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One Minute Screening of a Multiclass Drug Panel with Liquid Chromatography Coupled to High Resolution Mass Spectrometry

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Introduction

Due to regulatory changes in clinical urine drug testing, preliminary screening is now required before definitive testing can be performed. In many instances there are gaps in commercially available screening technologies such as EIA or ELISA. HRMS has emerged as an alternative technology to fill in the gaps. The purpose of this study was to validate a bioanalytical (LC-MS) method for preliminary screening of 9 drugs in urine using a Kinetex 2.6 µm EVO C18 LC column. This ultra-fast screening method (cycle time ~1 min) includes analytes where traditional screening methods, such as EIA and ELISA, could not be used because commercial kits are not available, or are cost prohibitive. Complete hydrolysis was possible with a 5-minute incubation utilizing a novel, purified hydrolysis enzyme. Linearity, LLOQ, Precision and Accuracy, Specificity, Selectivity, and Matrix Effects were evaluated and met all specifications.

Sample Preparation

In a 96-well plate, 75 µL of samples, calibrators, and controls were diluted with internal standard solution (40 µL), and buffered enzyme solution (300 µL). The plate was capped and incubated for 5 minutes at room temperature followed by centrifugation at 4000 rpm for 7 minutes. 7.5 µL was injected. Positive identity was confirmed by accurate mass, retention, time and isotope pattern.

LC Conditions

Column: Kinetex™ 2.6 µm EVO C18
Dimensions: 20 x 2.1 mm
Part No.: 00M-4725-AN
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile

Gradient:

Time (min)	%B
0	5
0.5	60
0.6	99
0.7	99
0.8	5

Flow Rate: 0.6 mL/min
Injection Volume: 7.5 µL
Temperature: 30 °C
LC System: Waters® ACQUITY® I Class UPLC
Detection: HRMS
Detector: Q Exactive™ Orbitrap™

HRMS Conditions

Polarity: Positive
Full Scan: 100-600 m/z
Sheath Gas Flow Rate: 65
Aux Gas Flow Rate: 20
Sweep Gas Flow Rate: 2
Spray Voltage: 3.5 kV
Collision Gas: 9 psi
Spray Current: 0 µA
Capillary Temperature: 300 °C
S-lens RF Level: 55
Aux Gas Heater Temperature: 420 °C
Inclusion: 10 ppm
Resolution: 140 K

Table 1. Full Scan: Exact Mass.

Compound	Chemical Formula	Extracted Mass	Internal Standard
Carisoprodol	$C_{12}H_{24}N_2O_4$	261.1809	Carisoprodol-D_7
Fentanyl	$C_{22}H_{28}N_2O$	337.2274	Fentanyl-D_5
Gabapentin	$C_9H_{17}NO_2$	172.1332	Gabapentin-D_{10}
Meprobamate	$C_9H_{18}N_2O_4$	219.1339	Meprobamate-D_7
Norbuprenorphine	$C_{25}H_{35}NO_4$	414.2639	Norbuprenorphine-D_3
Norfentanyl	$C_{14}H_{20}N_2O$	233.1648	Norfentanyl-D_5
Pregabalin	$C_8H_{17}NO_2$	160.1332	Pregabalin-D_6
Ritalinic Acid	$C_{13}H_{17}NO_2$	220.1332	Ritalinic Acid-D_{10}
Tapentadol	$C_{14}H_{23}NO$	222.1852	Tapentadol-D_3

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Results and Discussion

This preliminary qualitative screening method was developed to detect the presence of 9 drug analytes in urine samples. The use of the deuterated internal standards (**Table 1**) resulted in a matrix factor very close to 1. The concentration range for all analytes was 20 – 1000 ng/mL, except for Fentanyl and Norfentanyl, where was 2 – 100 ng/mL. Precision and accuracy was assessed at five levels with 3 preps of 6 replicate samples at each concentration: QC Neg (1.2/12.5 ng/mL), QC Pos (2.5/25 ng/mL), QC Mid (300 ng/mL – data not shown), QC High (700 ng/mL), and QC Gluc for analytes Norbuprenorphine and Tapentadol (free 147 and 117 ng/mL, respectively) (**Table 2**).

Calibration curves (**Figure 1**), with a regression model of 1/x showed good linearity with R^2 values ≥ 0.999 for all analytes (**Table 3**). The specificity assessment (**Figure 2**) included evaluation of interference from matrix/system components and internal standards; all acceptance criteria were met. A small subset of urine samples presented interferences for Gabapentin, Norfentanyl, Pregabalin, Ritalinic Acid, and Norbuprenorphine. These interferences are suspected to be endogenous substances present in urine. This phenomena explains the discrepancies observed between this screening method and the LC-MS/MS method used for confirmation.

Assessments of carryover, dilution linearity, and impact from hematuria were preformed as well as stability assessments including freeze/thaw, short-term, long-term, post-preparative, and autosampler stability. In all cases, results met acceptance criteria as per the 2001 Bioanalytical Guidance Document and current industry best practices.

Table 2. Inter-Assay Precision and Accuracy. Acceptance Criteria: ≤ 20 % Deviation from Nominal. (N=18)

Compound	QC Negative			QC Positive			QC High			QC Gluc		
	Nominal Conc. (ng/mL)	Mean % Nominal	%RSD	Nominal Conc. (ng/mL)	Mean % Nominal	%RSD	Nominal Conc. (ng/mL)	Mean % Nominal	%RSD	Nominal Conc. (ng/mL)	Mean % Nominal	%RSD
Carisoprodol	12.0	92.8	4.4	25.0	96.2	4.9	700	97.2	1.6	-	-	-
Fentanyl	1.20	100	3.0	2.50	96.2	5.6	70	98.7	2.3	-	-	-
Gabapentin	12.0	81.5	11.3	25.0	96.0	6.1	700	100	1.2	-	-	-
Meprobamate	12.0	90.0	10.0	25.0	94.0	10.8	700	98.0	2.2	-	-	-
Norbuprenorphine	12.0	93.8	4.0	25.0	99	4.3	700	98.7	1.3	147	98.9	1.4
Norfentanyl	1.20	101	1.8	2.50	93.6	7.7	70	90.3	6.0	-	-	-
Pregabalin	12.0	98.6	6.0	25.0	99.1	6.3	700	97.7	1.8	-	-	-
Ritalinic Acid	12.0	88.4	1.1	25.0	95.6	4.0	700	100	1.1	-	-	-
Tapentadol	12.0	90.8	0.4	25.0	96.7	3.0	700	98.1	1.2	117	106	0.8

Figure 1. Representative Calibration Curve.

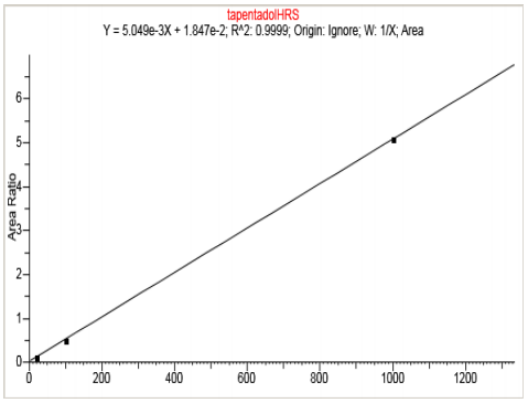


Table 3. Summary of Correlation Coefficient Calibration Results.

Compound	Run 1	Run 2	Run 3
Carisoprodol	0.999	1.000	0.999
Fentanyl	0.999	0.999	0.999
Gabapentin	0.999	1.000	1.000
Meprobamate	1.000	1.000	0.999
Norbuprenorphine	1.000	0.999	0.999
Norfentanyl	1.000	0.999	0.999
Pregabalin	1.000	1.000	1.000
Ritalinic Acid	0.999	0.999	0.999
Tapentadol	0.999	1.000	0.999

Figure 2 . Selectivity/Specificity of 100 Samples Confirmed Positive (50) and Negative (50) for the Presence of 9 Analytes Using Tandem Quadrapole Definitive LC-MS/MS Methods and Qualitatively Compared to Q Exactive™ Screen Results.

Carisoprodol		
QE Screen	Positive	Negative
QQQ Positive	50	0
QQQ Negative	0	50

Fentanyl		
QE Screen	Positive	Negative
QQQ Positive	50	0
QQQ Negative	0	50

Gabapentin		
QE Screen	Positive	Negative
QQQ Positive	50	0
QQQ Negative	1	49

Meprobamate		
QE Screen	Positive	Negative
QQQ Positive	50	0
QQQ Negative	0	50

Norbuprenorphine		
QE Screen	Positive	Negative
QQQ Positive	49	1
QQQ Negative	1	49

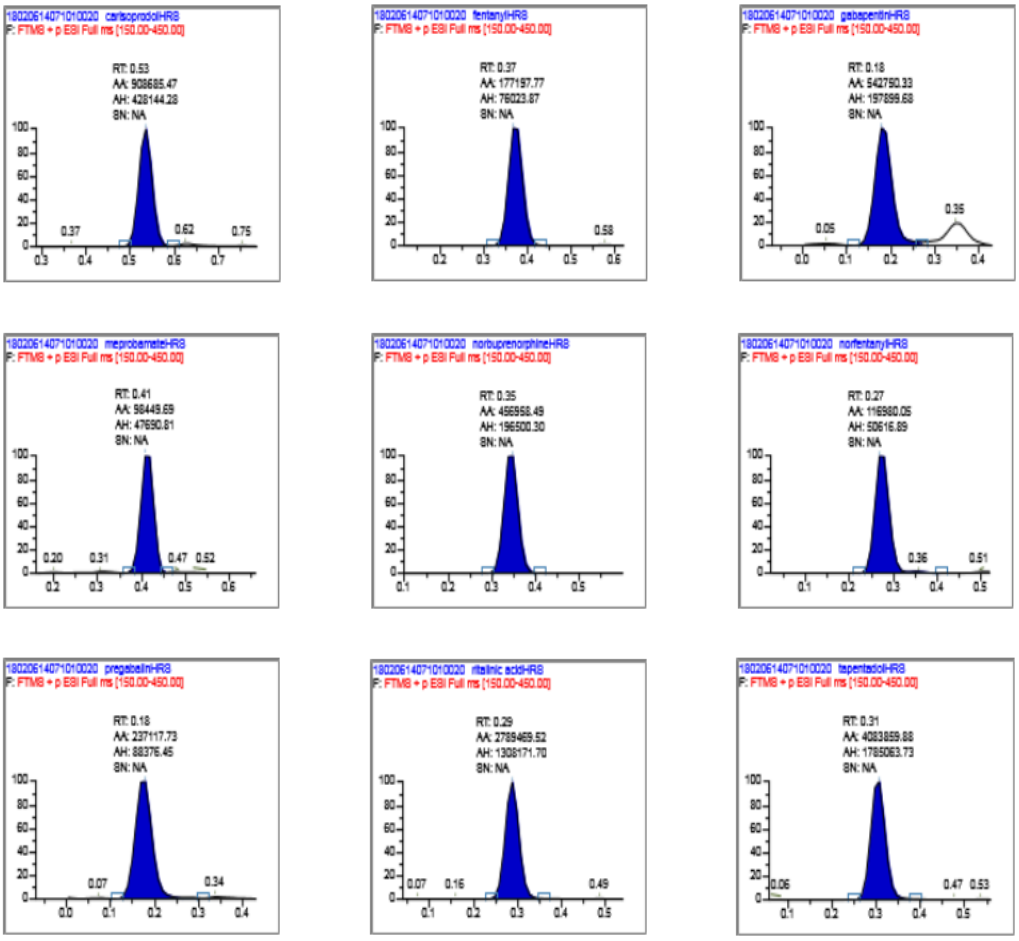
Norfentanyl		
QE Screen	Positive	Negative
QQQ Positive	47	3
QQQ Negative	0	50

Pregabalin		
QE Screen	Positive	Negative
QQQ Positive	48	2
QQQ Negative	0	50

Ritalinic Acid		
QE Screen	Positive	Negative
QQQ Positive	50	0
QQQ Negative	2	48

Tapentadol		
QE Screen	Positive	Negative
QQQ Positive	50	0
QQQ Negative	0	50

Figure 3 . Representative Chromatograms at LLOQ (2 ng/mL or 20 ng/mL).



Conclusions

This method provides a cost effective, highly selective and specific alternative to EIA screening. The use of a purified recombinant Glucuronidase enzyme and the use of a Kinetex™ 2.6 µm EVO C18 LC column, in conjunction with the high resolution of the Q Exactive™ mass spectrometer, allows for a fast Dilute-and-Shoot sample preparation for 9 drug panel analytes.

Ordering Information

Kinetex 2.6 µm Minibore Columns (mm)							SecurityGuard™ ULTRA Cartridges*
Phases	**20 x 2.1**	**30 x 2.1**	**50 x 2.1**	**75 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
EVO C18	00M-4725-AN	00A-4725-AN	00B-4725-AN	—	00D-4725-AN	00F-4725-AN	AJ0-9298
PS C18	—	00A-4780-AN	00B-4780-AN	—	00D-4780-AN	00F-4780-AN	AJ0-8951
Polar C18	—	00A-4759-AN	00B-4759-AN	—	00D-4759-AN	00F-4759-AN	AJ0-9532
Biphenyl	00M-4622-AN	00A-4622-AN	00B-4622-AN	—	00D-4622-AN	00F-4622-AN	AJ0-9209
XB-C18	—	00A-4496-AN	00B-4496-AN	00C-4496-AN	00D-4496-AN	00F-4496-AN	AJ0-8782
C18	00M-4462-AN	00A-4462-AN	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN	AJ0-8782
C8	—	00A-4497-AN	00B-4497-AN	00C-4497-AN	00D-4497-AN	00F-4497-AN	AJ0-8784
HILIC	—	00A-4461-AN	00B-4461-AN	00C-4461-AN	00D-4461-AN	00F-4461-AN	AJ0-8786
Phenyl-Hexyl	—	00A-4495-AN	00B-4495-AN	00C-4495-AN	00D-4495-AN	00F-4495-AN	AJ0-8788
F5	—	00A-4723-AN	00B-4723-AN	—	00D-4723-AN	00F-4723-AN	AJ0-9322

*SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

for 2.1 mm ID

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