Fully Automated Online Sample Preparation and LC-MS/MS Analysis of Drugs of Abuse in Oral Fluids

Joshua F. Emory[1], Nathan DeFreitas[2], Michael Roberts[1], Manoj Tyagi[2], M. Nazim Boutaghou[1], Brian J. Feild[1]

[1]Shimadzu Scientific Instruments, Columbia, MD, [2]Captiva Laboratory, Charlotte, NC

Novel Aspect

Fully automated sample preparation module that is seamlessly integrated online with LC/MS separation and analysis system

Introduction

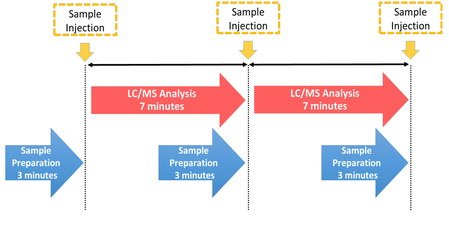
Despite recent advances in LC and MS technologies which enable faster and more robust analytical methods, advances in sample preparation for small molecule analysis have been slower to develop. Although there are robotic devices for offline sample preparation, there are no other fully automated/integrated online LC/MS sample preparation modules. The CLAM-2000 sample preparation module seamlessly integrates sample preparation, LC separation and MS detection of small molecules in an online platform. We have developed a fully automated method for sample preparation, LC separation and MS quantification of seventy-seven DRUGS in oral fluid. This system offers reproducibility of 5% RSD along with parallel processing for up to four samples to maximize mass spectrometer up time.

Methods

- Methanol, Formic Acid (Sigma-Aldrich, St. Louis, MO) and distilled water (in-house)
- Mobile Phase A (0.1% Formic in Water), Mobile Phase B (100% Methanol)
- Gradient: 10% to 60% Methanol over 4.5 minutes
- Matrix for Oral Fluid: Intercept i2HE oral fluid diluent (Orasure Technologies, Bethlehem, PA) which was used for all samples, calibrators and quality controls
- LC/MS system: Shimadzu Nexera LC system and a Shimadzu 8050 triple quadrupole

CLAM-2000 Sample Preparation and Workflow

- Pipette 15µL Methanol
- Pipette 40µL Sample
- Pipette 25µL ISTD
- Shake 30 seconds
- Filter 90 Seconds
- Pipette 240µL MPA



CLAM-2000 can parallel process four samples at once

Results and Discussion

CLAM-2000 LC/MS System

Pipetting
Shaking
Vacuum Filtration
Heating

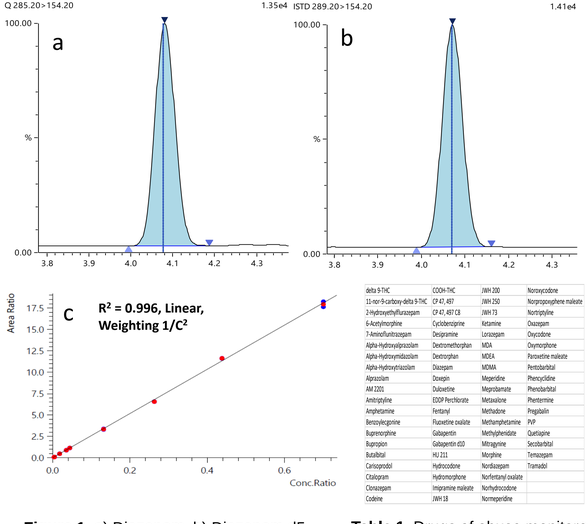


Figure 1. a) Diazepam, b) Diazepam d5, c) Calibration curve Diazepam

Table 1. Drugs of abuse monitored with the CLAM-2000 LC/MS method

delta 9-THC	COOH-THC	JWH 200	Noroxycodone
11-nor-9-carboxy-delta 9-THC	CP 47, 497	JWH 250	Norpropoxyphene maleate
2-Hydroxyethylflurazepam	CP 47, 497 C8	JWH 73	Nortriptyline
6-Acetylmorphine	Cyclobenzirpine	Ketamine	Oxazepam
7-Aminoflunitrazepam	Desipramine	Lorazepam	Oxycodone
Alpha-Hydroxyalprazolam	Dextromethorphan	MDA	Oxymorphone
Alpha-Hydroxymidazolam	Dextrorphan	MDEA	Paroxetine maleate
Alpha-Hydroxytriazolam	Diazepam	MDMA	Pentobarbital
Alprazolam	Doxepin	Meperidine	Phencyclidine
AM 2201	Duloxetine	Meprobamate	Phenobarbital
Amitriptyline	EDDP Perchlorate	Metaxalone	Phentermine
Amphetamine	Fentanyl	Methadone	Pregabalin
Benzoylecgonine	Fluoxetine oxalate	Methamphetamine	PVP
Buprenorphine	Gabapentin	Methylphenidate	Quetiapine
Bupropion	Gabapentin d10	Mitragynine	Secobarbital
Butalbital	HU 211	Morphine	Temazepam
Carisoprodol	Hydrocodone	Nordiazepam	Tramadol
Citalopram	Hydromorphone	Norfentanyl oxalate	
Clonazepam	Imipramine maleate	Norhydrocodone	
Codeine	JWH 18	Normeperidine	

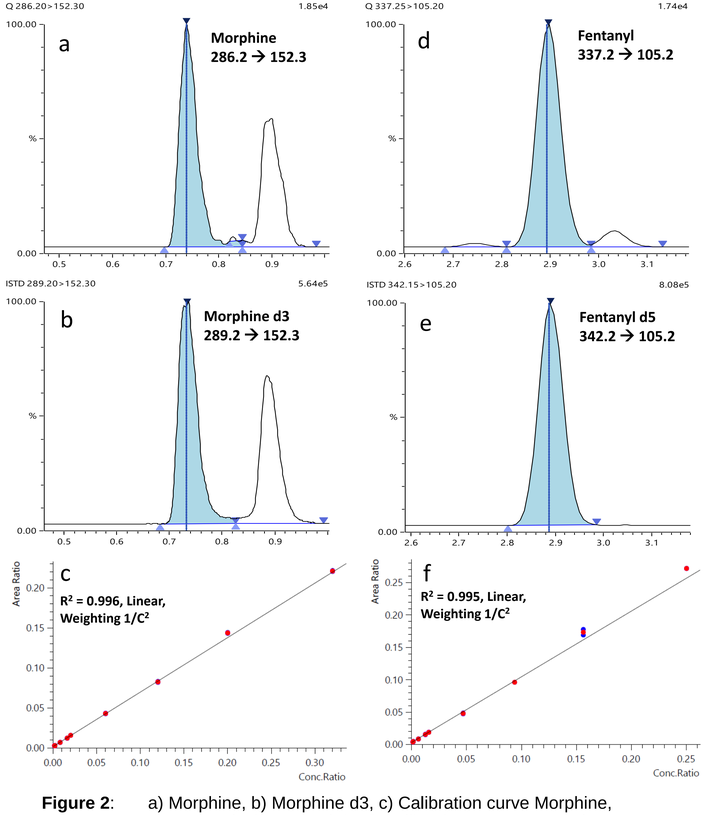
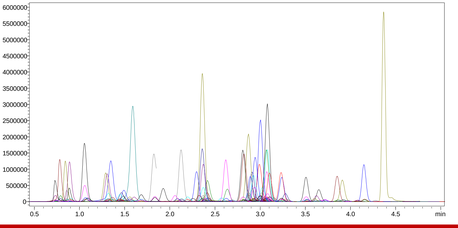


Figure 2: a) Morphine, b) Morphine d3, c) Calibration curve Morphine, d) Fentanyl, e) Fentanyl d5, f) Calibration curve Fentanyl

Acquisition of seventy-seven compounds in five minutes



Summary

- Fully automated sample preparation, LC separation, and MS analysis of seventy-seven drugs of abuse were performed using the CLAM-2000 LC/MS system.
- Real samples were loaded onto the CLAM-2000 instrument using Orasure oral fluid collection devices to avoid sample transfer steps.
- Total analysis time from sample preparation to MS analysis was eleven minutes, which corresponds to four minutes of sample preparation time and seven minutes of LC/MS analysis
- Calibration curves for the seventy-seven drugs of abuse exhibited R^2 values of 0.99 or greater with detection limits of 0.75 ng/mL for Fentanyl and 10 ng/mL for Morphine.
- Relative standard deviations of 5% or less were routinely observed.
- Considerable time savings for laboratory personnel were achieved via the use of the CLAM-2000 LC/MS system and elimination of errors associated with human sample preparation were avoided

Future Directions

Development of additional sample preparation procedures for drugs of abuse in various matrices as well development of analysis methods for other small molecule and peptide analytes by CLAM-2000 LC/MS system.