A Novel Benchtop Time-of-Flight GC-MS System for High Performance Analysis of Human Urine

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Introduction

The development of improved profiling workflows are critical for effective investigation of environmental exposure and its relationship to chronic diseases. Metabolomic methods are particularly useful in this endeavor due to the proximity of metabolites to system phenotype, and their relatively quick insight into system perturbations from diet, drugs, gut microbiota, etc. (Figure 1). Unfortunately, a major problem with these methods continues to be incomplete sample characterization due to metabolite diversity, wide concentration range, and the overall complexity of biological matrices.

Urine is an ideal bio-fluid for studying the effects of exposure, since it is easy to obtain in large volumes, and is relatively free from interfering proteins and lipids. In addition, high concentrations of drugs and other xenobiotic materials can be detected over extended periods of time. In this study, we used a high performance, benchtop gas chromatography time-of-flight mass spectrometer for comprehensive characterization of derivatized urine samples (Figure 2).

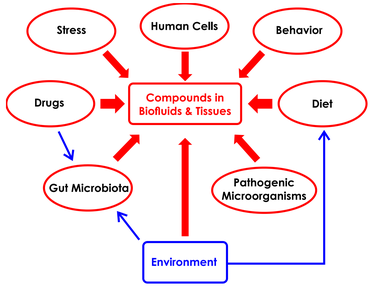


Figure 1. Human Exposure (Schantz and coworkers, *Anal. Bioanal. Chem.*, 2015, *407*, 2945-2954).

Figure 2. Pegasus® BT GC-TOFMS.

Objectives

- Develop and implement an effective workflow to facilitate the comparison of urine samples
- Use time-proven chemical protocol to expand metabolite coverage by producing stable, GC-MS amenable compounds
- Use high performance GC-TOFMS and powerful software tools to quickly and confidently annotate metabolites in urine

Sample Preparation

Urine (200 µL) was treated with urease (10 mg; 37°C for 15 minutes) to remove excess urea. This was followed by the addition of methanol (800 µL) to produce a solution that was vortexed for 10 minutes, and centrifuged at 12,000 rpm for 10 minutes. The supernatant was transferred to a GC vial with a 400 µL insert, and evaporated to dryness using a Speed Vac. 100 µL of MTBSTFA + 1% TBDMCS was added to the dry sample which was heated at 100°C for 1 hour, and then injected into a high performance GC-TOFMS using a split ratio of 20:1. The total acquisition time was 12 minutes (Figure 3).

Instrumentation Parameters and Methods

Gas Chromatograph	**Agilent 7890 with MPS2 Autosampler**
Injection	1µL, Split 20:1
Carrier Gas	He @ 0.8 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	60°C (0.5min), ramped 36°C/min to 330°C, held 4 min
Mass Spectrometer	**LECO Pegasus® BT**
Ion Source Temperature	250°C
Ionization Mode	EI
Mass Range (m/z)	45-600
Acquisition Rate	20 spectra/s

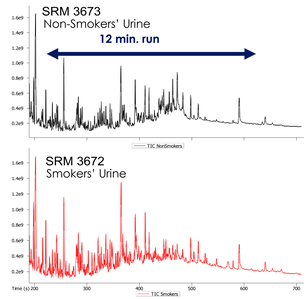
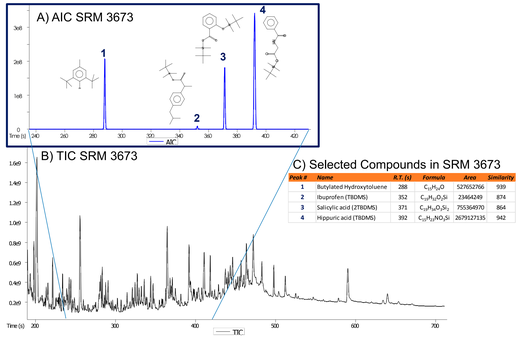


Figure 3. TICs for NIST SRMs 3673 (top) and 3672 (bottom).

Results (Non-Smokers' Urine)



Peak #	Name	R.T. (s)	Formula	Area	Similarity
1	Butylated Hydroxytoluene	288	$C_{15}H_{24}O$	527652766	939
2	Ibuprofen (TBDMS)	352	$C_{19}H_{32}O_2Si$	23466249	874
3	Salicylic acid (2TBDMS)	371	$C_{20}H_{36}O_3Si_2$	755366970	864
4	Hippuric acid (TBDMS)	392	$C_{19}H_{31}NO_3Si$	2679127135	942

Figure 4. (A) Analytical Ion Chromatogram (AIC) and (B) TIC of Non-Smokers' Urine (NIST SRM 3673), (C) Table of Selected Compounds in SRM 3673.

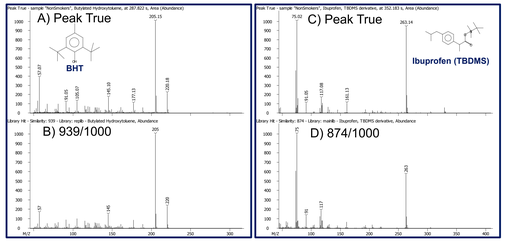


Figure 5. Peak True (Deconvoluted) and Library Spectra for BHT (A,B) and Ibuprofen (C,D).

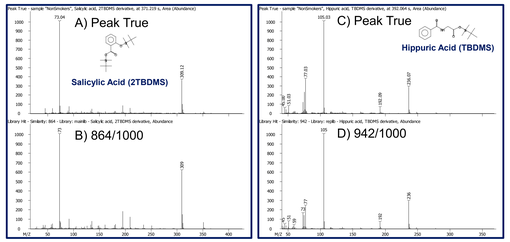


Figure 6. Peak True and Library Spectra for Salicyclic Acid (A,B) and Hippuric Acid (C,D).

Results (Smokers' Urine)

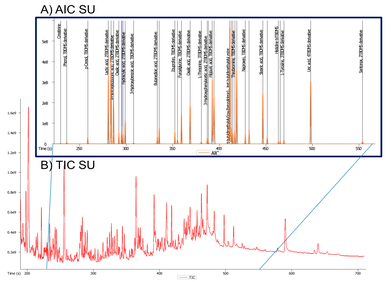


Table 1. Representative Compounds in Smokers' Urine.

Peak #	Name	R.T. (s)	Formula	Area	Similarity	Peak #	Name	R.T. (s)	Formula	Area	Similarity
1	Creatinine	230	$C_4H_7N_3O$	25029971	839	20	3-Hydroxyphenylacetic acid (2TBDMS)	387	$C_{20}H_{36}O_4Si_2$	47854536	880
2	Phenol (TBDMS)	236	$C_{12}H_{20}OSi$	31055125	767	21	Myristic acid (TBDMS)	388	$C_{20}H_{42}O_2Si$	50679422	807
3	p-Cresol (TBDMS)	259	$C_{13}H_{22}OSi$	98197697	768	22	Hippuric acid (TBDMS)	392	$C_{19}H_{31}NO_3Si$	2955526048	940
4	Lactic acid (2TBDMS)	281	$C_{15}H_{34}O_3Si_2$	935343073	842	23	4-Hydroxybenzeneacetic acid (2TBDMS)	394	$C_{20}H_{36}O_4Si_2$	1303589708	844
5	Glycolic acid (2TBDMS)	284	$C_{14}H_{32}O_3Si_2$	1653107770	885	24	Glycine Der (2TBDMS)	411	$C_{15}H_{33}NO_2Si_2$	1968590089	898
6	α-Hydroxyisobutyric acid (2TBDMS)	286	$C_{16}H_{36}O_3Si_2$	561335976	863	25	Hypoxanthine (2TBDMS)	413	$C_{16}H_{28}N_4OSi_2$	3043816577	901
7	Oxalic acid (2TBDMS)	292	$C_{14}H_{30}O_4Si_2$	271668319	850	26	Homovanillic acid (2TBDMS)	414	$C_{20}H_{36}O_4Si_2$	1213225512	853
8	Glycine (2TBDMS)	295	$C_{14}H_{31}NO_2Si_2$	161771909	832	27	Theobromine (TBDMS)	417	$C_{13}H_{22}N_4O_2Si$	3054003330	824
9	Amphetamine, TBDMS derivative	295	$C_{13}H_{23}NSi$	21617839	724	28	Palmitic acid (TBDMS)	419	$C_{22}H_{46}O_2Si$	1204500244	925
10	Glyceraldehyde (2TBDMS)	297	$C_{15}H_{34}O_3Si_2$	128666408	858	29	Naproxen (TBDMS)	428	$C_{20}H_{28}O_3Si$	108202368	809
11	Hydracrylic acid (2TBDMS)	299	$C_{15}H_{34}O_3Si_2$	217679170	908	30	3-Methylhistidine (2TBDMS)	432	$C_{19}H_{38}N_2O_2Si_2$	320704543	774
12	3-Hydroxybenzoic acid (TBDMS)	308	$C_{13}H_{20}O_3Si$	25036674	734	31	Stearic acid (TBDMS)	447	$C_{24}H_{50}O_2Si$	875617747	951
13	Cotinine	329	$C_{10}H_{12}N_2O$	67019591	739	32	Vanillylmandelic acid (3TBDMS)	451	$C_{23}H_{42}O_5Si_3$	63687352	866
14	Succinic acid (2TBDMS)	333	$C_{16}H_{34}O_4Si_2$	148327844	817	33	Histidine (2TBDMS)	463	$C_{18}H_{31}N_3O_2Si_2$	89723440	817
15	Uracil (2TBDMS)	335	$C_{16}H_{30}N_2O_2Si_2$	258514157	890	34	Citric acid (4TBDMS)	465	$C_{24}H_{52}O_7Si_4$	25299816	830
16	Ibuprofen (TBDMS)	352	$C_{19}H_{32}O_2Si$	188470403	894	35	L-Tyrosine (2TBDMS)	469	$C_{21}H_{39}NO_3Si_2$	165363614	867
17	Caffeine	355	$C_8H_{10}N_4O_2$	93756667	800	36	Uric acid (4TBDMS)	498	$C_{29}H_{68}N_4O_6Si_4$	2049217736	866
18	Furoylglycine (TBDMS)	359	$C_{13}H_{19}NO_4Si$	6225074778	910	37	Santonox (2TBDMS)	554	$C_{19}H_{30}O_3SSi_2$	78147841	827
19	Threonine (3TBDMS)	381	$C_{16}H_{37}NO_3Si_3$	33708837	809						

Figure 7. (A) Analytical Ion Chromatogram (AIC) and (B) TIC of Smokers' Urine (NIST SRM 3673).

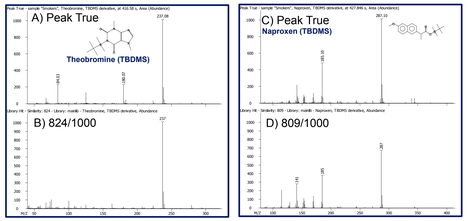


Figure 8. Peak True and Library Spectra for Theobromine (A,B) and Naproxen (C,D).

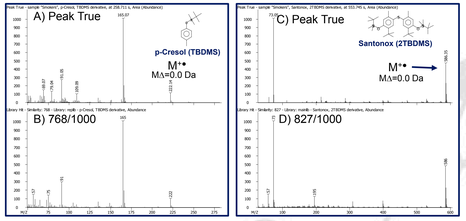


Figure 9. Peak True and Library Spectra for p-Cresol (A,B) and Santonox (C,D).

Target Analyte Finding (TAF) – Quick Processing for Sample Comparison

- Rapid processing
- Retrospective analysis of TOFMS data
- Leverage compound:
  - Retention Times
  - Ions

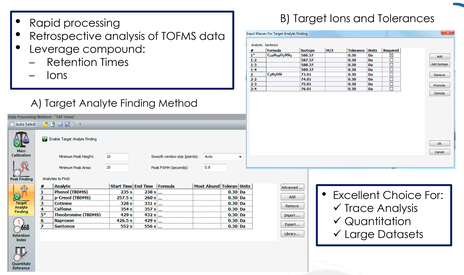
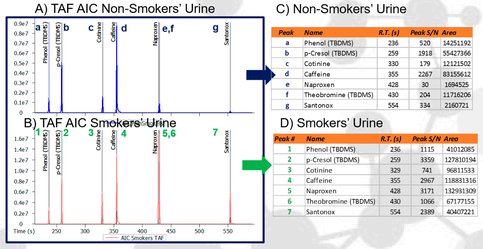


Figure 10. (A) ChromaTOF 5.0 TAF Method and (B) Target Ion(s).



Peak	Name	R.T. (s)	Peak S/N	Area
a	Phenol (TBDMS)	236	520	14251192
b	p-Cresol (TBDMS)	259	1918	55427366
c	Cotinine	330	179	12121502
d	Caffeine	355	2267	83155612
e	Naproxen	428	30	1694525
f	Theobromine (TBDMS)	430	204	11716206
g	Santonox	554	334	2160721

Peak #	Name	R.T. (s)	Peak S/N	Area
1	Phenol (TBDMS)	236	3115	410120365
2	p-Cresol (TBDMS)	259	3359	127810194
3	Cotinine	329	741	96811533
4	Caffeine	355	2967	118831316
5	Naproxen	428	3171	1329313039
6	Theobromine (TBDMS)	430	1066	67177155
7	Santonox	554	2389	40407221

Figure 11. TAF AICs (A,B) and Results (C,D), Showing Greater Quantities of Target Compounds in Smokers' versus Non-Smokers Urine.

Conclusions

A workflow was developed and implemented for the effective comparison of urine. It involved the preparation of stable compounds through derivatization, data acquisition using a high performance benchtop TOFMS, comprehensive data processing (NonTarget Deconvolution™), and quick retrospective analysis of rich datasets using Target Analyte Finding.

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