# Tip-on-Tip™ Solid-Phase Extraction of Viscous Oral Fluid Samples

William E. Brewer[1], Evan DiVirgilio[1], Kaylee R. Mastrianni[1]

1. DPX Technologies, Columbia, SC.

## INTRODUCTION

A forensic/clinical laboratory had viscous oral fluid samples that were unable to be processed due to clogging in their existing sample preparation procedure. We used a novel Tip-on-Tip™ Technology to develop a new method referred to as Tip-on-Tip Solid-Phase Extraction (ToT SPE™) for extracting drugs from viscous oral fluid samples. We previously presented an improved product for comprehensive analysis of drugs in oral fluid that utilized dSPE tips with mixed mode, strong cation and weak anion exchange sorbent (WAX/SCX). In this ToT SPE method, loose WAX/SCX sorbent is mixed with sample solutions in a well plate, and this mixing is more reproducible because the viscous solutions are not passed through porous frits. Pooled samples from the commercial laboratory were successfully analyzed. We report recovery studies for spiked Oral-Eze buffer solutions, and we report extraction results for the complex case study samples using this novel ToT SPE method.

## MATERIALS AND METHODS

The oral fluid samples (600 µL) were mixed loosely in a well plate on a Hamilton Heater Shaker (HHS) (alternatively wide bore tips can be used for mixing). In this manner, the sorbent could reproducibly mix with the viscous samples and allow for optimal binding. The solutions were mixed for 10 minutes. After mixing, the viscous solutions were allowed to settle, and then the supernatant (app. 500 µL) was aspirated and dispensed to waste. Then 500 µL of DI water was added to the samples, and the solutions were mixed by aspirating and dispensing 3 times. The 300 µL wide bore tips transferred the solution as a slurry (by over-aspirating the solution with air to collect all of the solution) to Single Phase Filtration Tips on a vacuum block from DPX Technologies. The vacuum block was custom outfitted on the deck of a Microlab Nimbus96 system, from Hamilton Company (shown in Figure 1). After collecting the sorbent and drying in the filtration tips, the sorbent was washed with 300 µL of DI water, then dried under vacuum for 5 minutes. Wide bore tips aspirated 300 µL of 4% $NH_4OH$ in methanol, and were inserted into the filtration tips on the vacuum block. The ToT device was moved over to the elution well plate, and the elution solvent was dispensed and pushed through the filtration tips and collected in the well plate. This ToT SPE method is depicted in Figure 2. The solutions were subsequently solvent evaporated and reconstituted in 125 µL 10% methanol in water.

This method was used to process spiked Oral-Eze buffer solutions for recovery, linearity and LOD/LOQs. This method was also used for analysis of pooled samples from a commercial laboratory, with all samples failing analysis due to clogging issues. All analyses were performed using a SCIEX 6500+ triple quadrupole MS system coupled to an Agilent 1260 LC system equipped with an Agilent C18 Poroshell (2.7 µm, 50 x 2.1 mm). LC conditions were the following: Mobile Phase A-0.1% Formic Acid in water; B-Acetonitrile.

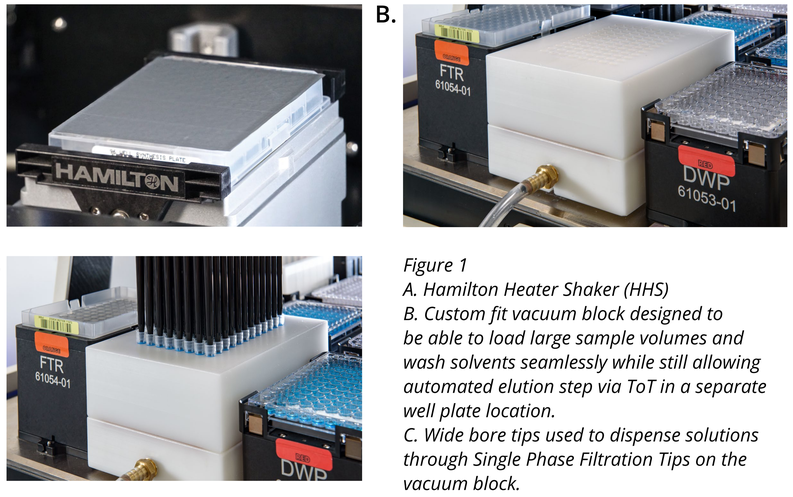


*Figure 1*
*A. Hamilton Heater Shaker (HHS)*
*B. Custom fit vacuum block designed to be able to load large sample volumes and wash solvents seamlessly while still allowing automated elution step via ToT in a separate well plate location.*
*C. Wide bore tips used to dispense solutions through Single Phase Filtration Tips on the vacuum block.*

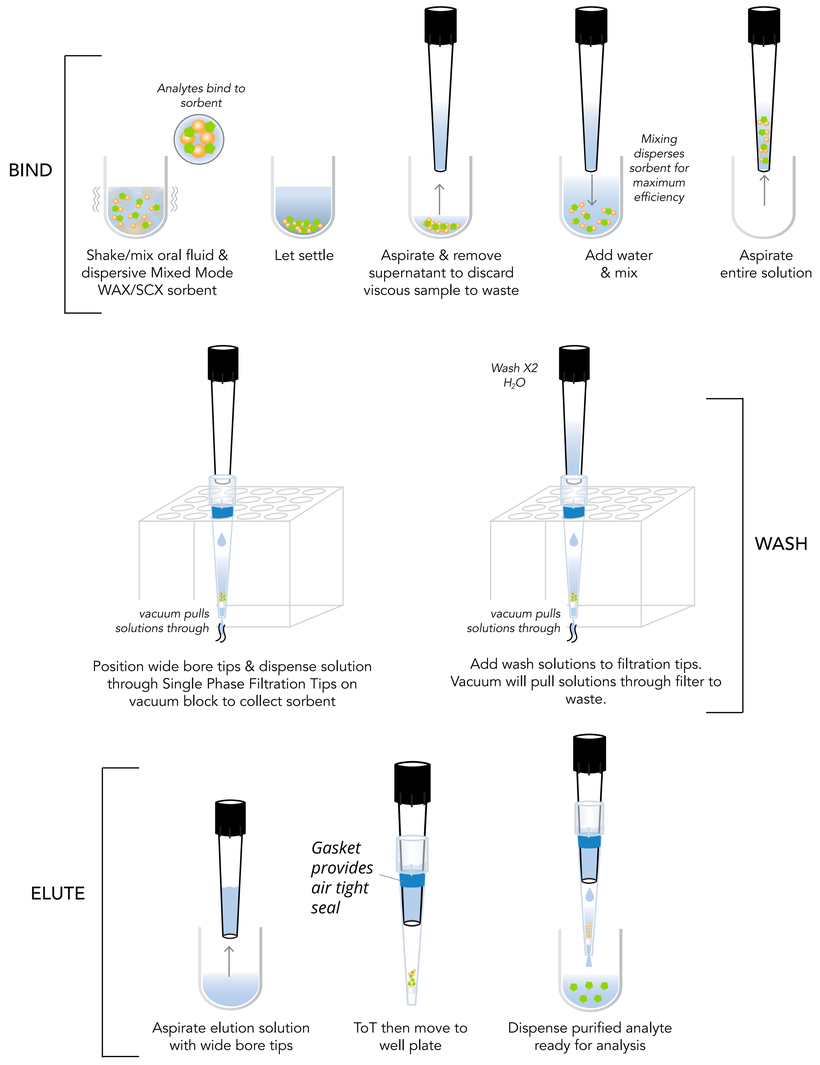


*Figure 2. Sample preparation using ToT SPE method.*

## RESULTS AND DISCUSSION

Initial tests using the ToT extractions were performed with various amounts of sorbent (SCX and WAX). It was determined that 5 mg SCX and 5 mg WAX sorbent provided recoveries greater than 80% for most of the drugs. Linearities were from as low as 0.0625 ng/mL to as high as 500 ng/mL, with linear regressions greater than 0.99 for all drugs. LODs ranged from about 0.05 ng/mL for fentanyl to less than 0.5 ng/mL for opiates, benzodiazepines and THC. Most of the compounds were extracted with less than 15% RSDs using this novel method.

The results are consistent with the validated method using Dispersive Pipette Extraction Technology with WAX/SCX, except recoveries were found to be high with less sorbent. SCX sorbent by itself worked well for most drugs. However, compounds like carisoprodol, meprobamate, and barbiturates had low recoveries without the addition of WAX sorbent. Higher recoveries for the amphoteric compounds can be obtained by decreasing the pH of the sample solution prior to extraction.

Recovery results for the drugs of abuse panel can be found in Figure 3. Results for the analyses of the samples that previously could not be analyzed by the commercial laboratory (due to clogging) are shown in Table 1.

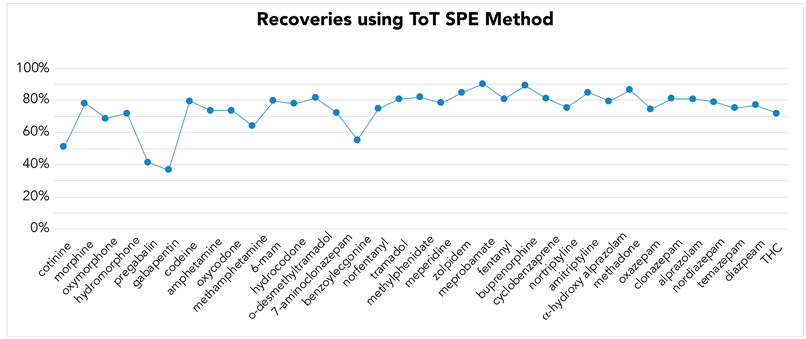


Figure 3. Percent recoveries for each analyte using the ToT SPE method.

Table 1. Quantitative results of problematic case samples that were analyzed using this reported ToT SPE method.

| Sample | Conc. (ng/mL) | Analyte |
|---|---|---|
| 17 | 492 | gabapentin |
| | 8.0 | 7-aminoclonazepam |
| | 3.8 | benzoylecgonine |
| | 325 | methadone |
| 19 | 267 | cotinine |
| | 583 | gabapentin |
| | 348 | norbuprenorphine |
| | 1808 | buprenorphine |
| 20 | 84 | cotinine |
| | 6.4 | morphine |
| | 0.14 | hydromorphone |
| | 74 | methadone |
| 21 | 67 | cotinine |
| | 6.7 | benzoylecgonine |
| | 210 | norbuprenorphine |
| | 2282 | buprenorphine |
| 22 | 226 | cotinine |
| | 91 | gabapentin |
| | 0.63 | nordiazepam |
| | 0.46 | diazepam |

| Sample | Conc. (ng/mL) | Analyte |
|---|---|---|
| 23 | 466 | cotinine |
| | 8.9 | amphetamine |
| | 4.3 | 7-aminoclonazepam |
| | 54 | norbuprenorphine |
| | 725 | buprenorphine |
| | 1.3 | clonazepam |
| | 20 | THC |
| 24 | 150 | cotinine |
| | 27 | hydrocodone |
| | 8.4 | 7-aminoclonazepam |
| | 186 | norbuprenorphine |
| | 1707 | buprenorphine |
| | 1.3 | clonazepam |
| | 0.55 | nordiazepam |
| 25 | 42 | cotinine |
| | 2.6 | morphine |
| | 0.14 | hydromorphone |
| | 400 | gabapentin |
| | 72 | oxycodone |

## CONCLUSIONS

This study demonstrates a rapid, efficient and automated method for analyzing comprehensive drugs in viscous oral fluid samples using a novel ToT SPE method. Very difficult sample solutions that have failed analyses by a forensic/clinical laboratory were able to be readily processed.

## REFERENCES

1. *W. E. Brewer and E. S. DiVirgilio, "Automated, High Throughput Quantitative Analysis of Drugs of Abuse in Oral Fluid Using DPX Extraction and LC-MS/MS", SOFT Annual Meeting, Oct. 2018, Minneapolis, MN.*