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An SLE-Based Workflow for the Analysis of the SAMHSA Oral Fluid Drug List by LC/TQ

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Overview

This research study outlines a simple cleanup workflow for oral fluid samples that enables analytical sensitivity on par with the guidelines set forth by SAMHSA for workplace drug testing while minimizing the amount of instrument maintenance that would be required with dirtier samples. Herein, this study aims to outline the typical analytical performance of a panel of drugs in oral fluid via an SLE cleanup and detection with an Ultivo LC/TQ system. Lower limits of quantitation, precision and linearity, range, and accuracy will be discussed.

Introduction

The introduction and implementation of guidelines from SAMHSA for oral fluid testing offers a newer and easier option for workplace drug testing. While use of oral fluid is less invasive and more tamper-resistant, samples can suffer from suppression due to the matrix when analyzed via mass spectrometry. Historically, sample preparation involved compound class-based cleanups using solid phase extraction (SPE), which can increase cost and decrease throughput in the analysis process. In an effort to minimize cost and to increase throughput while using a cleaner matrix than would be achieved through simple dilute and shoot, samples were prepared using Agilent's Chem Elut S supported liquid extraction (SLE) cartridges and analyzed on the Ultivo LC/TQ. The 16 compounds included in this study were 6-acetylmorphine, amphetamine, benzoylecgonine, cocaine, codeine, hydrocodone, hydromorphone, MDA, MDEA, MDMA, methamphetamine, morphine, oxycodone, oxymorphone, phencyclidine (PCP), and THC. Calibration concentrations ranged from 0.1 ng/mL to 125 ng/mL in vial, corresponding to an in-mouth concentration range of 0.4 ng/mL to 500 ng/mL The injection to injection cycle time was about 8 minutes, and multiple transitions were monitored for each of the analytes of interest.

Calibration curve accuracies were within 20% of the expected concentration at the lowest calibration level, and reproducibility across all levels was acceptable with CVs less than 15%. R^2 values were all greater than 0.992, and all but one of the compounds displayed linear responses throughout the concentration range, while the remaining one required a quadratic fit.

Experimental

Reagent and Chemicals

All reagents used in this application were HPLC or LCMS grade. Acetonitrile and methanol were purchased from Honeywell (Morristown, NJ, USA) and ultrapure water was sourced from a Milli-Q Integral system with an LC-Pak Polisher and a 0.22 µm point-of-use membrane filter cartridge (EMD Millipore, Billerica, MA, USA). Formic acid and ammonium formate were purchased from Fluka (Sigma-Aldrich Corp., St. Louis, MO, USA). Chemical standards were purchased from Cerilliant (Sigma-Aldrich Corp., Round Rock, TX, USA).

Sample Preparation

Negative synthetic oral fluid prediluted with extraction buffer was spiked with drug standards of the 16 compounds to achieve the top concentration, while the rest of the calibration standards were created by serial dilution. Each sample was combined with an internal standard solution and pretreated with ammonium hydroxide as per collection device instructions. Samples were applied to the extraction cartridges and allowed to equilibrate on the sorbent bed for at least 5 minutes before elution with a DCM:MTBE mixture under gravity. The eluate was dried under nitrogen and reconstituted in chromatographic starting conditions prior to introduction into the LCMS system.

Analytical Method and Data Analysis

The LC/MS/MS system consisted of a 1290 binary pump, a thermostatted autosampler, a temperature-controlled column compartment, and a triple quad mass spectrometer. Separation conditions are given in Tables 1 and 2. System control and data acquisition were performed by Agilent MassHunter Acquisition Software (Version 1.1 for Ultivo LC/TQ). Data were analyzed using Agilent MassHunter Quantitative Analysis Software (Version 10.0) and Qualitative Analysis Software (Version 10.0).

Table 1. The 1290 Infinity II HPLC conditions.

Column	Poroshell 120 EC-C18 2.1 x 100 mm, 2.7µm	
Mobile phase	A: 10 mM ammonium formate + 0.01%formic acid in water B: Methanol + 0.01% formic acid	
Flow rate	0.500 mL/min	
Gradient	Time 0 0.5 1.0 4.0 5.0 7.0 **7.01**	B% 10 10 15 50 95 95 10

Experimental

Capillary voltage on the Agilent Jet Stream ESI source was set at 2500 V with 0 V for the nozzle. The sheath gas temperature was 400ºC coupled with a drying gas temperature at 300ºC. The sheath gas and drying gas flows were 11 L/min and 12 L/min, respectively. The nebulizer pressure was set to 50 psi. Positive ionization was utilized.

Table 2. Transitions for amino acid detection in MRM mode

Compound Name	Precursor (m/z)	Product (m/z)	RT (min)	Frag (V)	CE (V)
6MAM	328.2	211.1 165	2.22	130	24 48
6MAM-D6	334.2	165.1 152.1	2.20	130	44 80
Amphetamine	136.1	119 90.9	2.27	55	4 16
Amphetamine-D8	144.2	127 97	2.23	60	4 16
Benzoylecgonine	290.1	168.1 104.9	2.97	105	16 32
Benzoylecgonine-D8	298.2	171.1 81.9	2.93	95	20 76
Cocaine	304.2	182.1 81.9	3.39	95	16 32
Cocaine-D3	307.2	185.2 76.9	3.39	95	20 72
Codeine	300.2	165.1 114.9	1.82	120	52 80
Codeine-D6	306.2	152.1 114.9	1.79	125	80 80
Hydrocodone	300.2	199.1 171.1	2.13	135	32 44
Hydrocodone-D3	303.2	199 127.9	2.12	135	32 72
Hydromorphone	286.2	185.1 128	1.00	135	32 72
Hydromorphone-D3	289.2	185 156.9	0.99	130	32 48
MDA	180.1	163 105	2.35	60	4 20
MDA-D5	185.1	168.1 110.1	2.33	60	8 24
MDEA	208.1	163 105	2.73	70	12 28
MDEA-D6	214.2	166.1 108	2.72	70	12 28
MDMA	194.1	163 105	2.42	65	8 24
MDMA-D5	199.1	165.1 107	2.40	65	8 24
Methamphetamine	150.1	119 91	2.39	65	8 20
Methamphetamine-D5	155.2	121 92	2.38	65	8 20
Morphine	286.2	165 128	0.78	120	52 72
Morphine-D6	292.2	151.9 127.8	0.78	120	72 72
Oxycodone	316.2	298.1 256.2	1.99	100	16 24
Oxycodone-D6	322.2	304.2 247.2	1.97	95	16 32
Oxymorphone	302.1	284.1 227.1	0.87	95	16 28
Oxymorphone-D3	305.2	287.1 232.3	0.86	105	20 28
PCP	244.2	159.1 86	4.16	60	12 8
PCP-D5	249.2	95.9 86	4.14	60	44 8
THC	315.2	193 123	6.03	110	24 36
THC-D3	318.2	196.1 135	6.03	170	28 24

Results and Discussion

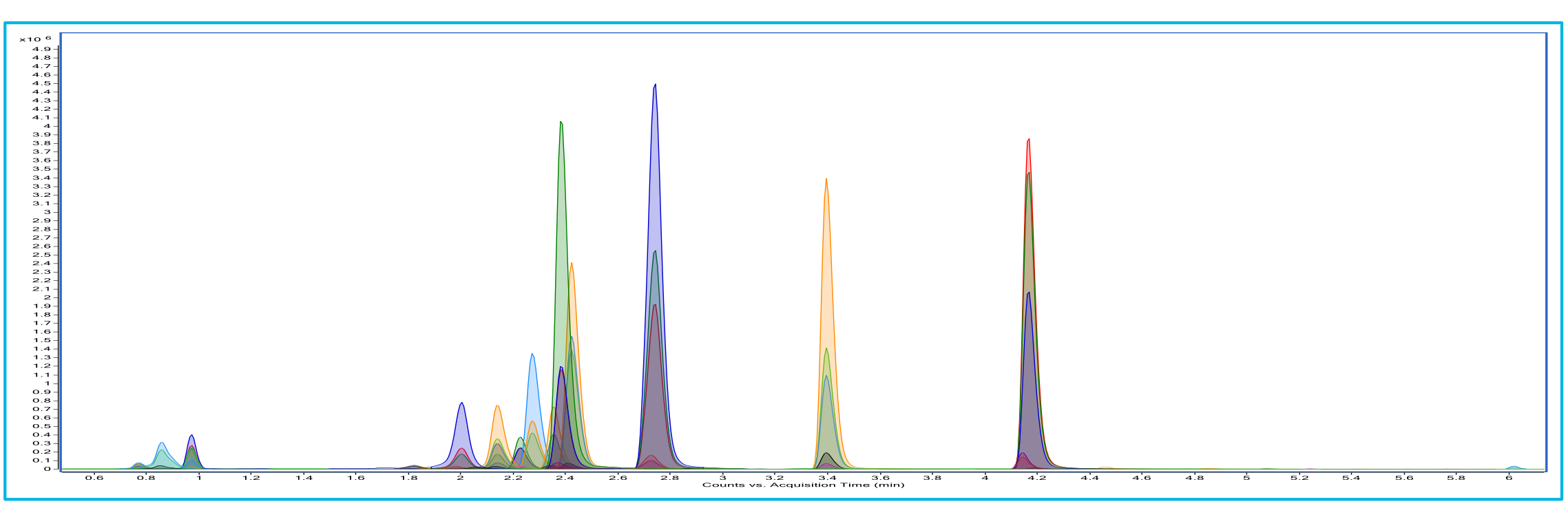

Figure 1. Composite MRM chromatogram showing 16 analytes.

Results and Discussion

Linearity, Accuracy, and Reproducibility

The calibration concentrations ranged from 0.1 ng/mL to 125 ng/mL for the various analytes, corresponding to an in-mouth concentration range of 0.4 ng/mL to 500 ng/mL. Limits of quantitation (LOQs), along with curve fit parameters, are given in *Table 3*. Each curve had an R^2 value greater than 0.992 and responses showed excellent reproducibility from run to run. Calibration curve accuracies were within 13.5% of the expected concentration at the lowest level, while RSDs were within 16% at the LOQs and within 5% at the higher levels.

Table 3. Calibration curve fit, LOQs (in-vial), and signal-to-noise (S/N).

Compound Name	Curve Fit	R^2	LOQ (ng/mL)	S/N at LOQ
6MAM	Linear	0.9987	0.25	300.92
Amphetamine	Linear	0.9989	0.25	38.06
Benzoylecgonine	Linear	0.9927	0.5	145.95
Cocaine	Linear	0.9961	0.25	566.20
Codeine	Linear	0.9974	0.5	58.39
Hydrocodone	Linear	0.9994	0.25	759.83
Hydromorphone	Linear	0.9923	0.25	468.17
MDA	Linear	0.9948	0.25	22.68
MDEA	Linear	0.9966	0.25	464.67
MDMA	Linear	0.9984	0.25	323.41
Methamphetamine	Linear	0.9972	0.1	137.74
Morphine	Quadratic	0.9920	2.0	40.37
Oxycodone	Linear	0.9996	0.1	38.94
Oxymorphone	Linear	0.9955	0.25	46.97
PCP	Linear	0.9983	0.25	2132.89
THC	Linear	0.9938	0.5	44.95

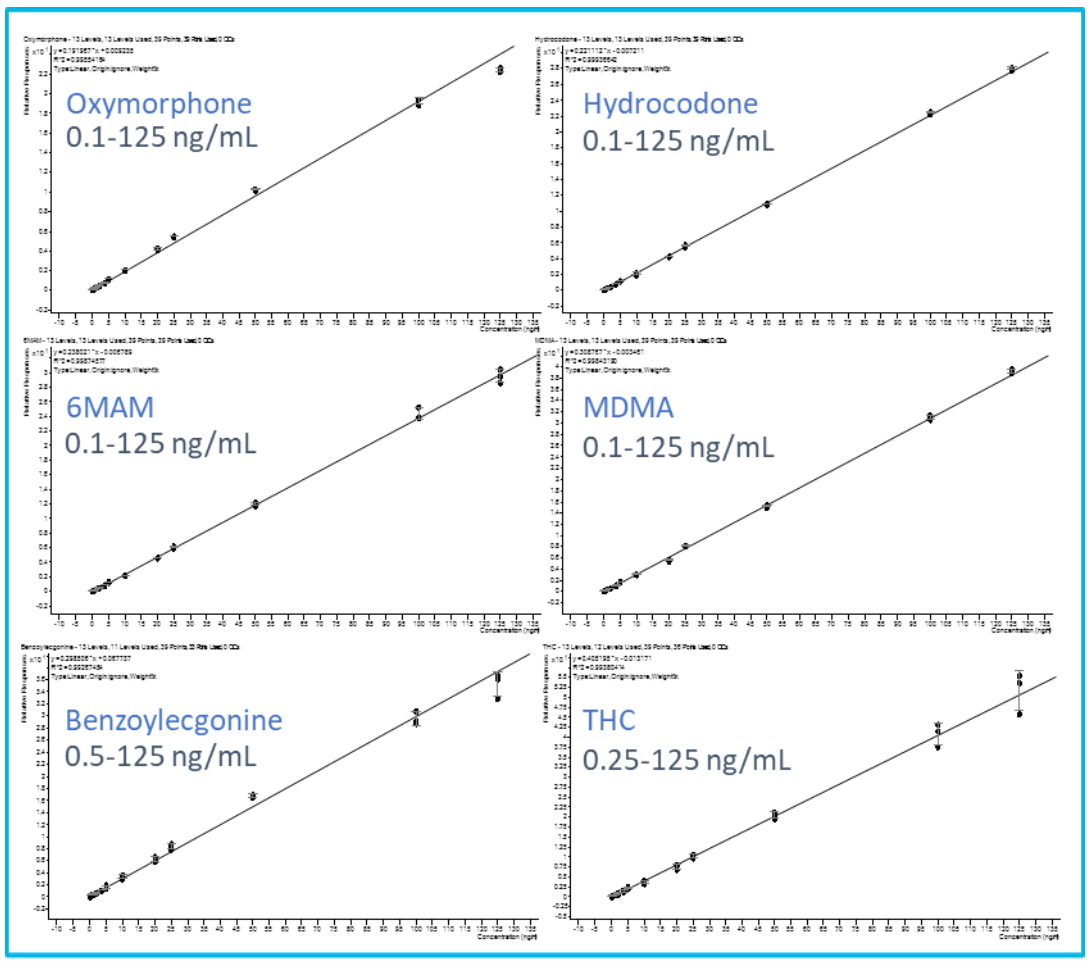

Figure 2. Calibration curves of selected compounds.

Figure 2 shows examples of calibration curves for 6 selected compounds, while replicate injections of 4 selected compounds in matrix are shown in *Figure 3*, demonstrating excellent precision and chromatographic separation of the isomers.

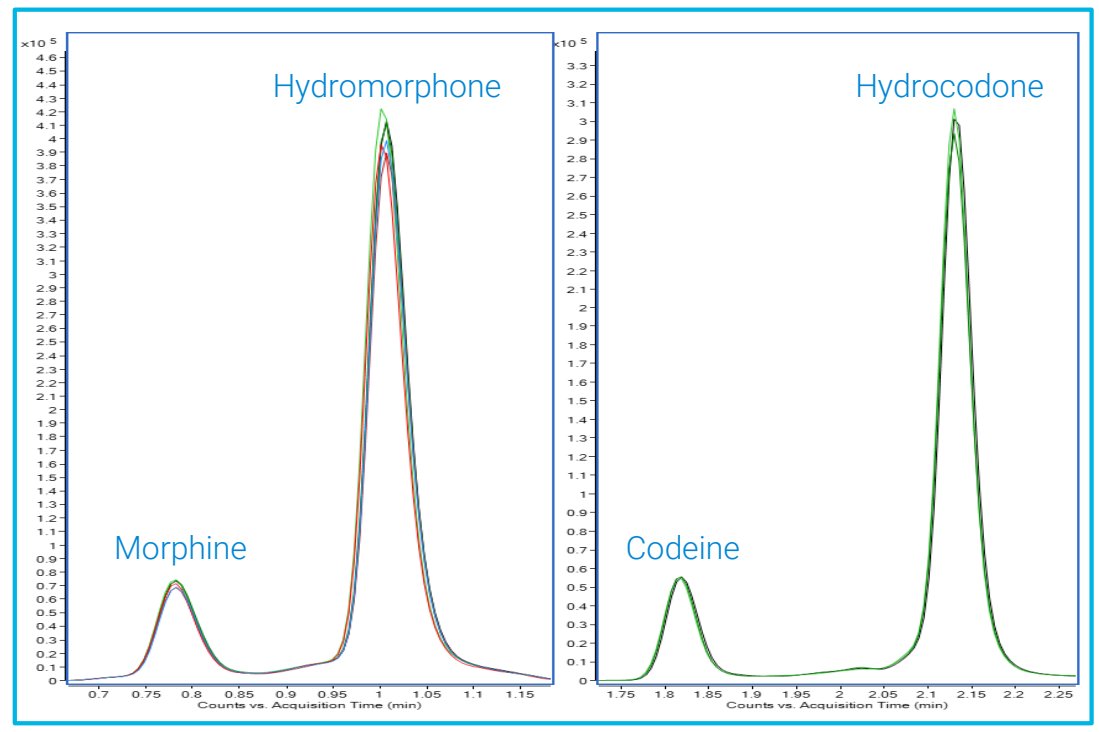

Figure 3. Excellent precision demonstrated for replicate injections of selected isomers in sample matrix.

Conclusions

A simple cleanup workflow for oral fluid samples can decrease matrix effects and downtime for maintenance without dramatically increasing cost. This study demonstrated an efficient and simple cleanup process and showed analytical sensitivity that met or exceeded the guidelines set forth by SAMHSA for workplace drug testing in oral fluid.

References

- Agilent Application Note 5991-1667EN—Comprehensive LC/MS Analysis of Opiates, Opioids, Benzodiazepines, Amphetamines, Illicits, and Metabolites in Urine
- Agilent Application Note 5994-0950EN—Drug of Abuse Analysis in Human Urine Using Agilent Chem Elut S Supported Liquid Extraction by LC/MS/MS

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