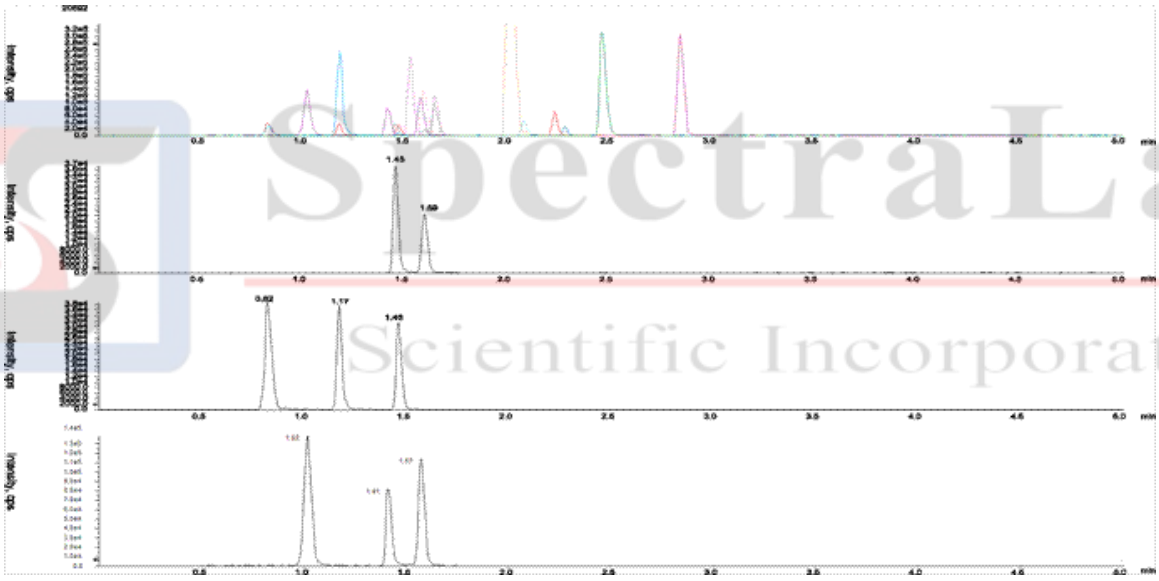
Analysis of Opiates by LC-MS-MS

Method 1: Pain Panel Opiates by LC-MS-MS

Instrumentation: Shimadzu LC System with AB Sciex LC/MS/MS System
Column: Waters XBridge C18 2.5µm 2.1 X 50mm Column (Ctrl + Click to follow link)
Elution Type: Gradient
Mobile Phase A: 10mM Ammonium formate
Mobile Phase B: Methanol with 0.1% formic acid
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	95	5
2	3	5	95
3	3.1	95	5

Flow Rate: 0.5 mL/min
Col. Temp: Ambient
Detection: Tandem Mass Spec (MS-MS) @ amu (22 °C) (Ctrl + Click to follow link)
Detector Info: AB Sciex API 4000 (Ctrl + Click to follow link)



1: Morphine
2: Oxymorphone
3: Hydromorphone
4: Codeine
5: Norhydrocodone
6: Oxycodone
7: Noroxycodone
8: Hydrocodone
9: 6-MAM
10: Norfentanyl

11: Tramadol
12: Meperidine
13: Normeperidine
14: Norbuprenorphine
15: Fentanyl
16: Buprenorphine
17: Methadone

Method 2: Opiates in Urine by LC-MS-MS

Instrumentation: Agilent HPLC system with AB Sciex LC/MS/MS System
Column: Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8-µm particle size
Elution Type: Gradient
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Gradient Profile:

Step No.	Time (min)	Pct A	Pct B
1	0	100	2
2	4	60	40
3	4.1	10	90
4	6	10	90
5	6.1	98	2

Flow Rate: 0.4 mL/min
Col. Temp: 50 °C
Detection: Tandem Mass Spec (MS-MS) @ amu (350°C) (Ctrl + Click to follow link)
Detector Info: AB Sciex API 4000 (Ctrl + Click to follow link)

MS Conditions

Gas temperature: 350 °C
Gas Flow: 11 L/min
Nebulizer: 60 psi
Capillary Ion spray voltage: 2000 V
Fragmentor: 110 V
Dwell: 50 msec
Polarity: Positive ESI

MRM Mode Parameters for Opiates

Segment	Compound	Collision Transition	Retention energy (V)	time (min)
1 (0 min)	D3-morphine	289.2 > 152.1	75	1.851
	Morphine	286.2 > 152.1 (128.0)	75 (73)	1.862
	D3-oxymorphone	305.2 > 230.1	33	2.138
	Oxymorphone	302.2 > 227.1 (198.0)	33 (55)	2.146
	D3-hydromorphone	289.2 > 157.1	50	2.379
	Hydromorphone	286.2 > 185.0 (157.0)	33 (50)	2.385

MRM Mode Parameters for Opiates

Segment	Compound	Collision Transition	Retention energy (V)	time (min)
2 (2.65 min)	D3-codeine	303.2 > 152.0	75	2.908
	Codeine	300.2 > 152.0 (115.0)	75 (85)	2.912
	D3-oxycodone	319.2 > 244.1	30	3.109
	Oxycodone	316.2 > 241.0 (256.0)	30 (27)	3.120
	D6-6-MAM	334.2 > 165.1	40	3.161
	6-MAM	328.2 > 165.0 (211.0)	40 (27)	3.168
	D3-hydrocodone	303.2 > 199.1	28	3.245
	Hydrocodone	300.2 > 199.0 (128.0)	28 (73)	3.249

Sample Preparation:

1. Start with 250-µL sample size
2. Add 500 µL sodium acetate buffer
3. Add 20 µL glucuronidase
4. Add 75 µL of internal standard mixture at 500 ng/mL concentration (de-ionized water)
5. Vortex
6. Incubate at 60 °C for 20 minutes
7. Add 850 µL de-ionized water
8. Vortex and spin down
9. Place 200 µL of supernatant in sample vial

Results:

Morphine, oxymorphone, hydromorphone, codeine, oxycodone, hydrocodone, and 6-acetylmorphine (6-MAM), all have calibration curves with linearity coefficients of at least 0.99, and show good reproducibility and accuracy at the lowest concentration levels. This corresponds to 1 to 150 ppb for all analytes except 6-MAM; for 6-MAM this range is 0.067 to 10 ppb. This range corresponds to 0.74 to 110.6 pg on-column (49 fg to 7.5 pg for 6-MAM). For all seven compounds linearity and detection levels are below the 10 ng/mL confirmatory cutoff level for workplace testing proposed by the U.S. Substance Abuse Mental Health Services Administration (SAMHSA). The lowest calibration levels correspond to levels of 1 pg/µL in urine with the exception of oxycodone and 6-MAM, the confirmation ion ratio of ± 20% for oxycodone and 6-MAM are 5 and 0.3 pg/µL, respectively. Limits of detection are less than 1 pg on-column for all analytes.

References: http://www.phenomenex.com/Application/Detail/20692
http://www.chem.agilent.com/Library/applications/5989-7213EN.pdf