Rapid Chromatography-Free Confirmatory Screening of Stimulant Drugs in Human Urine Using DART-MS Analysis

DART-MS screening of urine-based stimulant drugs provides rapid and accurate confirmatory screening results in a chromatography-free workflow, offering an easy and cost-effective alternative to immunoassays that overcomes the limitations of lower throughput overall and presumptive false-positive results associated with immunoassay evaluation of human urine samples.

Abstract

Immunoassays (IA) are most commonly used as a test method in initial Urine Drug Screening (UDS) tests for drugs of abuse in the field of forensic toxicology. This is in part due to the rapid generation of results and ease of adaptability to automation. However, IA results are considered to be presumptive and not confirmatory in their accuracy due to the high frequency of false positives attributed to cross-reactivities with other ubiquitous co-analytes. Due to the number of potential interferents in these assays, a positive IA result must be confirmed by another analytical approach, typically a chromatography-based method. LC-MS and GC-MS are most commonly used as confirmatory assays due to their high degree of sensitivity, specificity, and accuracy. While chromatography-based approaches are well established and commonly achieve sub-ng/mL detection limits, they often rely on costly carrier gases and solvents and are limited in throughput with time-consuming chromatography steps and sample preparation. In this work, we report the development of a chromatography-free method using direct analysis in real time-mass spectrometry (DART-MS) that is shown to accurately identify and measure four illicit phenylethylamine drugs: amphetamine, methamphetamine, 3,4-Methylenedioxyamphetamine (MDA), and 3,4-Methylenedioxy methamphetamine (MDMA). The detection of these common illicit compounds commonly suffers from interferences in immunoassay-based urine screens. The optimized DART-MS based workflow achieves a throughput rate of 96 samples in 40 minutes that is roughly equivalent to IA. This chromatography-free workflow meets the low limits of detection and low % RSD for high repeatability in urine matrices and avoids interference from matrix or co-analytes.

Introduction

Phenylethylamines are a class of synthetic substances which act as central nervous system stimulants that induce the effects of euphoria, increased energy, distortion of time, and enhanced enjoyment of tactile experiences – to name a few[1]. These compounds are classified as Schedule I substances under the Controlled Substances Act, and the related illicit drugs amphetamine, methamphetamine, MDA, and MDMA are commonly monitored in the field of toxicology (DEA) typically within the context of urine testing[2]. Traditional Urine Drug Monitoring (UDM) is comprised of two types of tests: presumptive Urine Drug Screening (UDS) by immunoassay followed by a confirmatory test using a spectrometric analytical technique such as LC-MS or GC-MS[3].

A limitation of testing for these small analyte compounds arises from their simple structure which leads to significant cross-reactivities with other analytes when using antibody-based immunoassays[1]. Cross-reactivity occurs with structurally related sympathomimetics commonly used as anti-hypertensive, anti-diabetic, antihistamine, antibiotic, and psychiatric drugs and is well documented, often leading to false positive test results within traditional UDS testing. It has been shown that false positive results occur in as many as 15% of samples, resulting in unnecessary and expensive confirmatory testing[5].

Compared to presumptive and subjective IA techniques, MS-based techniques are capable of identification and quantitation of trace-level analytes with a high degree of specificity and accuracy. Tandem-MS (MS^n) provides enhanced levels of specificity and structural information about analytes of interest. Conventionally, MS and MS^n approaches are preceded by a chromatographic separation to further improve the performance and detection of analytes in complex mixtures[6].

While chromatography improves specificity and sensitivity, analysis often takes between 10 and 30 minutes per sample which leads to severe bottlenecks in analytical workflows[7]. Now, with the availability of ambient ionization techniques such as DART, the requirement for a chromatography separation step prior to MS analysis when monitoring appropriate analytes is no longer necessary. DART-MS generates a signal which is smoothed to be similar to LC data that includes molecular fragment ion information specific to the illicit compound. Because desorption conditions can be altered to favor lower boiling point and higher boiling point substances, DART is effective in separating compounds simply by changing parameters that control desorption and ionization. DART-MS offers a rapid chromatography-free alternative with higher selectivity, specificity that significantly reduces high false-positive screening results in IA urine drug screens.

In this work, we perform a liquid-liquid extraction on urine samples containing the four common illicit drugs amphetamine, methamphetamine, MDA, and MDMA. Samples were processed using ToxBox® custom drug panel (PinPoint® Testing) and analyzed via DART-MS to successfully measure all four compounds with good linearity ($R^2 > 0.99$) and repeatability (3-6% RSD) across the linear range for each analyte.

Methods

Sample Preparation

500 µL of certified drug-free urine and 300 µL DI water were added to each well in a 96 deep-well ToxBox® customized Stimulants Validation plate from PinPoint Testing. The ToxBox custom drug panel contains reagent solutions A-C, a preloaded 96 well plate with selected analytes for an 8-point triplicate calibration curve, triplicate QC samples, along with sample and calibration blanks. The entire plate was then agitated for 10 minutes at 500 RPM on a horizontal plate shaker after which, 600 µL of PinPoint Solution B was added to each well and aspirated 10X to mix. Samples were allowed to separate for 10 minutes. Next, the aqueous layer (800 µL) was removed from each well and discarded. The remaining organic layer was evaporated under nitrogen at 60 psi for 40 minutes followed by reconstitution in 50 µL of PinPoint Solution C. Reconstituted samples were agitated at 500 RPM on a horizontal plate shaker after which a 1 µL aliquot from each well was transferred onto a Bruker DART QuickStrip HTS-96 screen and allowed to dry under nitrogen gas at 40°C for 15 minutes.

DART-MS Analysis

After spots were fully dried, the prepared QuickStrip HTS-96 screen was loaded onto the automated XY transmission stage of a JumpShot DART source (Bruker Daltonics) mounted to an EVOQ™ Elite (Bruker Daltonics) triple quadrupole mass spectrometer and analyzed in pulse mode via MS/MS with each analysis taking approximately 20s/sample. Samples were analyzed and processed using tqControl software (Bruker Daltonics), a single interface for instrument control and data analysis. Each calibration level was analyzed in duplicate and data were fitted to a linear regression model with QCs at two levels presented in tables below.

DART and MS Parameters

Tables 1 and 2 detail the DART and MS parameters used to analyze the four samples.

Table 1
DART method parameters

DART Parameter	Value
Gas flow temperature	250°C
Grid Voltage	100 V
Pulse time	2 sec
Ionization gas	He
Polarity	Positive

Table 2
EVOQ Elite MS method parameters

MS Parameter	Value
Cone temperature	350°C
Cone gas pressure	20 psi
CID cell pressure	1.25 mTorr
Collision gas	Ar
Detector voltage	1.36 kV
Polarity	Positive

Compound Transitions

For all four analytes, the MRM transitions are shown in the table below, as well as the optimized collision energies and scan times used.

Table 3
EVOQ™ Elite MS method compound transitions

Analyte	MRM Transition (m/z)	CE (V)	Scan Time (msec)	Q1 Res	Q3 Res
Amphetamine	136 → 91	5	30	0.7	1.5
Methamphetamine	150 → 91	21	30	0.7	1.5
MDA	180 → 135	18	30	0.7	1.5
MDMA	194 → 163	9	30	0.7	1.5
PCP	244 → 86	12	30	0.7	1.5
D5-amphetamine	141 → 93	10	30	0.7	1.5
D5-methamphetamine	155 → 92	10	30	0.7	1.5

Results

DART-MS analysis of the panel of compounds resulted in good linear correlation with respect to the QC samples that were run and adequate for use in screening, all demonstrating an $R^2 > 0.99$. Additionally, the Lower Level of Quantitation (LLOQ) was shown to be 125 ng/mL for each of the four analytes, indicating that this simple chromatography-free workflow is sufficient in detecting these compounds at levels at or below the common cutoffs within urine matrix[8]. Performance of this quantitative screening workflow is as good as or better than commonly used UDS assays, without the high rate of false positives associated with UDS assays.

Table 4
Chromatography-free stimulants workflow quantitative data performance

Analyte	Range (ng/mL)	R^2	LLOQ (ng/mL)	Slope	Accuracy	Repeatability (%RSD)
Amphetamine	125-25,000	.998	125	.001	92%	6%
Methamphetamine	125-25,000	.997	125	.005	97%	6%
MDA	125-5,000	.990	125	.003	92%	9%
MDMA	125-5,000	.999	125	.04	94%	8%
PCP	6.25-1250	.995	6.25	.02	98%	6%

An example of the DART-MS data that was collected for Amphetamine is shown in Figure 1 below. This figure shows the raw 'unsmoothed' signal that is generated by DART alongside the smoothed data that was used to quantify this sample. This shows that while the nature of the DART signal is not identical to that produced by chromatography-based MS measurements, similar levels of quantitative accuracy can be generated from this signal.

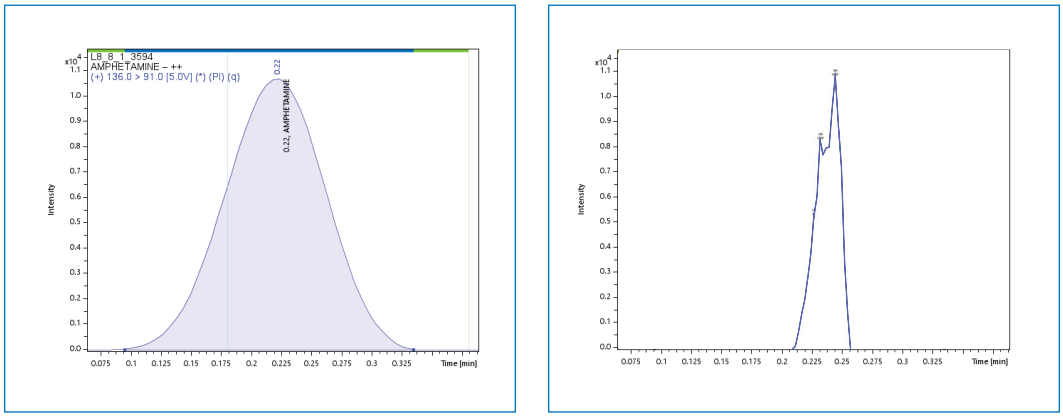

Fig. 1
Amphetamine DART signal smoothed data alongside raw 'unsmoothed' data

Figure 2 shows an example of the calibration curve that was generated for Amphetamine, where a linear R^2 correlation value > 0.998 was realized. Again, this shows the strength of DART-MS and its ability to detect Amphetamine accurately and sensitively at confirmatory levels with high confidence.

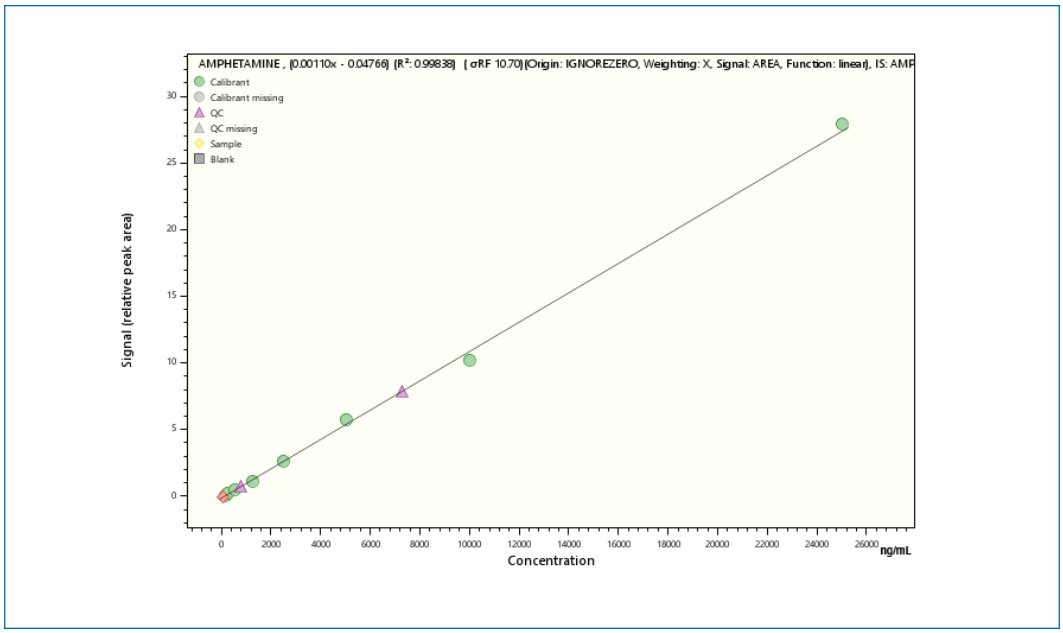

Fig. 2
8 Point calibration for Amphetamine

Conclusions

The work presented demonstrates the utility of DART-MS in rapid quantitative drug screening for urine as a viable alternative to current UDS assays. The chromatography-free workflow is faster, more accurate, and quantitative. In addition, the chromatography-free workflow has the benefit of minimizing false positives typically associated with immunoassay screening avoiding non-valued added work to yield higher productivity. This high performance workflow also eliminates the need for expensive and time-consuming chromatography based confirmatory tests.

References

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