

series	cap color	membrane	pore size	part #
eXtreme FV®	●	PVDF	0.2µm	85531

## Clinical Urine Mega Method by LC-MS/MS

Nadine Koenig<sup>1</sup>, Crystal Xander<sup>1</sup>, Melanie Stauffer<sup>1</sup>, Dean Fritch<sup>2</sup>

<sup>1</sup> Health Network Laboratories, 794 Roble Road, Allentown, PA 18109

<sup>2</sup> Analytical Associates, 225 Millwood Drive, East Greenville, PA

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### 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
Benzoylcegonine	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 Filter Vials, 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 55°C±2°C. 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 according to table 1. Cap and vortex for 5 minutes at 850 rpm using the Eppendorf Mix Mate. Incubate at 55°C±2°C for 30 minutes uncapped. Allow tubes to come to room temperature.

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

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

Table 1

TUBE	AMOUNT OF CLINICAL/FUMM2 WORKING I.S.(μL)	AMOUNT OF CUTOFF CALIBRATOR SPIKING STANDARD A(μL)	AMOUNT OF CUTOFF CALIBRATOR SPIKING STANDARD B(μL)	AMOUNT OF QUALITY CONTROL Spiking Standard A(μL)	AMOUNT OF QUALITY CONTROL Spiking Standard B(μL)	AMOUNT OF NEGATIVE URINE(μL)	AMOUNT OF SPECIMEN(μL)
Blank	20	-	-	-	-	200	-
Level 1	20	1	1	-	-	198	-
Level 2	20	2	2	-	-	196	-
Level 3	20	5	5	-	-	190	-
Level 4	20	20	20	-	-	160	-
Level 5	20	50	50	-	-	100	-
Low Control	20	-	-	2	2	196	-
High Control	20	-	-	30	30	140	-
Specimens	20	-	-	-	-	-	200
LOD (when applicable)	20	0.5	0.5	-	-	199	-

## Results

Final concentrations (ng/mL) including linearity for the various analytes including controls can be found in Table 2.

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, see Fig. 1 were evaluated for interference. Fig. 2 shows the list of analytes that show interference with one or more analytes in the above mixes. The analytes in Fig. 2 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. 3-10.

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

Table 2. 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
Amphetamine	100	200	500	2000	5000	200	3000
Methamphetamine	100	200	500	2000	5000	200	3000
MDA	100	200	500	2000	5000	200	3000
MDMA	100	200	500	2000	5000	200	3000
Gabapentin	500	1000	2500	10000	25000	1000	15000
Pregabalin	500	1000	2500	10000	25000	1000	15000
2-Hydroxyethylflurazepam	75	150	375	1500	3750	150	2250
7-Aminoclonazepam	75	150	375	1500	3750	150	2250
aOH-Alprazolam	75	150	375	1500	3750	150	2250
Diazepam	75	150	375	1500	3750	150	2250
Nordiazepam	75	150	375	1500	3750	150	2250
Oxazepam	75	150	375	1500	3750	150	2250
Temazepam	75	150	375	1500	3750	150	2250
aOH-midazolam	75	150	375	1500	3750	150	2250
Lorazepam	75	150	375	1500	3750	150	2250
Codeine	50	100	250	1000	2500	100	1500
Morphine	50	100	250	1000	2500	100	1500
6-MAM	5	10	25	100	250	10	150

Analyte	Level 1 (LOD/LOQ/CUTOFF CONCENTRATION)	Level 2	Level 3	Level 4	Level 5 (LINEARITY)	Low Control	High Control
Hydrocodone	50	100	250	1000	2500	100	1500
Hydromorphone	50	100	250	1000	2500	100	1500
Norhydrocodone	50	100	250	1000	2500	100	1500
Dihydrocodeine	50	100	250	1000	2500	100	1500
Oxycodone	50	100	250	1000	2500	100	1500
Oxymorphone	50	100	250	1000	2500	100	1500
Noroxycodone	50	100	250	1000	2500	100	1500
Buprenorphine	5	10	25	100	250	10	150
Norbuprenorphine	5	10	25	100	250	10	150
Fentanyl	1	2	5	20	50	2	30
Norfentanyl	1	2	5	20	50	2	30
Acetylfentanyl	1	2	5	20	50	2	30
Meperidine	50	100	250	1000	2500	100	1500
Normeperidine	50	100	250	1000	2500	100	1500
Methadone	100	200	500	2000	5000	200	3000
EDDP	100	200	500	2000	5000	200	3000
Mitragynine	10	20	50	200	500	20	300
7-Hydroxymitragynine	10	20	50	200	500	20	300
Tapentadol	100	200	500	2000	5000	200	3000
N-Desmethyl Tapentadol	100	200	500	2000	5000	200	3000
Tramadol	100	200	500	2000	5000	200	3000
O-desmethyltramadol	100	200	500	2000	5000	200	3000
Carisoprodol	100	200	500	2000	5000	200	3000
Meprobamate	100	200	500	2000	5000	200	3000
Cyclobenzaprine	50	100	250	1000	2500	100	1500
Benzoylcegonine	100	200	500	2000	5000	200	3000
PCP	25	50	125	500	1250	50	750
Nortriptyline	50	100	250	1000	2500	100	1500
Duloxetine	50	100	250	1000	2500	100	1500
Ketamine	100	200	500	2000	5000	200	3000
Norketamine	100	200	500	2000	5000	200	3000
Methylphenidate	10	20	50	200	500	20	300
Ritalinic Acid	100	200	500	2000	5000	200	3000
Zolpidem	75	150	375	1500	3750	150	2250
Carboxyzolpidem	25	50	125	500	1250	50	750
Butalbital	200	400	1000	4000	10000	400	6000
Pentobarbital	200	400	1000	4000	10000	400	6000
Phenobarbital	200	400	1000	4000	10000	400	6000
Secobarbital	200	400	1000	4000	10000	400	3000
THC-COOH	15	30	75	300	750	30	450
Nicotine	100 (LOD/LOQ)	200(Cutoff Conc)	500	2000	5000	200	3000
Cotinine	100 (LOD/LOQ)	200 (Cutoff Conc)	500	2000	5000	200	3000
3-trans-OH-Cotinine	100 (LOD/LOQ)	200 (Cutoff Conc)	500	2000	5000	200	3000

## Analyte mixtures that were used to evaluate interfering substances

### Mix 1

- Acetaminophen
- Caffeine
- Cotinine
- Ibuprofen
- Naproxen
- Nicotine
- Phentermine
- Pseudoephedrine

### Mix 2

- Gabapentin
- Pregabalin
- Salicylic acid
- Valproic Acid
- Vigabatrin

### Mix 3

- Aripiprazole
- Lacosamide
- Oxcarbazepine
- Propoxyphene
- Rufinamide
- Warfarin

### Mix 4

- Alprazolam
- Fluconazole
- Cimetidine
- Hydrochlorothiazide
- Citalopram
- Lamotrigine
- Clopidogrel bisulfate
- Levothyroxine
- Clonazepam
- Methylphenidate
- Dextromethorphan
- Omeprazole

### Mix 5

- Carbamazepine
- Phenytoin
- Levetiracetam
- Sertraline
- Metformin
- Topiramate
- Phenobarbital
- Zolpidem Tartrate
- Phenylephrine
- Zonisamide

### Mix 6

- Amlodipine besylate
- Atorvastatin Calcium Salt
- Azithromycin
- Bupivacaine HCL
- Cetirizine diHCL
- Dimenhydrinate
- Lisinopril
- Loratidine

### Mix 7

- Montelukast Sodium
- Pioglitazone HCl
- Prednisolone
- Prednisone
- Procainamide HCl
- Simvastatin

## Analytes that interfered with other analytes

### Interfering Peaks

- Acetylfentanyl
- Cotinine
- Gabapentin
- Methylphenidate
- Mitraginine
- Nicotine
- o-desmethyl tramadol
- OH-mitraginine
- Phenobarb
- Pregabalin
- Ritalinic acid
- Tapentadol
- Temazepam
- Tramadol
- Zolpidem
- 3-OH-cotinine

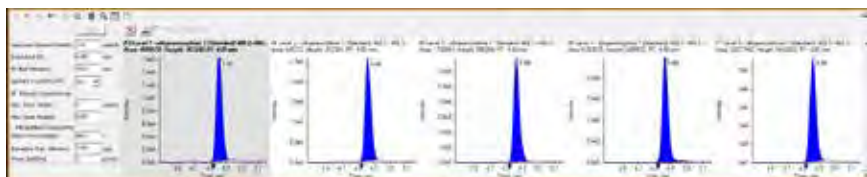


Fig 3. Buprenorphine

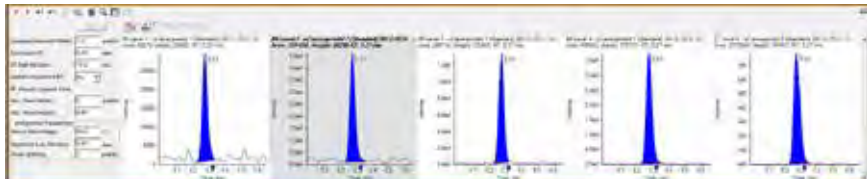


Fig 4. Carisoprodol

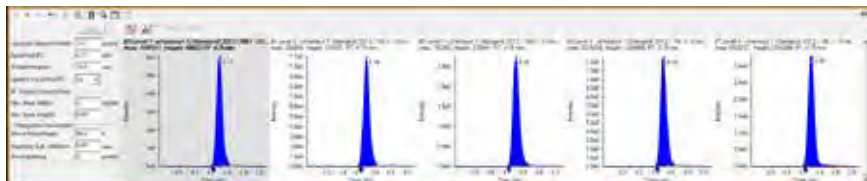


Fig 5. Fentanyl

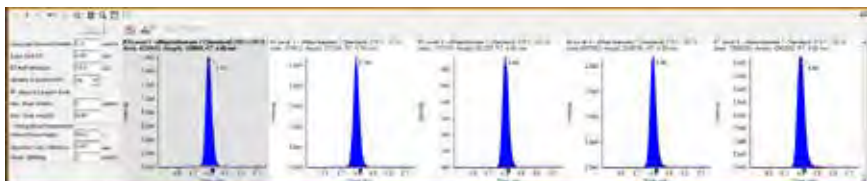


Fig 6. Meprobamate

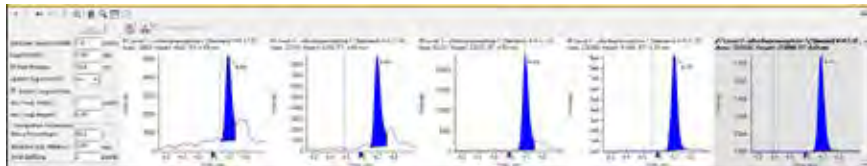


Fig 7. Norbuprenorphine

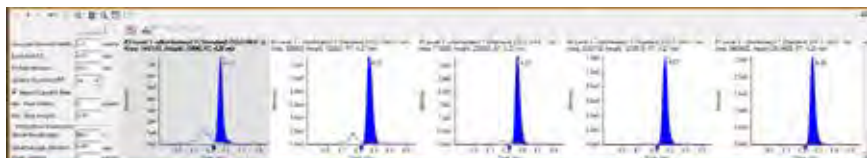


Fig 8. Norfentanyl

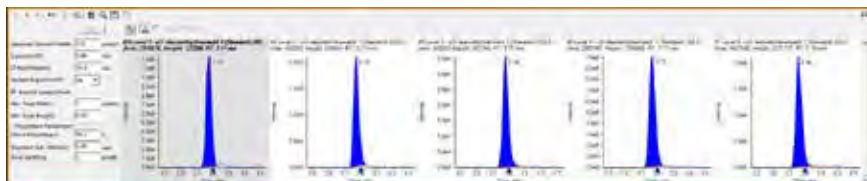


Fig 9. O-desmethyiltramadol

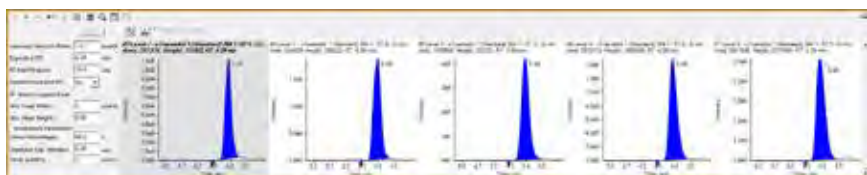


Fig 10. Tramadol

## 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.