Can Reducing Sample Volume and Desorption Time Lead during Ambient Ionization lead to Improved Drug Detection from Biological Fluids

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Abstract

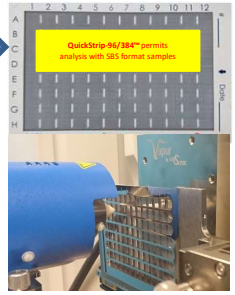
Direct Analysis in Real Time (DART) is a temperature tunable atmospheric ionization technology that couples to LC/MS systems. In prior attempt to complete bioanalytical assays using the method significant matrix effects were encountered when direct analysis of urine, plasma, and oral fluid were analyzed. More recently high throughput sample analysis has been completed in combination with acoustic sampling and now low volume nanoliter-pipetting.

Using low volume, high throughput spotting techniques (TTP Labtech Mosquito) for sample deposition onto wire mesh screens, we have demonstrated a significant decrease in matrix related interference, increased analysis times to faster than 1 well / second and increase throughput by using sample deposition in 384-well format spacing to considerable reduce the time loss due to movement of the stage between samples.

DART approach to small molecule analytics provides a rapid, cost effective sample introduction system, alleviating a number of bottlenecks caused by liquid chromatography or MALDI plate preparation, while retaining the sensitivity and data quality expected from MS data.

Automated Low Volume Pipettor

High-throughput analysis was enabled with the low volume liquid handling pipettor, the mosquito® X1 (TTP LabTech). The mosquito® X1 was used to spot sub-microliter volumes of sample onto mesh screens in a 384 and 1536 well format, for the automated direct introduction to DART-MS for analysis.

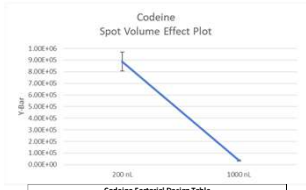


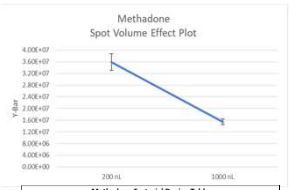
TTPLabtech Mosquito® Robotics for rapid simultaneous low volume sample pipetting onto the QuickStrip-HT

With this workflow we are able to show;
1) Speeds of 2 seconds per sample allowing for analysis of 384 samples in under 10 minutes
2) Accurate mass and quantitation with low %RSD values when using labelled internal std.

Thanks to Ben STTP LabTech for technical support with the mosquito® X1 and for their collaboration

DART Biological Fluids: The DART source was operated with the helium as the ionizing gas set to a temperature of 350C for generating precursor ion of drugs of abuse. A narrow-bore (1mm) exit cap was used to limit the desorption area. Replicates analysis of two different volume spots (200nl and 1000nl) were completed . Speed of presentation was constant. The signal generated by the same amount of drug in each sample is shown





Codeine Factorial Design Table

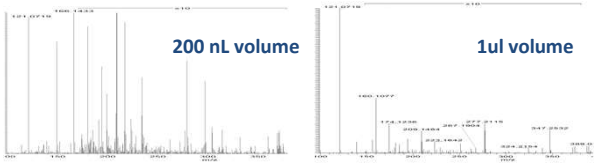
Run	Rail Speed	Sample Volume	Interaction	Average	Range
1	1 mm s^{-1}	200 nL	1 mm s^{-1} & 200 nL	7.31E+05	6.71E+04
2	3 mm s^{-1}	200 nL	3 mm s^{-1} & 200 nL	1.57E+05	9.11E+04
3	1 mm s^{-1}	1000 nL	1 mm s^{-1} & 1000 nL	2.39E+04	1.61E+04
4	3 mm s^{-1}	1000 nL	3 mm s^{-1} & 1000 nL	1.20E+04	1.97E+02
$\bar{x}_{Y\text{-}Bar+}$	1.81E+05	3.59E+04	8.88E+05	σ	3.86E+04
$\bar{x}_{Y\text{-}Bar-}$	-7.43E+05	-8.88E+05	-3.59E+04	σ_{Effect}	2.73E+04
Effect	-2.81E+05	-4.26E+05	4.26E+05	CL_{Effect}	7.58E+04

Methadone Factorial Design Table

Run	Rail Speed	Sample Volume	Interaction	Average	Range
1	1 mm s^{-1}	200nL	1 mm s^{-1} & 200 nL	2.53E+07	5.17E+06
2	3 mm s^{-1}	200 nL	3 mm s^{-1} & 200 nL	1.06E+07	1.05E+06
3	1 mm s^{-1}	1000 nL	1 mm s^{-1} & 1000 nL	8.84E+06	4.79E+05
4	3 mm s^{-1}	1000 nL	3 mm s^{-1} & 1000 nL	6.60E+06	9.68E+05
$\bar{x}_{Y\text{-}Bar+}$	1.95E+07	1.54E+07	3.60E+07	σ	1.70E+06
$\bar{x}_{Y\text{-}Bar-}$	-3.19E+07	-3.60E+07	-1.54E+07	σ_{Effect}	1.20E+06
Effect	-6.24E+06	-1.03E+07	1.03E+07	CL_{Effect}	3.3E+06

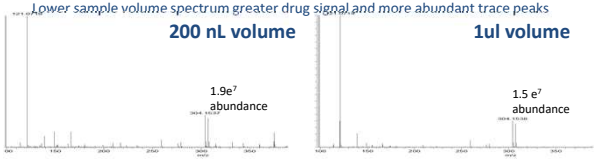
Direct analysis of untreated urine

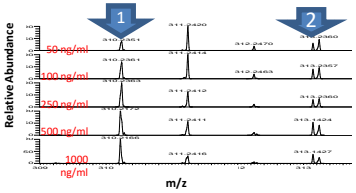
Lower sample volume spectrum much richer in abundant peaks



Direct analysis of drug spiked urine

Lower sample volume spectrum greater drug signal and more abundant trace peaks



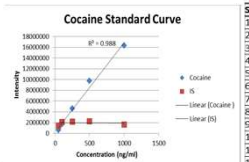


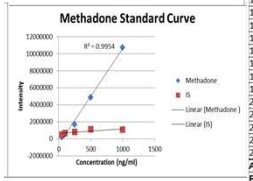
Partial MS for (1)Methadone and (2) Methadone-d_3

Dynamic range is good with sub-50ng/ml detection possible even from very low volume samples

Quantitative Analysis: Reliable and consistent sample deposition and ionization was determined utilizing DART MS/MS even at high sampling speed

- Urine samples spotted by Mosquito and analyzed by DART allow for quantitation of target drugs, with calibration curves showing R2 values greater than 0.988
- Sample deposition and analysis is consistent, with the %RSD for a row of 24 samples 1% with internal standard correction

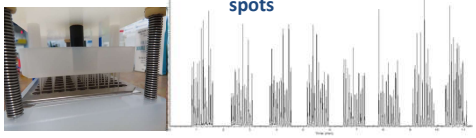




Spot#	Methadone Area	Methadone D_3 Area	Ratio
1	9192477	4726356	1.94
2	13028064	6749966	1.93
3	14941131	7774225	1.92
4	15509356	8041628	1.93
5	11757203	6139805	1.91
6	10719603	5645525	1.90
7	14423181	7510870	1.92
8	13923088	7310156	1.90
9	15124953	7831709	1.93
10	10997691	5706301	1.93
11	7235249	3758329	1.93
12	8623697	4513035	1.91
13	14441789	7552207	1.91
14	19970879	10523812	1.90
15	21354895	11123363	1.92
16	13833300	7208351	1.92
17	11805493	6105707	1.93
18	10768935	5668568	1.90
19	20385612	10633185	1.92
20	18965875	9801654	1.93
21	20347835	10523441	1.93
22	15675004	8142748	1.93
23	15658559	8088413	1.94
24	16790430	8661522	1.94
Average	14394762	7489203	1.92
RSD	27%	27%	1%

Future Prospects: **(1)** Integrated sample processing robotics to enable continuous operation and more accurate positioning of ultralow volume spots in the desorption ionization region. (2) utilization of plastic pin tools as low cost sample deposition devices (0.57 cents/sample)

DART analysis of 100 (estimate) nanoliter coffee spots



Disposable Pin tool

SIM Protonated Caffeine HRMS

Conclusions:

- Low volume pipetting enabled desorption ionization and detection of trace levels of drugs in urine without sample clean up or processing
- Spotting of sub-microliter volumes of urine reduced the matrix effect when analysis by DART ambient ionization
- Quantitation of drugs in urine is possible with low volume samples with consistent with RSD 1% when using labelled IS
- Low volume spotting and high speed sample positioning combine to decrease analysis time to same time per plate (96, 384, or 1536)
- Utility of disposable pin tools as sample dispensers being investigated as an alternative to advanced robotics