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Clinical Urine Mega Method by LC-MS/MS

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Presented at MSACL 2019

Introduction

This improved sample preparation method allows for the quantitative measurement of over 60 drugs of different classes in urine for clinical purposes. Drugs of abuse include naturally occurring, semi-synthetic and synthetic drugs. The use of hydrolysis in the analysis of natural and synthetic drugs in urine has become standard practice in toxicology labs. Many laboratories currently use solid phase extraction or solid liquid extraction techniques in the sample preparation of urine. This method quantitatively measures multiple drugs of different classes in urine for clinical purposes. This method is known as the CLUMM (Clinical Urine Mega Method) and run on the Sciex 4500 using the Phenomenex Phenyl-hexyl Kinetex analytical column. The samples are hydrolyzed, then prepared using a dilute and filter technique followed by LC-MS/MS analysis.

Amphetamine	Codeine
Meperidine	Nortriptyline
Methamphetamine	Morphine
Normeperidine	Duloxetine
MDA	6 MAM
Methadone	Ketamine
MDMA	Hydrocodone
EDDP	Norketamine
Gabapentin	Hydromorphone
Mitragynine	Methylphenidate
Pregabalin	Norhydrocodone
7-Hydroxymitragynine	Ritalinic Acid
2-Hydroxyethylflurazepam	Dihydrocodeine
Tapentadol	Zolpidem
7 Aminoclonazepam	Oxycodone
N-Desmethyl Tapentadol	Carboxyzolpidem
aOH-Alprazolam	Oxymorphone
Tramadol	THC-COOH
Diazepam	Noroxycodone
O-desmethyltramadol	Nicotine
Nordiazepam	Buprenorphine
Carisoprodol	Cotinine
Oxazepam	Norbuprenorphine
Meprobamate	3-OH-Cotinine
Temazepam	Fentanyl
Cyclobenzaprine	Butalbital
aOH-midazolam	Norfentanyl
Benzoylecgonine	Pentobarbital (qualitative only)
Lorazepam	Acetylfentanyl
PCP	Phenobarbital (qualitative only)
Secobarbital (qualitative only)	

Equipment

- Sciex 4500 LC-MS/MS System
- Phenomenex Phenyl-hexyl Kinetex analytical 100A 50 x 4.6 mm column
- Eppendorf Mix Mate
- Thomson eXtreme|FV®s, 0.2µm

Sample Preparation

A. Urine Specimens are 1.5mL and are kept refrigerated. Allow standards, specimens and controls to come to room temperature. Turn Block Heater on to 55ºC±2ºC. Label one 1.5 mL Safe-Lock Tube and one Thomson vial for each blank, standard, control and client specimen. For samples falling outside the calibration range, make appropriate dilutions using Negative Urine and record on the run sheet. The goal is to prevent mass spectral distortion (failing ion ratios) that occurs in a sample that is too concentrated while keeping the concentration of the diluted sample above the cutoff (or a least the limit of quantitation).

NOTE: The maximum dilution allowed for this analysis is 1:20. This dilution is for all analytes with the exception of THC. Perform this dilution in a separate 12x75 mm glass tube. Place 950 µL of Negative Urine into the tube using the 200-1000 µL and add 50µL of sample requiring dilution into the same tube. Vortex for 20-30 seconds.

For the LC Check, place 400 µL of 2% Methanol into a 12 x 75 mm glass culture tube. Add 20 µL of working IS and 1 µL of Cutoff Calibrator Spiking Standard A and 1 µL of Cutoff Calibrator Spiking Standard B. Vortex and transfer to an autosampler vial with insert. To each 1.5 mL Safe-Lock Tube add 90 µL of Rapid Hydrolysis Mixture. Prepare 1.5 mL Safe-Lock Tubes for analysis. Cap and vortex for 5 minutes at 850 rpm using the Eppendorf Mix Mate. Incubate at 55ºC±2ºC for 30 minutes uncapped. Allow tubes to come to room temperature.

Add 200 µL of 2% Methanol to each Thomson outer shell vial. Give each Eppendorf tube a quick vortex and add 200 µL of the hydrolyzed urine sample to its respective Thomson outer shell vial. Place Thomson Filter Plunger on top of Thomson outer shell vial. Press filter plunger down approximately ¼ of the way into each of the Thomson outer shell vial. Vortex for 5 minutes at 1750 rpm using the Eppendorf Mix Mate.

B. Add 200 µL of 2% Methanol to each Thomson outer shell vial. Briefly vortex each sample tube. 200 µL of the hydrolyzed urine sample should be added to its respective Thomson outer shell vial. Place Thomson Filter Plunger on top of Thomson outer shell vial. Press filter plunger down approximately ¼ of the way into each of the Thomson outer shell vials. Vortex for 5 minutes at 1750 rpm using the Eppendorf Mix Mate.

Results

Final concentrations (ng/mL) including linearity for the various analytes including controls can be found in Table 1.

Validation of any method must include evaluation of interfering substances/co-eluting peaks. There may be unknown substances in certain specimens which co-elute with the analyte or the internal standard and may cause low recovery or cause ion ratios to fail. Seven analyte mixes, were evaluated for interference. The analytes in table 1 had % accuracies exceeding 60-140% when spiked into the low control. There are unknown substances that interfere with Barbiturates*. Examples of mass spectrum of some of the analytes can be seen in Fig. 1-8.

*Note: If any of these analytes appears positive in any patient sample they will be reflexed and repeated by an appropriate alternate method.

Table 1. Concentrations of the various analytes

Analyte	Level 1 (LOD/LOQ/CUTOFF CONCENTRATION)	Level 2	Level 3	Level 4	Level 5 (LINEARITY)	Low Control	High Control
Buprenorphine	5	10	25	100	250	10	150
Carisoprodol	100	200	500	2000	5000	200	3000
Fentanyl	1	2	5	20	50	2	30
Meprobamate	100	200	500	2000	5000	200	3000
Norbuprenorphine	5	10	25	100	250	10	150
Norfentanyl	1	2	5	20	50	2	30
O-desmethyltramadol	100	200	500	2000	5000	200	3000
Tramadol	100	200	500	2000	5000	200	3000

For more information, see the full application note at https://htslabs.com/technical/urine-mega-method

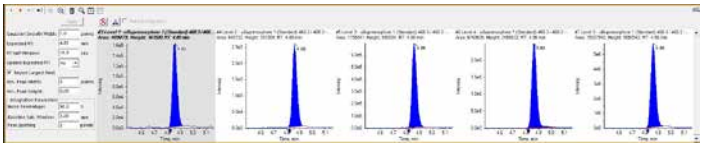

Fig 1. Buprenorphine

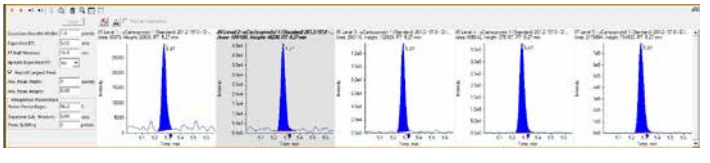

Fig 2. Carisoprodol

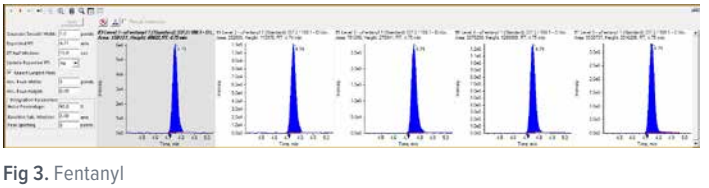

Fig 3. Fentanyl

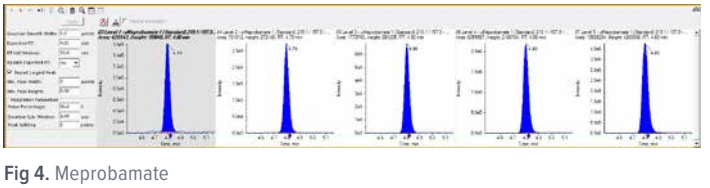

Fig 4. Meprobamate

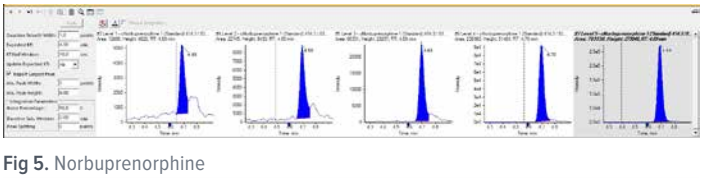

Fig 5. Norbuprenorphine

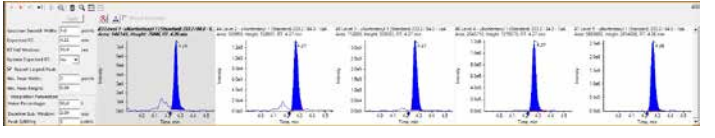

Fig 6. Norfentanyl

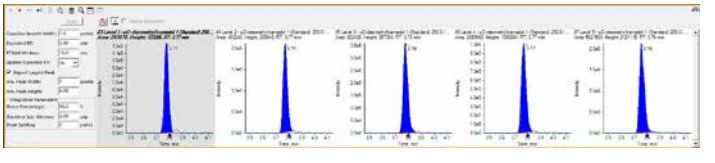

Fig 7. O-desmethyltramadol

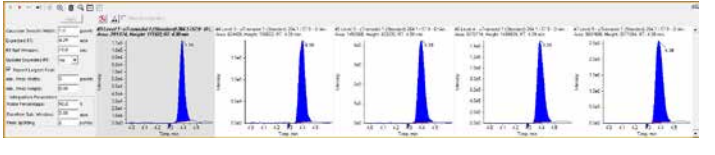

Fig 8. Tramadol

Conclusion

This method quantitatively measures multiple drugs of different classes in urine for clinical purposes. This method is known as the CLUMM (Clinical Urine Mega Method). This new improved method allows for a large sample panel, reduces sample prep time, limits transfer steps, improves column life, and reduces instrument downtime.