

## APPLICATION

## Rapid Analysis of a 48 Drug Research Panel in Less Than 4 Minutes Using Kinetex<sup>®</sup> Biphenyl Core-Shell Technology HPLC/UHPLC Columns

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*Using a 30 x 2.1 mm Kinetex 2.6 $\mu$ m Biphenyl LC Column, our team successfully analyzed 48 drug analytes in less than 4 minutes. A full ESI+/ESI- 52 drug panel was performed in 7 minutes and 30 seconds—including re-equilibration. This rapid analysis showed great resolution and no loss in sensitivity compared to slower methods.*

### Introduction

Abuse of pain management drugs has reached an epidemic level across the United States. According to the US Center for Disease Control, use of and non-intentional deaths from opioids have quadrupled since 1999<sup>1</sup>. In the first 3 months of 2016, deaths from heroin and fentanyl-laced heroin have surpassed 2015 death tolls in several cities in the United States<sup>2,3</sup>. These increases have led to increased pressure on researchers.

Labs demand methods that can help them analyze these samples as fast as technology will allow without compromising confidence in their results. Large research panels, from urine, covering several classes of drugs have become standard in the industry. In order to satisfy their customers' needs, labs commonly test for amphetamines, benzodiazepines, opioids, drugs of abuse, tricyclic antidepressants, barbiturates, nicotine, and THC metabolites. A list of analytes is outlined in **Tables 1** and **2**.

Sample preparation, LC/MS/MS analysis, and data review are all steps which contribute to a sample's turnaround time. In our work, we focused on shortening the LC runtime to under 8 minutes per sample while maintaining resolution of all critical pairs including isobaric/isomeric compounds like morphine and hydromorphone, codeine and hydrocodone, and 6-MAM and naloxone.

The method was then tested and fully validated at TriEpiq Lab Group, Oklahoma City, OK.

### Materials and Methods

#### Reagents and Chemicals

Analytical reference standards and Surine negative synthetic urine were purchased from Cerilliant Corporation (Round Rock, TX, USA). IMCSzyme purified  $\beta$ -glucuronidase was purchased from IMCS (Columbia, SC, USA).

#### Sample Preparation

Calibrators were prepared in synthetic urine.

Urine samples underwent a 60 minute incubated enzymatic hydrolysis at 55 °C and were diluted 40-fold with mobile phase prior to injection. Samples were centrifuged for 20 minutes at 18,000 rcf before and after incubation.

Sample prep methods, such as sorbent-based  $\beta$ -glucuronidase removal or protein precipitation, were outside of the scope of this method development. To avoid system downtime and premature column death, we recommend more extensive sample prep procedures.

### Experimental Conditions

UHPLC analysis was performed using a Shimadzu<sup>®</sup> Nexera<sup>®</sup> X2 LC-30 (Kyoto, Kyoto Prefecture, Japan) with an upper pressure limit of 1300 bar, equipped with a binary pump and autosampler. Detection by tandem mass spectrometry was performed using a SCIEX QTRAP<sup>®</sup> 6500 (Framingham, MA, USA) with ESI configuration. Two separate methods were developed for analysis of compounds by positive and negative electrospray ionization. Method conditions are listed on page 2. The Kinetex 2.6 $\mu$ m Biphenyl Core-Shell HPLC/UHPLC column was used to perform the separation. The Biphenyl chemistry was chosen for its robustness, excellent peak shape, and strong selectivity for this group of analytes. By using a 30 x 2.1 mm column, instead of the 50mm column typically used for these types of panels, we decreased retention times and system backpressure thereby allowing the flow rate to be increased.



## Positive ESI Panel

### LC Conditions

**Column:** Kinetex® 2.6 µm Biphenyl  
**Dimensions:** 30 x 2.1 mm  
**Part No.:** 00A-4622-AN  
**SecurityGuard™ ULTRA Cartridge:** AJ0-9209  
**SecurityGuard Holder:** AJ0-9000  
**Mobile Phase:** A: 10 mM Ammonium Formate in Water (pH unadjusted)  
 B: 1.0% Formic Acid in Methanol  
**Flow Rate:** 1.0 mL/min  
**Gradient:**

Time (min)	% B
0.00	5
1.20	30
1.30	55
2.30	70
2.40	95
2.70	95
2.72	5
4.00	5

  
**Temperature:** 30 °C  
**Injection Volume:** 15 µL

### MS/MS Conditions

**Conditions:** SCIEX 6500 QTRAP®  
**Mode:** Positive electrospray ionization  
**Scan Type:** MRM  
**Curtain Gas (CUR):** 30  
**Gas 1 (GS1):** 70  
**Gas 2 (GS1):** 80  
**IS:** 2000 V  
**Temperature (TEM):** 550 °C  
**Interface Heater:** ON  
**Collision Gas (CAD):** Medium  
**Entrance Potential (EP):** 10 V

**Table 1. (continued)**

List of ESI+ Analytes and Transitions.

Analyte	Q1 (m/z)	Q3 (m/z)
Carisoprodol 2	261.1	97.0
Citalopram 1	325.1	109.1
Citalopram 2	325.1	262.0
Codeine 1	300.1	115.1
Codeine 2	300.1	152.1
Cotinine 1	177.1	80.0
Cotinine 2	177.1	98.0
Diazepam 1	285.1	193.1
Diazepam 2	285.1	154.1
EDDP 1	278.1	234.1
EDDP 2	278.1	186.1
Fentanyl 1	337.2	188.1
Fentanyl 2	337.2	105.1
Fluoxetine 1	310.1	44.1
Fluoxetine 2	310.1	148.2
Gabapentin 1	172.1	137.2
Gabapentin 2	172.1	95.1
Hydrocodone 1	300.0	199.1
Hydrocodone 2	300.0	128.1
Hydromorphone 1	286.1	185.1
Hydromorphone 2	286.1	128.1
Imipramine 1	281.1	86.0
Imipramine 2	281.1	58.1
Lorazepam 1	321.1	275.1
Lorazepam 2	321.1	229.1
MDMA 1	194.1	163.1
MDMA 2	194.1	135.1
Meperidine 1	248.1	174.1
Meperidine 2	248.1	220.1
Meprobamate 1	219.1	158.1
Meprobamate 2	219.1	97.0
Methadone 1	310.2	105.1
Methadone 2	310.2	265.1
Methamphetamine 1	150.1	91.0
Methamphetamine 2	150.1	119.1
Methylphenidate 1	234.1	84.1
Methylphenidate 2	234.1	56.0
Morphine 1	286.1	152.1

**Table 1.**

List of ESI+ Analytes and Transitions.

Analyte	Q1 (m/z)	Q3 (m/z)
6-MAM 1	328.1	165.1
6-MAM 2	328.1	211.1
7-Aminoclonazepam 1	286.0	222.1
7-Aminoclonazepam 2	286.0	121.0
alpha-Hydroxyalprazolam 1	325.2	297.1
alpha-Hydroxyalprazolam 2	325.2	216.1
Alprazolam 1	309.1	205.1
Alprazolam 2	309.1	281.0
Amitriptyline 1	278.1	191.0
Amitriptyline 2	278.1	202.0
Amphetamine 1	136.1	119.1
Amphetamine 2	136.1	91.0
Benzoylcegonine 1	290.1	168.1
Benzoylcegonine 2	290.1	105.1
Buprenorphine 1	468.1	396.2
Buprenorphine 2	468.1	55.2
Carisoprodol 1	261.1	176.1

**Table 1. (continued)**

List of ESI+ Analytes and Transitions

Analyte	Q1 (m/z)	Q3 (m/z)
Morphine 2	286.1	165.2
Naloxone 1	328.2	212.0
PCP 1	244.1	91.0
PCP 2	244.1	159.1
Pregabalin 1	160.1	55.1
Pregabalin 2	160.1	97.1
Sertraline 1	306.1	159.0
Sertraline 2	306.1	275.1
Tapentadol 1	222.1	107.0
Tapentadol 2	222.1	121.0
Temazepam 1	301.1	255.1
Temazepam 2	301.1	177.1
Tramadol 1	264.1	58.1
Tramadol 2	264.1	42.1
Zolpidem 1	308.1	235.1
Zolpidem 2	308.1	219.0
Zolpidem-4-carboxylic Acid 1	338.1	265.0
Zolpidem-4-carboxylic Acid 2	338.1	292.9
6-MAM-D3	331.1	165
7-Aminoclonazepam-D4	290.1	226.2
Alpha-hydroxyalprazolam-D5	330.1	302.0
Alprazolam-D5	314.1	286.0
Amitriptyline-D3	281.1	202.0
Amphetamine-D5	141.1	96.0
Benzoyllecgonine-D3	293.1	171.1
Buprenorphine-D4	472.3	400.1
Carisoprodol-D7	268.1	183.1
Citalopram-D6	331.1	109.0
Codeine-D3	303.2	152.1
Cotinine-D3	180.1	80.0
Diazepam-D5	290.1	198.1
EDDP-D3	281.0	234.1
Fentanyl-D5	342.2	105.1
Gabapentin-D10	182.2	164.2
Hydrocodone-D3	303.0	199.1
Hydromorphone-D3	289.0	185.0

**Table 1. (continued)**

List of ESI+ Analytes and Transitions

Analyte	Q1 (m/z)	Q3 (m/z)
Imipramine-D3	284.1	89.0
Lorazepam-D4	325.0	105.9
MDMA-D5	199.1	165.0
Meperidine-D4	252.2	224.1
Meprobamate-D7	226.1	165.1
Methadone-D3	313.2	105.0
Methamphetamine-D5	155.1	92.0
Methylphenidate-D9	243.2	93.0
Morphine-D3	289.2	152.0
Naloxone-D5	333	258.0
Norbuprenorphine-D3	417.2	83.2
Nordiazepam-d5	276.1	140.1
Norfentanyl-D5	238.2	84.0
Norhydrocodone-D3	289.1	202.0
Noroxycodone-D3	305.1	190.0
Nortriptyline-D3	267.1	233.0
O-Desmethyl-cis-tramadol-D6	256.3	64.1
Oxycodone-D3	319.1	244.1
Oxymorphone-D3	305.1	230.0
Phencyclidine-D5	249.1	96.0
Pregabalin-D6	166.1	148.3
Tapentadol-D3	225.1	107.0
Temazepam-D5	306.1	260.1
Tramadol-13C, D3	268.3	58.1
Zolpidem-D7	315.1	242.1



## Negative ESI Panel

### LC Conditions

**Column:** Kinetex® 2.6 µm Biphenyl  
**Dimensions:** 30 x 2.1 mm  
**Part No.:** 00A-4622-AN  
**SecurityGuard™ ULTRA Cartridge:** AJ0-9209  
**SecurityGuard Holder:** AJ0-9000  
**Mobile Phase:** A: 10 mM Ammonium Formate in Water (pH unadjusted)  
 B: 0.1 % Formic Acid in Methanol  
**Flow Rate:** 1.0 mL/min  
**Gradient:**

Time (min)	% B
0.0	10
0.2	10
2.5	90
2.9	90
3.0	10
3.5	10

  
**Temperature:** 40 °C  
**Injection Volume:** 25 µL

### MS/MS Conditions

**Detector:** SCIEX 6500 QTRAP®  
**Mode:** Negative Electrospray Ionization  
**Scan Type:** MRM  
**Curtain Gas (CUR):** 30  
**Gas 1 (GS1):** 60  
**Gas 2 (GS1):** 65  
**IS:** -4500 V  
**Temperature (TEM):** 650 °C  
**Interface Heater:** ON  
**Collision Gas (CAD):** Medium  
**Entrance Potential (EP):** -10 V

**Table 2.**

List of ESI- Analytes and Transitions

Analyte	Q1 (m/z)	Q3 (m/z)
Butalbital 1	223.0	42.0
Butalbital 2	223.0	180.0
Phenobarbital 1	231.1	42.1
Phenobarbital 2	231.1	188.0
Secobarbital 1	237.0	42.0
Secobarbital 2	237.0	194.0
THC-COOH 1	343.1	245.0
THC-COOH 2	343.1	191.2
Butalbital-D5	228.0	42.0
Secobarbital-D5	242.0	42.0
THC-COOH-D9	352.1	254.0

## Results and Discussion

The chromatographic conditions produced good peak shape and separation of analytes. Total run time for both panels was 7 minutes 30 seconds, with 48 of the 52 drugs analyzed in positive ESI mode in just under 4 minutes (**Figure 1**), and the remaining analytes analyzed in negative ESI mode in 3 minutes and 30 seconds (**Figure 2**). **Figures 3-5** demonstrate baseline resolution for isobaric compounds morphine/hydromorphone/norhydrocodone, codeine/hydrocodone, and oxycodone/noroxycodone, and near baseline resolution for naloxone/6-MAM and EDDP/amitriptyline (**Figures 6 and 7**).

A six-point calibration curve was prepared in synthetic urine with a linear range of 40% to 10x the cutoffs listed in **Tables 3 and 4**. An additional two points at 25x and 50x the cutoff were run to extend the range for selected opiates. Data analysis was performed using SCIEX MultiQuant™ Software.

The methods were fully validated at TriEpiq Lab, with the parameters tested including precision and accuracy, LOD, and carryover studies.

**Table 3.**  
ESI+ Retention Times and Cutoffs

	List of Analytes (ESI+)	Retention Time (min)	Cutoff (ng/mL)
1	6-MAM	1.7	10
2	7-Aminoclonazepam	2.0	100
3	alpha-hydroxyalprazolam	2.5	100
4	Alprazolam	2.7	100
5	Amitriptyline	2.4	50
6	Amphetamine	1.1	100
7	Benzoylcegonine	1.8	50
8	Buprenorphine	2.2	20
9	Carisoprodol	2.1	200
10	Citalopram	2.1	50
11	Codeine	1.6	100
12	Cotinine	1.7	100
13	Diazepam	2.8	100
14	EDDP	2.3	100
15	Fentanyl	2.1	10
16	Fluoxetine	2.2	50
17	Gabapentin	0.7	100
18	Hydrocodone	1.7	100
19	Hydromorphone	1.2	100
20	Imipramine	2.3	50
21	Lorazepam	2.3	100
22	MDMA	1.6	50
23	Meperidine	1.9	100
24	Meprobamate	1.9	100
25	Methadone	2.5	100
26	Methamphetamine	1.4	100
27	Methylphenidate	1.9	10
28	Morphine	1.0	100
29	Naloxone	1.6	50
30	Norbuprenorphine	2.0	20
31	Nordiazepam	2.5	100
32	Norfentanyl	1.8	10
33	Norhydrocodone	1.6	100
34	Noroxycodone	1.5	100
35	Normorphine	0.4	100
36	Nortriptyline	2.3	50
37	O-Desmethyltramadol	1.6	100
38	Oxycodone	1.7	100
39	Oxymorphone	1.1	100
40	Paroxetine	2.3	50

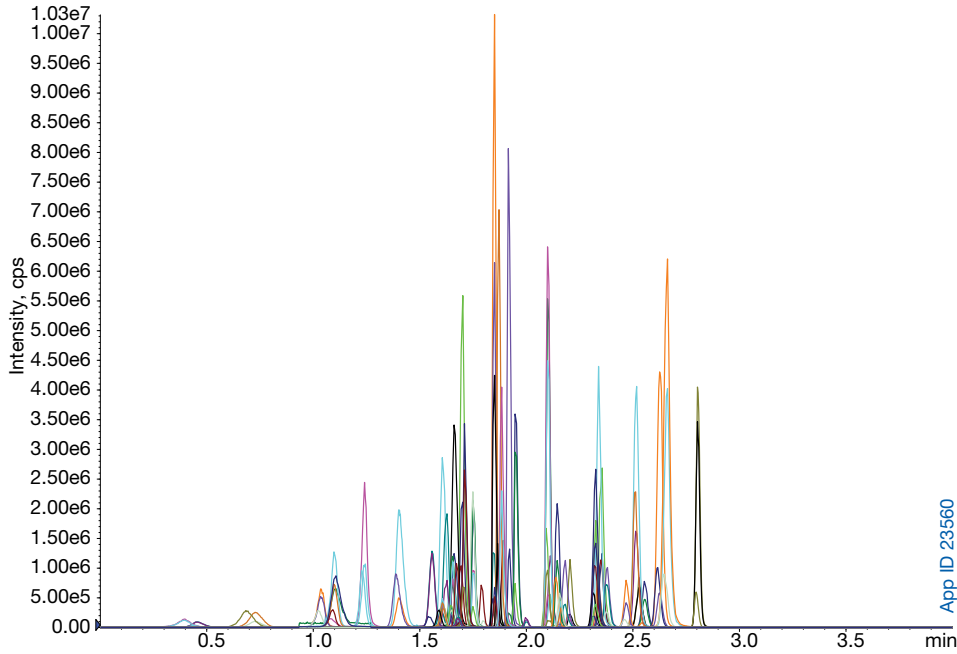
	List of Analytes (ESI+)	Retention Time (min)	Cutoff (ng/mL)
41	PCP	2.2	50
42	Pregabalin	0.4	100
43	Sertraline	2.5	50
44	Tapentadol	1.8	100
45	Temazepam	2.6	100
46	Tramadol	1.9	100
47	Zolpidem	2.1	20
48	Zolpidem-4-carboxylic Acid	1.9	20

**Table 4.**  
ESI- Retention Times and Cutoffs.

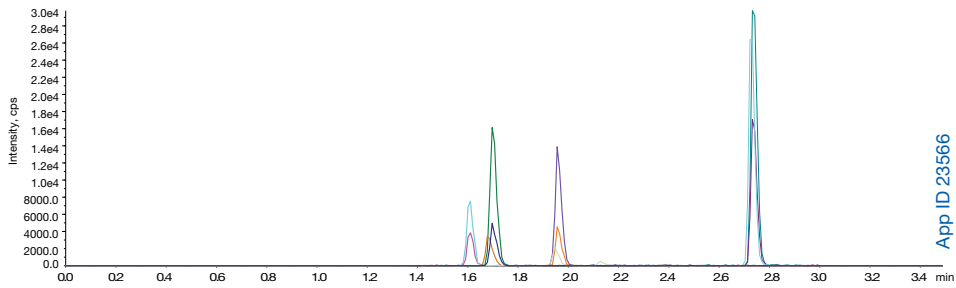
	List of Analytes (ESI-)	Retention Time (min)	Cutoff (ng/mL)
1	Butalbital	1.7	100
2	Phenobarbital	1.6	100
3	Secobarbital	2	100
4	THC-COOH	2.7	50



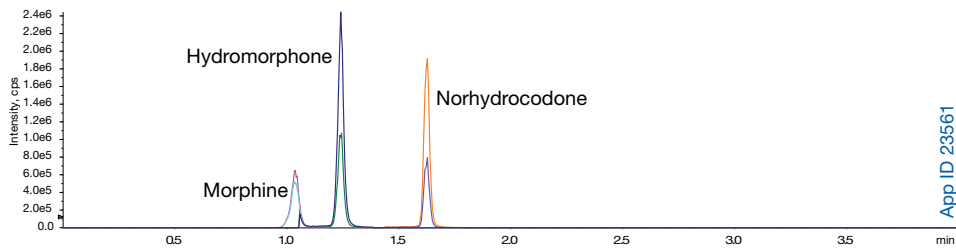
**Figure 1.**  
TIC of ESI+ panel



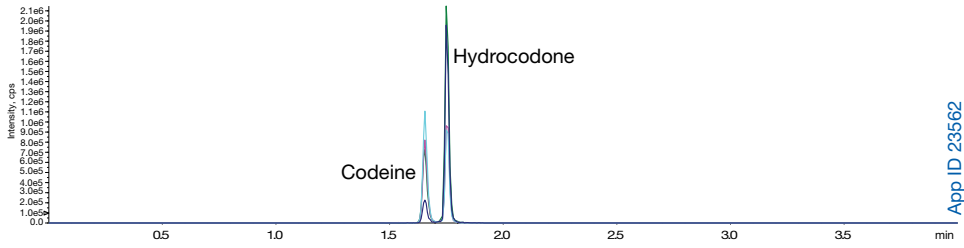
**Figure 2.**  
TIC of ESI- panel



**Figure 3.**  
XIC of morphine, hydromorphone, norhydrocodone ( $m/z = 286.1$ )

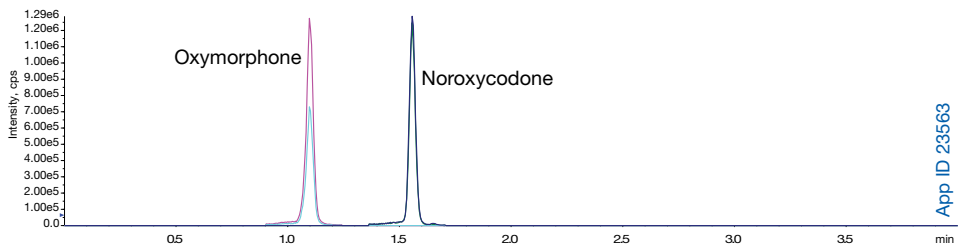


**Figure 4.**  
XIC of codeine & hydrocodone (m/z = 300.1)



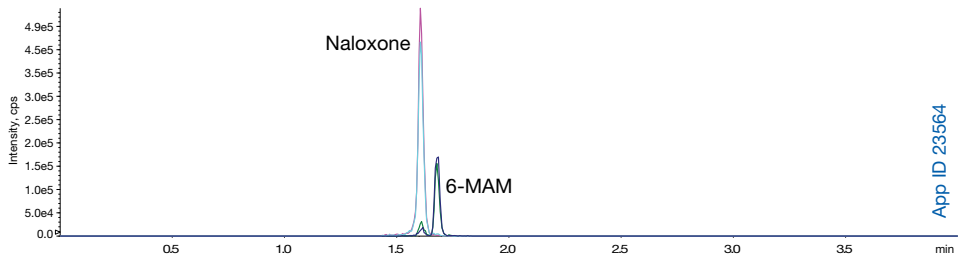
App ID 23562

**Figure 5.**  
XIC of oxymorphone & noroxycodone (m/z = 302.1)



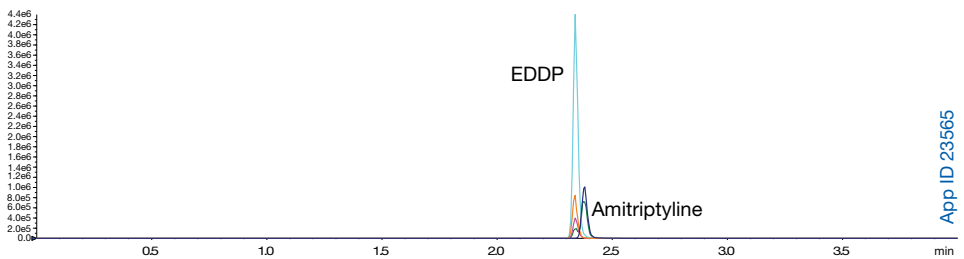
App ID 23563

**Figure 6.**  
XIC of naloxone & 6-MAM (m/z = 328.2)



App ID 23564

**Figure 7.**  
XIC of EDDP & amitriptyline (m/z = 278.1)



App ID 23565

(continued)



## APPLICATION

**Conclusion**

In this work we demonstrated how research facilities can improve runtimes without sacrificing results. The increases in throughput may allow labs to better respond to the increases in demand without costly investment in capital equipment.

**References**

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	30 x 2.1	3/pk
Biphenyl	00A-4622-AN	AJ0-9209

\*SecurityGuard ULTRA cartridges require holder, Part No. AJ0-9000

**Verex<sup>™</sup> Certified Vial Products**

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