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

David E. Alonso[1], Petra Gerhards[2], Charles Lyle[1] and Joe Binkley[1] | [1]LECO Corporation, St. Joseph, MI; [2]LECO European LSCA Centre, Moenchengladbach, Germany

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"

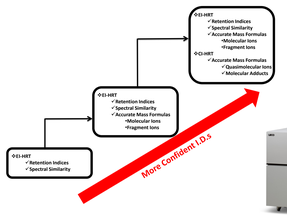
Fig. 1: A Physician Examining Urine through a Matula (Left). A Urine Color Chart (Right).

- 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



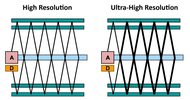


Fig. 2: The Utilization of EI- and CI-HRT Technology for Confident Compound Characterization.

Experimental

Samples

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

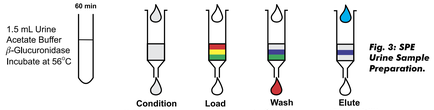


Fig. 3: SPE Urine Sample Preparation.

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:

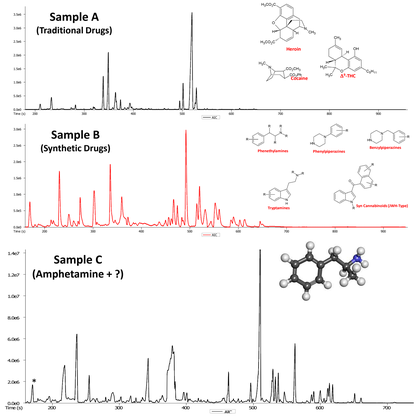
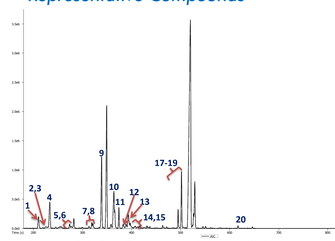


Fig. 4: Illustrative Urine Samples A, B, C.

Sample A (Traditional Drugs)

Representative Compounds



Peak #	Name	Formula	R.T. (s)	Area	Similarity	Mass Delta (mDa)	MA (ppm)
1	Creatinine ME	$C_5H_9N_3O$	210	1328229	800	-0.05	-0.43
2	o-Ethynylaniline	C_8H_7N	219	1674346	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	940734	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	$C_9H_8O_3$	323	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	1179948	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	$C_5H_7NO_2$	397	441603	872	0.04	0.2
15	4-Methyl-5H-carboline	$C_{12}H_{12}N_2$	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

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

Table 1: Compounds in Sample A.

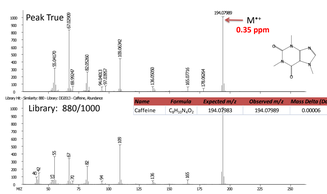


Fig. 6: Peak True, Deconvoluted (Top) and Library Mass Spectra (Bottom) for Caffeine in Sample A.

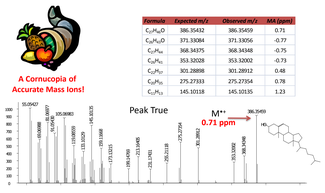
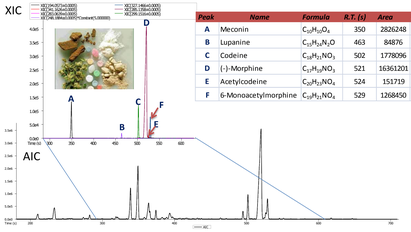


Fig. 7: Peak True Mass Spectrum and Table of Accurate Mass Ions for Cholesterol in Sample A.

Traditional Drugs



Peak	Name	Formula	R.T. (s)	Area	Similarity MA(ppm)	
A	Meconin	$C_{11}H_{10}O_4$	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.

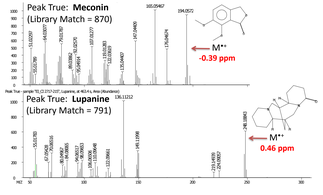


Fig. 9: Peak True Mass Spectra for Meconin (Top) and Lupanine (Bottom) in Sample A.

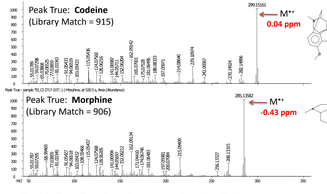
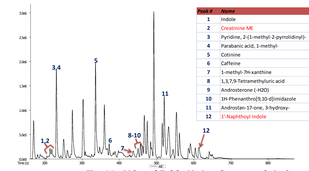


Fig. 10: Peak True Mass Spectra for Codeine (Top) and Morphine (Bottom) in Sample A.

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	2'-Naphthoyl Indole	$C_{19}H_{13}NO$	613	1056803	879

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

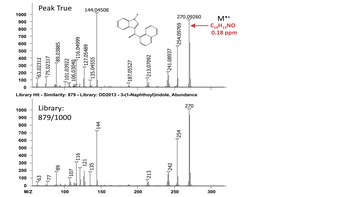
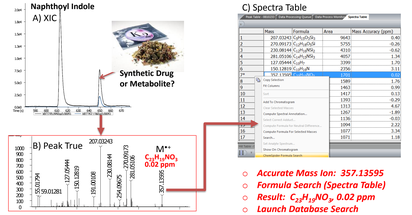


Fig. 12: Peak True (Top) and Library Mass Spectra (Bottom) for 3-(1-Naphthoyl)indole in Sample B.

Synthetic Drugs



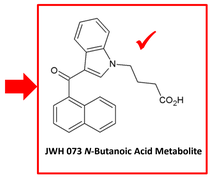
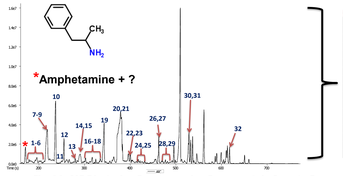


Fig. 13: An XIC showing an unknown synthetic drug or metabolite not present in the NIST or Wiley libraries (A). Peak True mass spectrum for the unknown (B). The ChromaTOF-HRT® Spectra Table (C) showing the formula $C_{23}H_{19}NO_2$ for m/z = 357.13595 (mass accuracy = 0.02 ppm) and the initiation of 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)



Peak	Name	Formula	R.T. (s)	Area	Similarity	MA(ppm)
1	Amphetamine	$C_9H_{13}N$	170	991599	841	-0.14
2	3-Pyridinol	C_5H_5NO	183	6185856	812	-0.09
3	2-Ethylbutyrophe	$C_{12}H_{16}O$	187	62464	746	0.73
4	N,3-Dimethylimidazole	$C_5H_8N_2$	188	178643	813	-0.47
5	Ethanol, 2-phenoxy-	$C_8H_{10}O_2$	194	4463665	809	-0.68
6	Octanoic acid, 2-methyl-	$C_9H_{18}O_2$	204	3662983	726	-0.29
7	Dihydrobenzofuran	C_8H_8O	208	399549	737	0.00
8	Creatinine ME	$C_5H_9N_3O$	218	36175929	773	-0.92
9	o-Ethynylaniline	C_8H_7N	221	3330856	792	-0.19
10	5-methylhydantoin	$C_4H_6N_2O_2$	232	2465388	793	-0.46
11	Nicotin	$C_{10}H_{14}N_2$	237	43628594	889	-1.04
12	1H-Indole, 3-methyl-	C_9H_9N	249	1481899	808	-1.11
13	2-(Methylmercaptobenzoxazole	C_8H_7NOS	255	5421426	868	-0.78
14	Creatinine, 1-acetyl-	$C_6H_9N_3O_2$	276	1017562	767	-0.13
15	Methyl 4-hydroxyphenylacetate	$C_9H_{10}O_3$	288	1417270	738	-0.68
16	4 - vinyl - syringol	$C_{10}H_{12}O_3$	292	207732	734	-0.91

Peak	Name	Formula	R.T. (s)	Area	Similarity	MA(ppm)
17	Hexahydropyrrolizin-3-one	$C_7H_{11}NO$	302	3545305	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	23921115	865	-0.91
20	methyl 1H-indole-3-acetate	$C_{11}H_{11}NO_2$	373	1792478	792	-0.85
21	Hydroxycotinine	$C_{10}H_{12}N_2O_2$	382	63350156	795	-0.57
22	Theobromine	$C_7H_8N_4O_2$	397	5647784	790	-0.31
23	Proline anhydride	$C_{10}H_{16}N_2O_2$	401	5113606	854	-0.52
24	Acridine, 9-methyl-	$C_{14}H_{11}N$	410	519170	707	-0.47
25	Imidazo[1,5-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	Trimeprazine	$C_{18}H_{22}N_2S$	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	$C_{19}H_{30}O_2$	505	252430	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

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

Table 2: Representative Compounds in Sample C.

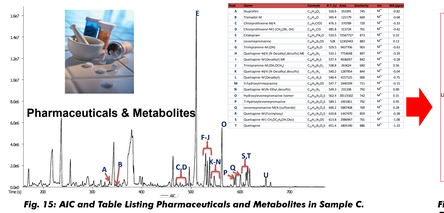


Fig. 15: AIC and Table Listing Pharmaceuticals and Metabolites in Sample C.

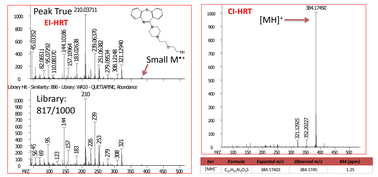


Fig. 16: EI-HRT Peak True (Top) and Library Mass Spectra (Bottom) for Quetiapine (anti-psychotic). CI-HRT Peak True Spectrum for Quetiapine (Right).

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