Comparison of HPLC - QTOF MS/MS Analysis to a Routine EMIT, HPLC, GC/NPD and GC/MS Workflow for Forensic Drug Screening

Curtis Hedman[1], Xiang He[2], Daniel McManaway[1], Ryan Pieters[1], Amy Miles[1], Josh Plath[2] and **Adrian Taylor[2]**

[1]University of Wisconsin-Madison/Wisconsin State Laboratory of Hygiene, 2601 Agriculture Drive, Madison, WI 53718
[2]SCIEX, 1201 Radio Road, Redwood City, CA 94065

INTRODUCTION

The Wisconsin State Laboratory of Hygiene (WSLH) Toxicology Section currently uses multiple EMIT, HPLC (with wavelength detection) and GC based screening methods to analyze for more than 300 forensically related drug compounds in over 18,000 medical examiner and operating while intoxicated samples each year. These workflows are time and labor intensive. In addition, the particular drugs used in newer drug classes, such as synthetic cannabinoids and other novel psychoactive substances can vary widely, making their identification and confirmation difficult. The WSLH would like to investigate the use of HPLC coupled to QTOF detection for both targeted and unknown identification of drugs in forensic screening workflows. The newest iterations of HPLC and QTOF instruments are sufficiently sensitive and reliable to achieve these goals (Figure 1).

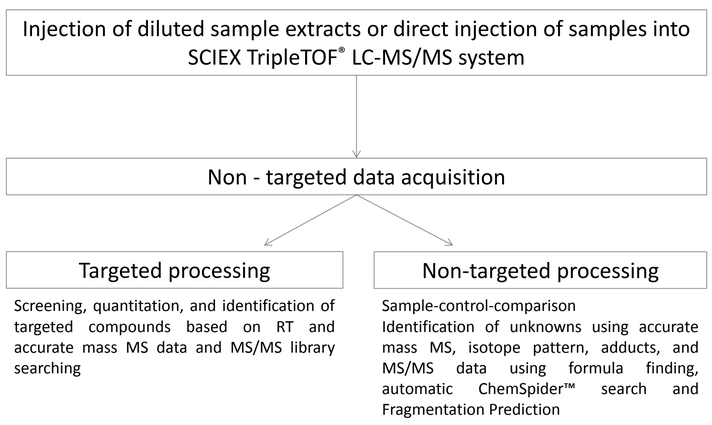

Figure 1: Proposed HPLC QTOF sample workflow.

METHODS

Ten blinded whole blood samples with protein precipitation which were previously analyzed by the EMIT, HPLC, GC/NPD and GC/MS workflows at the WSLH were shipped to SCIEX (Redwood City, California) and analyzed by a Shimadzu Prominence HPLC coupled to a SCIEX TripleTOF® 5600+ LC-MS/MS system with both TOF-IDA-MS/MS and MS/MS^{All} with SWATH® acquisition modes in non-targeted fashion (Figure 3). For HPLC separation, a 10µL aliquot was injected onto a reversed-phase 50×2.1 mm column held at 30°C and separated at 500 mL/minute by a binary mobile phase gradient. Figure 2 summarizes the sample preparation process used and Figures 4 and 5 list pertinent SCIEX TripleTOF® 5600+ LC-MS/MS instrument parameters for TOF-IDA-MS/MS and MS/MS^{All} with SWATH® acquisition modes.

Sample Preparation at WI State Lab:

1. Pipette standards and internal standards into tubes.
2. Pipette quality control materials and subject samples.
3. Fill all tubes to 1 mL with blank blood.
4. Allow to rest at room temp for 30 minutes (Note: these were left overnight in the hood).
5. Slowly add 2 ml cold acetonitrile (dropwise while vortexing).
6. Vortex on multi-tube vortexer for 1 minute.
7. Centrifuge for 15 minutes at 4750 rpm.
8. Transfer supernatant to clean centrifuge tube.
9. Dry down at 15 psi and 50° for 40 minutes, 20 psi and 60° for 10 minutes.
10. Reconstitute with 100 uL 20% MeOH in H20.
11. Vortex, cap and multi-vortex for 1 minute.
12. Centrifuge for 5 minutes at 4750 rpm.
13. Transfer supernatant to labeled autosampler vials with inserts.
14. Cap and vortex vials.

Additional Sample Processing at SCIEX:

1. Transfer to microcentrifuge tubes.
2. Centrifuge at 16,000 x g for 5 minutes.
3. Dilute 20 µL clear solution with 180 µL 20% MeOH in water in autosampler vial.
4. Cap and vortex vials.

Figure 2: Sample preparation method used for HPLC-QTOF MS/MS samples.

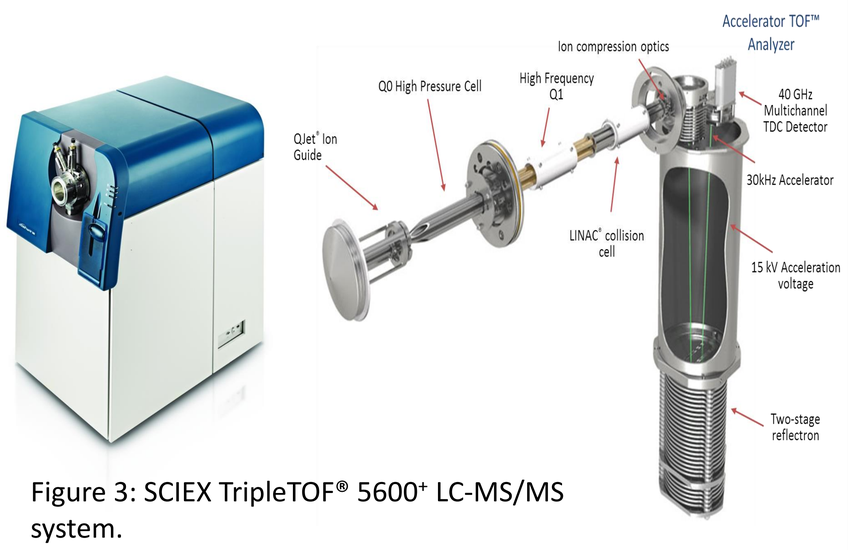

Figure 3: SCIEX TripleTOF® 5600+ LC-MS/MS system.

- **MS Detection**
  - DuoSpray™ ion source
- **Source/gas parameters**
  - ISVF: 2500 V (-2500 V for negative mode)
  - Cur: 35 psi
  - TEM: 600°C (500°C for negative mode)
  - GS1: 60
  - GS2: 60
- **TOF-MS**
  - 100 to 1000 m/z; 100 ms scan time; CE: 10; DP: 90
- **IDA-MS/MS**
  - 40 TO 1000 m/z; DP: 90
  - IDA: 12 candidate ions scan, each at 30 ms using CE spread (20 to 50 V)
  - Dynamic Background Subtraction™ algorithm

Figure 4: SCIEX TripleTOF® 5600+ LC-MS/MS system parameters.

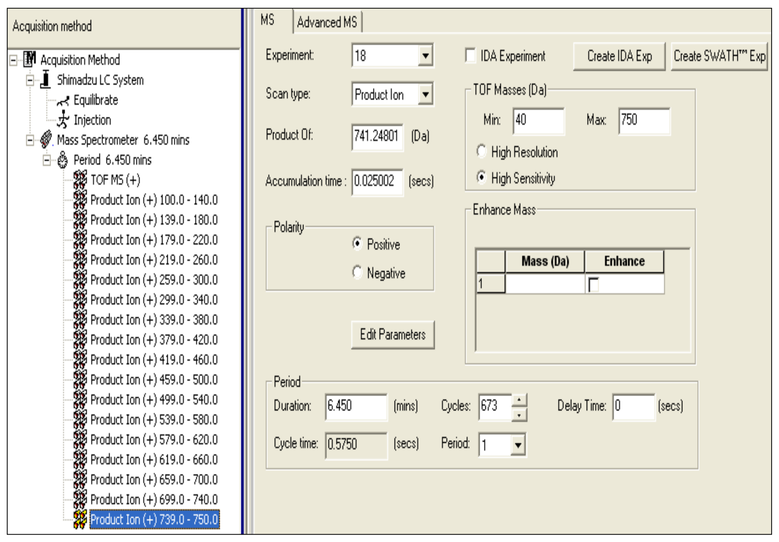

Figure 5: MS/MS^{ALL} with SWATH® acquisition settings

RESULTS

The results of the targeted screening by HPLC-QTOF MS/MS versus the EMIT, HPLC and GC workflow is summarized in Figure 6. There were 112 compounds detected using both methods. Seven compounds reported by the EMIT, HPLC, GC/NPD and GC/MS workflow were not reported in the blinded HPLC-QTOF MS/MS analysis. This is likely due to the difference in sample preparation procedures used. The WSLH method extraction procedure uses a double solvent extraction/cleanup and the samples for the HPLC-QTOF MS/MS analysis were prepared by a simple protein precipitation procedure and were further diluted 1:10 prior to analysis. Moreover, the HPLC-QTOF MS/MS analysis also detected five compounds in higher confidence with MS/MS matching that were not detected in the original WSLH screening analysis methods.

Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
THC-COOH	Primidone	Venlafaxine	Trazodone	Carisoprodol
Nicotine	Phenytoin	O-Desmethylvenlafaxine	Tramadol	MDPV
THC	**Amobarbital/pento**	Doxylamine	Citalopram	Cocaine
11-OH-THC	**Butalbital**	Chlorpheniramine	Amitriptyline	Zolpidem
	Phenobarbital	Cyclobenzaprine	Mirtazapine	Methadone
	Phenytoin	Fluoxetine	MDMA	Diazepam
	Secobarbital	Sertraline	Desmethylcitalopram	Meprobamate
		Amphetamine	Nortriptyline	Alprazolam
	Sample 7	*Norsertraline*	Dextromethorphan	Nordiazepam
	Zolpidem		Bupropion	Mephedrone
	Alprazolam	Sample 8	MDA	Benzoylecgonine
	Diazepam	Naloxone	Paroxetine	N-Ethylcathinone
	Midazolam	Buprenorphine	Phentermine	Acetaminophen
	Triazolam	Acetaminophen		Methamphetamine
Sample 6	Estazolam	Levetiracetam	Sample 9	EDDP
Hydrocodone	Clobazam	Norbuprenorphine	Trazodone	BZP
Codeine	Flunitrazepam	Nicotine	Quetiapine	**Ibuprofen**
Oxycodone	Alpha-hydroxymidazolam	**Ibuprofen**	Alprazolam	*Meperidine*
Morphine	Prazepam	**Salicyclic acid**	Gabapentin	*Lidocaine*
Hydromorphone	2-Hydroxyethylflurazepam		Quetiapine metabolite (+2O, -2H)	
6-MAM	Chlordiazepoxide		Morphine	Sample 10
Oxymorphone	7-Aminoflunitrazepam		Quetiapine metabolite (-88)	Carbamazepine
Acetaminophen	Temazepam		Acetominophen	Zolpidem
	Alpha-Hydroxyalprazolam		**Ibuprofen**	Carisoprodol
	Alpha-Hydroxytriazolam		*THC*	Chlordiazepoxide
	Nordiazepam		*THC-COOH*	Fentanyl
	Nitrazepam			Metaxalone
	7-Aminoclonazepam			Meprobamate
	Clonazepam			Demoxepam
	Phenazepam			Promethazine
	Oxazepam			*Olanzapine*
	Lorazepam			

Key:
Italicized font = Compounds reported by the WSLH ELISA and GC/MS screen that were not detected by targeted HPLC Q-TOF MS/MS (NOTE: some compounds were detected in QTOF mode, but without MSMS info, such as 11-OHTHC and olanzapine).
Underlined font = Compounds that were not on WSLH list but with good MSMS matching.
Bolded font = Compounds that were detected in negative ion mode.

Figure 6: Results of the targeted screening by HPLC-QTOF MS/MS versus the EMIT, GC/NPD, HPLC and GC/MS workflows.

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Figure 7 shows an example of how MS/MS^{ALL} with SWATH® acquisition compared to targeted screening by HPLC-QTOF MS/MS acquisition for compound detection in one of the submitted blinded samples. Both TOF-IDA-MS/MS and TOF-MS/MS^{All} with SWATH® data acquisition will provide MS/MS information for unknown targets. While IDA-MS/MS offers more selective precursor isolation for MS/MS data acquisition, the SWATH® acquisition approach makes sure the MS/MS information will be recorded for everything all the time. This is of key importance for surveillance of novel psychoactive substances, such as synthetic cannabinoids, as new variations of these compounds are being introduced frequently.

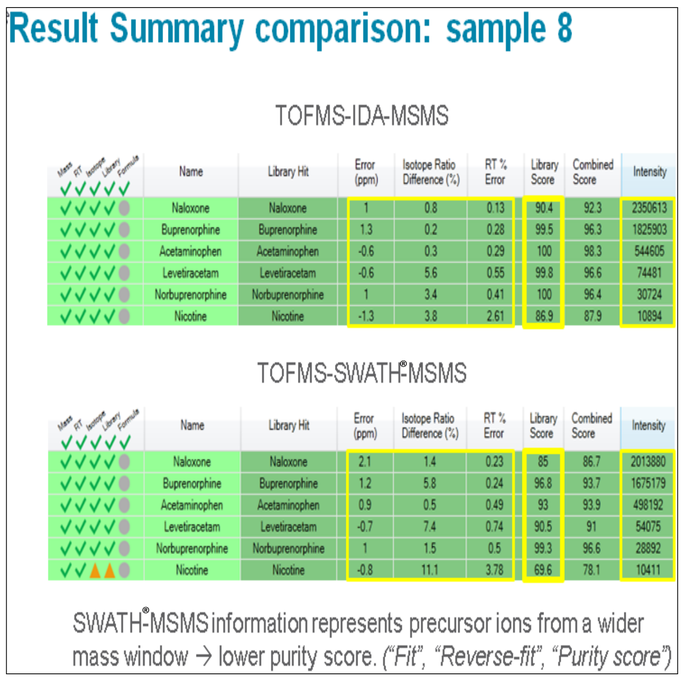

Figure 7: Comparison of IDA-MS/MS to MS/MS^{ALL} with SWATH® acquisition.

CONCLUSION

In summary, the use of HPLC-QTOF MS/MS shows great promise for streamlining routine forensic drug screening workflows, and has the added benefit of being able to also detect and identify true unknowns making this a very powerful analysis technique for a forensic toxicology laboratory.

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