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

Kavinda De Silva, Mariko Nakano, Siobhan McKenney-Hara

Molecular Testing Labs. Vancouver, WA

## Abstract

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.

## Introduction

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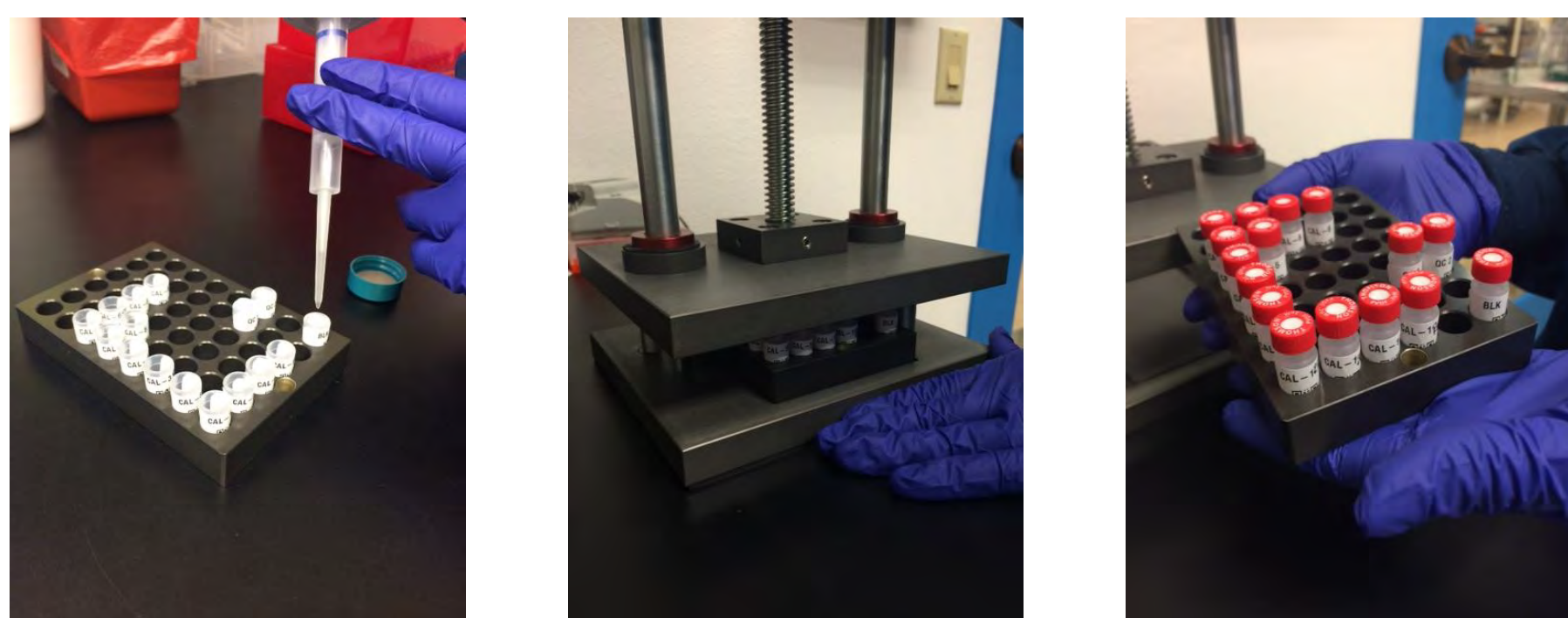
Analytes were separated with a Phenomenex Biphenyl 1.7μm column on SCIEX 6500 QQQ coupled with Shimadzu 30 HLPC. The total run time was 6.5 minutes with a simple gradient utilizing 0.1% formic acid in water as mobile phase A and 0.1% formic acid in methanol as mobile phase B. The LC-MS/MS method was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

## Results and Discussion

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 ($R^2$) for all the analytes were also greater than 0.990.

Table 1: Linearity

| Transition name | LOQ (ng/mL) | Linear range (ng/mL) | Calibrator %CV | Calibrator 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 |

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%.

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 |
| 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 |

Interferences were evaluated using the analytes shown in Table 3. No interference was observed with assay.

Figure 1: Chromatograms of the LOQ Standards
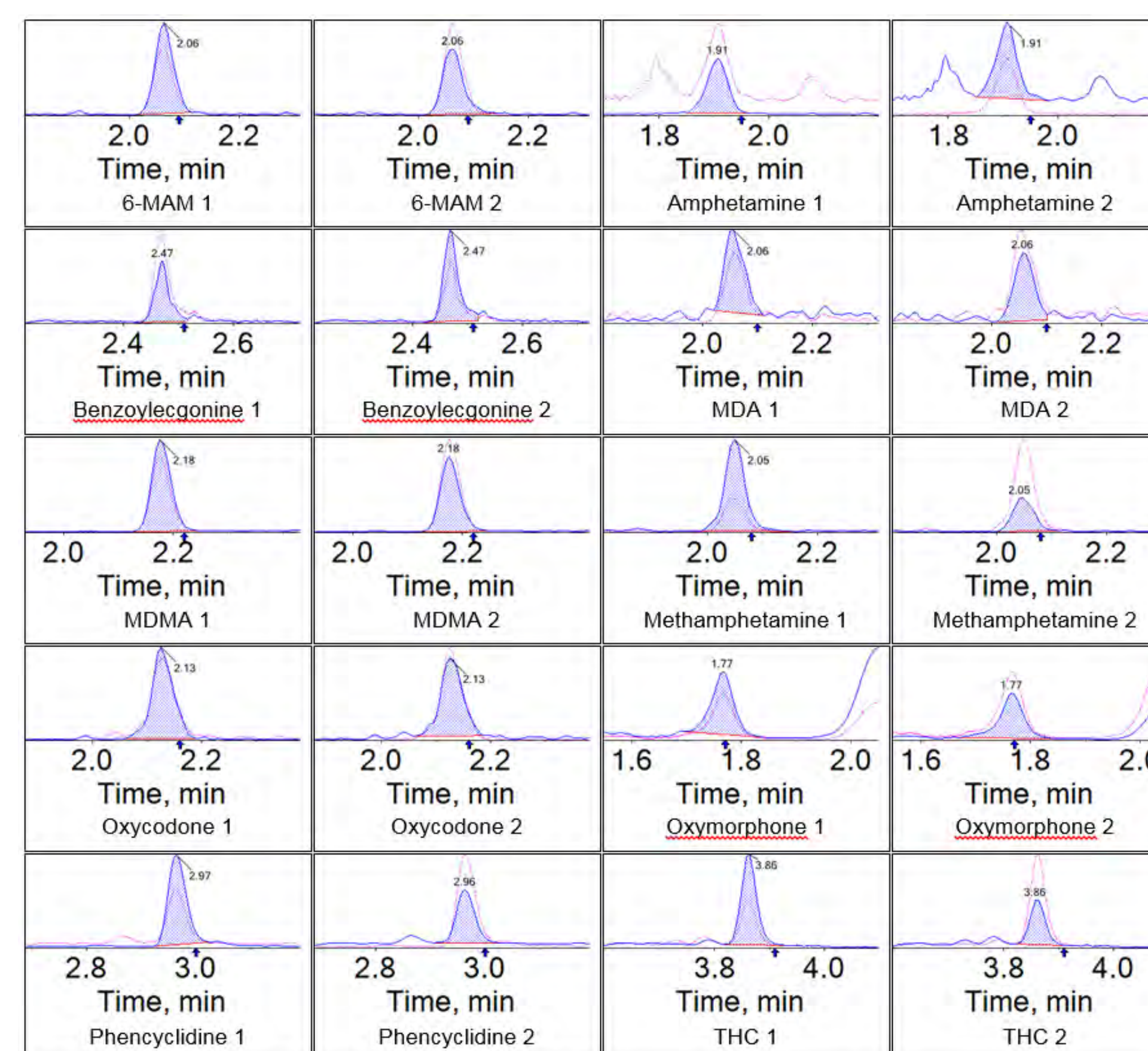


## Results & Discussion (continued)

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 a 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

- Dams, Riet, Robin E. Choo, Willy E. Lambert, Hendree Jones, and Marilyn A. Huestis. "Oral Fluid as an Alternative Matrix to Monitor Opiate and Cocaine Use in Substance-abuse Treatment Patients." *Drug and Alcohol Dependence* 87.2-3 (2007): 258-67.
- Heltsley, R., A. Depriest, D. L. Black, T. Robert, L. Marshall, V. M. Meadors, Y. H. Caplan, and E. J. Cone. "Oral Fluid Drug Testing of Chronic Pain Patients. I. Positive Prevalence Rates of Licit and Illicit Drugs." *Journal of Analytical Toxicology* 35.8 (2011): 529-40.
- Koenig, N., Xander, C., et al. Improved method for the analysis of 31 drugs of abuse/pain management panel in oral fluid samples using the Thomson eXtreme® filter vials by LC-MS/MS. (provided by OraSure).

Molecular® Testing Labs
Diagnostic Services

Clinical Laboratory Improvement Amendments

cap ACCREDITED™

TIC-PL-082-278