Quantitation with Direct Analysis in Real Time Mass Spectrometry by Utilization of Less Sample

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Overview

- Direct Analysis in Real Time (DART) - MS is a versatile technique that can be used to ionize samples in their native states
- Quantitation is believed to be difficult, or not possible with DART-MS
- Utilization of less sample or volume with internal standard enables quantitation

Introduction

DART is an ambient ionization source that can be interfaced directly to liquid chromatography compatible mass spectrometers. The versatility of DART enables analysis of nonconventional samples such as solid powder or an intact leaf from a plant. However DART does not provide any separation of samples and is affected by the matrix of the sample or the ambient ionizable species. There are some misconceptions of DART suggesting that the technique is strictly qualitative. The misconception does not take into consideration the linear dynamic range nor the detector saturation level with the analyzed samples.

Methods

- A DART-SVP ionization source was interfaced to a Waters QDa, and an Agilent QTOF.
- Solutions of Fentanyl ranging from 5 ppb to 10 ppm were spiked with 1 ppm of Fentanyl D_5 for quantitation
- Samples were deposited as 200 nL and 1 µL sample spots on QuickStrip™ utilizing SPT Labtech's Mosquito HTS Liquid Handler.
- Samples were analyzed with both MS systems

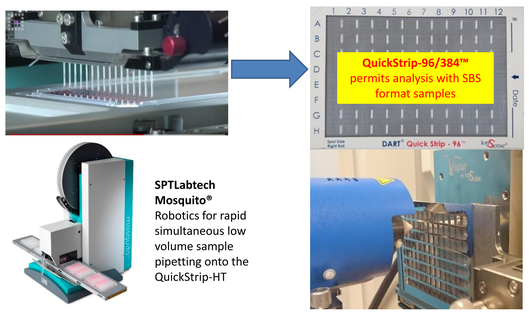


Figure 1 – Workflow Diagram

Results

Initial Findings

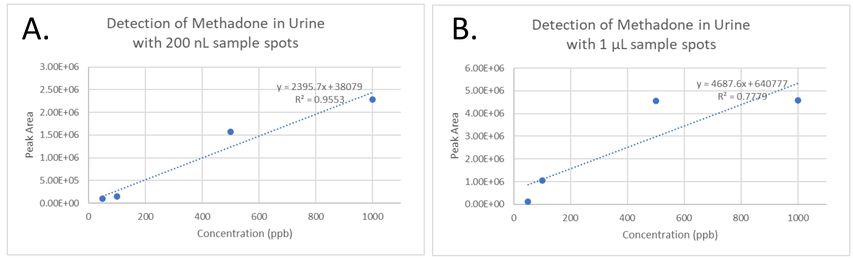
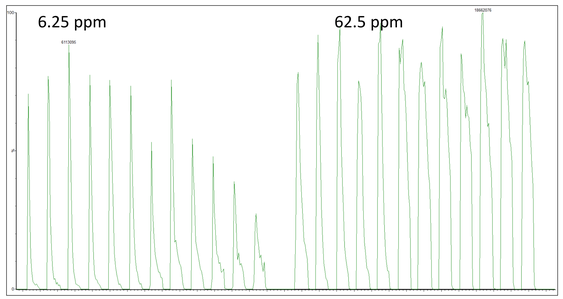


Figure 2 – Linearity of data acquired with 200 nL droplets (A) and 1 µL droplets (B)

- Earlier work from 2019 evaluated the effect of smaller sample volume on DART-MS[1]
- It was concluded that using small sample spots of 200 nL improved detection sensitivity of methadone in pooled urine matrix
- Linearity was also improved from an R^2 of 0.7729 to 0.9553 by utilizing 200 nL spots without internal standard as shown in the graphs in **Figure 2**

Detector Saturation

Figure 3 – Fentanyl extracted ion chromatograms of 6.25 and 62.5 ppm concentrations



- Detector saturation was observed for samples in the range of 62.5 ppm to 500 ppm with no difference in area counts
- Low ppm samples are near detector limit as shown in **Figure 3**, peak areas differ by a factor of 3 (6.1E6 vs 1.8E7) when concentrations differ by a factor of 10
- Quantitation at these levels will be impossible without sample preparation, i.e. sample dilution

Linearity and Quantitation

- R^2 coefficients of the standard curve and log scale were both collected to compare linearity on the QTOF with internal standard correction
- The following factors improved linearity:
  - MS Systems with higher resolution
  - Internal standard correction
  - Spotting less volume on mesh
- Quantitation of the data acquired on the QTOF data is shown in **Figure 2**
- Adjusting response factor to the center of the standard curve at 370 ppb resulted in low percent errors between 100 ppb to 10 ppm
  - Low ppb levels between 5 and 50 ppb have high percent errors and require a different standard curve for quantitation
  - 5 ppb concentrations was not detected when using 1 µL deposits

Instrument	Sample	Volume	R^2	R^2 (Log Scale)
Orbitrap	Methadone in Urine	200 nL	0.9553	0.9773
Orbitrap	Methadone in Urine	1 µL	0.7779	0.8518
Single Quadrupole	Fentanyl	200 nL	0.9424	0.7712
Single Quadrupole	Fentanyl	1 µL	0.9385	0.9376
Single Quadrupole	Fentanyl w/ IS Correction	200 nL	0.9732	0.9710
Single Quadrupole	Fentanyl w/ IS Correction	1 µL	0.9459	0.9610
QTOF	Fentanyl	200 nL	0.9908	0.9521
QTOF	Fentanyl	1 µL	0.9969	0.8705
QTOF	Fentanyl w/ IS Correction	200 nL	0.9999	0.9914
QTOF	Fentanyl w/ IS Correction	1 µL	0.9999	0.9748

Table 1 – Linearity comparison of 200 nL sample spot with 1 µL sample spot

Concentration	200 nL	1 µL
5	266%	100%
14	110%	81%
41	88%	36%
123	15%	5%
370	0%	0%
1111	0%	14%
3333	5%	9%
10000	8%	8%

Table 2 – Quantitation and Percent Error (%)

Conclusion

- Sample quantitation can be performed with DART-MS and the performance will be optimized with the following:
  - Concentrations are within the linear range
  - Concentrations are not at saturation level
  - Internal standard, preferably deuterated
  - Utilization of low sample volume

Reference

1. Liang, P., Li, F., Laramee, B., Musselman, B.: Effect of reducing sample volume on the detection of drugs in urine by transmission mode direct analysis in real time mass spectrometry. Rapid Commun. Mass Spectrom. DOI: 10.1002/rcm.8688