Rapid Quantitative Screening of 16 Synthetic Cannabinoids in Urine Using DART-MS Analysis

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Introduction

Immunoassay-based (IA) detection for drugs of abuse is commonly used as an initial screening step in urine-based drugs testing due to rapid generation of results and ease of automation. However, IAs suffer from significant issues with cross-reactivity leading to false positive and negative results, requiring costly and time-consuming chromatography-based confirmatory testing or missing a positive sample entirely. As a cost-effective alternative, DART-MS provides quantitative and highly selective results greatly reducing or eliminating false positives compared to conventional IA-based drug screening. The easily automated liquid-liquid extraction preparation is well suited for DART-MS and LC-MS based analyses. In this work, we report on the development of a rapid, chromatography-free screening approach for fifteen synthetic cannabinoids in urine requiring less than 30 seconds per sample and perform a validation with urine samples confirmed positive by LC-MS measurement.

Results

DART and EVOQ® DART-TQ+ MS parameters were optimized for to maximize sensitivity, selectivity, precision, and achieve rapid analysis time. With DART gas temperature and grid voltage optimized at 350°C and 50 V, respectively, unique MS/MS transitions, collision energies, and MS scan times were successfully identified for **1-16**. DART-MS analysis of the synthetic cannabinoid panel resulted in good linear correlation of $R^2 > 0.99$ for all measured analytes and a recovery between 89 and 110% for all 16 analytes across the defined calibration ranges. The reported lower level of quantitation (LLOQ) for all analytes is at or below common IA screening cutoff values of between 0.1 to 5 ng/mL for the synthetic cannabinoid panel. In a representative cross-validation plot of positive urine samples (n=20) the butanoic acid metabolite of MDMB 4-en PINACA concentrations ranged from undetectable to 362 ng/mL with a mean value of 170.5 ng/mL ± 15.2. Measurements using The newly developed DART-MS method were well correlated with LC-MS measurements (R^2=0.995, $p<0.05$) with a slope of near unity at 0.95. The results in this work indicate that this rapid chromatography-free workflow using DART-MS for quantitative screening is sufficient at detecting all 16 analytes at or below the common cutoff values without the high rate of false positives associated with IA based screening approaches.

Methods

Calibration series were prepared in certified drug-free urine by spiking with standards **1-16** (0.1-2500 ng/mL) using deuterated AB-PINACA as an internal standard. Enzyme hydrolysis was performed using 500 µL pre-spiked urine. After hydrolysis, 0.1 M Borax buffer (pH=10.4) and 30:70 ethyl acetate:n-chlorobutane were added to each sample followed by centrifugation. Next, the organic layer was transferred to glass vials and evaporated to dryness under N_2 at followed by reconstitution MeOH. 2 µL aliquots of each reconstituted sample were transferred onto a Bruker DART QuickStrip™ HTS-96 screen and allowed to dry under N_2 gas. For analysis, the prepared QuickStrip HTS 96 screen was loaded onto the EVOQ® DART-TQ+ (Bruker Daltonics) triple quadrupole mass spectrometer for DART-MS-MS analysis. Accuracy was determined in triplicate using certified drug-free urine without detectable levels of 1-16 at 2 levels for each analyte within the linear range of each calibration series. Results were validated against LC-MS that was performed using 20 urine samples confirmed as positive for one or more analytes.

Analyte	Range (ng/mL)	R^2	Cutoff (ng/mL)	Slope	LOD (ng/mL)	LLOQ (ng/mL)	Recovery (QC 1)	Recovery (QC 2)	Repeatability (%RSD)
(1) 4-cyano-cumyl-butinaca	0.1-50	0.991	0.1	5455	0.02	0.06	105	99	5%
(2) 4-F-abutinaca-N-(4-hydroxylbutyl) metabolite	0.5-250	0.994	0.5	8172	0.08	0.27	89	92	14%
(3) 4-F-MDMB butica	0.2-50	0.999	0.2	1202	0.04	0.16	101	98	2%
(4) 4-F-butica-N-butanoic acid metabolite	0.25-125	0.991	0.25	657	0.02	.06	102	100	4%
(5) 4-F-butinaca-N-butanoic acid metabolite	1.25-125	0.997	1.25	881	0.35	1.2	104	93	2%
(6) 5-F-ADB metabolite	1-500	0.995	1	1933	0.05	0.14	98	102	5%
(7) 5-F-MDMB pica	1-500	0.995	1	2724	0.08	0.27	101	97	5%
(8) 5-F-MDMB-pica metabolite	1-500	0.996	1	545	0.1	0.5	110	93	5%
(9) ADB-4en-pinaca	0.25-125	0.995	0.25	12994	0.001	0.03	101	96	5%
(10) MDMB-4en-pinaca butanoic acid metabolite	1-500	0.995	1	4618	0.04	0.12	89	110	6%
(11) ADB-binaca	0.5-250	0.995	0.5	4126	0.01	0.03	109	85	6%
(12) ADB-butinaca	0.5-250	0.995	0.5	6348	0.03	0.1	92	87	12%
(13) ADB-hexinaca	0.5-250	0.997	0.5	3332	0.2	0.7	92	94	10%
(14) AMP-4en-pinaca	0.25-125	0.998	0.25	1777	0.03	0.1	95	93	7%
(15) JWH-N-pentanoic acid metabolite	0.25-125	0.999	0.25	140	0.05	0.14	102	92	9%
(16) MDMB-CHMICA metabolite	1-500	0.997	1	403	0.17	0.58	100	108	3%

Fig. 1 Figure of merit for DART-MS analysis of 16 synthetic cannabinoids in urine

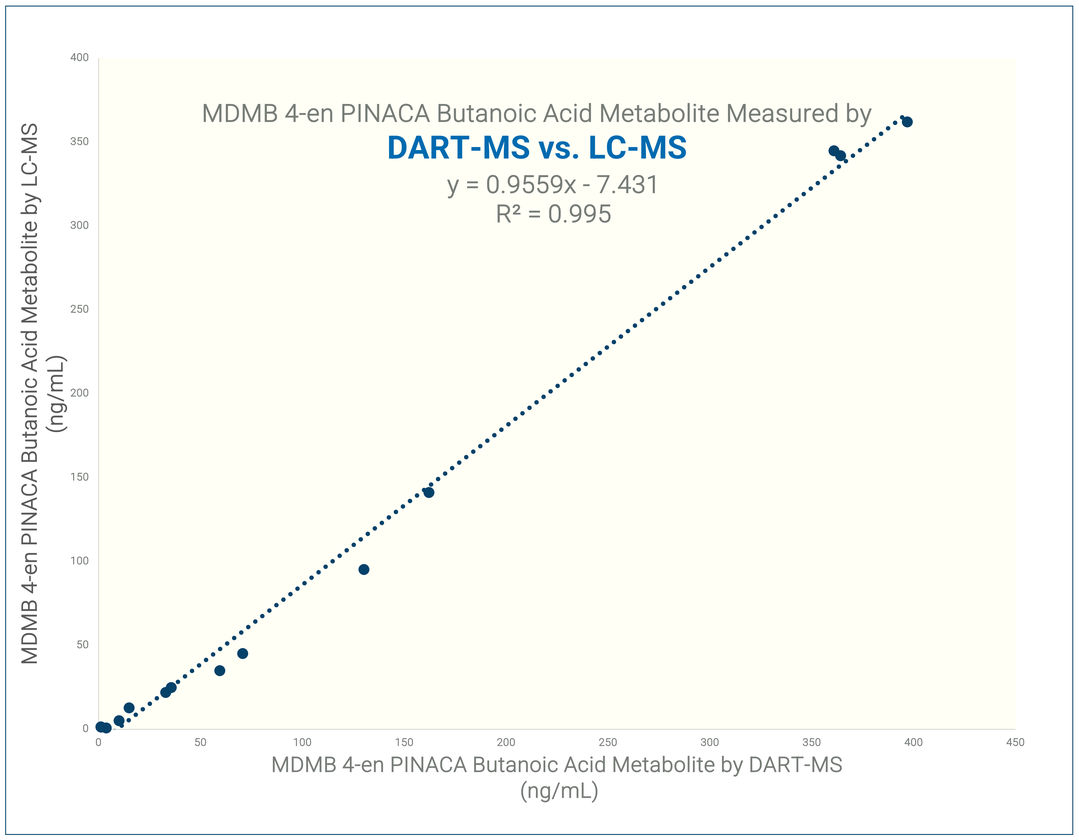

Fig. 3 Correlation between MDMB 4-en PINACA butanoic acid metabolite (10) measured by DART-MS and LC-MS (n=20)

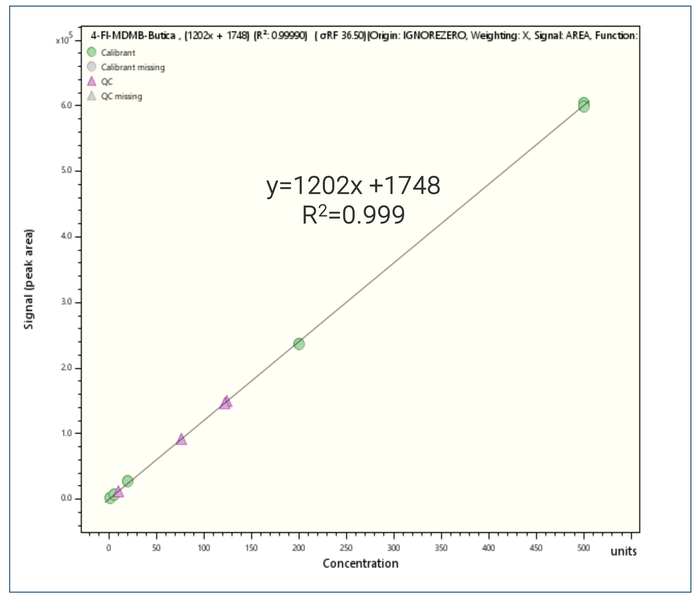

Fig. 2 Calibration curve for 4-Fl-MDMB Butica (3) in urine

Summary

The results presented herein demonstrate the suitability of the DART-MS workflow as a rapid, quantitative, and selective alternative to conventional IA-based urine screening by offering a quantitative screening method with the benefits of minimizing false positives typically associated with IA based screening, avoiding costly and unnecessary chromatography-based confirmatory testing.

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Conclusion

- **Chromatography-free workflow** is viable and dynamic alternative to current IA UDS assays
- Quantitative screening method **cross-validated** with LCMS measurements
- Maximize **throughput**
- **Minimal** solvent and gas use
- **96 samples in less than 45 minutes**

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