Sensitive Analyses of Opiates and Opioids in Whole Blood Using Tip-on-Tip™ Technology with LC-MS/MS

William E. Brewer[1], Evan DiVirgilio[1], Kaylee R. Mastrianni[1], Kevin W.P. Miller[2]

1. DPX Technologies, Columbia, SC. 2. Hamilton Company, Reno, NV.

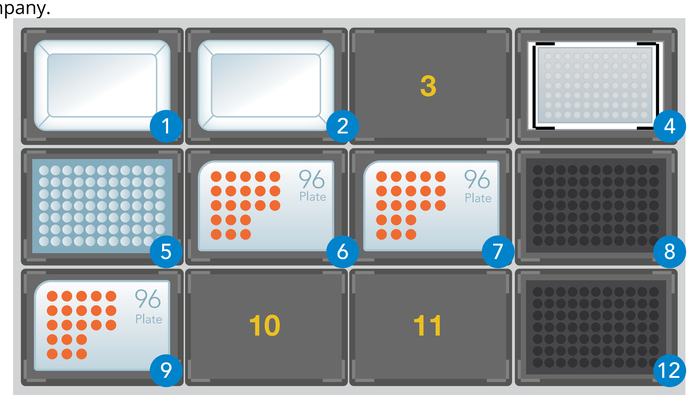
INTRODUCTION

LC/MS systems have made a significant impact on sample preparation requirements in forensic toxicology. In particular, highly sensitive LC/MS triple quadrupole instruments allow for low volumes of sample solutions, even when trying to achieve very low detection limits. In addition, the efficiency of HPLC separations minimizes the need for rigorous extraction processes to purify samples for analysis. We demonstrate an automated protein precipitation method that provides rapid and sensitive analyses of opiates and opioids in whole blood. The procedure uses a novel Tip-on-Tip (ToT) Filtration method to process up to 96 samples in less than 10 minutes.

MATERIALS AND METHODS

Blank whole blood samples (from Utak) were spiked at various concentrations of opiates and opioids, including morphine, oxymorphone, hydromorphone, codeine, oxycodone, hydrocodone, 6-MAM, tramadol, meperidine, fentanyl, and methadone. Using just 0.2 mL of whole blood, the samples were added directly to well plates containing 100 mg of anhydrous $MgSO_4$ and 600 µL of acetonitrile. The samples were placed in a Hamilton Heater Shaker (HHS), from Hamilton Company, Reno, NV., and mixed for 5 minutes. After mixing, 300 µL wide bore tips aspirated the supernatant, then moved and positioned into Low Porosity Filtration Tips, from DPX Technologies, Columbia, SC., for ToT Filtration. The solution was dispensed into a clean well plate. The wells were solvent evaporated using nitrogen and heat, and reconstituted using 300 µL of 10% methanol. The automation setup is shown in Figures 1 and 2, and the schematic of the method is shown in Figure 3.

All analyses were performed using a SCIEX 6500+ triple quadrupole MS system coupled to an Agilent 1260 LC system equipped with a Phenomenex Biphenyl column (3 x 50 mm, 2.6 µm). LC conditions were the following: Mobile Phase A-0.1% Formic Acid in water; B-Methanol. All extractions were performed using a Microlab Nimbus96 liquid handler from Hamilton Company.



1. Acetonitrile (ACN)
2. Internal Standard (I.S.)
4. Hamilton Heater Shaker (HHS)
5. Low Porosity Filtration Tips
6. $MgSO_4$
7. Blood
8. 1 mL tips
9. Filtrate
12. 300 µL tips

(3, 10, and 11 blank)

Figure 1. Deck layout for Nimbus96 for the method utilized.

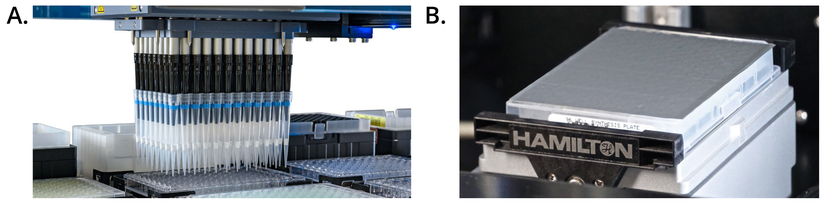


Figure 2 A. Top wide bore tip and bottom Low Porosity Filtration Tip for ToT Filtration. Low Porosity Filtration Tips provide <1 µm, high efficiency matrix filtration.
B. The HHS automates heating and shaking of ANSI/SLAS footprint microplates.

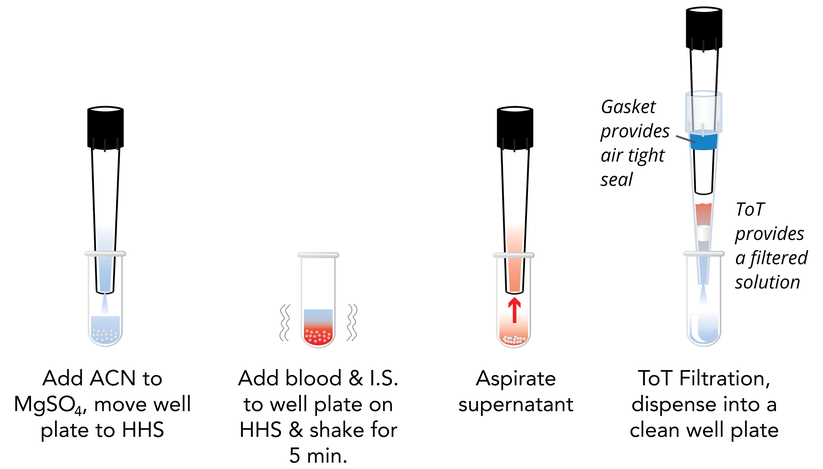


Figure 3. The method involves first precipitating the blood by mixing 0.2 mL blood with 0.6 mL ACN and 100 mg anhydrous $MgSO_4$ using the automated HHS. After shaking, 0.3 mL of ACN supernatant is aspirated into 300 µL wide bore tips, and then fitted into Low Porosity Filtration Tips for ToT Filtration. The solution is then dispensed through the Low Porosity Filtration Tips and collected in a well plate for subsequent solvent evaporation and LC/MS analysis.

RESULTS AND DISCUSSION

The automated protein precipitation and ToT Filtration was performed in less than 10 minutes. This method can process up to 96 samples simultaneously. Recoveries of the automated method are compared to manual vortex mixing in Figure 4. The recoveries for the automated method ranged from 78.4% (hydromorphone) to 88.8% (codeine). Samples were spiked at concentrations ranging from 0.1 ng/mL to 5.0 ng/mL. Limits of detection for all compounds were found to be 0.1 ng/mL (and 0.05 ng/mL for fentanyl). LOQs were approximately 0.2 ng/mL for all of the drugs except fentanyl, which was 0.1 ng/mL. All linear regression values were greater than 0.99.

Matrix effects were also studied with and without the use of $MgSO_4$, and the results are shown in Figure 5. With the exception of meperidine, all matrix effects using $MgSO_4$ were less than 30%. Further studies are being performed to minimize matrix effects.

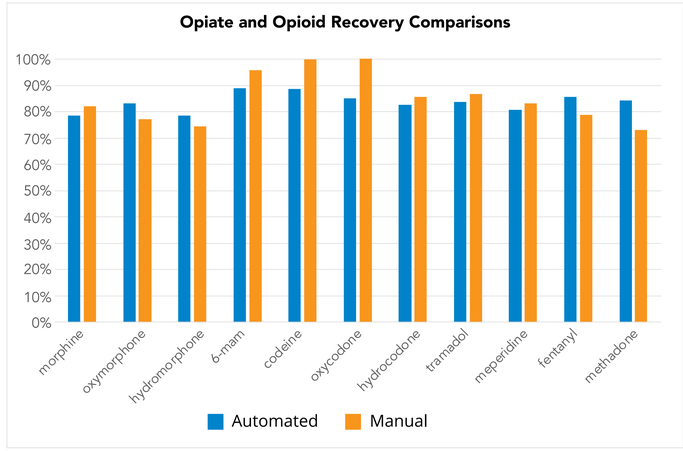


Figure 4. Recoveries of the automated method compared to manual vortex mixing

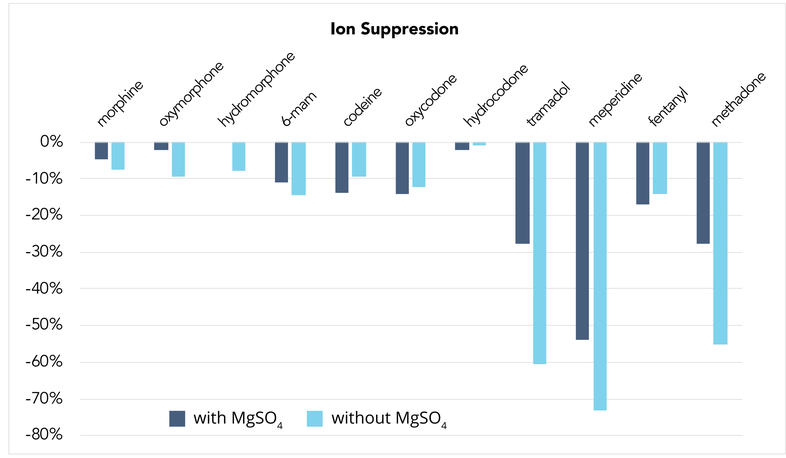


Figure 5. Matrix effects for the protein precipitation and ToT filtration with $MgSO_4$ (series 1, navy) and without $MgSO_4$ (series 2, light blue).

CONCLUSIONS

This study demonstrates a rapid, efficient and automated method for analyzing opiates and opioids in whole blood. Further validation studies will be performed through collaboration with a forensic toxicology laboratory.

REFERENCES

1. "Rapid Automated Protein Crash Method Using Novel Tip-on-Tip Filtration", K. R. Mastrianni, E. DiVirgilio, and W. E. Brewer, SOFT Annual Meeting, Sept. 2018.