Sample Preparation for over 60 Analytes in Urine using the Thomson eXtreme Filter Vials® by LC-MS/MS

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Abstract

This improved sample preparation method allows for the quantitative measurement of over 60 different drugs 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 Supported 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 Clinical Urine Mega Method and run on the Sciex 4500 using the Phenomenex Kinetex Phenyl-Hexyl analytical column. The samples are hydrolyzed, then prepared using a dilute and filter technique followed by LC-MS/MS analysis.

Experimental

Table 1. Drugs analyzed

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
α-OH-Alprazolam	Oxymorphone	Tramadol	THC-COOH
Diazepam	Noroxycodone	O-desmethyltramadol	Nicotine
Nordiazepam	Buprenorphine	Carisoprodol	Cotinine
Oxazepam	Norbuprenorphine	Meprobamate	3-OH-Cotinine
Temazepam	Fentanyl	Cyclobenzaprine	Butalbital
α-OH-midazolam	Norfentanyl	Benzoylecgonine	Pentobarbital (qualitative only)
Lorazepam	Acetylfentanyl	PCP	Phenobarbital (qualitative only)
			Secobarbital (qualitative only)

Equipment

- Sciex Triple Quad™ 4500 LC-MS/MS System
- Phenomenex Kinetex® Phenyl-Hexyl 100A 50 x 4.6 mm analytical column
- Eppendorf MixMate®
- IMCSzyme® genetically modified ß-glucuronidase
- Thomson eXtreme Filter Vials, 0.2µm

Method

1) Urine Specimens: Minimum of 1.5mL, refrigerated.
2) Allow standards, specimens and controls to come to room temperature. Turn Block Heater on to 55ºC±2ºC.
3) 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. 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).
4) To each 1.5 mL Safe-Lock Tube add 90 µL of Rapid Hydrolysis Mixture.
5) Cap and vortex for 5 minutes at 850 rpm using the Eppendorf Mix Mate. Incubate at 55ºC±2ºC for 30 minutes uncapped.
6) Allow tubes to come to room temperature.
7) Add 200 µL of 2% Methanol to each Thomson Vial.
8) Vortex each sample tube.
9) Add 200 µL of the hydrolyzed urine sample to its respective Thomson Vial.
10) Place Thomson Filter Plunger on top of Thomson Vial.
11) Press filter plunger down approximately ¼ of the way into each of the Thomson Vials.
12) Vortex for 5 minutes at 1750 rpm using the Eppendorf Mix Mate.
13) Depress the plungers completely using the Thomson Vial Press.
14) The vials are ready for injection on the LC-MS/MS.

Validation Summary

Final concentrations (ng/mL) including linearity for the various analytes including controls can be found in Table 2. Examples of mass spectrum of some of the analytes can be seen in Fig. 1-8.

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
α-OH-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
α-OH-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
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
Benzoylecgonine	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

Linearity:

To evaluate linearity five standard curves of five concentrations (cutoff (X), 2x, 5x, 20x and 50x) were analyzed over the course of five separate runs.

Regression analysis (R) was performed in each run for each analyte's quantification ion and was greater than 0.98 as specified in the Analytical Method Validation SOP.

Accuracy:

Accuracy studies were analyzed concurrently with linearity studies, but also included testing at the cutoff (limit of quantitation) in triplicate on each of the three days and the controls with three replicates over five days. Accuracy for all of the analytes was within 80-120% for concentrations of the cutoff to the ULOQ (Upper limit of quantification.

In addition, past proficiency samples and 12 previously analyzed patient samples were analyzed and all analytes tested met quantification acceptance criteria.

Precision:

Precision studies were analyzed concurrently with linearity studies, but also included LOQ in triplicate on each of the three days and the controls with three replicates over five days. Between run precision for all of the analytes was less than 20 CV% for concentrations at the LOQ up to the ULOQ (Upper limit of quantification). Phenobarbital, Pentobarbital and Secobarbital samples will be reflexed to a urine barbiturate specific method for identification.

Ion suppression/enhancement:

Ion suppression /enhancement studies were conducted by analyzing ten different samples spiked at the 2x and 50x concentration.

There was less than 25% CV for any of the analytes at 50x concentration. Buprenorphine, Norfentanyl and Butalbital had higher %CV at the 2x concentration.

Carryover:

Carryover was evaluated by analyzing a blank sample after a high calibrator in each of the validation runs samples.
None of the blanks for any of the analytes following the highest calibrator met the LOD requirement for a positive result.

Data

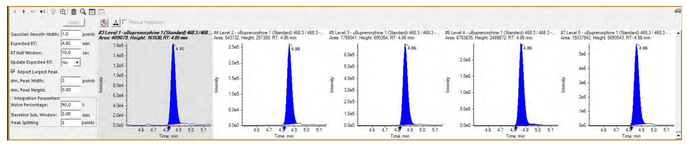

Fig 1. Buprenorphine

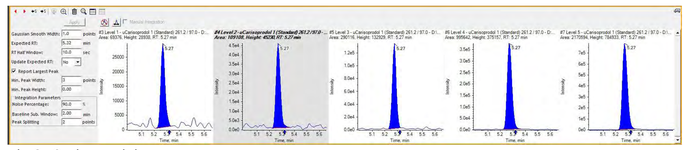

Fig 2. Carisoprodol

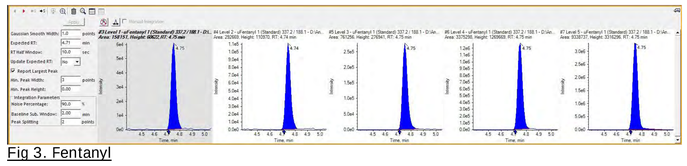

Fig 3. Fentanyl

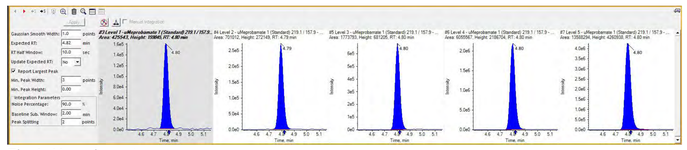

Fig 4. Meprobamate

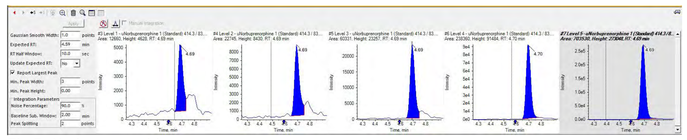

Fig 5. Norbuprenorphine

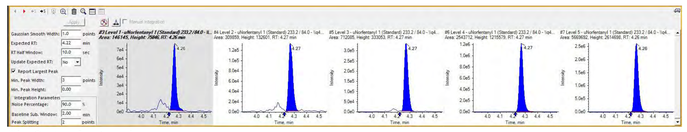

Fig 6. Norfentanyl

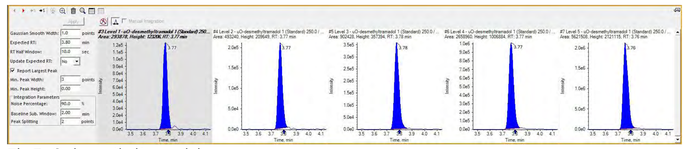

Fig 7. O-desmethyltramadol

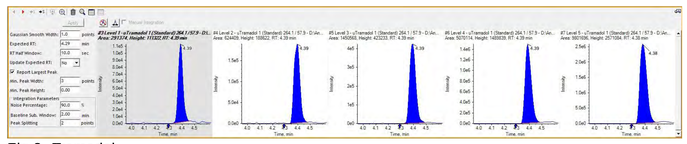

Fig 8. Tramadol

Conclusion

This method quantitatively measures multiple drugs of different classes in urine for clinical purposes. This method allows for a large sample panel, reduces sample prep time, limits transfer steps, improves column life, and reduces instrument downtime.