

Rapid Screening of Submicroliter Urine Samples for Drugs of Abuse utilizing Pulsed Gas Ambient Ionization

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Overview

Rapid detection of trace drugs-of-abuse in urine is demonstrated using a pulsed gas direct analysis in real time ambient ionization equipped time-of-flight mass spectrometer. Submicroliter sampling combined with analysis at multiple temperatures serve to minimize the matrix effects normally encountered in direct analysis of urine without pre-processing to remove salts, proteins, metabolic products including creatinine and urea. Accurate control of the temperature of ionization exiting the DART source and limiting thermal desorption time to one second per sample serve to permit rapid sensitive detection of drugs-of-abuse. Addition of isotopically labelled reference standards and their ionization serve to provide semi-quantitative determination for those drugs detected making the method suitable for high throughput screening of raw urine.

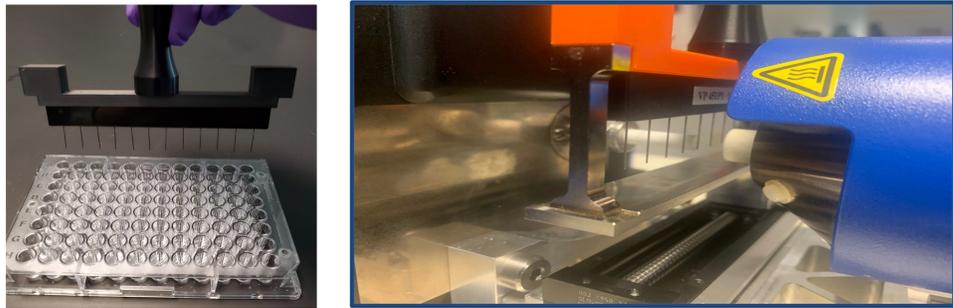


Figure 1. 12-pin tool sampling from 96-well microtiter plate containing urine samples (left). Pin tool mounted on automated presentation robotics of JumpShot equipped DART-MS (right).

Method

Analysis of urine samples presented in 96-well SBS format plates were completed by sampling using a 12-pin replicator (VP Scientific) with the 45-micron diameter pins serving as both sample collector and desorption support. The replicator was mounted on the linear rail presentation system of a JumpShot DART-MS to permit automated presentation of each sample (Figure 1).

The release of a one second pulse of heated ionizing gas from the DART source was coordinated with the introduction of each sample into the desorption ionization region. Ion detection was completed using a JEOL AccuTOF-DART high-resolution time-of-flight MS with DART gas temperature of 350C.

Experimental

Analysis of Urines spiked with DOA standards

100µl of Urine including untreated and those spiked with varying concentrations of DOA's were sequentially. DART-MS results for detection of Methadone shown.

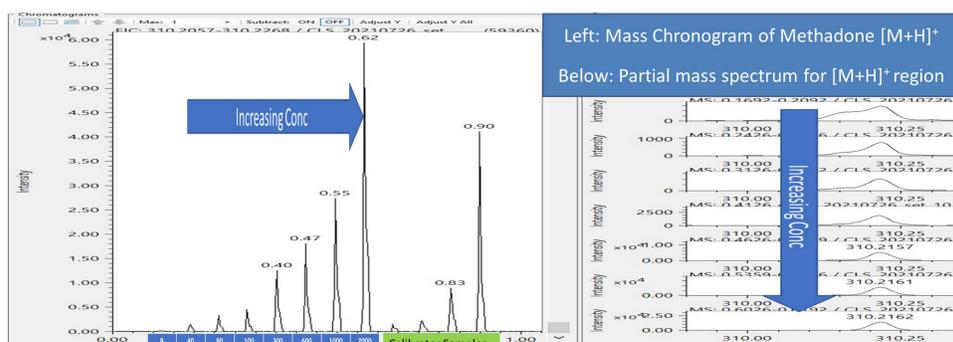
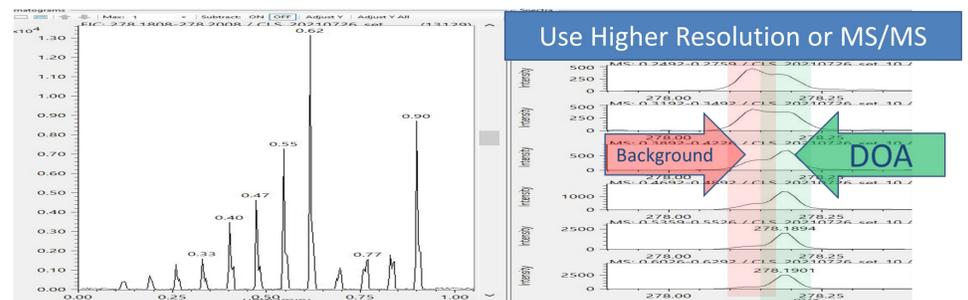


Figure 2. Plots of Pulse DART-MS results from simultaneous analysis of 26 DOA's added to either untreated or glucuronidase treated urine.

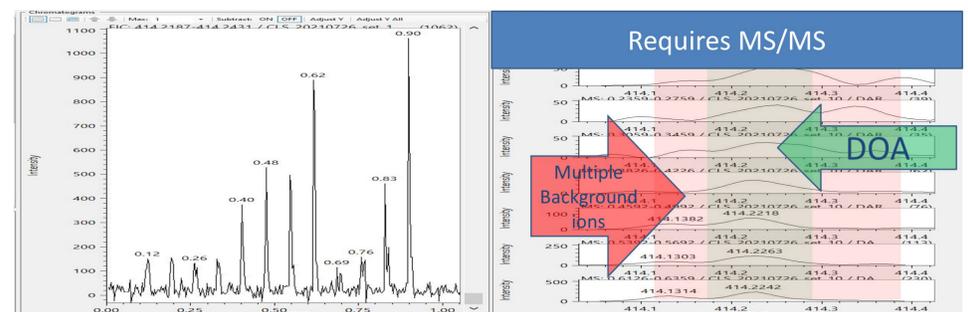
	Cal Levels			L3 = Confirmation cutoff							
	L1	L2	L3	L4	L5	L6	L7				
EDDP	24	48	60	180	360	600	1200	MHR			
Hydromorphone	20	40	50	150	300	500	1000	MSMS-ISOMER			
Temazepam	20	40	50	150	300	500	1000	HR			
Methadone	40	80	100	300	600	1000	2000	MHR			
Methamphetamine	60	120	150	450	900	1500	3000	VHR			
Morphine	20	40	50	150	300	500	1000	MSMS-ISOMER			
Nordiazepam	20	40	50	150	300	500	1000	HR			
Oxycodone	20	40	50	150	300	500	1000	HR			
Amphetamine	60	120	150	450	900	1500	3000	MHR			
Oxymorphone	20	40	50	150	300	500	1000	VHR			
Hydrocodone	20	40	50	150	300	500	1000	MSMS-ISOMER			
Oxazepam	20	40	50	150	300	500	1000	MSMS			
Benzoylcegonine	16	32	40	120	240	400	800	MHR			
Codeine	20	40	50	150	300	500	1000	MSMS			
7-Aminoceclonazepam	10	20	25	75	150	250	500	VHR			
a-Hydroxylprazolam	20	40	50	150	300	500	1000				
Buprenorphine	10	20	25	75	150	250	500				
6-Acetylmorphine	2	4	5	15	30	50	100	MHR			
Norbuprenorphine	10	20	25	75	150	250	500				
Lorazepam	20	40	50	150	300	500	1000	MSMS/HR			
THC-COOH	8	16	20	60	120	200	400	Negative Ion MS			
Norfentanyl	0.2	0.4	0.5	1.5	3	5	10	VHR			
Phenobarbital	60	120	150	450	900	1500	3000	VHR			
Secobarbital	60	120	150	450	900	1500	3000	MSMS			
Butalbital	60	120	150	450	900	1500	3000				

Figure 3. Results of analysis classified as either Feasible using 10K resolution (Green), requiring either MSMS or higher resolution (Blue), and requiring additional clean-up (Red).

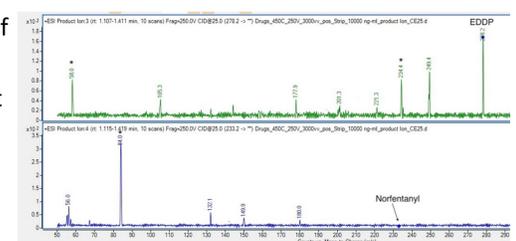
High-Throughput Screening for Drugs: Higher Resolution or MSMS required – Detection of EDDP [M+H]⁺



Detection of ions from background observed in mass chromatograms of 10ppm mass windows (left) and easily observed in the partial mass spectrum. Despite background some drugs are detectable down to their cut-off concentration.



Pulsed ion MS/MS results for detection of readily detectable EDDP (right top) and Norfentanyl which requires MS/MS (right bottom) as measured.



Conclusion

- The 24-Pin Sampler with DART-MS analysis permits rapid detection and quantitative analysis of some drugs-of-abuse using high resolution mass spectrometers.
- No sample preparation is required as the narrow metal pins collect only a small volume of urine from either 96- or 384-well plates facilitating ultrahigh speed analysis at very low cost per sample.
- Utilization of higher resolution and MS/MS methods are underway in order to increase the number of DOA's detectable.
- Method has potential for screening of >1000 samples per hour with no solvent or chromatographic materials.

Acknowledgement DART-MSMS completed with Agilent Ultiva courtesy of Joan Zou, and Nandhini Sokkalingam, MassWerk, Inc.