



Enzyme Hydrolysis Workflow for Analyzing Drugs of Abuse in Urine by LC-MS/MS

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Abstract

The analysis of drugs of abuse in urine can be complicated by matrix components, drug metabolites, and isobaric compounds. In the method developed here, 70 drugs of abuse, including novel psychoactive substances, were analyzed in urine following a simple enzyme hydrolysis sample preparation step. The fast, 8-minute LC-MS/MS method separated all isobars and generated accurate quantitative results at trace levels for all compounds.

Introduction

Testing for drugs of abuse is a necessary task, whether it be for postmortem toxicology, pain management, workplace testing, or a host of other reasons. A variety of biological specimens can be used, but urine is often preferred because it is relatively easy to collect, sample volumes typically exceed what is needed, and target analyte concentrations tend to be higher than in other matrices [1]. In addition, detection windows in urine are typically 1–7 days for most drugs of abuse and can be even longer in samples from chronic users [2]. For these reasons, urine has been used for decades to test for drugs of abuse.

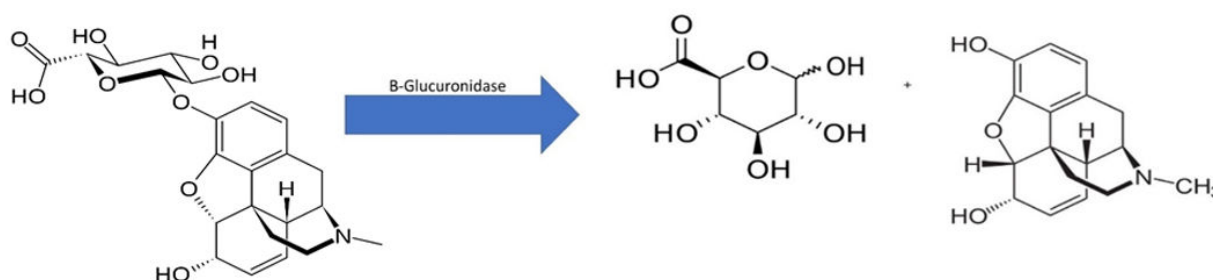
While urine is a common matrix for drugs of abuse analysis, salt concentrations and matrix effects can present analytical challenges. Dilute-and-shoot methods are one approach, but low-level detection can be difficult to achieve because target analytes are diluted along with matrix components. In addition to matrix issues, drugs of abuse analysis can be complicated by the transformation of some drug compounds into glucuronide metabolites. Through this metabolic pathway, which occurs primarily in the liver, glucuronic acid binds to the parent drug, which increases its solubility in water and enables more efficient urinary excretion [3]. The glucuronide forms can be analyzed directly, but because it can be difficult and expensive to source glucuronide standards, LC-MS/MS methods that target the parent drug instead of the glucuronide forms may be advantageous, although effective chromatographic separation is essential when isobars are present.

In order to measure parent drugs directly, a hydrolysis step is required prior to analysis to release glucuronic acid from the drugs of interest [3]. One way of achieving this is through enzymatic hydrolysis using β -glucuronidase, and an example of this reaction is shown in Figure 1. The method developed here employs a simple enzyme hydrolysis sample preparation using β -glucuronidase and LC-MS/MS analysis on a Raptor Biphenyl column in order to accurately quantify 70 drugs of abuse, including isobars, at trace levels in urine.

Related Products

- Raptor Biphenyl column, 2.7 μ m, 50 x 2.1 mm (cat.# 9309A52)
- Raptor Biphenyl EXP guard column cartridge, 2.7 μ m, 5 x 2.1 mm (9309A0252)
- EXP direct connect holder for EXP guard cartridges (cat.# 25808)
- Amber vial, 2 mL (cat.# 21142)
- Vial insert, glass (cat.# 21776)
- Vial cap (cat.# 24497)

Figure 1: β -Glucuronidase Reaction with Morphine-3-Glucuronide



When performing enzyme hydrolysis, it is best practice to use a hydrolysis control, but there are some things to consider first. A hydrolysis control is a urine sample that has been fortified at a known concentration with a glucuronide standard, and its purpose is to demonstrate that the hydrolysis reaction is complete. Because it is more difficult to fully hydrolyze a sample when the analytes are present at high concentrations, it is important that the hydrolysis control be fortified at the higher end of the linearity range [4]. Another consideration when using a hydrolysis control is making sure that the fortified concentration is calculated and prepared correctly. This means taking into account the molecular weight of the glucuronide form in addition to the molecular weight of the parent drug. For example, the molecular weight of morphine is 285.3 g/mol, and the molecular weight of morphine-3- β -glucuronide is 461.4 g/mol. If the molecular weight of the glucuronide form is not considered, then the actual concentration of parent drug in the hydrolysis control will be lower than the target concentration. Accurate fortification levels can be calculated using Formula 1.

Formula 1: Fortification Calculation for Hydrolysis Control Samples

$$\text{Glucuronide Stock Spiking Volume } (\mu\text{L}) = \frac{[(\text{Target Concentration } \frac{\text{ng}}{\text{mL}} * \text{Total Sample Volume } \mu\text{L}) * (\frac{\text{MW of Glucuronide}}{\text{MW of Parent}})]}{\text{Concentration of Glucuronide Stock } \frac{\text{ng}}{\text{mL}}}$$

Experimental

Master Mix

The following sample preparation uses a master mix containing IMCSzyme RT genetically modified β -glucuronidase (IMCS Irmo, SC). The basic ratio for this master mix is 4 μL of IMCS RT enzyme, 8 μL of IMCS RT buffer, 4.7 μL of water, and 3.3 μL of internal standard (Table I). The total volume of master mix that is prepared should be adjusted to accommodate the number of samples in a batch as each sample is spiked with 20 μL of master mix.

Table I: Internal Standard Concentrations in Urine

Internal Standard	Concentration ng/mL
Norbuprenorphine-D4	50
Fentanyl-D5	50
Buprenorphine-D4	50
6-Acetylmorphine-D3	100
Norfentanyl-D5	100
LSD-D3	100
PCP-D5	250
THC-COOH-D9	250
EDDP-D3	400
Paroxetine-D6	400
Amitriptyline-D3	400
Methadone-D3	400
Oxazepam-D5	400
Alpha-hydroxyalprazolam-D5	400
Nordiazepam-D5	400
Temazepam-D5	400
Morphine-D3	400
Oxymorphone-D3	400
Hydromorphone-D3	400
MDMA-D5	400
Haloperidol-D4	400
Oxycodone-D6	400
Tramadol-13C-D3	400
Ketamine-D4	400
7-Aminoclonazepam-D4	400
Methamphetamine-D5	750
Phenobarbital-D9	750

Calibrators, Quality Control Samples, and Urine Sample Preparation

Control urine or sample (20 µL) was added to a 1.5 mL microcentrifuge tube along with 20 µL of the premade master mix. Samples were vortexed for 10 seconds and left to incubate at room temperature for 20 minutes. After incubation, 260 µL of diluent (90:10 0.1% formic acid in water:0.1% formic acid in methanol) was added to each sample. The samples were vortexed for 10 seconds and centrifuged for 10 minutes at 3700 rpm. A 100 µL aliquot of supernatant was added to a 200 µL vial insert, and the samples were moved to the LC-MS/MS for analysis. Fortified calibration standards and quality control samples were prepared at the concentrations shown in Table II.

Table II: Analytical Ranges for Drugs of Abuse in Urine

Analyte	Analytical Range (ng/mL)								QC Range (ng/mL)			
	Cal H	Cal G	Cal F	Cal E	Cal D	Cal C	Cal B	Cal A	QC LOQ	QC Low	QC Med	QC High
6-β-Naltrexol	2	5	20	30	40	60	80	100	5	15	35	75
Acetyl fentanyl												
Buprenorphine												
Fentanyl												
Sufentanil												
6-Monoacetylmorphine	4	10	40	60	80	120	160	200	10	30	70	150
7-Hydroxymitragynine												
LSD												
Norbuprenorphine												
Norfentanyl												
Naloxone	8	20	80	120	160	240	320	400	20	60	140	300
Benzoylcegonine	10	25	100	150	200	300	400	500	25	75	175	375
PCP												
THC-COOH (delta-9-COOH)												
7-Aminoclonazepam	20	50	200	300	400	600	800	1000	50	150	350	750
9-Hydroxyrisperidone												
Alpha-OH-alprazolam												
Amitriptyline												
Amphetamine												
Carisoprodol												
Citalopram												
Codeine												
Cyclobenzaprine												
Dehydroaripiprazole												
Desmethyldoxepin												
Dextromethorphan												
Duloxetine												
EDDP												
Haloperidol												
Hydrocodone												
Hydromorphone												
Hydroxybupropion												
Lamotrigine												
Lorazepam												
MDMA												
Meprobamate												
Methadone												
Mirtazapine												

Table II: Analytical Ranges for Drugs of Abuse in Urine (cont.)

Analyte	Analytical Range (ng/mL)								QC Range (ng/mL)			
	Cal H	Cal G	Cal F	Cal E	Cal D	Cal C	Cal B	Cal A	QC LOQ	QC Low	QC Med	QC High
Morphine	20	50	200	300	400t	600	800	1000	50	150	350	750
Naltrexone												
N-Desmethyltapentadol												
Nordiazepam												
Norfluoxetine												
Norhydrocodone												
Norketamine												
Normeperidine												
Noroxycodone												
Nortriptyline												
O-Desmethyltramadol												
O-Desmethyl-venlafaxine												
Oxazepam												
Oxycodone												
Oxymorphone												
Paroxetine												
7-Hydroxyquetiapine												
Ritalinic acid												
Norsertraline												
Temazepam												
Tramadol												
Trazodone												
Venlafaxine												
Xylazine												
Zolpidem phenyl-4-carboxylic acid												
Butalbital	40	100	400	600	800	1200	1600	2000	100	300	700	1500
Cotinine												
Methamphetamine												
Phenobarbital												
Phentermine												
Gabapentin	100	250	1000	1500	2000	3000	4000	5000	250	750	1750	3750
Pregabalin												

Hydrolysis Control

The hydrolysis control prepared in this method contained four analytes fortified to equal 1000 ng/mL of parent drug when liberated. Morphine-3- β -D-glucuronide; hydromorphone-3- β -D-glucuronide; amitriptyline-N- β -D-glucuronide; and oxazepam glucuronide were spiked into urine using Formula 1. After fortification, 20 μ L of the hydrolysis control was added to a 1.5 mL microcentrifuge tube along with 20 μ L of the premade master mix. Samples were vortexed for 10 seconds and left to incubate at room temperature for 20 minutes. After incubation, 260 μ L of diluent (90:10 0.1% formic acid in water:0.1% formic acid in methanol) was added to each sample. The samples were vortexed for 10 seconds and centrifuged for 10 minutes at 3700 rpm. A 100 μ L aliquot of supernatant was added to a 200 μ L vial insert, and the samples were moved to the LC-MS/MS for analysis.

Instrument Conditions

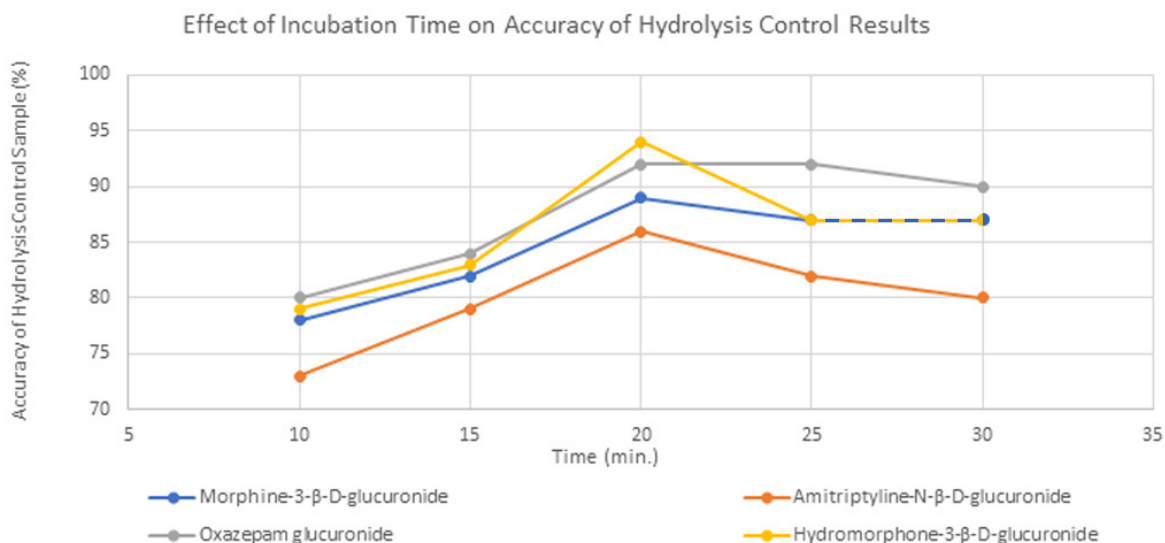
Analytical column:	Raptor Biphenyl 2.7 μ m, 50 mm x 2.1 mm (cat.# 9309A52)	
Guard column:	Raptor Biphenyl EXP guard column cartridge 5 x 2.1 mm, 2.7 μ m (cat.# 9309A0252)	
Mobile phase A:	0.1% Formic acid in water	
Mobile phase B:	0.1% Formic acid in methanol	
Gradient	Time (min)	%B
	0.00	10
	6.00	75
	7.00	100
	7.01	10
	8.00	10
Flow rate:	0.6 mL/min	
Injection volume:	2 μ L	
Column temp.:	45 $^{\circ}$ C	
Ion mode:	Positive and negative ESI	

Results and Discussion

Optimization of Incubation Time

Different incubation times were tested to determine the optimal time for IMCSzyme RT to perform maximum hydrolysis. This test was performed using a hydrolysis control sample, and incubation times of 10, 15, 20, 25, and 30 minutes were evaluated. An incubation time of 20 minutes produced results closest to the nominal value for all four compounds (Figure 2).

Figure 2: Effect of Hydrolysis Enzyme on Control Samples



Optimization of Diluent Volume

In order to reduce the amount of matrix injected on the column and still achieve the required sensitivity for the method, different diluent volumes (260 μ L, 360 μ L, 460 μ L, and 560 μ L) were examined. Overall, 260 μ L of diluent was chosen as the best compromise when considering matrix loading, peak shapes, and sensitivity.

Evaluation of Hydrolysis Efficiency

To assess hydrolysis efficiency, three hydrolysis controls were prepared and analyzed over the course of three days (n=9). The control samples showed acceptable results with all analytes falling within $\pm 15\%$ of the expected value for both intraday and interday repeatability studies (Table III). Precision results also passed with %RSD values of $<10\%$.

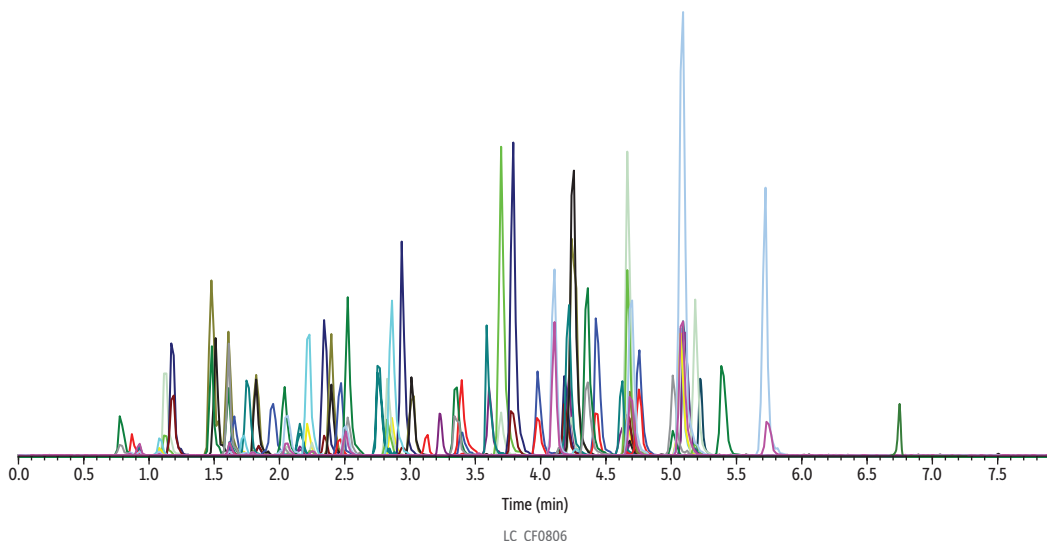
Table III: Interday Repeatability of the Hydrolysis Control (Average Across Three-Day Study)

Morphine				
Sample	Expected (ng/mL)	Average (ng/mL)	% Difference	% RSD
1	1000	1110	11.0	7.6
2	1000	915	8.5	9.3
3	1000	950	5.0	8.9
Hydromorphone				
Sample	Expected (ng/mL)	Average (ng/mL)	% Difference	% RSD
1	1000	1040	4.0	2.5
2	1000	1100	10.0	2.4
3	1000	1090	9.0	2.4
Amitriptyline				
Sample	Expected (ng/mL)	Average (ng/mL)	% Difference	% RSD
1	1000	1150	15.0	6.9
2	1000	962	3.8	8.3
3	1000	1010	1.0	0.0
Oxazepam				
Sample	Expected (ng/mL)	Average (ng/mL)	% Difference	% RSD
1	1000	977	2.3	4.1
2	1000	940	6.0	4.2
3	1000	880	12.0	0.0

Chromatographic Performance

The analysis and separation of 70 drugs of abuse in urine was achieved in a fast, 8-minute cycle time on a Raptor Biphenyl 50 x 2.1 mm, 2.7 μ m column by LC-MS/MS as demonstrated in Figure 3.

Figure 3: 70 Drugs of Abuse in Urine Analyzed by LC-MS/MS Following Enzymatic Hydrolysis



Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion 1	Product Ion 2	Polarity	Peaks	t_R (min)	Conc. (ng/mL)	Precursor Ion	Product Ion 1	Product Ion 2	Polarity
1. Cotinine	0.78	800	177.1	80.0	98.1	+	36. Phenobarbital	3.23	800	230.8	187.8	85.0	-
2. Morphine	0.85	400	286.2	152.1	165.0	+	37. Venlafaxine	3.35	400	278.4	260.4	195.1	+
3. Pregabalin	0.88	2000	160.2	142.1	55.0	+	38. Mirtazapine	3.39	400	266.1	72.1	195.1	+
4. Oxycodone	0.92	400	302.1	227.2	198.2	+	39. Butalbital	3.47	800	222.9	180.0	84.9	-
5. Hydromorphone	1.08	400	286.2	184.9	156.9	+	40. Norbuprenorphine	3.51	80	414.3	152.2	165.2	+
6. Amphetamine	1.13	400	136.2	91.0	65.1	+	41. LSD	3.61	80	324.2	223.1	208.0	+
7. Gabapentin	1.18	2000	172.2	154.0	137.1	+	42. 7-Hydroxymirtazapine	3.65	80	415.5	190.1	174.9	+
8. Methamphetamine	1.48	800	150.2	91.1	119.0	+	43. 9-Hydroxyrisperidone	3.77	400	427.2	110.2	207.1	+
9. Phentermine	1.62	800	150.2	91.1	133.1	+	44. Acetyl fentanyl	3.79	40	323.2	188.0	105.0	+
10. Noroxycodone	1.62	400	302.1	227.0	197.9	+	45. Citalopram	3.94	400	325.1	109.1	262.0	+
11. Naloxone	1.65	160	328.3	310.1	212.3	+	46. Desmethyldoxepin	3.99	400	266.1	107.1	115.0	+
12. Norhydrocodone	1.73	400	286.1	199.0	128.2	+	47. Trazodone	4.10	400	372.3	148.0	260.4	+
13. O-Desmethyltramadol	1.75	400	250.1	58.0	42.0	+	48. Dextromethorphan	4.18	400	272.1	215.1	170.9	+
14. Codeine	1.80	400	300.2	152.0	165.1	+	49. Haloperidol	4.18	400	377.2	170.9	123.0	+
15. MDMA	1.82	400	194.1	163.0	135.1	+	50. Fentanyl	4.20	40	337.2	188.0	105.1	+
16. 6-Acetylmorphine	1.84	80	328.2	211.0	165.0	+	51. Norfluoxetine	4.23	400	296.3	134.3	104.9	+
17. Oxycodone	1.95	400	316.2	298.0	169.0	+	52. PCP	4.25	200	244.1	86.1	159.1	+
18. Naltrexone	2.04	400	342.2	324.0	267.0	+	53. Buprenorphine	4.27	40	468.3	55.1	414.2	+
19. Hydrocodone	2.06	400	300.2	199.0	128.0	+	54. Carisoprodol	4.43	400	261.1	176.0	62.0	+
20. O-desmethylvenlafaxine	2.16	400	164.1	58.1	107.0	+	55. EDDP	4.64	400	278.1	234.3	249.2	+
21. 6-β-Naltrexol	2.21	40	344.2	326.1	308.1	+	56. Duloxetine	4.65	400	298.1	154.1	188.2	+
22. Lamotrigine	2.24	400	255.9	211.1	145.0	+	57. Paroxetine	4.65	400	330.1	192.2	70.1	+
23. Ritalinic acid	2.34	400	220.1	84.1	56.2	+	58. Nortriptyline	4.67	400	264.1	91.1	115.2	+
24. N-Desmethylpentadrol	2.40	400	208.1	121.2	107.1	+	59. Cyclobenzaprine	4.67	400	276.2	215.0	189.0	+
25. Norketamine	2.46	400	224.1	125.0	89.1	+	60. Sufentanil	4.70	40	387.2	238.1	111.1	+
26. Hydroxybupropion	2.50	400	256.0	130.2	166.0	+	61. Amitriptyline	4.82	400	278.1	91.1	202.1	+
27. Norfentanyl	2.53	80	233.1	84.1	55.0	+	62. Norsertraline HCl	5.02	400	292.0	275.0	159.0	+
28. 7-Hydroxyquetiapine	2.71	400	400.2	269.0	208.0	+	63. Lorazepam	5.02	400	321.1	229.0	275.0	+
29. Tramadol	2.76	400	264.2	58.0	77.1	+	64. Methadone	5.08	400	310.2	264.9	105.1	+
30. Xylazine	2.84	400	221.9	90.1	71.9	+	65. Oxazepam	5.08	400	287.1	268.8	241.2	+
31. Zolpidem Phenyl-4-carboxylic acid	2.84	400	338.1	265.1	219.0	+	66. Dehydro aripiprazole	5.18	400	446.2	285.0	98.1	+
32. Benzoyllecgonine	2.85	200	290.1	168.1	77.1	+	67. alpha-Hydroxyalprazolam	5.33	400	325.1	297.0	216.2	+
33. Normeperidine	2.94	400	234.1	160.2	91.0	+	68. Nordiazepam	5.40	400	271.0	139.9	208.0	+
34. Meprobamate	3.01	400	219.1	158.2	97.0	+	69. Temazepam	5.71	400	301.1	255.1	282.9	+
35. 7-Aminoclonazepam	3.07	400	286.1	121.2	250.1	+	70. THC-COOH	6.73	200	343.0	298.9	244.8	-

Column Raptor Biphenyl (cat.# 9309A52)
Dimensions: 50 mm x 2.1 mm ID
Particle Size: 2.7 µm
Pore Size: 90 Å
Guard Column: Raptor Biphenyl EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9309A0252)
Temp.: 45 °C

Standard/Sample
Diluent: 90:10 Water, 0.1% formic acid:methanol, 0.1% formic acid
Inj. Vol.: 2 µL

Mobile Phase
A: Water, 0.1% formic acid
B: Methanol, 0.1% formic acid

Time (min)	Flow (mL/min)	%A	%B
0.00	0.6	90	10
6.00	0.6	25	75
7.00	0.6	0	100
7.01	0.6	90	10
8.00	0.6	90	10

Detector SCIEX Triple Quad 4500
Ion Source: Electrospray
Ion Mode: ESI+/ESI-
Instrument Shimadzu Nexera X2
Sample Preparation 20 µL of a calibrator control in urine was added to a 1.5 mL microcentrifuge tube along with 20 µL of a premade enzyme hydrolysis master mix. The sample was vortexed for 10 seconds and left to incubate at room temperature for 20 minutes. After the incubation, 260 µL of the diluent [water, 0.1 % formic acid: methanol, 0.1 % formic acid 90:10 (v:v)] was added. The sample was vortexed for 10 seconds and centrifuged for 10 minutes at 3700 rpm. One hundred microliters was added to a vial insert (cat. #21776) in a 2.0 mL, amber, short-cap vial (cat.# 21142) and capped with a 9 mm short cap (cat.# 24497) and injected on the LC-MS/MS for analysis.

Resolution of Isobars

Drugs of abuse assays often contain multiple isobars that require chromatographic separation. These analytes must be resolved in order to accurately quantitate each compound because they cannot be distinguished by the MS alone. A target resolution of 1.5 (baseline) or greater is ideal for quantitative work. Resolution can be calculated using Formula 2 where t_R is retention time and W is peak width.

Formula 2: Calculation of Resolution

$$R = \frac{tR_1 - tR_2}{0.5 (W_1 + W_2)}$$

As shown in Table IV, nine groups of isobars were analyzed, and the resolution values within each group were calculated using Formula 2. The Raptor Biphenyl column provided good selectivity and effectively separated the compounds within all nine isobar groups.

Table IV: Isobar Resolution at High QC

Isobar Group	Name	Molecular Weight (g/mol)	Retention Time (min)	Peak Width	Resolution
1	Morphine	285.3	0.87	0.105	1.9
	Hydromorphone		1.11	0.150	
	Norhydrocodone		1.75	0.122	8.7
	7-Aminoclonazepam		3.07	0.180	
2	Oxymorphone	301.3	0.95	0.12	5.8
	Noroxycodone		1.65	0.12	
3	Methamphetamine	149.2	1.41	0.15	1.2*
	Phentermine		1.56	0.11	
4	Naloxone	327.3	1.65	0.15	1.5
	6-Acetylmorphine		1.84	0.1	
5	Codeine	299.4	1.80	0.113	1.7
	Hydrocodone		2.07	0.201	
6	O-desmethylenlafaxine	263.4	2.11	0.15	3.3
	Tramadol		2.70	0.203	
	Mirtazapine		3.39	0.201	7.2
	Nortriptyline		4.66	0.150	
7	Lamotrigine	256.1	2.24	0.150	1.6
	Hydroxybupropion	255.7	2.50	0.185	
8	Citalopram	324.4	3.98	0.2	6.6
	Alpha-hydroxyalprazolam		5.36	0.22	
9	EDDP	278.1	4.64	0.11	1.5
	Amitriptyline		4.81	0.12	

*If additional resolution of methamphetamine and phentermine is required, a Raptor Biphenyl 50 x 4.6 mm, 2.7 μ m column is recommended.

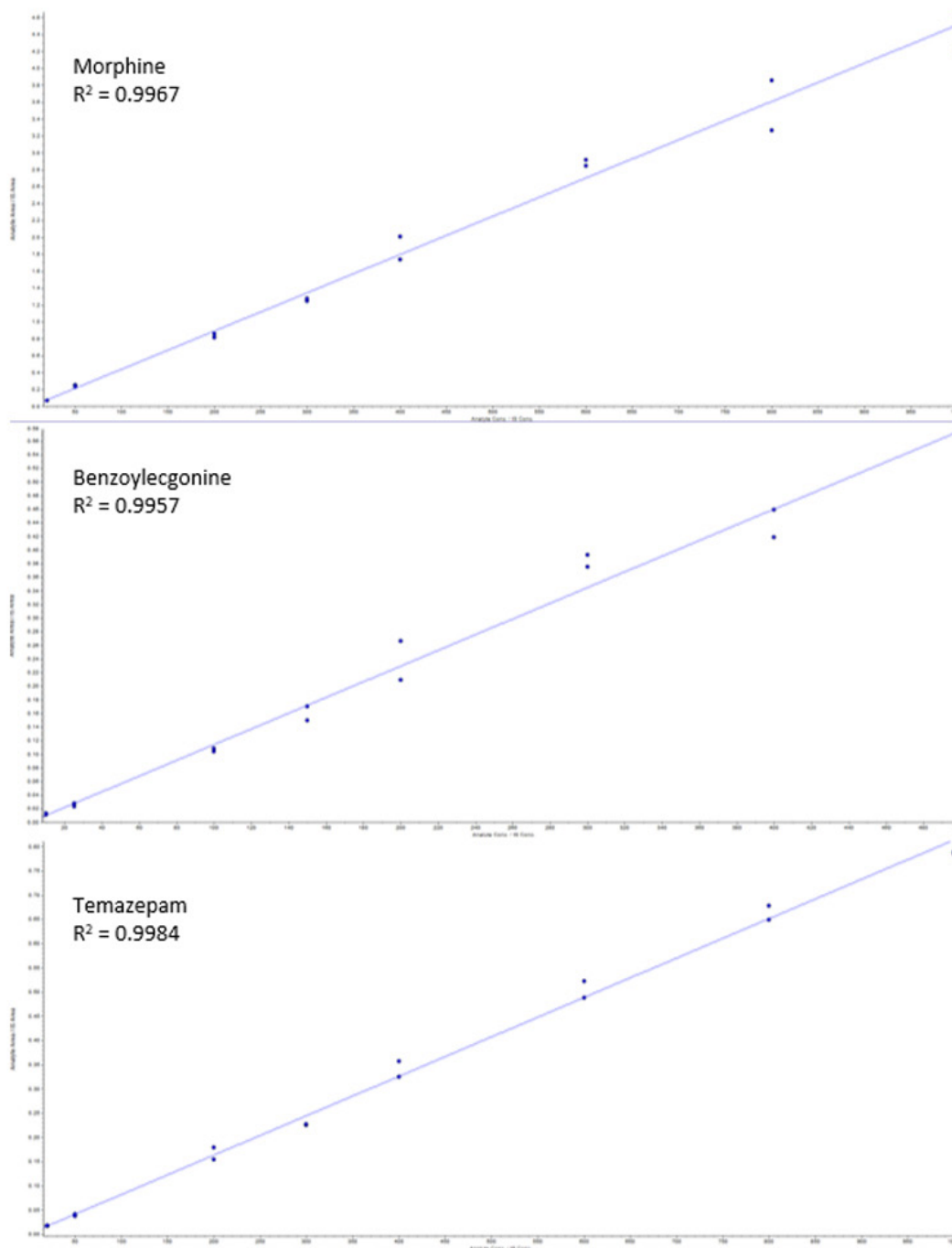
Accuracy and Precision

Precision and accuracy analyses were performed over the course of three days using three sets each day (n=9). Method accuracy was successfully demonstrated with QC LLOQ, QC Low, QC Med, and QC High results falling within $\pm 15\%$ of the expected values for all analytes. The percent relative standard deviation (%RSD) for intraday and interday testing fell below 9.69%, indicating acceptable method precision.

Linearity

Calibration curves were built using standard over internal standard ratios. Linearity was demonstrated using a $1/x^2$ weighted linear regression, and all analytes showed acceptable R^2 values of 0.991 or greater. The example calibration curves shown in Figure 4 highlight three analytes that represent early, middle, and late eluting compounds: morphine (0.85 min); benzoylecgonine (2.85 min); and temazepam (5.71 min). The linear ranges varied across the different drugs of abuse and are presented above in Table II.

Figure 4: Calibration Curves for Selected Drugs of Abuse



Column Robustness

Because urine is a relatively dirty matrix, using a guard column is recommended to remove matrix components and protect the analytical column from contamination. Column robustness was tested by running more than 250 matrix injections on the same guard column and analytical column. This evaluation showed good results, with the retention times of the first and last injections having a percent difference of 4.52% or less for all analytes with no observed increase in back pressure. This demonstrates that the performance of both the guard and analytical columns is robust over many injections.

Conclusion

The method developed here provides a quick, effective approach for sample preparation and LC-MS/MS analysis of 70 drugs of abuse in urine. Separation of all drugs, including isobars, was achieved with this rapid and reliable 8-minute method, allowing high-throughput, quantitative analysis at trace levels. This method demonstrated successful precision, accuracy, and linearity for all analytes. It also showed that enzymatic hydrolysis was effective in cleaving the glucuronide from the analyte of interest, allowing lower limits of detection and the ability to report total concentrations.

References

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This method has been developed for research use only; it is not suitable for use in diagnostic procedures without further evaluation.

Raptor Biphenyl HPLC Column

Product Name	Units	Cat.#
Raptor Biphenyl, 2.7 µm, 50 x 2.1 mm HPLC Column	ea.	9309A52



Raptor Biphenyl EXP Guard Column Cartridge

Product Name	Units	Cat.#
Raptor Biphenyl EXP Guard Column Cartridge, 2.7 µm, 5 x 2.1 mm	3-pk.	9309A0252



EXP Direct Connect Holder

Product Name	Units	Cat.#
EXP Direct Connect Holder for EXP Guard Cartridges, Includes Fitting & Ferrules	ea.	25808



Short-Cap Vial with Grad Marking Spot

Product Name	Units	Cat.#
Short-Cap Vial with Grad Marking Spot, 9-425 Screw-Thread, 2.0 mL, 9 mm, 12 x 32 (vial only)	100-pk.	21142



Vial Inserts

Product Name	Units	Cat.#
Vial Inserts, Glass, Big Mouth w/Bottom Spring, 250 µL	100-pk.	21776



Vial Caps

Product Name	Units	Cat.#
Short Screw Cap, Polypropylene, Screw-Thread, PTFE/Silicone/PTFE Septa, Blue, Preassembled, 2.0 mL, 9 mm	100-pk.	24497





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