N_3O_2S$	610.8	1447470	859	$M^{\bullet+}$	-0.38
S	Quetiapine-M (-CH₂OC₂H₄OH,Oxo)	$C_{18}H_{17}N_3OS$	613.8	2986947	761	$M^{\bullet+}$	-1.08
T	Quetiapine	$C_{21}H_{25}N_3O_2S$	651.4	1805190	886	$M^{\bullet+}$	-1.33

Fig. 15: AIC and Table Listing Pharmaceuticals and Metabolites in Sample C.



Ion	Formula	Expected m/z	Observed m/z	MA (ppm)
$[MH]^+$	$C_{21}H_{25}N_3O_2S$	384.17402	384.1745	1.25

Fig. 16: EI-HRT Peak True (Top) and Library Mass Spectra (Bottom) for Quetiapine (anti-psychotic). CI-HRT Peak True Spectrum for Quetiapine (Right).

Summary

- GC-HRT analysis provides an effectual "molecular profile" of samples
- Confident compound identification through spectral similarity searches & robust formula determinations for fragment, molecular and adduct ions

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