Automated Hydrolysis and Sample Preparation for the Analysis of 12 Opiates in Urine using the Thomson eXtreme Filter Vials® by LC-MS/MS

Nadine Koenig[1], Crystal Xander[1], Melanie Stauffer[1], Dean Fritch[2], Lisa Wanders[3], Dennis Peterson[3], Sam Ellis[3]
[1] Health Network Laboratories, 794 Roble Road, Allentown, PA 18109
[2] Analytical Associates, 225 Millwood Drive, East Greenville, PA
[3]Thomson Instrument Company, 1121 South Cleveland Street Oceanside, CA 92054

Abstract

This improved sample preparation method allows for the quantitative measurement of Opioids in urine. Opioids are highly addictive and affects nearly 5 million people in the U.S. Opioids include naturally occurring Opiates, semi-synthetic opioids derived from morphine and synthetic opioids. are analgesic alkaloids found naturally in *Papaver somniferum*, poppy plant. The use of hydrolysis in the analysis of natural and synthetic opiates in urine has become standard practice in forensic toxicology. Many laboratories currently use solid phase extraction or solid liquid extraction techniques in the sample preparation of urine for the analysis for opiates. This improved automated sample preparation method evaluates the robustness for the quantitative measurement of opiates in urine without the need for SPE/SLE thereby reducing pipetting errors. The sample preparation of incurred urine, controls, standards and internal standard additions as well as the hydrolysis step are performed by a liquid handler. The Thomson eXtreme Filter Vials provide a simple and efficient extraction technique that has demonstrated adequate analyte recovery, reduced matrix interferences and the reduction of solvent and consumable waste.

Experimental

Table 1. Drugs analyzed in this Opiate Panel

6-Monoacetylmorphine	Hydrocodone	Norhydrocodone
B-Naltrexone	Hydromorphone	Noroxycodone
Codeine	Morphine	Oxycodone
Dihydrocodeine / Hydrocodol	Naltrexone	Oxymorphone

Equipment/Reagents:

ß-Glucuronidase - IMCSzyme™, genetically engineered beta-glucuronidase, p/n #04-E1F-010 (IMCS - Integrated Micro- Chromatography Systems)
ABI 4500 Mass Spectrometer
Shimadzu Prominence HPLC
- Flow Rate: 0.5 mL/min
- Run Time: 8.5 minutes
- Injection Volume: 15µL
- Mobile Phases:
  - A: 0.1% Formic Acid in HPLC Water
  - B: 0.1% Formic Acid in Methanol
- Column: Restek Ultra Biphenyl Columns (5µm 50 x 2.1 mm)

Hamilton Automated Liquid Handler
Thomson eXtreme|FV® 0.2µm PVDF (p/n 85531)
Thomson 48 position Vial Filter Press (p/n 35010)

Method:

The method created for the Hamilton Liquid Handler will replace manual steps currently performed by lab personnel. The method includes the pipetting of blanks, patient samples (dilution if necessary), controls, standards, internal standard and enzyme from 12x75mm glass tubes into a 96 well plate for the hydrolysis step. The final step in the robotic method is the transfer of the hydrolyzed urine samples to a 48 position plate containing the outer shell vial of the eXtreme Filter Vials. The plungers are than added to the outer shell vials of the eXtreme Filter Vial and the plate is transferred to the Multi-Press. The samples are analyzed by LC-MS/MS.

The opiates analyzed in this method include Codeine, Oxycodone, Dihydrocodeine/Hydrocodol, Hydrocodone, Hydromorphone, 6-Monoacetylmorphine, Morphine, Noroxycodone, ß-Naltrexone, Naltrexone, Norhydrocodone, Oxycodone, and Oxymorphone. Stock Solutions for each analyte and internal standards are made in methanol. These stock solutions are diluted in negative urine by the Liquid Handler to generate working solutions for a 6-point calibration curve, generate controls and add Internal Standards to Specimens. Limit of Detection (LOD) and Limit of Quantification (LOQ) will be compared to the existing validated method for opiate analysis by LC-MS/MS. The 6-point standard curve for 6-Monoacetylmorphine includes the LOQ, Level 1 concentration of 5ng/mL with upper limit, Level 6 concentration of 1000ng/mL. For all the other analytes the LOQ, Level 1 concentration of 50ng/mL with upper limit, Level 6 concentration of 20000ng/mL.

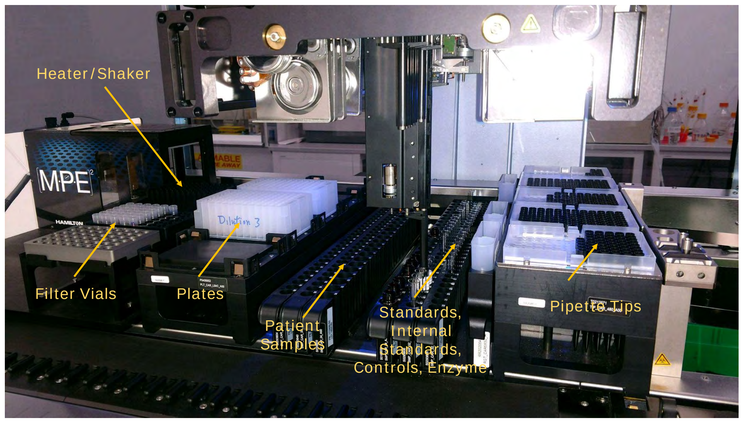
Improved Automated Sample Preparation - 12 Drugs

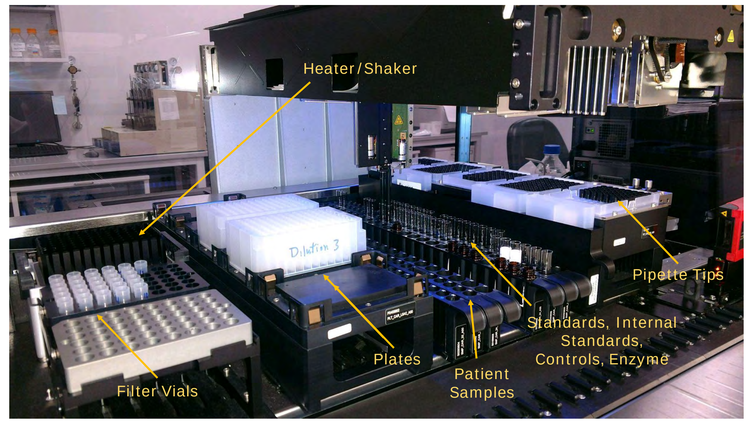
Liquid Handler Process:

- Reads sample barcodes to ensure proper tracking.
- 12 x 75mm glass culture tubes are used for: Urine Standards, LC Checks, Controls, Internal Standard and Enzyme
- Eight channel pipette head is utilized for adding standards, internal standards and enzyme to each of the 12x75mm culture tubes to create blanks, Standard Curve, patient samples and controls.
- All samples are transferred to a 96 well plate and the plate is transferred to the heater/shaker unit.
  - Vortexed for 2 minutes
  - Hydrolyzes for 30 minutes at 55º +/- 2°C
- Upon completion the plate is removed from the heater/shaker and returned to the deck for a 10 minute cool down.
- During cool down, 2% Methanol is added to each eXtreme Filter Vial outer shell.
- All hydrolyzed samples are transferred to the eXtreme Filter Vial outer shell.
- The rack containing the eXtreme Filter Vial outer shells is removed from the deck of the robot and the plungers are added ¼ of the way down.
  - The rack containing the eXtreme Filter vials is vortexed for 5 minutes.
- The plungers are completely depressed using the Thomson Multi-Press.
- Samples are ready for analysis by LC-MS/MS

Final concentrations for the various analytes are as follows:

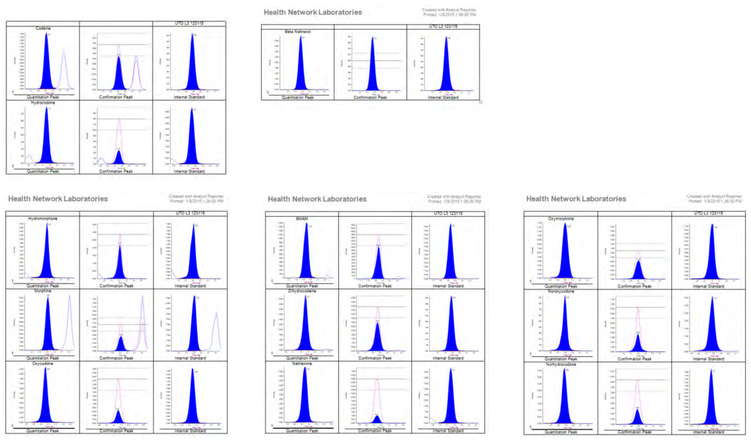
	Final Concentration (ng/mL) Opiates	Final Concentration (ng/mL) 6-MAM
Level 1	50	5
Level 2	200	20
Level 3	1000	50
Level 4	5000	250
Level 5	10000	500
Level 6	20000	1000



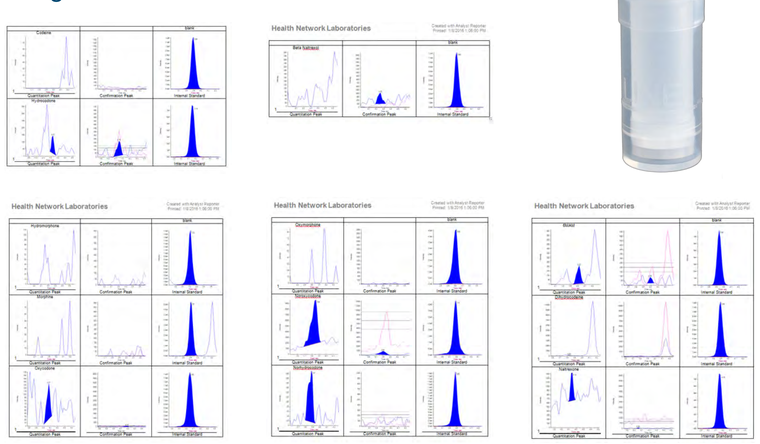


Data

Positive Results



Negative Results



Conclusion

This validated automated method reduces the risk of contamination and alleviates the risk of human pipetting errors during specimen transfer steps. Samples are diluted, receive Internal Standards, hydrolyzed and dispensed into the vial going onto the LC-MS/MS. All sample/specimen containers are barcoded and tracked throughout the process significantly reducing the need for repeat sampling.

Thomson Instrument Company is not affiliated with Health Network Laboratories®, Analytical Associates, Eppendorf®, IMCS - Integrated Micro-Chromatography System, Zymark or their products