A Novel Benchtop Time-of-Flight GC-MS System For High Throughput Qualitative And Quantitative Analysis of Drugs of Abuse in Human Urine

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Introduction

Illicit drug use by teenagers and young adults continues to increase in the United States. Drug abuse is costly to our nation, exacting more than $700 billion annually in costs related to crime, lost work productivity, and health care. At the same time, many forensic laboratories are experiencing increased workloads and reduced resources. There is an immediate need for high throughput methods resulting in conclusive identification of controlled substances such as opiates, barbiturates, and amines. In this study, preparation and analysis times were significantly reduced without sacrificing thorough characterization of samples.

Sample Preparation

Urine Extraction and Standard Addition

Urease (100 mg) was added to 2 mL of urine sample which was then incubated at 37°C for 15 minutes. The mixture was treated with 2 mL of 5M NaOH and extracted with 7 mL of CH_2Cl_2: 1) vortex for 1 minute, 2) centrifuge at 12,000 rpm for 5 minutes, and 3) separate the layers. The organic layer was dried with anhydrous $CaCl_2$ and drug standards were spiked into the solution at the following levels: 5, 10, 50, 100, 500, and 1000 ng/mL. The CH_2Cl_2 was removed using N_2 (g) and the residue was reconstituted in $CHCl_3$.

Instrument Parameters

Gas Chromatograph	Agilent 7890 with MPS2 Autosampler
Injection	1μL, Split 20:1
Carrier Gas	He @ 1.4 ml/min, Constant Flow
Column	Rxi-5ms, 20 m x 0.18 mm i.d. x 0.18 μm (Restek, Bellefonte, PA, USA)
Temperature Program	50°C (0.5 min), ramped 50°C/min to 320°C, held 5 min
Mass Spectrometer	LECO Pegasus® BT
Ion Source Temperature	250°C
Ionization Mode	EI
Mass Range (m/z)	35-650
Acquisition Rate	20 spectra/s

Table 1: Peak Table of Drug Abuse Compounds in Human Urine

| Name	RT (s)	Formula	Peak S/N	Similarity
Amobarbital	237.28	$C_{11}H_{18}N_2O_3$	22305	943
Amphetamine	142.52	$C_9H_{13}N$	1371	963
Buprenorphine	489.49	$C_{29}H_{41}NO_4$	834	898
Butalbital	230.81	$C_{11}H_{16}N_2O_3$	3852	933
Codeine	323.82	$C_{18}H_{21}NO_3$	6212	935
Fentanyl	356.73	$C_{22}H_{28}N_2O$	376	804
Hydrocodone	330.82	$C_{18}H_{21}NO_3$	2153	929
Hydromorphone	332.66	$C_{17}H_{19}NO_3$	652	885
MDEA	218.06	$C_{12}H_{17}NO_2$	19917	916
Meperidine	244.10	$C_{15}H_{21}NO_2$	13085	933
Methadone	294.56	$C_{21}H_{27}NO$	5333	917
Methamphetamine	153.35	$C_{10}H_{15}N$	28971	912
Morphine	329.50	$C_{17}H_{19}NO_3$	3186	855
MDMA	211.25	$C_{11}H_{15}NO_2$	282	894
Oxycodone	339.64	$C_{18}H_{21}NO_4$	1892	895
Oxymorphone	341.85	$C_{17}H_{19}NO_4$	498	807
Pentobarbital	241.17	$C_{11}H_{18}N_2O_3$	14092	949
Phenobarbital	271.80	$C_{12}H_{12}N_2O_3$	9466	910
Phentermine	149.41	$C_{10}H_{15}N$	1882	922
Secobarbital	247.90	$C_{12}H_{18}N_2O_3$	11180	928
Tenamfetamine	203.32	$C_{10}H_{13}NO_2$	6651	889
Tramadol	270.50	$C_{16}H_{25}NO_2$	14976	801
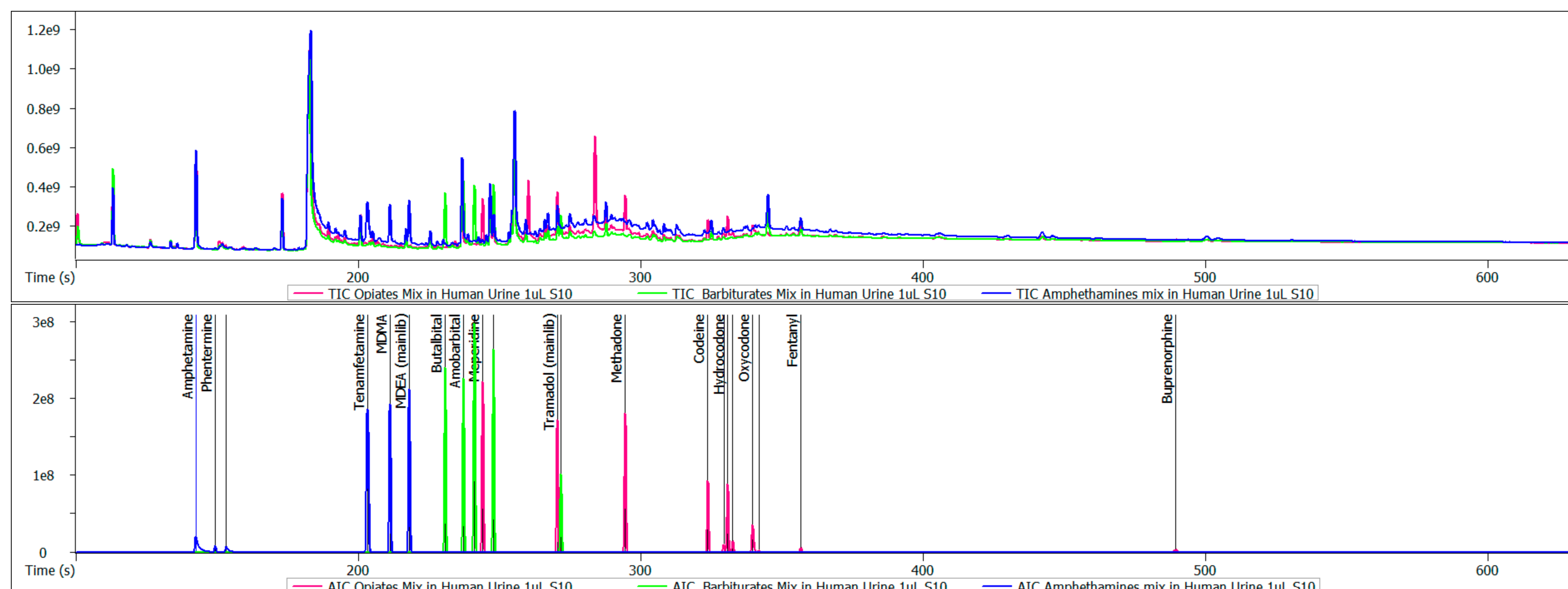
Figure 1. TIC and AIC overlap of Drug Abuse Mixes in Human Urine. Mixes include Opiates, Barbiturates, and Amphetamines.
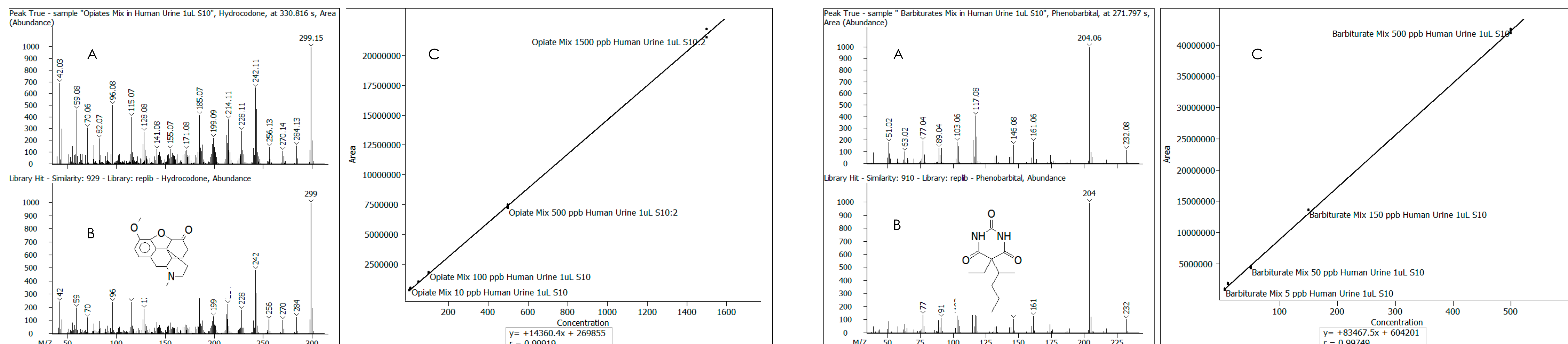

Figure 2. (A,B) Peak True and Library Mass Spectral Data for Hydrocodone in Smoker Urine. (C) Calibration Curve of Hydrocodone in Human Urine.

y= +14360.4x + 269855
r = 0.99919

Figure 3. (A,B) Peak True and Library Mass Spectral Data for Pentobarbital in Human Urine, (C) Calibration Curve of Pentobarbital in Human Urine

y= +83467.5x + 604201
r = 0.99749

Table 2: Peak table of Compounds in Barbiturate Spike Human Urine

Name	R.T. (s)	Formula	Area	Similarity	Mass Delta (Da)
3-Methoxyamphetamine	203	$C_{10}H_{15}NO$	4141165	913	N/A
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	265	$C_{11}H_{18}N_2O_2$	19262066	827	N/A
10,18-Bisnorabieta-8,11,13-triene	280	$C_{18}H_{26}$	32302999	840	0.00
N-Desmethyl-cis-tramadol	274	$C_{15}H_{23}NO_2$	35880970	839	-0.01
Phenobarbital	272	$C_{12}H_{12}N_2O_3$	97649461	866	0
Butalbital	231	$C_{11}H_{16}N_2O_3$	231882170	932	N/A
Secobarbital	248	$C_{12}H_{18}N_2O_3$	248340909	941	-0.02
Amobarbital	237	$C_{11}H_{18}N_2O_3$	387855631	960	-0.01
Methyl stearate	284	$C_{19}H_{38}O_2$	437237841	947	-0.01
Pentobarbital	241	$C_{11}H_{18}N_2O_3$	453729166	938	-0.02
Caffeine	255	$C_8H_{10}N_4O_2$	1106666278	951	0.00
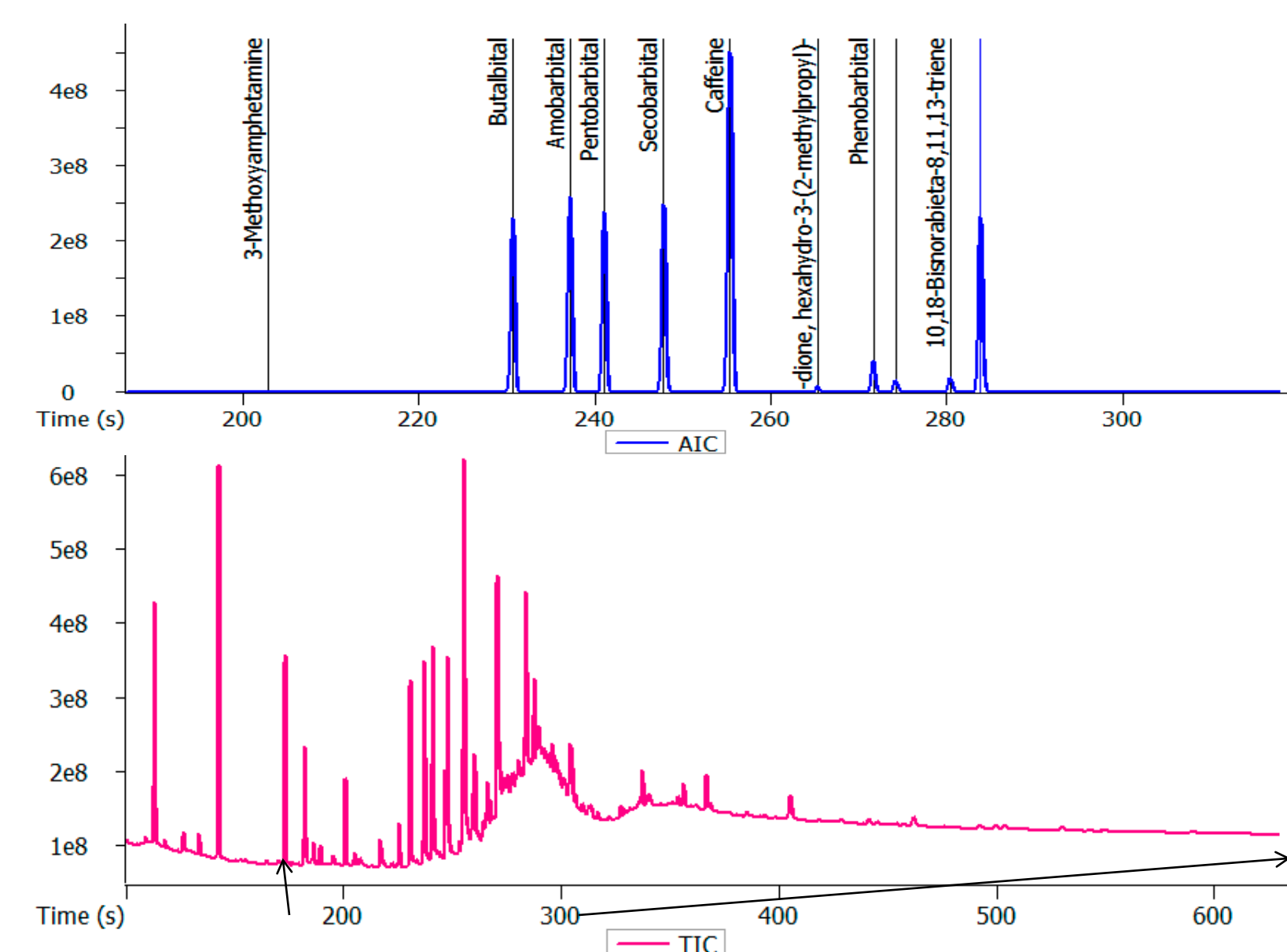
Figure 4. AIC and TIC of Barbiturates in Human Urine

Table 3. Detection limits of Drug Abused Compounds in Human Urine and Government Cut-Off Levels[1]

Compound names	Detection limits (ng/mL)	Government cut-off level (ng/mL)
Amobarbital	0.5	200
Phenobarbital	5	200
Butabarbital	1	200
Secobarbital	5	200
Pentobarbital	0.5	200
Amphetamine	10	500
Methamphetamine	10	500
MDEA	0.5	250
Tenamfetamine	50	250
MDMA	0.5	250
Phentermine	10	250
Buprenorphine	1	250
Hydrocodone	0.5	300
Methadone	10	300
Naltrexone	100	300
Tramadol	0.5	100
Codeine	50	2000
Hydromorphone	50	300
Morphine	120	2000
Oxycodone	15	100
Fentanyl	100	300
Meperidine	10	20
Naloxone	100	300
Oxymorphone	60	100

Data Acquisition and Processing

Data was processed using comprehensive NonTarget Deconvolution™, with Peak True spectra compared to reference spectra registered in the NIST library. Target Analyte Finding was used to search for compounds of interest in urine samples and to generate calibration curves for drugs of abuse.
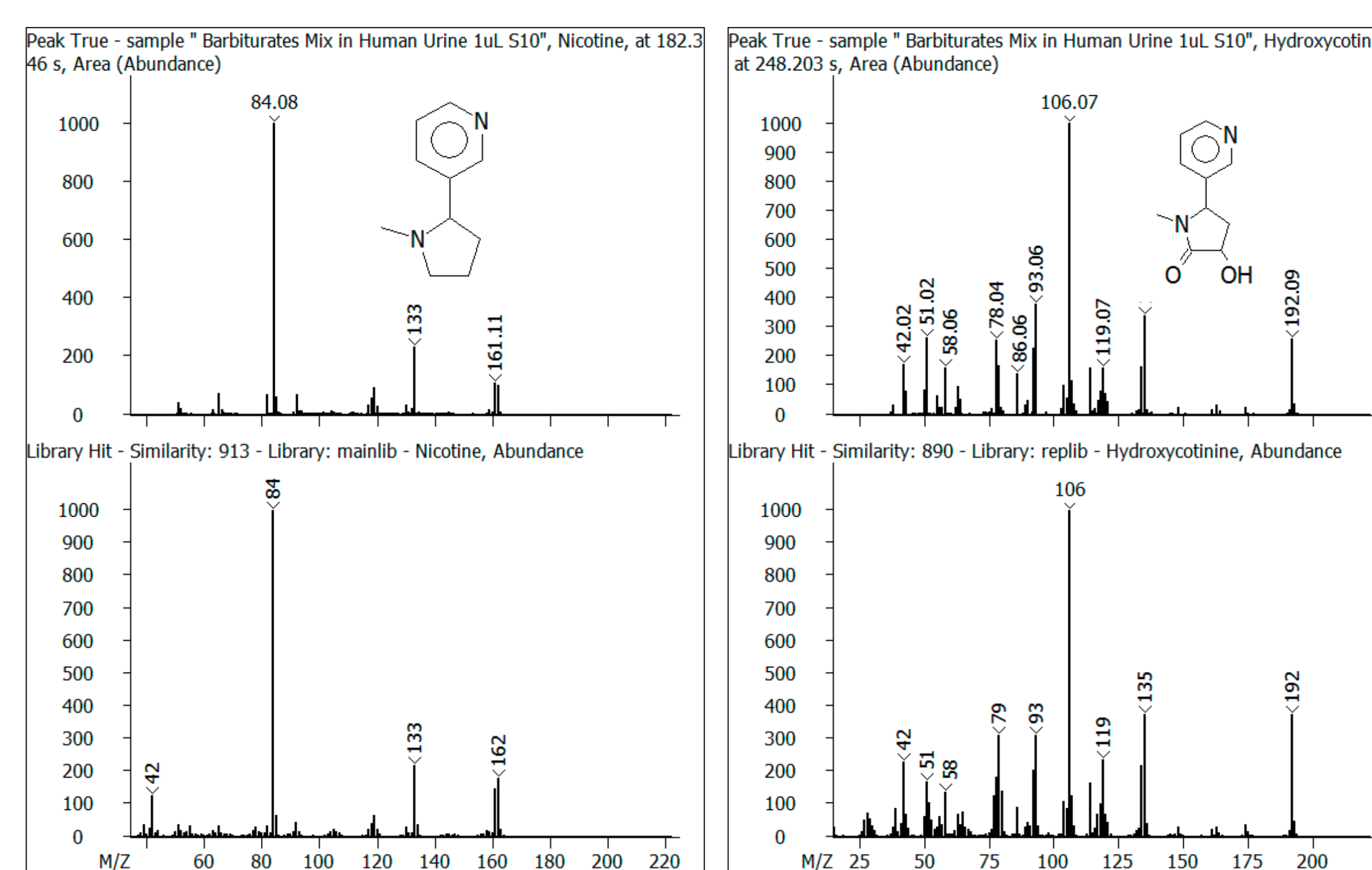

Figure 5. Peak True and Library Mass Spectral Data for Nicotine
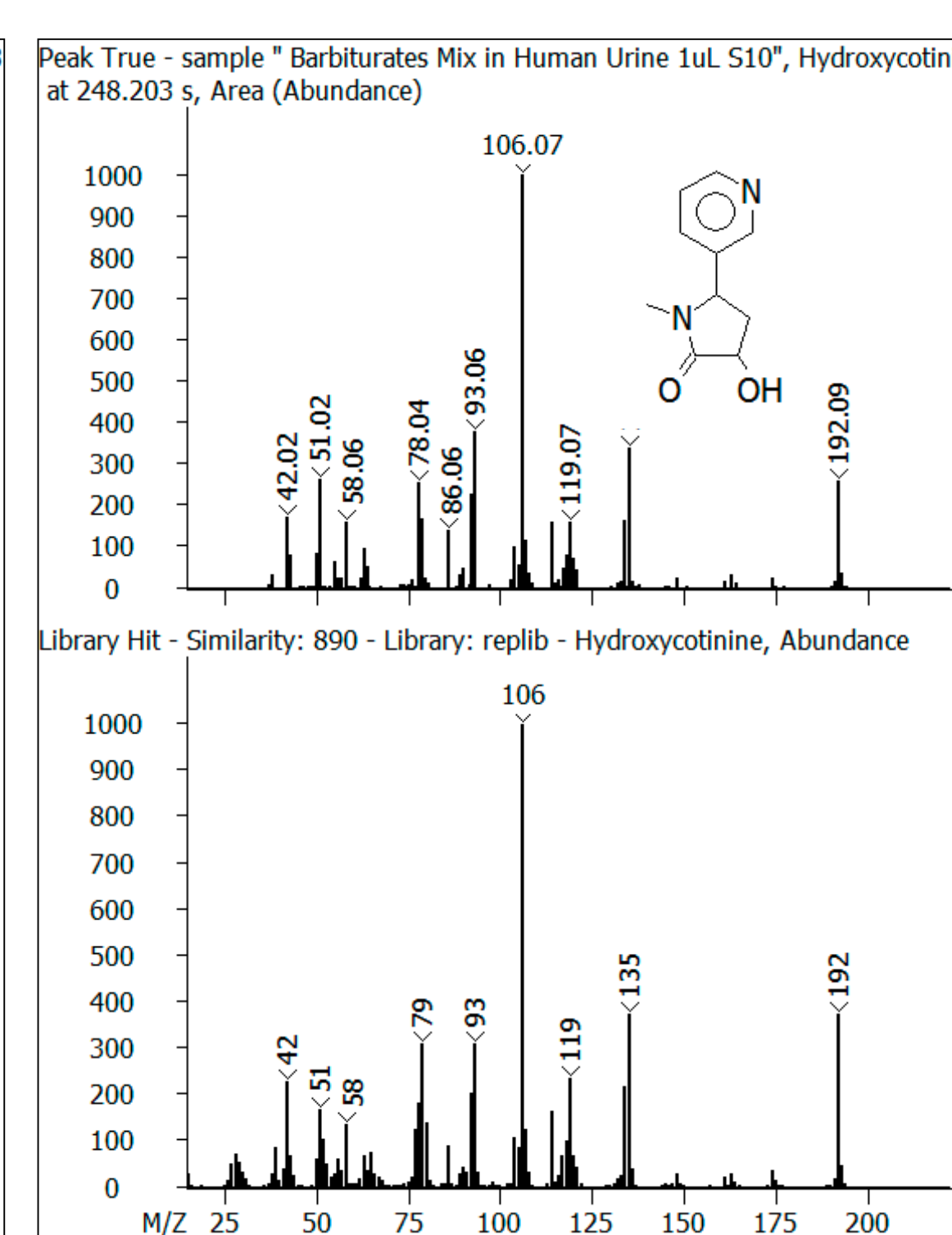
Figure 6. Peak True and Library Mass Spectral Data for Hydroxycotonine
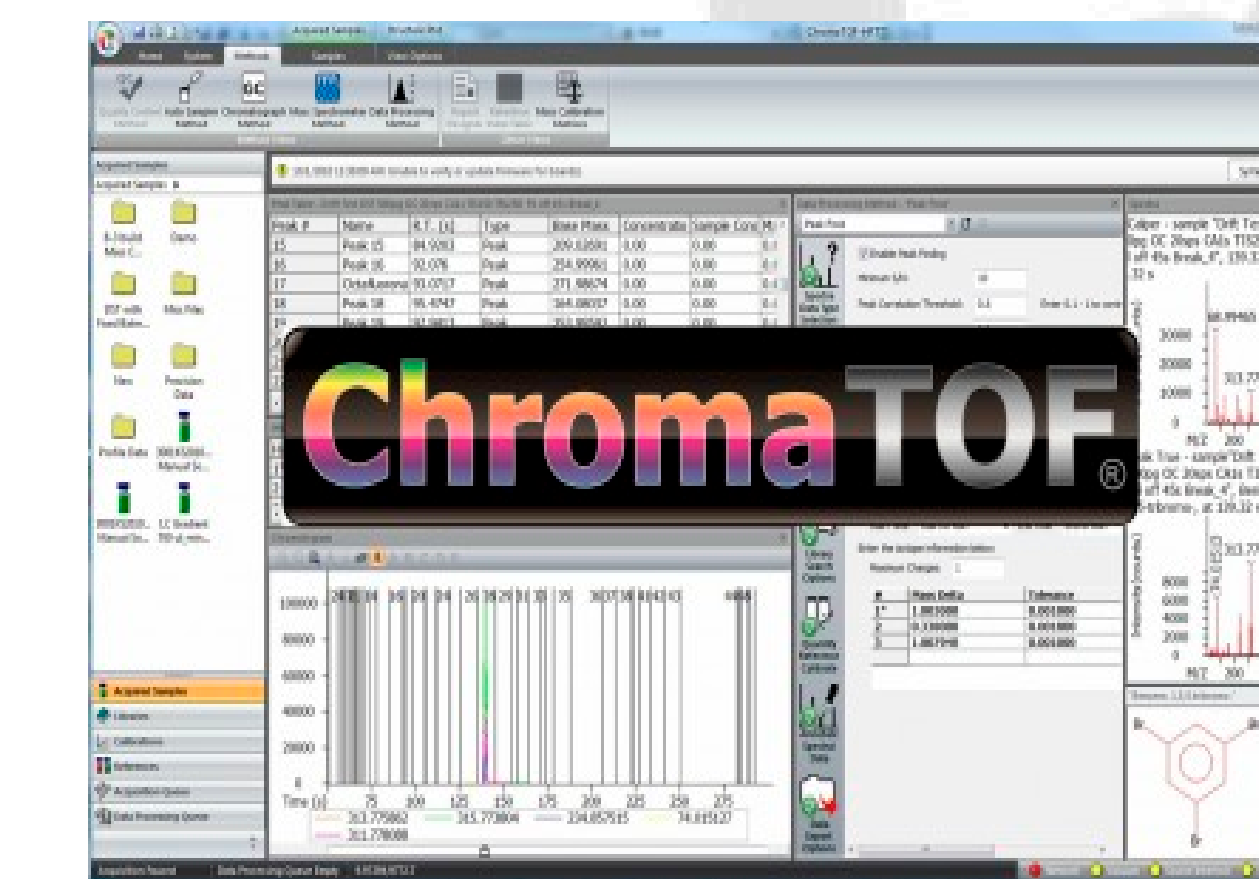
Conclusion

- High performance GC-TOFMS analysis facilitated drug identification through the acquisition of full range, non-skewed mass spectral data.
- Detection limits of non-derivatized drugs were well below the government cut-off levels.
- Runtime for barbiturates, opiates, and amines are under 10 minutes.
- Excellent calibration linearity with a concentration range from 5 ng/mL to 1000 ng/mL for opiates, barbiturates, and amines.
- Comprehensive data processing resulted in identification of heterocyclic compounds such as alkaloids including cotinine and nicotine, as well as narcotics, steroids, and pharmaceuticals in urine samples.

Reference

[1]Pizzo, Pat. "INTERPERTATION OF DRUG TEST RESULTS." *National Association of Drug Court Professionals*. N.p., **2015**. Web. 03 Aug. 2016.