Analysis of Urine SRMs Using Solid Phase Micro Extraction, Dynamic Headspace and Liquid Injection with Comprehensive Two-Dimensional Gas Chromatography (GCxGC) High Resolution Time-of-Flight Mass Spectrometry

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Introduction

Laboratory medicine began thousands of years ago through urine analysis. Urine was a "Divine Fluid and Window to the Body". During Babylonian, Egyptian, and through Victorian times, urine was the primary diagnostic tool (Uroscopy).

Today urine is still a favored biofluid for diagnostic testing (Urinalysis) because large volumes are easily obtained. In addition, urine is relatively free from interfering proteins and lipids, and it tends to "hold" high concentrations of drugs and metabolites over extended periods of time. Modern, routine clinical tests include the determination of specific gravity, measurement of glucose, nitrates, etc.

Objectives

- Evaluate different methods of sample introduction
- Implement the use of enhanced, comprehensive (GCxGC) chromatography for the separation of urine compounds
- Use modern-day, high resolution time-of-flight mass spectrometry (HRT) and powerful processing software to quickly and confidently identify compounds in urine

Technology

Sample Introduction

Data Acquisition and Processing

LECO Pegasus® GC-HRT

Instrument Attribute	Value
Mass Accuracy	<1ppm
Mass Range	10-1500 m/z
Resolving Power	up to 50,000
Data Acquisition Speed	Up to 200 sps
Ionization	EI, PCI

Experimental

Sample Preparation

a) Solid Phase Micro Extraction (SPME), Twister, and Dynamic Head Space (DHS) analysis.

1 mL aliquots of smoker's (NIST SRM 3673) and non-smoker's (SRM 3672) urine were placed in 10 mL vials. Salt (200 mg NaCl) was added to increase the ionic strength of the mixture. The urine was sampled without further treatment, or after the addition of either acid (60 µL 12M HCl) or base (100 µL 5M NaOH).

b) Liquid sampling.

200 µL of urine incubated with 10 mg of urease at 37°C for 15 min. 800 µL of methanol was added and the mixture was vortexed and then centrifuged (12,000 g for 10 minutes). 500 µL aliquots were evaporated to dryness and derivatized using a two-step process.

1) Methoximation (30 µL of MEOX, 60°C, 1 hour) and
2) Silylation (100 µL of ACN, 200 µL MSTFA, 60°C, 1 hour)

Instrument Parameters

- *SPME*: Extracted samples with a 85 µm polyacrylate coated SPME fiber for 30 minutes in an agitator at 80°C (5s on, 2s off, 250 rpm). Desorbed for 3 minutes in a CIS4 injector at 280°C.
- *DHS*: Sampled urine for 1 hour using Tenax® desorption tubes (Complete Evaporation). Desorbed compounds using a TDU (40°C to 280°C at 720°C/min, 5 min hold) and CIS4 injector (-120°C to 280°C at 12°C/s, 5 min hold).
- *Twister:* Extracted samples with PDMS coated stir bars for 1 hour and transferred bars to TDU tubes.

  TDU Desorption (30°C to 280°C at 12°C/s, 5 min hold) and CIS4 injector (-30°C to 280°C at 12°C/s, 5 min hold).
- *Liquid:* 1 µL injection, split 20:1, CIS4 injector (35°C to 280°C at 12 °C/s, 5 min hold).

GC-HRT 4D

Gas Chromatograph	Agilent 7890B with Gerstel MPS Autosampler
Injection	1µL, Split 20:1, CIS 4 (35°C to 280°C at 12°C/s, hold 5 minutes)
Carrier Gas	He @ 1.0 ml/min, Constant Flow
Column 1 Column 2 (GCxGC-HRT)	Rxi-5 Sil MS, 30 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA) Rxi-17 Sil MS, 0.6 m x 0.25 mm i.d. x 0.25 µm (Restek, Bellefonte, PA, USA)
Temperature Program Modulation (GCxGC-HRT)	Liq.: 50°C (1 min) to 150°C @ 10°C/min, to 320°C @ 15°C/min (10 min) SPME, DHS, Twister: 50°C (1 min) to 300°C @ 10°C/min, to 320°C @ 30°C/min (6 min) 3s with 5°C temperature offset relative to oven temperature program and 15°C modulator offset relative to secondary oven
Mass Spectrometer	**LECO Pegasus HRT**
Transfer Line	300°C
Ion Source Temperature	250°C (EI)
Acquisition Mode	High Resolution, R = 25,000 at m/z = 219
Ionization and Mass Range (m/z)	EI35-510
Acquisition Rate	10 spectra/s; (200 spectra/s GCxGC-HRT)

Results: SPME

Peak #	Name	R.T. (s)	Formula	Area	Similarity
1	3-Furaldehyde	295	$C_5H_4O_2$	10091769	965
2	p-Cresol	495	C_7H_8O	198616139	961
3*	L-Borneol	582	$C_{10}H_{18}O$	5832814	725
4	o-Thymol	691	$C_{10}H_{14}O$	59556821	906
5*	Corocalene	959	$C_{15}H_{20}$	1074563	764
6	Oxybenzone	1198	$C_{14}H_{12}O_3$	37787078	944
7	Androst-2-en-17-one	1331	$C_{19}H_{28}O$	27645425	846

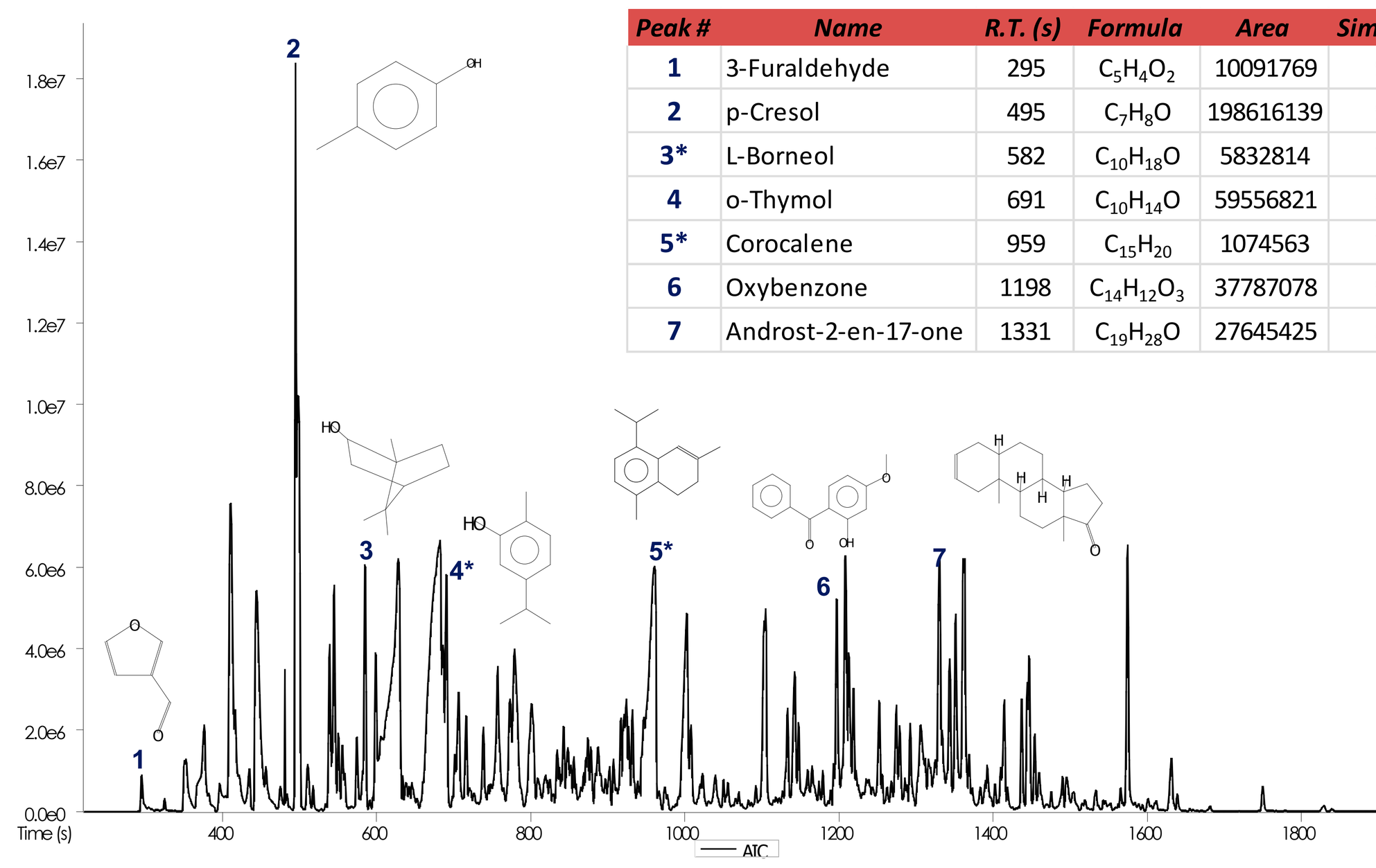


Figure 1. AIC and Table of Representative Compounds in Smoker's Urine.

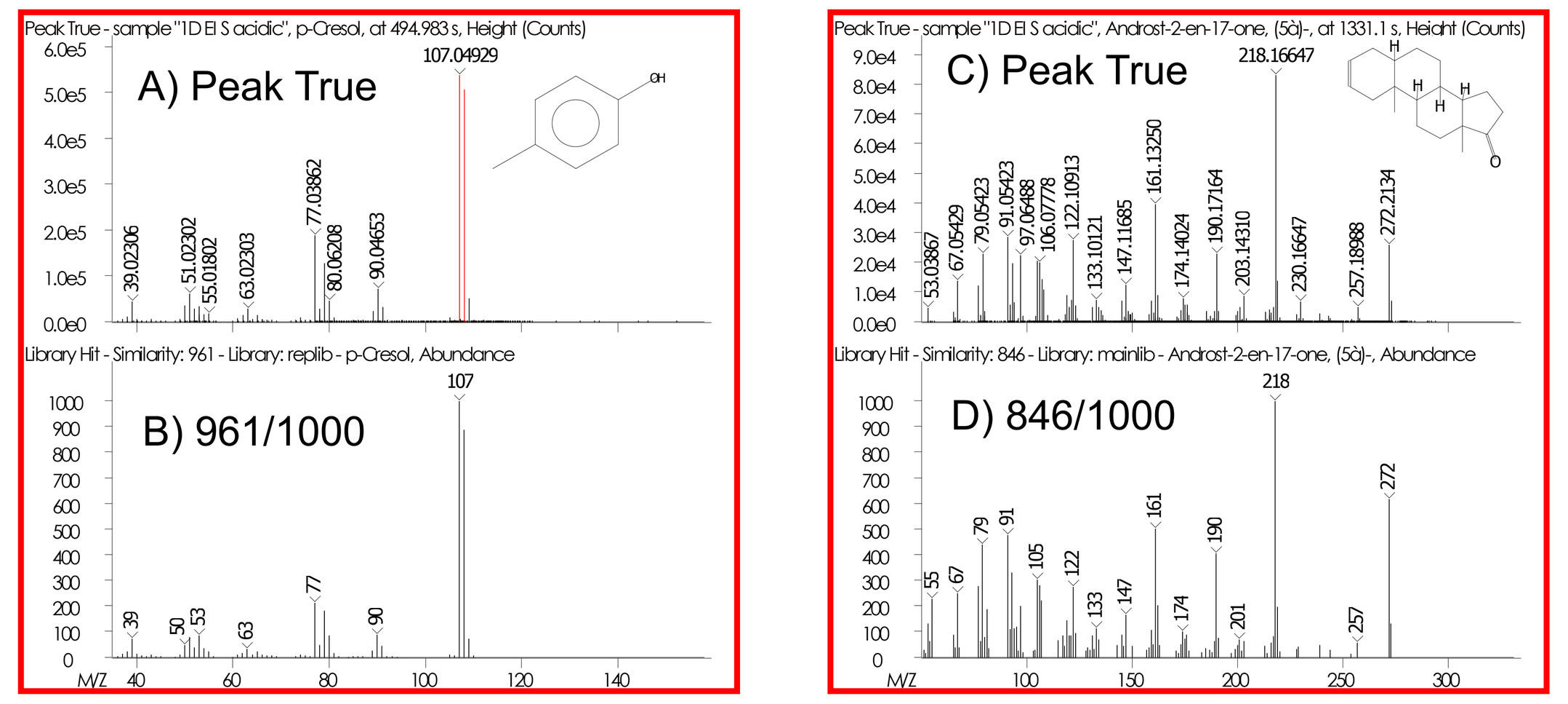


Figure 2. Peak True (Deconvoluted) and Library Mass Spectral Data for p-Cresol and Androst-2-en-17one.

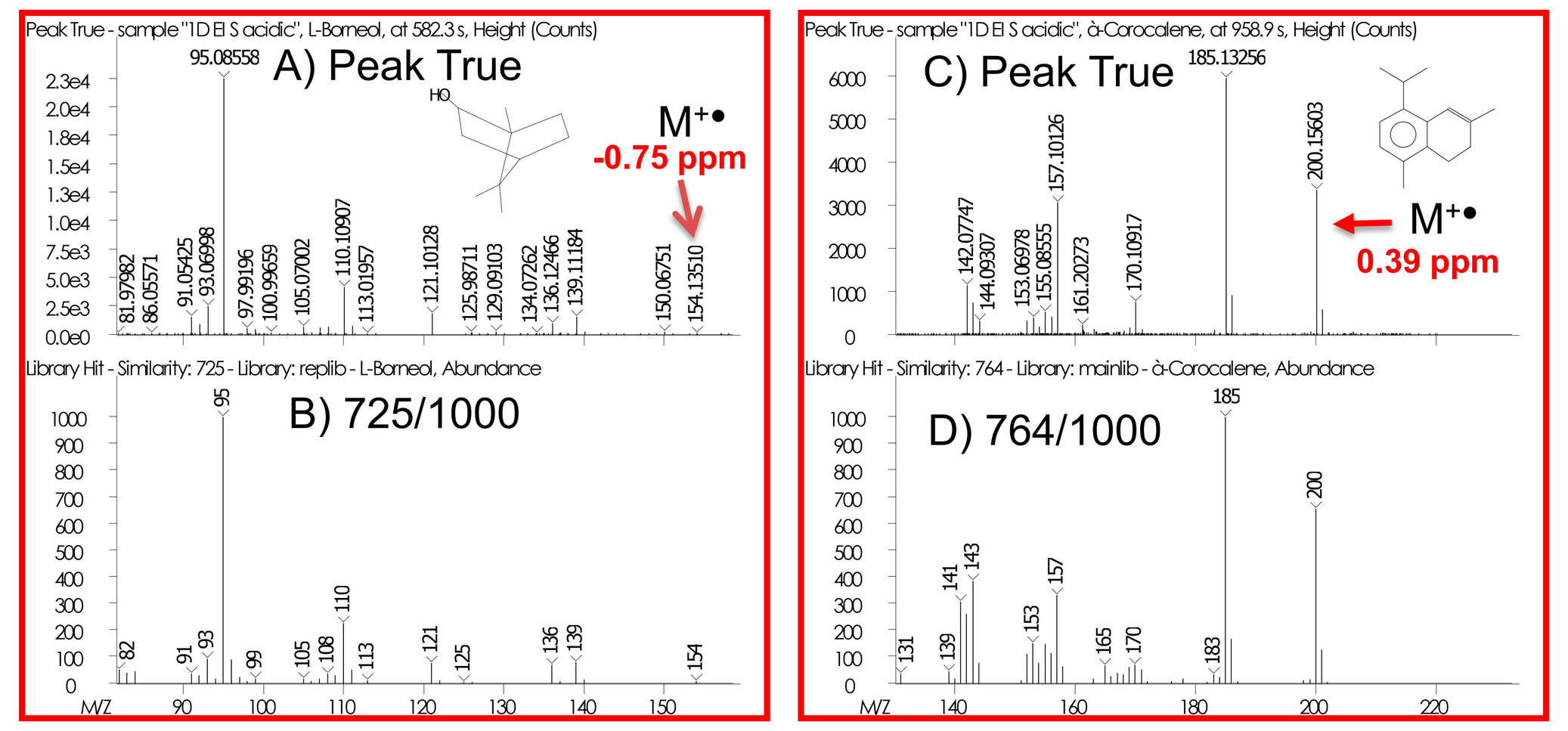


Figure 3. Peak True and Library Mass Spectral Data for L-Borneol and α-Corocalene.

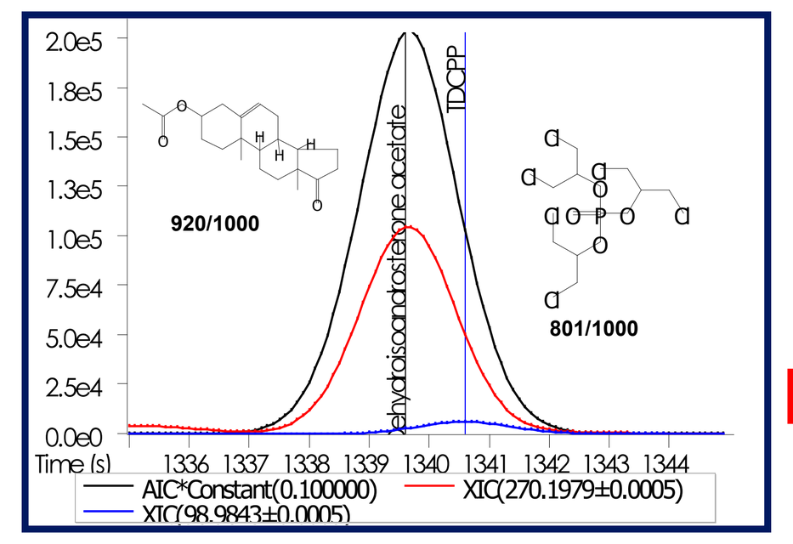
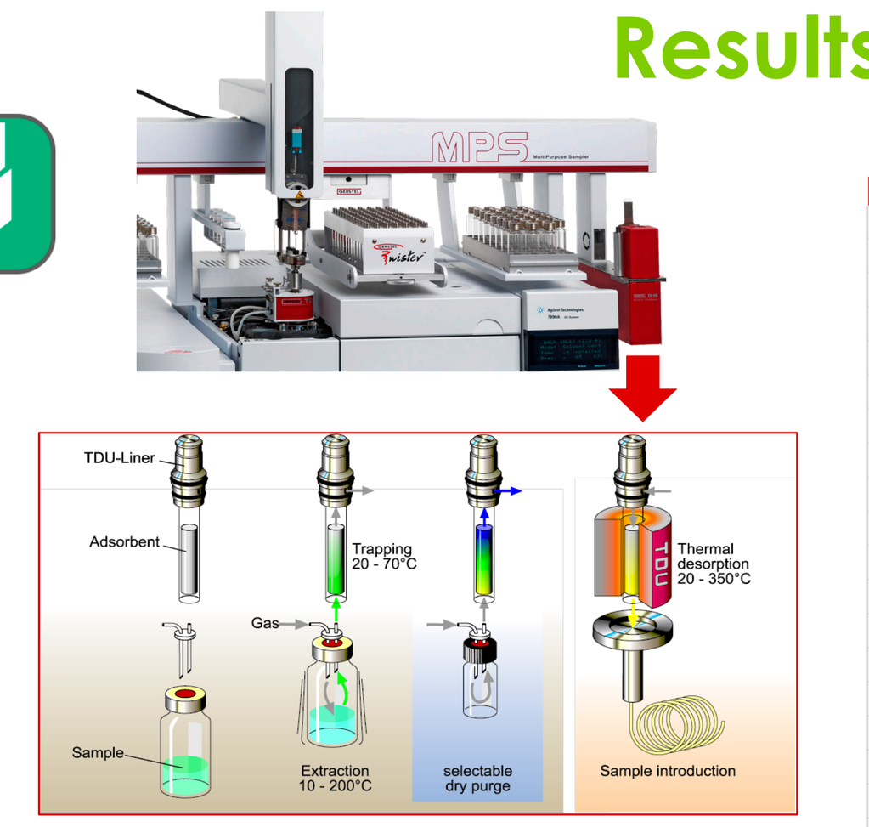


Figure 7. AIC and XIC Expansion of Smoker's Urine.

Results: DHS



Peak #	Name	R.T. (s)	Formula	Area	Similarity
1	Ethyl 2-methyllactate	304	$C_6H_{12}O_3$	1671140	863
2	4-Heptanone	309	$C_7H_{14}O$	9633829	895
3	Ethanone, 1-(2-furanyl)-	345	$C_6H_6O_2$	43956532	931
4	Phenol	400	C_6H_6O	220036706	932
5	α-Cymene	445	$C_{10}H_{14}$	13171425	950
6	Maple lactone	462	$C_6H_8O_2$	5608045	860
7	p-Cresol	491	C_7H_8O	505346237	956
8	Mequinol	506	$C_7H_8O_2$	24175466	710
9	Methyl caprolactone	511	$C_7H_{12}O_2$	15120252	965
10	Maltol	533	$C_6H_6O_3$	4554001	871
11	(+)-Isomenthol	581	$C_{10}H_{20}O$	23827800	948
12	Benzeneacetic acid	655	$C_8H_8O_2$	26194161	908
13	3-Methoxycatechol	662	$C_7H_8O_3$	5849249	917
14	Nonanoic acid	669	$C_9H_{18}O_2$	50648943	930
15	o-Thymol	685	$C_{10}H_{14}O$	6481339	940
16	2-Acetyl-4-methylphenol	697	$C_9H_{10}O_2$	1302967	829
17	2,4-Di-tert-butylphenol	845	$C_{14}H_{22}O$	3529693	938
18	Kodaflex txib	907	$C_{16}H_{30}O_4$	5130216	866
19	Diethyl Phthalate	907	$C_{12}H_{14}O_4$	13582179	947
20	Ibuprofen	929	$C_{13}H_{18}O_2$	15798221	937
21	Myristic acid	1011	$C_{14}H_{28}O_2$	3136089	736
22	Caffeine	1079	$C_8H_{10}N_4O_2$	309544	872
23	Palmitic acid	1136	$C_{16}H_{32}O_2$	26732524	883
24	Dibutyl phthalate	1138	$C_{16}H_{22}O_4$	1120513	958
25	Stearic acid	1245	$C_{18}H_{36}O_2$	17809730	905
26	Androst-2-en-17-one, (5α)-	1319	$C_{19}H_{28}O$	101901	802
27	Dehydroisoandrosterone acetate	1341	$C_{21}H_{30}O_3$	75445	810
28	Dehydroabietic acid	1397	$C_{20}H_{28}O_2$	77045	785

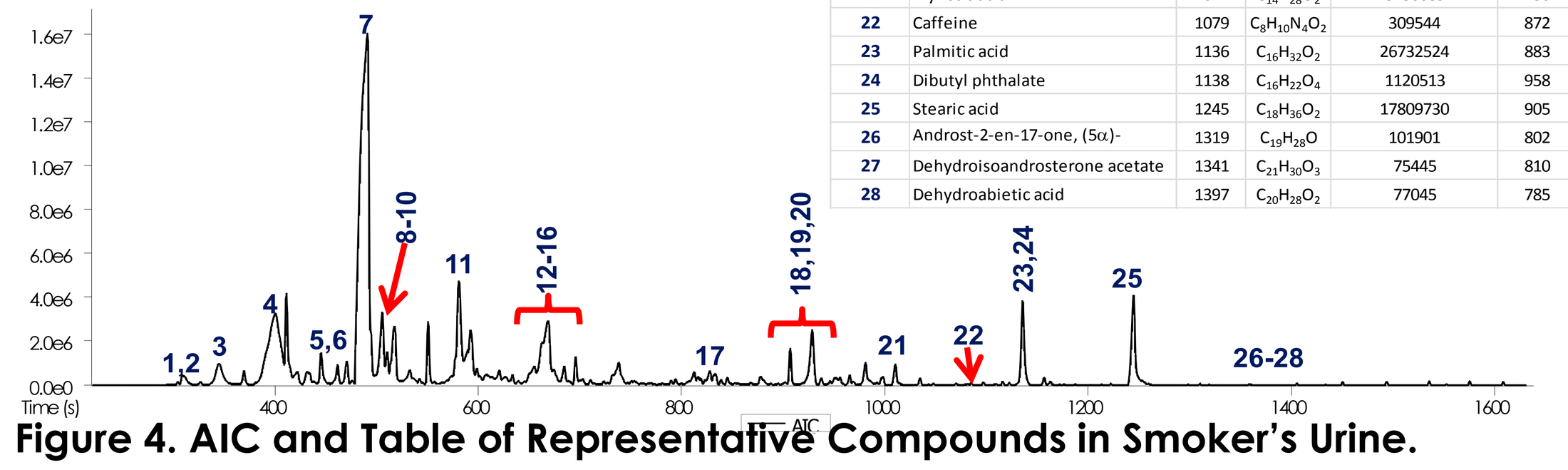


Figure 4. AIC and Table of Representative Compounds in Smoker's Urine.

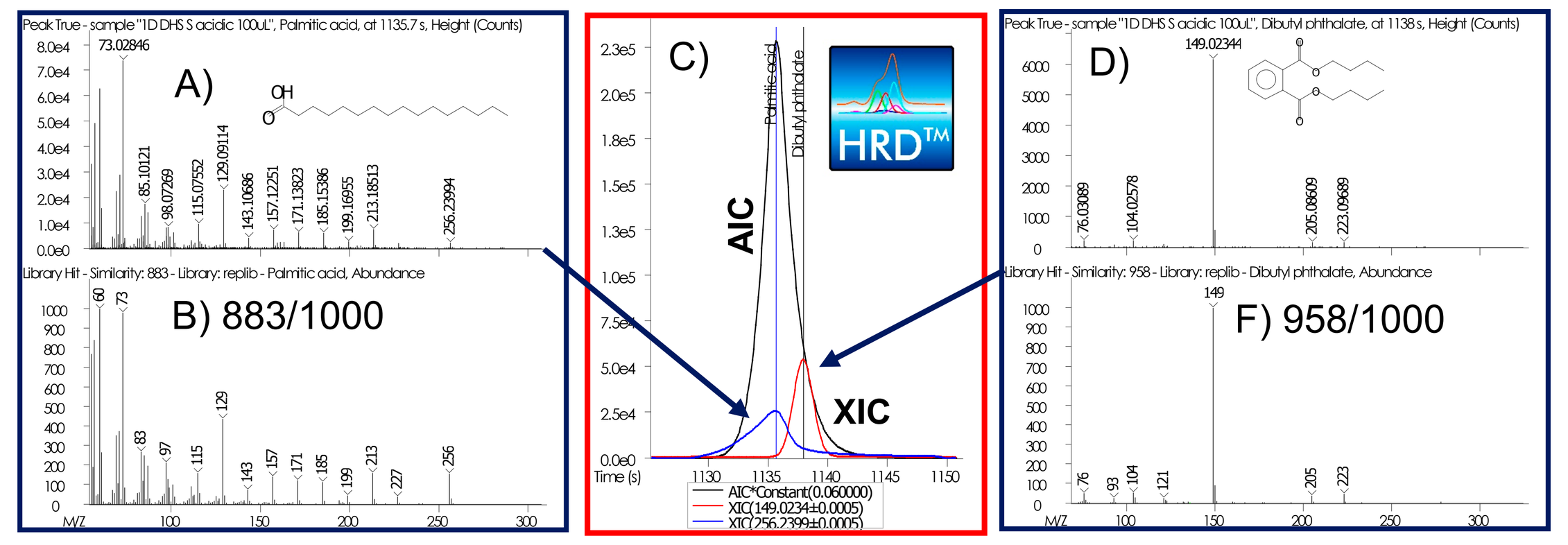
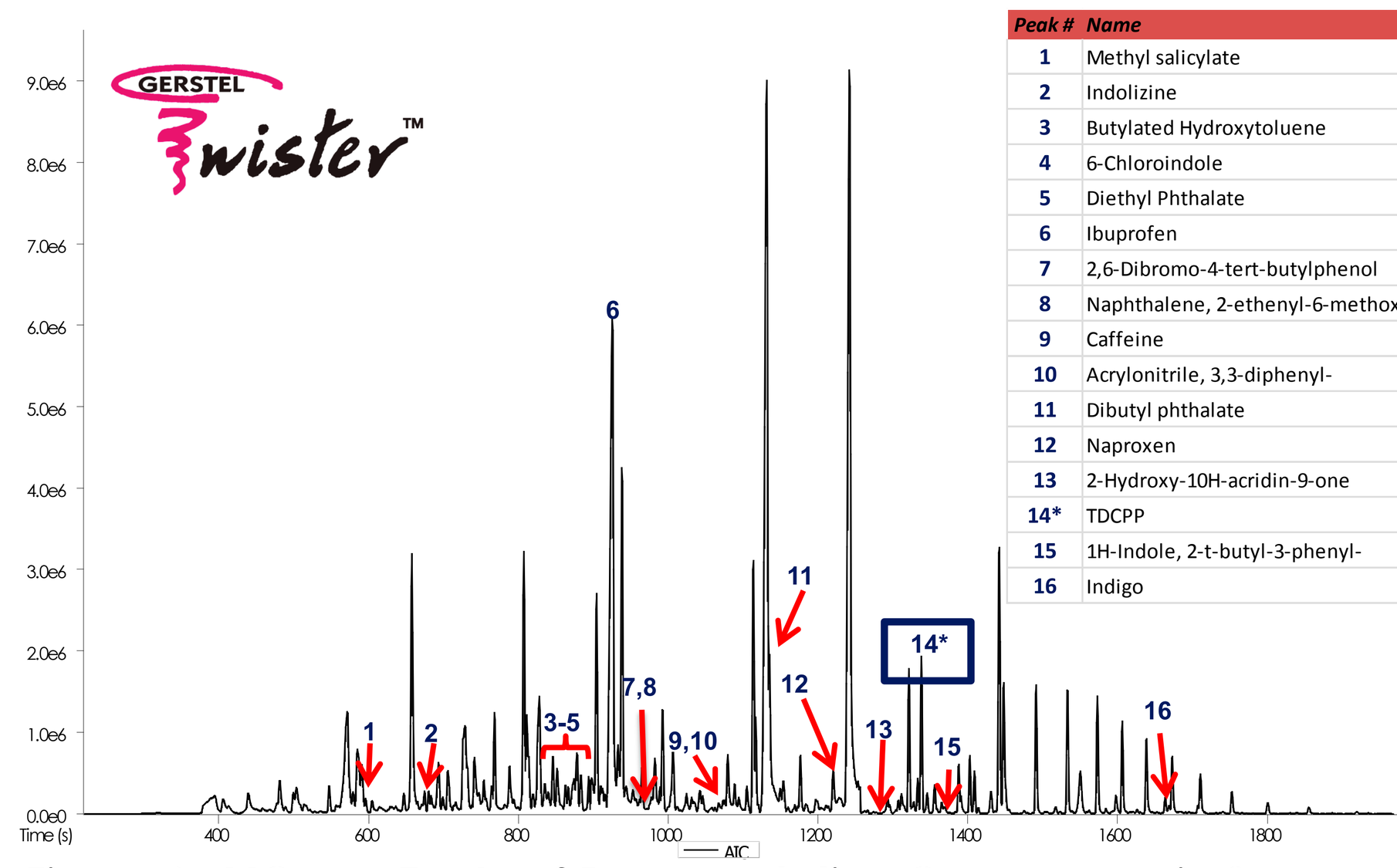


Figure 5. AIC and XIC Expansion of Smoker's Urine Demonstrating Benefits of High Resolution Deconvolution. Peak True and Library Mass Spectral Data for Palmitic Acid and Dibutyl Phthalate.

Results: Twister



Peak #	Name	R.T. (s)	Formula	Area	Similarity	ppm
1	Methyl salicylate	596	$C_8H_8O_3$	1187677	899	0.30
2	Indolizine	681	C_8H_7N	1573629	747	0.35
3	Butylated Hydroxytoluene	847	$C_{15}H_{24}O$	3214774	917	0.17
4	6-Chloroindole	867	C_8H_6ClN	1847775	894	-0.07
5	Diethyl Phthalate	905	$C_{12}H_{14}O_4$	25958062	959	-1.02
6	Ibuprofen	927	$C_{13}H_{18}O_2$	36222585	943	0.61
7	2,6-Dibromo-4-tert-butylphenol	978	$C_{10}H_{12}Br_2O$	129860	758	0.44
8	Naphthalene, 2-ethenyl-6-methoxy-	985	$C_{13}H_{12}O$	618867	814	0.61
9	Caffeine	1077	$C_8H_{10}N_4O_2$	641926	880	0.61
10	Acrylonitrile, 3,3-diphenyl-	1093	$C_{15}H_{11}N$	183868	831	-0.99
11	Dibutyl phthalate	1137	$C_{16}H_{22}O_4$	16641478	937	-0.96
12	Naproxen	1222	$C_{14}H_{14}O_3$	1925593	816	0.86
13	2-Hydroxy-10H-acridin-9-one	1275	$C_{13}H_9NO_2$	173092	717	0.62
14*	TDCPP	1341	$C_9H_{15}Cl_6O_4P$	121539	801	N/A
15	1H-Indole, 2-t-butyl-3-phenyl-	1369	$C_{18}H_{19}N$	134526	783	0.16
16	Indigo	1602	$C_{16}H_{10}N_2O_2$	301880	850	0.64

Figure 6. AIC and Table of Representative Compounds in Smoker's Urine.

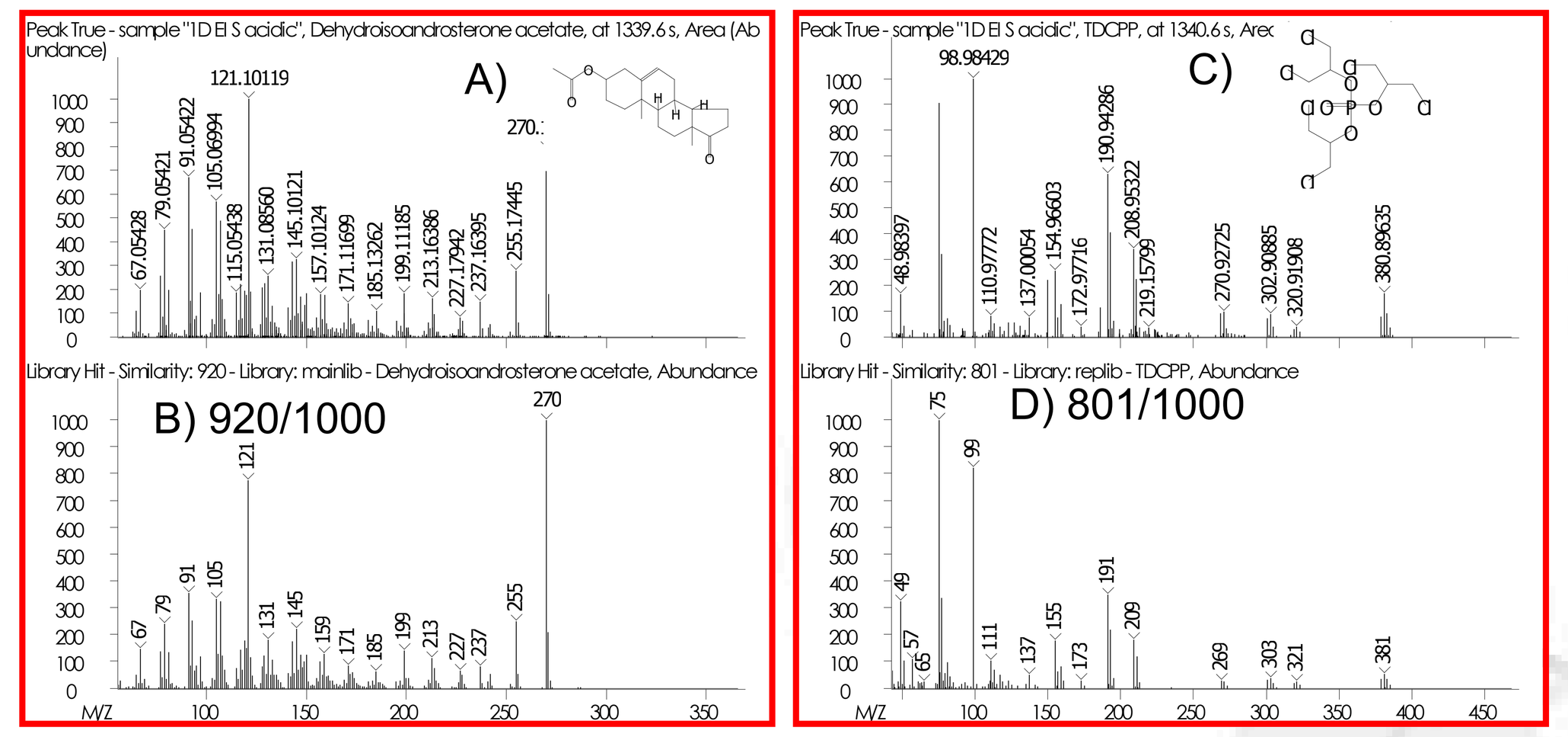


Figure 8. Peak True and Library Mass Spectral Data for Dehydroisoandrosterone Acetate and TDCPP in Smoker's Urine.

Results: Derivatization/Liquid Injection

HRT 4D: GCxGC-HRTOFMS

- Enhanced Chromatographic and Mass Spectral Resolution
- Group Clustering – Structured Chromatograms
- Improved Characterization of Compounds

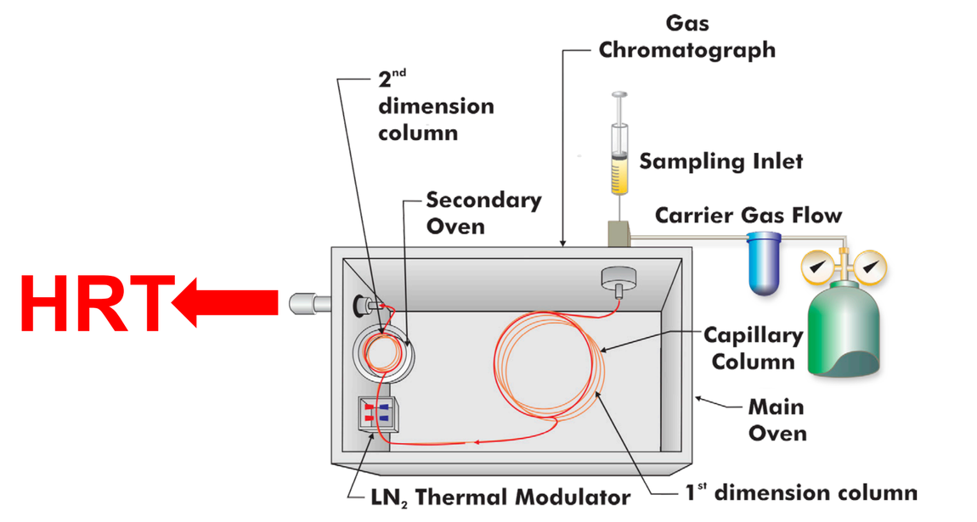


Figure 9. GCxGC-HRT 4D Instrument

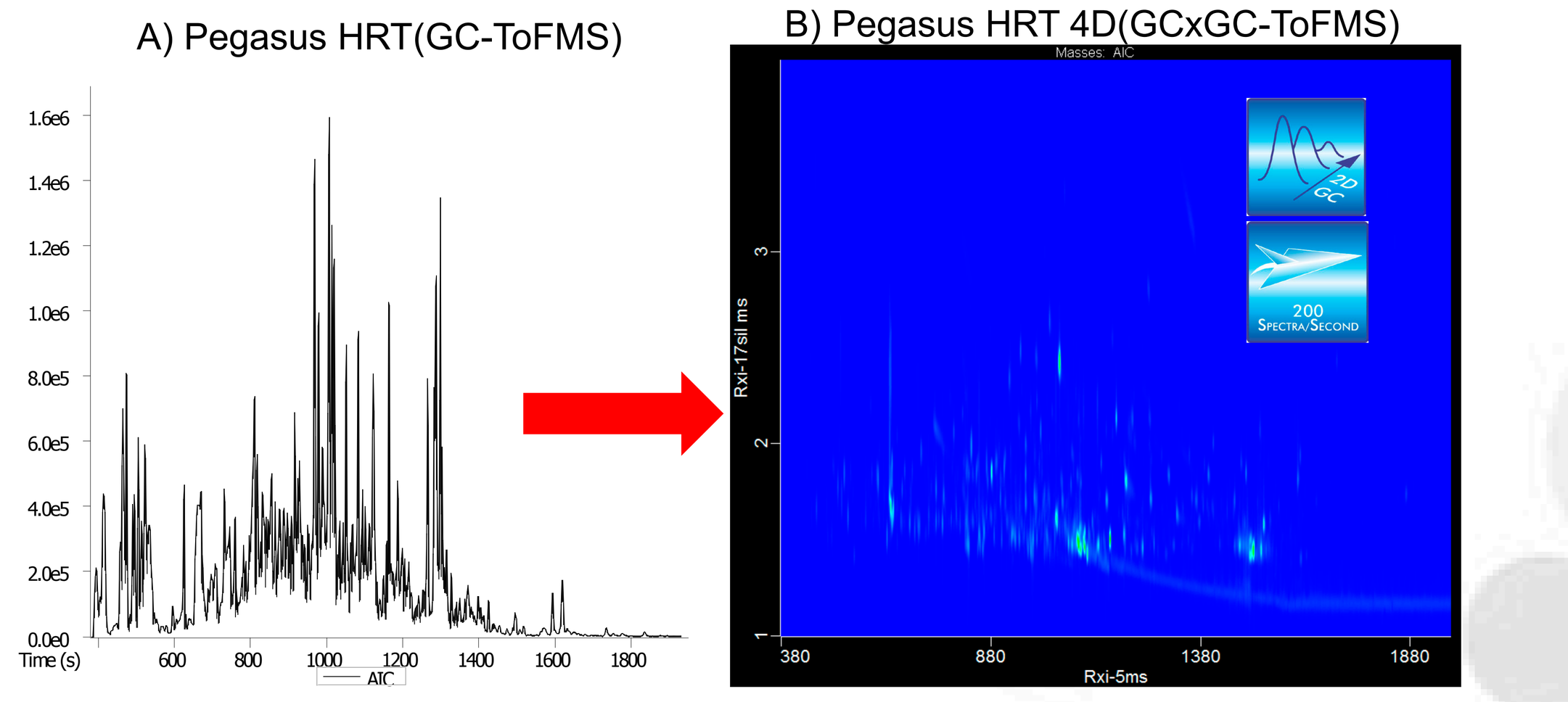
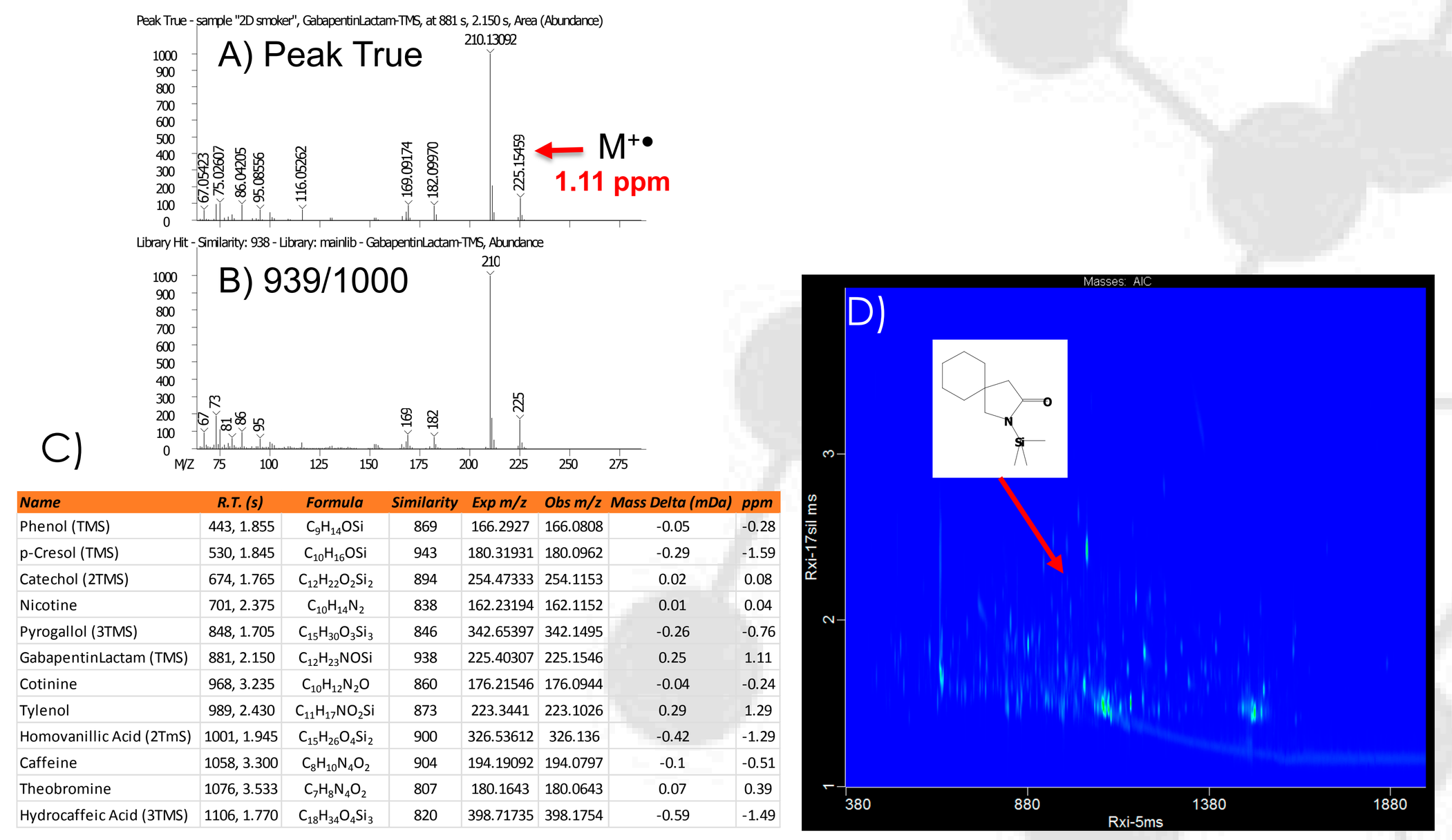


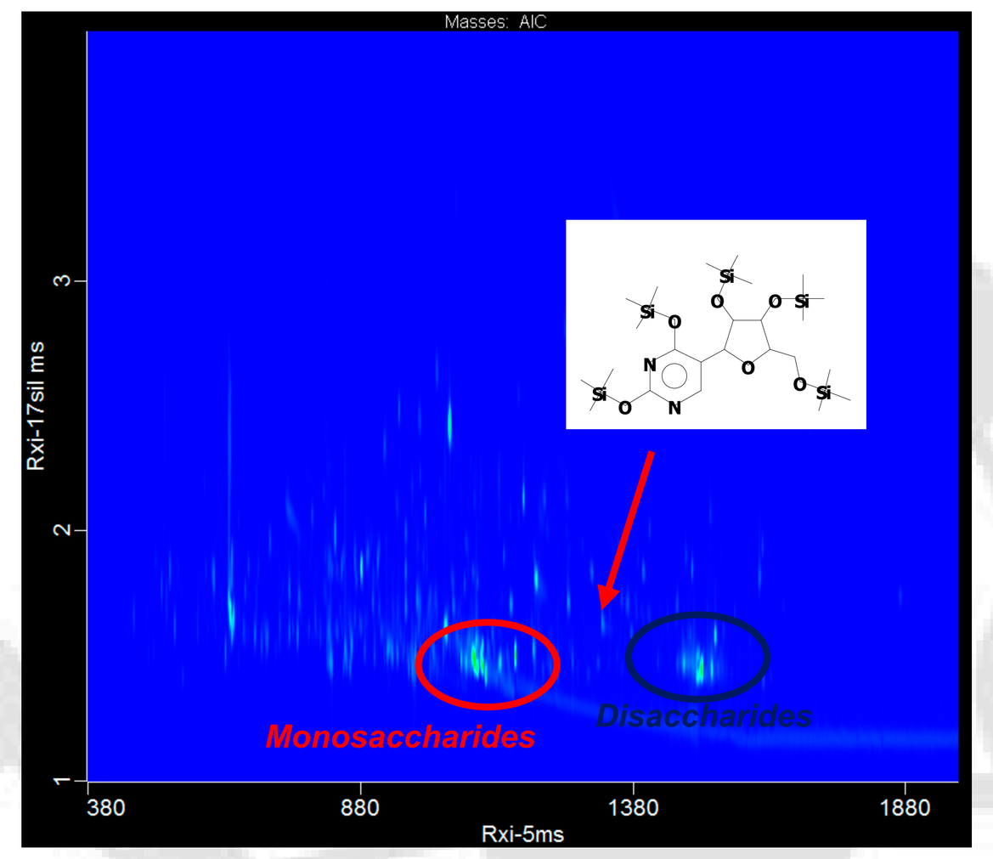
Figure 10. A) GC-HRT AIC and B) GCxGC-HRT 4D Contour Plot Smoker's Urine.



C)

Name	R.T. (s)	Formula	Similarity	Exp m/z	Obs m/z	Mass Delta (mDa)	ppm
Phenol (TMS)	443, 1.855	$C_9H_{14}OSi$	869	166.2927	166.0808	-0.05	-0.28
p-Cresol (TMS)	530, 1.845	$C_{10}H_{16}OSi$	943	180.31931	180.0962	-0.29	-1.59
Catechol (2TMS)	674, 1.765	$C_{12}H_{22}O_2Si_2$	894	254.47333	254.1153	0.02	0.08
Nicotine	701, 2.375	$C_{10}H_{14}N_2$	838	162.23194	162.1152	0.01	0.04
Pyrogallol (3TMS)	848, 1.705	$C_{15}H_{30}O_3Si_3$	846	342.65397	342.1495	-0.26	-0.76
GabapentinLactam (TMS)	881, 2.150	$C_{12}H_{23}NOSi$	938	225.40307	225.1546	0.25	1.11
Cotinine	968, 3.235	$C_{10}H_{12}N_2O$	860	176.21546	176.0944	-0.04	-0.24
Tylenol	989, 2.430	$C_{11}H_{17}NO_2Si$	873	223.3441	223.1026	0.29	1.29
Homovanillic Acid (2TmS)	1001, 1.945	$C_{15}H_{26}O_4Si_2$	900	326.53612	326.136	-0.42	-1.29
Caffeine	1058, 3.300	$C_8H_{10}N_4O_2$	904	194.19092	194.0797	-0.1	-0.51
Theobromine	1076, 3.533	$C_7H_8N_4O_2$	807	180.1643	180.0643	0.07	0.39
Hydrocaffeic Acid (3TMS)	1106, 1.770	$C_{18}H_{34}O_4Si_3$	820	398.71735	398.1754	-0.59	-1.49

Figure 11. A) Peak True and B) Library Mass Spectral Data for GabapentinLactam in Smokers Urine. C) Table of Representative Compounds. D) Contour Plot.



Monosaccharides

Name	R.T. (s)	Formula	Area	Similarity
Lyxose (MEOX, 4TMS)	932, 1.530	$C_{18}H_{45}NO_5Si_4$	250293	936
Fructose (MEOX, 5TMS)	947, 1.512	$C_{22}H_{55}NO_6Si_5$	241485	872
Myristic Acid (TMS)	1040, 1.710	$C_{17}H_{36}O_2Si$	15320	776
Galactose (MEOX, 5TMS, Z)	1082, 1.475	$C_{22}H_{55}NO_6Si_5$	686835	880
Glucose (MEOX, 5TMS, Z)	1103, 1.462	$C_{22}H_{55}NO_6Si_5$	1038745	924
Palmitic Acid (TMS)	1154, 1.710	$C_{19}H_{40}O_2Si$	184742	929
Stearic Acid (TMS)	1259, 1.720	$C_{21}H_{44}O_2Si$	106005	913

Disaccharides, Nucleosides

Name	R.T. (s)	Formula	Area	Similarity
Pseudo Uridine (5TMS)	1322, 1.635	$C_{24}H_{52}N_2O_6Si_5$	449480	820
Maltose (8TMS)	1493, 1.460	$C_{36}H_{86}O_{11}Si_8$	133270	802
Lactose (MEOX, 8TMS, Z)	1505, 1.455	$C_{37}H_{89}NO_{11}Si_8$	575135	916
Lactose (8TMS)	1523, 1.449	$C_{36}H_{86}O_{11}Si_8$	581935	946
Galactinol (9TMS)	1535, 1.450	$C_{39}H_{94}O_{11}Si_9$	333231	860
Cellobiose (MEOX, 8TMS, Z)	1868, 1.741	$C_{37}H_{89}NO_{11}Si_8$	100819	895

Figure 12. Contour Plot of Non-Smoker's Urine. Table of Representative Mono-, Nucleosides and Disaccharides in Non-Smoker's Urine.

Conclusion

- The Pegasus GC-HRT 4D facilitates fast and confident compound identification through enhanced two-dimensional chromatographic resolution, spectral similarity searches of large, well-established databases, and formula determinations using high resolution accurate mass ions.
- SPME, DHS, and Twister compound sampling techniques are a viable alternative for the analysis of volatiles, semi-volatiles, and hydrophilic compounds in urine samples.
- Derivatization and liquid injections greatly increase the number of compound classes amenable to GC-HRT 4D analysis.

Delivering the Right Results