Development of a SPE/DART Assay for Rapid Narcotics Screening in Urine

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Overview

•The use of DART MS (direct analysis in real time) has been investigated for the analysis of drugs of abuse in urine in the past. Removal of creatinine is critical for ionization by DART.

• Benzoylecgonine (BZE) and 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), the primary metabolites of cocaine and Δ9-tetrahydrocannabinol, were used as representative narcotic analytes.

•Creatinine, a major component of urine with a typical physiological concentration of 1 mg/mL, virtually eliminates the ionization of many narcotics for detection by DART[1].

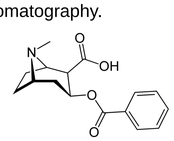
•Creatinine concentrations need to be reduced to less than 10 µg/mL to limit ionization suppression[1].

•An automated method to reduce creatinine concentrations by greater than 99% while providing quantitative recovery of BZE and THC-COOH has been developed on Instrument Top Sample Prep (ITSP) Solid Phase Extraction (SPE) cartridges.

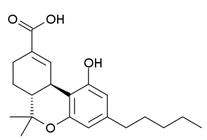
Introduction

DART is a new ionization method for rapid, non-contact surface sampling of compounds. Operating at ambient pressure with the sample at ground potential, the source enables near instantaneous determination of sample composition by using mass spectrometry. Electronic or vibronic excited-state species generated in the source interact with reagent molecules and polar or non-polar analyte present near the inlet of the mass spectrometer.

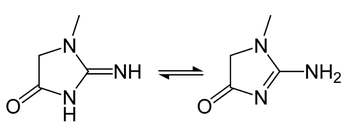
Detection and quantitation of narcotics in urine by using DART without sample prep has not been successful. Production of ions from creatinine, normally found in urine at high concentrations, virtually eliminated the ionization of narcotics. A quantitative analytical method has been developed for the determination of benzoylecgonine (BZE) and 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) in human urine after removal of creatinine. BZE and THC-COOH are the primary metabolites of cocaine and Δ9-tetrahydrocannabinol found in urine and are the species used to confirm the presence of cocaine and Δ9-tetrahydrocannabinol. Standards of BZE and THC-COOH were prepared in both water containing creatinine and in urine and the analytes were confirmed and quantified using reversed phase (C_{18}) ITSP SPE and liquid chromatography mass spectrometry (LC-MS) following removal of the creatinine. Single ion monitoring (SIM) was used for the analysis and deuterated analogs of the two analytes were used for quantitation. The SPE-treated samples have been successfully analyzed by using DART-MS thus demonstrating the utility of the method for rapid determination of drugs of abuse without chromatography.



BZE Exact Mass 289.1



THC-COOH Exact Mass 344.2



Creatinine Exact Mass 113.1

Methods

Sample Preparation

BZE standard and deuterated analog were obtained from Sigma-Aldrich. THC-COOH standard and deuterated analog were obtained from Cerilliant. Creatinine standard was obtained from Acros Organics. Two sets of standards of BZE and THC-COOH were prepared, one set in water with 1 mg/mL creatinine and one set in urine. Calibration standards were prepared at nominal concentrations of 0.1, 0.5, 1, 5, 10 and 20 µg/mL. Deuterated analogs were prepared in isopropanol with a concentration of 250 ng/mL. A 10 µg/mL standard of creatinine in water was prepared.

ITSP SPE Method

ITSP Cartridges: SPE µLplate w/C18 10mg (Product No.: 07-C1810-20A)

A CTC Analytics PAL HTS sample handler was used to prepare the samples. The PAL was configured with a 100 µL L-Mark syringe and two tray holders. Each tray holder held 2 microplates, one of which was designed to hold the ITSP hardware kit (Product No.: 07-ITSP-HW). The extraction protocol was as follows:

Step	Solvent	Volume (µL)	Flowrate (µL/sec)
Condition	B	100	15
Condition	A	100	15
Load	Sample	50	5
Wash	A	3 x 100	15
Wash	C	3 x 100	15
Aspirate	Air	80	100
Elute	D	2 x 50	5
Aspirate	Air	80	100

Solvent A: Water
Solvent B: Methanol
Solvent C: Water with 0.1% TFA
Solvent D: Isopropanol with deuterated analogs

Analysis Method for BZE and THC-COOH

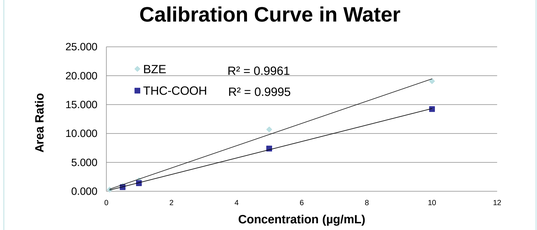
Instrument:	Agilent 1956B single quadrupole with 1200 Rapid Resolution HPLC
Solvent A:	Water with 0.05% TFA
Solvent B:	Methanol with 0.05% TFA
Column:	Shimadzu Shim-pack XR-ODS, 3.0x30mm, 2.2 µm particles
Column Temp.:	50 °C
Flowrate:	1.5 mL/min
Gradient:	0.00 min (5% B), 3.0 min (100% B), 3.30 min (100% B), 3.35 min (5% B)
Ionization Mode:	Positive Ion Electrospray
SIM Channels:	m/z 290 (BZE), m/z 293 (BZE-d3), m/z 345 (THC-COOH), m/z 348 THC-COOH-d3

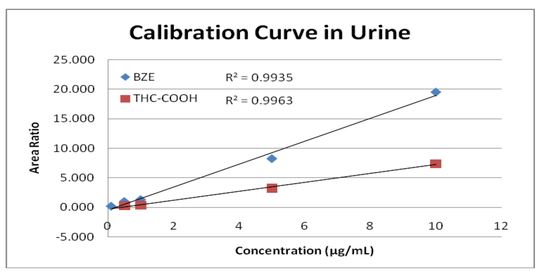
Analysis Method for Creatinine

Instrument:	Agilent 1956B single quadrupole with 1200 Rapid Resolution HPLC
Solvent A:	10 mM NH_4OAc pH 2.3
Solvent B:	10 mM NH_4OAc pH 7.2
Column:	Agilent Zorbax 300-SCX, 2.1x50mm, 5 µm particles
Column Temp.:	Ambient
Flowrate:	1.0 mL/min
Gradient:	0.00 min (5% B), 2.0 min (66% B), 2.1 min (66% B), 2.4 min (5% B)
Ionization Mode:	Positive Ion Electrospray
SIM Channels:	m/z 114 (Creatinine)

Results

Standards of BZE and THC-COOH in water containing creatinine and in urine were processed with the ITSP cleanup procedure and analyzed by LC/MS. The calibration curves that were obtained for each are shown below, plotting the analyte to internal standard area ratio versus the concentration.



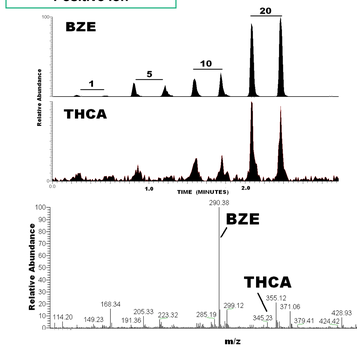


Creatinine concentrations in processed water and urine standards were confirmed to have been reduced from 1 mg/mL in water to less than 10 µg/mL in all samples by comparison of sample peak areas to that of a 10 µg/mL creatinine standard.
When using C18 ITSP cartridges, creatinine had very different absorption characteristics from BZE and THC-COOH. It was still necessary however, to wash the cartridge intensely to reduce the creatinine concentration in water from 1 mg/mL to less than 10 µg/mL. Fewer washes were investigated but the creatinine concentrations increased as the number of washes decreased. Following the washes a pulse of air was used to remove residual wash solvent from the cartridge prior to elution to increase the effectiveness of the elutions and to decrease dilution of the eluted sample. Two elutions of 50 µL isopropanol were found to be more effective than a single 100 µL elution possibly because of the increased residency time of isopropanol on the cartridge. Additional pulses of air were administered after each 50 µL elution to dislodge residual drops of eluant remaining on the tip of the ITSP cartridge.

DART-MS

Analysis of Urine Samples by
Thermo LCQ DECA equipped with DART ionization source

Positive Ion



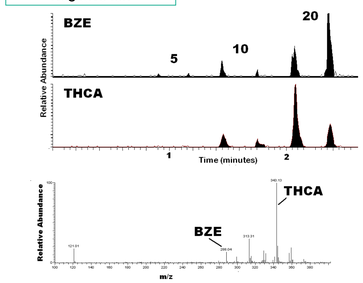
TOP: Mass chromatograms for protonated BZE and THCA from duplicate analysis of sample at each concentration ranging from 1 to 20 ug/ml

Analysis completed in scan mode with approximately 1.5 ul per sample in each sample

8 separate samples were analyzed in under 3 minutes time using 250 degree Centigrade He carrier gas

BOTTOM: POSITIVE ION DART-MS of urine sample after removal of creatine and dry down for concentration

Negative Ion



TOP: Mass chromatograms for protonated BZE and THCA from duplicate analysis of sample at each concentration

Analysis completed in scan mode with approximately 1.5 ul per sample in each sample

8 separate samples were analyzed in under 3 minutes time using 250 degree Centigrade He carrier gas

BOTTOM: NEGATIVE ION DART-MS of urine sample after removal of creatine and dry down for concentration

Conclusion

One of the advantages of ITSP SPE is the ability to perform automated sample cleanup on-line. A method for removal of greater than 99% of creatinine from human urine prior to the determination of 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid and benzoylecgonine has been demonstrated using reversed phase (C_{18}) ITSP solid phase extraction (SPE) for sample cleanup and LC-MS for detection. A linear response was observed for 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid over the concentration range of 0.5 - 10 µg/mL and for benzoylecgonine over the concentration range of 0.1 - 10 µg/mL by LC/MS. In the case of DART-MS, direct analysis of these samples did not yield significant ion abundances until after dry down with nitrogen gas and reconstitution with methanol suggesting that other components in the urine sample may be playing a role in suppression of ionization with metastable atoms.

References

1. Ropero-Miller, J.D., Stout, P. R., National Criminal Justice Reference Service, Doc. Num. 224522

For Further Information

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