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Cost Effective Dilute and shoot Approach For Determination of Illicit Drugs in Oral Fluids Using LC-MS/MS

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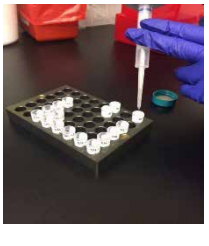
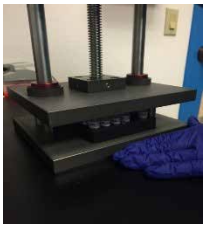
Introduction

Due to a recent increase in the demand of oral fluid analysis, many challenges have been set forth in developing robust and cost effective assays for determination of illicit drugs. Forensic testing on oral fluids has been increasingly appreciated due to reduction in time, simplicity of collection and reduction of adulteration and substitution. Thus, we developed a simplified and robust assay using filtering vials.

Demand for alternative matrices for drug testing has increased in the recent years. Even though urine, blood and hair have been utilized as the most common specimen, oral fluid is a more promising matrix for forensic testing. The use of oral fluid as an alternate matrix has a variety of advantages more so than disadvantages due to less pathogenicity and easier accessibility. In addition, oral fluid sample collection is an easy and non-invasive techniques and reduces the chances for sample substitutions or adulteration. Oral fluid analysis in the field of toxicology has had enormous growth recently. The techniques and instrumentations have evolved to meet the growing demands. Early analytical methods for oral fluid testing were developed primary based on gas chromatography - mass spectrometry (GC-MS or GC-MS/MS). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the preferred analytical instrument in recent years. This assay demonstrates an easy and cost effective method to analyze illicit drugs in an oral fluid matrix.

Method and Materials

Sample preparation was developed with minimum and easy steps that did not involve the traditional and time consuming clean ups (e.g., SPE columns). Standards and samples were diluted in methanol – water diluent fortified with internal standards.These diluted samples were filtered by 0.2µm eXtremeIFV® (Thomson).


Analytes were separated with a Phenomenex® Biphenyl 1.7µm column on SCIEX 6500 QQQ coupled with Shimadzu 30 HLPC.The total run time was 6.5 minutes with a simple gradient utilizing 0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B. The LC-MS/MS method was validated according to the CLIA guidelines.

Results

We were able to achieve three orders of magnitude in linear dynamic range. Table 1 shows the linear ranges and LOQ of all the analytes. The % coefficient of variation (%CV) was less than 20% and the coefficient of determination (R2) for all the analytes were also greater than 0.990. As depicted in Table 2, the day-to-day precision was determined with the low quality control (LQC) and high quality control (HQC). The % coefficient of variation for all the analytes were less than 10%. Interferences were evaluated using the analytes shown in Table 3. No interference was observed with assay

Table 1. Linearity

			Calibrator	
Transition name	LOQ (ng/mL)	Linear range (ng/mL)	%CV	R value
6-MAM 1	1	1-300	< 8.7	0.99580
6-MAM 2	1	1-300	< 16.9	0.99371
Amphetamine 1	5	5-1500	< 6.5	0.99719
Amphetamine 2	5	5-1500	< 7.5	0.99699
Benzoylecgonine 1	1	1-300	< 9.6	0.99691
Benzoylecgonine 2	1	1-300	< 13.7	0.99058
MDA 1	1	1-300	< 17.3	0.99285
MDA 2	1	1-300	< 12.9	0.99109
MDMA 1	10	10-3000	< 5.6	0.99516
MDMA 2	10	10-3000	< 7.5	0.99406
Methamphetamine 1	5	5-1500	< 11.3	0.99314
Methamphetamine 2	5	5-1500	< 12.2	0.99344
Oxycodone 1	2.5	2.5-750	< 7.0	0.99698
Oxycodone 2	2.5	2.5-750	< 13.5	0.99601
Oxymorphone 1	2.5	2.5-750	< 12.8	0.99297
Oxymorphone 2	2.5	2.5-750	< 13.9	0.99251
Phencyclidine 1	1	1-300	< 13.3	0.99359
Phencyclidine 2	1	1-300	< 13.3	0.99352
THC 1	5	5-1500	< 9.0	0.99533
THC 2	5	5-1500	< 12.7	0.99479

Table 2. Day-to-Day Precision

Transition name		% CV
6-MAM	HQC	4.9
6-MAM	LQC	6.1
Amphetamine	HQC	1.7
Amphetamine	LQC	4.4
Benzoylecgonine	HQC	3.4
Benzoylecgonine	LQC	6.3
MDA	HQC	4.9
MDA	LQC	8.6
MDMA	HQC	2.8
MDMA	LQC	1.9
Methamphetamine	HQC	2.3
Methamphetamine	LQC	3.8
Oxycodone	HQC	4.1
Oxycodone	LQC	5.3

Transition name		% CV
Oxymorphone	HQC	7.4
Oxymorphone	LQC	7.8
Phencyclidine	HQC	6.3
Phencyclidine	LQC	8.8
THC	HQC	6.7
THC	LQC	8

Table 3. Interference Compounds

Interference Compounds	
Acetaminophen	Caffeine
CPAM	Ibuprofen
Naproxen	Pseudoephedrine
Trazodone	Tizanidine
Salicilic Acid	Venlafaxine
Diphenhydramine	Lisinopril
Dextromethorphan	Hydromorphone
Hydrocodone	Naloxone

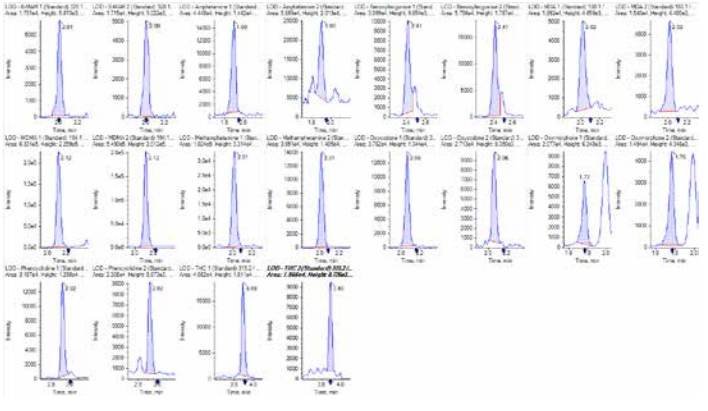


Fig 1. Chromatograms of the LOQ Standards

Table 4. Analyte Recoveries after with filter vials

Analyte Name	% Recovery
6-MAM	110
Amphetamine	106
Benzoylecgonine	106
MDA	106
MDMA	103
Methamphetamine	102
Oxycodone	107
Oxymorphone	102
Phencyclidine	87
THC	105

Analyte recoveries at LQC concentrations were compared in HPLC vials against filtered samples. Table 4 shows the percent recovery of each analyte. The recoveries for all the analytes were in a range of between 87%-110%.

Conclusion

We were able to develop a robust, simple and easy assay to determine illicit drugs in oral fluids. We were also able to cut the cost greater than half compared to the traditional sample preparation techniques, as this assay remarkably reduced the sample preparation time, the necessity of extra equipment (e.g. SPE system, evaporators) and drastic reduction of solvent uses. Further cost reductions could be achieved by automating the sample preparation.

References

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