

A Novel and Fast Workflow for Forensic Toxicological Screening and Quantitation Using QTOF LC-MS/MS System

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Introduction:

Forensic toxicological screening is challenging in that: (1) the target compound list can exceed hundreds with drastically varying chemical properties, (2) new compounds are constantly introduced which are unrecognizable by the targeted approaches, (3) the current major detection techniques such as immunoassay lacks the promptness and flexibility to adapt to the new analytes, and (4) these same techniques often yield significant false-positive and false-negative rates due to insufficient sensitivity and specificity. In this study, we aim to develop a sensitive and selective screening workflow in a forensic toxicological setting by utilizing TripleTOF® 4600 LC-MS/MS system and the novel MSMS^{All} with SWATHTM acquisition.

Methods:

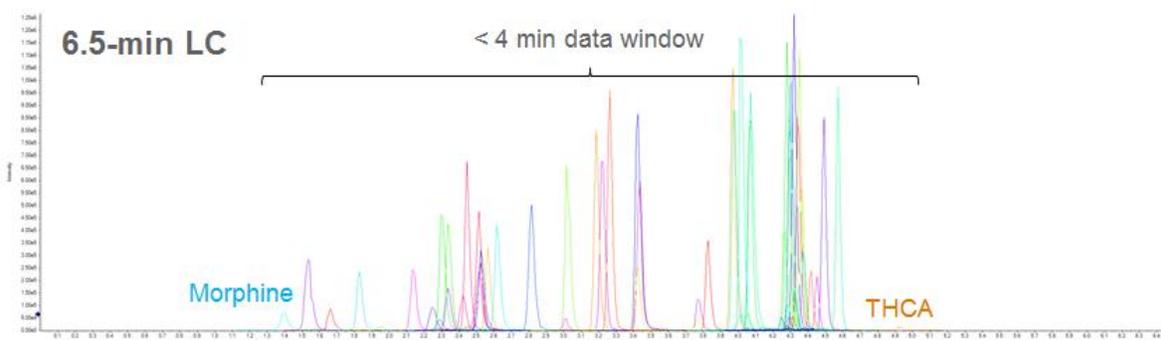
Blank human urine samples were spiked with more than 50 common drugs found in forensics setting at different concentration levels. Along with the unspiked blank urine sample, these samples were diluted 10-fold in 10% methanol and centrifuged. The clear supernatants were transferred to autosampler vials and 10 µL sample was injected each. HPLC separation was performed on two different reverse-phase columns (dimensions: 50 × 2.1 and 20 × 2.1 mm) at 30 °C with. Two different LC gradients (6.5-min and 2-min) were developed for these two columns. Data was collected on a TripleTOF® 4600 mass spectrometer with Analyst® TF software 1.7 using: 1) TOF-MS survey scan with Information Dependent Acquisition (IDA)-triggering of up to 16 product ion scans or 2) SWATH acquisition. For SWATH acquisition, the precursor isolation window width was either fixed or varied for each MS/MS experiment. The source (DuoSpray) parameters were: 2500 V or -2500 V spray voltage, 35 psi curtain gas, 600 °C source temperature, 60 psi nebulizing and desolvation gas. Data was processed in MasterViewTM software 1.1. Quantitation was performed with MultiQuantTM software 3.0.

Preliminary Results:

Screening

1. Both the 6.5 and 2.0-min LC gradients using both IDA-MSMS and SWATH acquisition were tested. Multiple confidence criteria: mass accuracy, RT, isotope, and most importantly, library match (Purity and Fit), were used.

2. The availability of MS/MS information for identification was required in this study. Due to better LC separation and reduced matrix effects, the 6.5-min method yielded better MS/MS library matching score than 2-min method (87% vs 81% with Purity score, and 96% vs 87% in Fit score) and more true positive rate (89 vs 84% with IDA and 98 vs 93% with SWATH).
3. IDA-MS/MS data acquisition was associated with the risk of occasionally missing MS/MS acquisition especially with very fast LC, still, 84% true positive rate with MS/MS matching was observed for 2-min method. For SWATH acquisition, this performance was further improved to 93% with library matching using Fit score.
4. Extra settings such as threshold of ratio (vs control), intensity, and weighted combined score were used to ensure maximum true positive and minimum false identification rate.



Quantitation

1. Both TOF-MS and MS/MS modes were used to evaluate the quantitation performance. Overall, the MS/MS mode provided better limit of detection amid higher specificity.
2. In TOF-MS mode, the quantitation performance was better with longer LC. Only a few drugs screening compounds (amphetamine, PCP, and THC-COOH) showed slightly better sensitivity with shorter LC.
3. In MS/MS mode, SWATH acquisition ensures that all data to be acquired at all times. Due to the fast scanning speed (up to 100 Hz at 35K resolution) in MS/MS mode, a fast cycletime (0.5 sec) was used for the 6.5-min LC method that allowed for TOF-MS scan followed by 16 MS/MS scans. Further, variable-SWATH-window approach was used to improve the MS/MS selectivity resulting in unambiguous quantitation at MS/MS level.
4. For SWATH acquisition with 2-min LC method, the acquisition setup was adjusted due to narrower LC peak width to ensure sufficient data points across the LC peak.

SWATH acquisition with variable window

Mass Spectrometer 6.502 mins

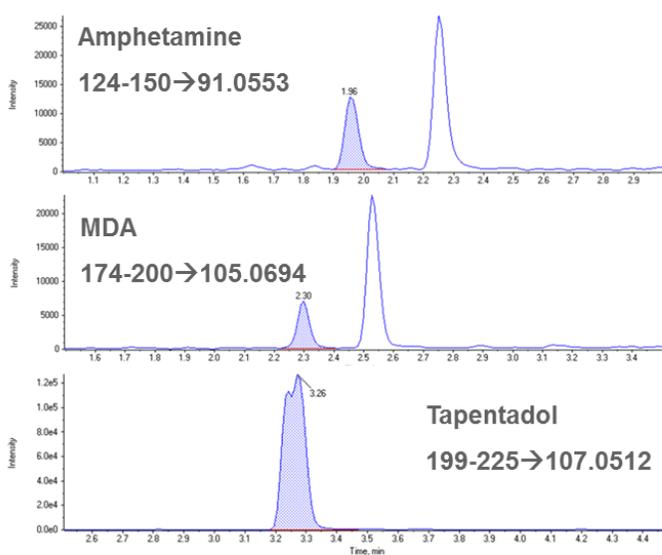
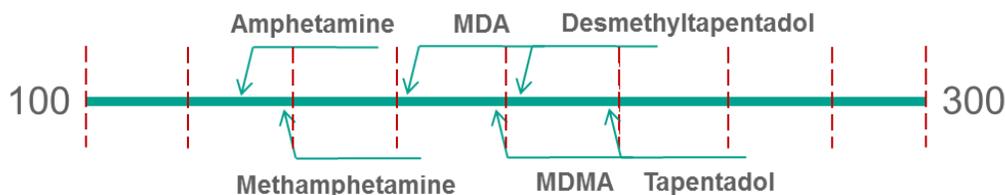
Period 6.502 mins

- TOF MS (+)
- Product Ion (+) 120.0 - 140.0
- Product Ion (+) 139.0 - 165.0
- Product Ion (+) 164.0 - 190.0
- Product Ion (+) 189.0 - 205.0
- Product Ion (+) 204.0 - 218.0
- Product Ion (+) 217.0 - 237.0
- Product Ion (+) 236.0 - 258.0
- Product Ion (+) 257.0 - 273.0
- Product Ion (+) 272.0 - 290.0
- Product Ion (+) 289.0 - 304.0
- Product Ion (+) 303.0 - 313.0
- Product Ion (+) 312.0 - 325.0
- Product Ion (+) 324.0 - 338.0
- Product Ion (+) 337.0 - 350.0
- Product Ion (+) 349.0 - 450.0
- Product Ion (+) 449.0 - 500.0

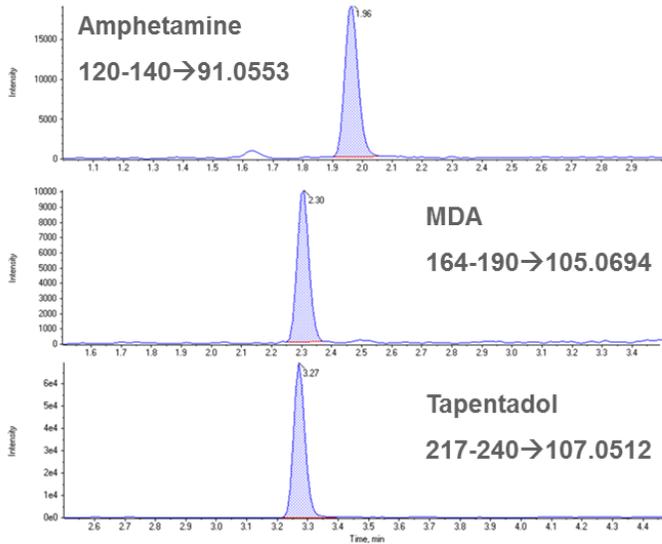
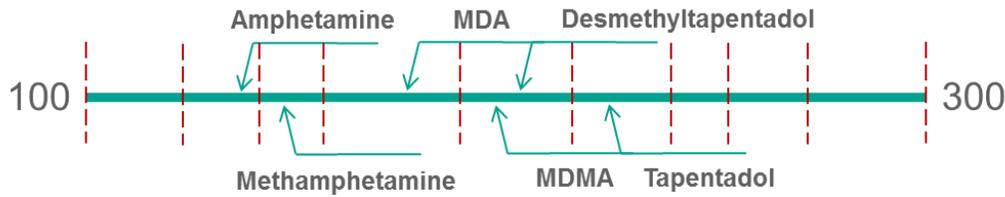
Pairs to separate (not a complete list)				
Amphetamine (136)				
Methamphetamine (150)				
MDA (180)				
MDMA (194)	Nortapentadol (204)			
	Tapentadol (222)			
Norketamine (224)				
Ketamine (238)	Nortramadol (250)			
	Tramadol (264)	Norvenlafaxine (264)	Nordoxepin (266)	Nordiazepam (271)
Morphine (286)	Norchlordiazepoxide (286)	Venlafaxine (278)	Doxepin (280)	Diazepam (285)
Codeine (300)	Chlordiazepoxide (300)	Norsertaline (292)	Norfluoxetine (296)	
Alprazolam (309)	Norcitalopram (311)	Sertraline (306)	Fuoxetine (310)	
Hydroxylalprazolam (325)	Citalopram (325)	Norclozapine (313)	THC (315)	
Norpropoxyphene (326)		Clozapine (327)	THC-OH (331)	
Propoxyphene (340)	Triazolam (343)			
Norbuprenorphine (414)	Hydrotriazolam (359)	Noverapamil (441)		
Buprenorphine (468)		Verapamil (455)		

Shimadzu LC System

- Equilibrate
- Injection



Fixed SWATH window



Variable SWATH window

Conclusion:

A sensitive and selective workflow was developed for toxicological drug screening in a forensic setting using the TripleTOF® 4600 system. Both the IDA-MS/MS and the novel MS/MS^{All} with SWATH acquisition were used for screening task and both acquisition approaches yielded excellent performance. Further, with SWATH which acquired all MS/MS data at all times in a looped fashion, consistent number of data points across the LC peak were acquired, and the resulted MS/MS data can be not only used for screening but also for quantitation when lower detection limit was desired with complex matrix content in the samples.