

Analysis of 26 Natural and Synthetic Opioids in Blood and Urine Using Clean Screen® DAU SPE and a Selectra® DA UHPLC Column



UCT Part Numbers

CSDAU206Clean Screen® DAU
200 mg, 6mL Column**Abalonase™ Ultra Purified
β-Glucuronidase**
UASBETA-GLUC-10**SLDAGDC20-18UMOPT**
Selectra® DA Guard Column
5 X 2.1 mm, 1.8 μm**SPPHO6001-10**Select pH Buffer Pouch
100 mM Phosphate, pH 6.0**SLDA50ID21-18UM**
Selectra® DA UHPLC Column
50 X 2.1 mm, 1.8 μm**SLGRDHLDR-HPOPT**
Guard Column Holder

Summary:

In recent years, drug abuse has become one of the leading causes of accidental deaths across the country. The opioid crisis was one of the main manifestations of drug-related addictions that caused severe dependency and, in too many cases, fatal overdoses. In this application note, UCT offers a simple yet effective procedure to extract and monitor an extensive panel of opiates in blood and urine using solid-phase extraction (SPE) combined with ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLCMS/MS). Clean Screen® DAU is UCT's flagship SPE column that can be used to extract a wide range of natural and synthetic opioids with excellent recoveries and overall precision. In addition, UCT's Selectra® DA UHPLC column provides excellent retention and peak shape for all the opioids in the panel, including baseline separation of the critical isobaric compounds. Furthermore, all compounds eluted in less than 8 minutes from the Selectra® DA UHPLC column. The simple protocol outlined in this application note can be readily implemented in pain management, clinical diagnostics, and forensic analysis.



Sample Pretreatment:

Urine Specimens*:

- To 1 mL of urine add 1 mL of pH 6 phosphate buffer (0.1M) and appropriate volume of internal standard(s)
- Mix/vortex briefly

***A hydrolysis protocol is required if conjugated compounds are added into the drug panel**

Blood Specimens:

- To 1 mL of blood add 4 mL of pH 6 phosphate buffer (0.1M) and appropriate volume of internal standard(s)
- Mix/vortex briefly
- If necessary (e.g. postmortem blood), centrifuge the sample for 10 minutes at 3000 rpm (discard pellet after loading sample onto SPE column)

SPE Procedure:

1. Condition Column

- a) 1 x 3 mL MeOH
- b) 1 x 3 mL pH 6 phosphate buffer (0.1M)

2. Apply Sample

- a) Load at 1-2 mL/minute

3. Wash Column

- a) 1 x 3 mL 1% Formic Acid in DI H₂O
- b) 1 x 3 mL MeOH
- c) Dry cartridges under full vacuum or pressure for 1 minute to remove residual MeOH

4. Elute Analytes

- a) 1 x 3 mL MeOH + 5% Ammonium Hydroxide (MeOH:NH₄OH, 95:5, v/v)
- b) Collect at 1-2 mL/minute

5. Dry Eluate

- a) Evaporate to dryness under a gentle stream of nitrogen at <40°C

6. Reconstitute

- a) Reconstitute sample in 1 mL of mobile phase (alternative volumes may also be used)



LC-MS/MS Parameters:

HPLC Parameters		
MS System		Thermo Scientific TSQ Vantage
HPLC System		Thermo Scientific Dionex Ultimate 3000
UHPLC Column		Selectra® DA (50 X 2.1 mm, 1.8 µm) (UCT P/N: SLDA50ID21-18UM)
Guard Column		Selectra® DA Guard Column (5 X 2.1 mm, 1.8 µm) (UCT P/N: SLDAGDC20-18UMOPT)
Column Temperature		40°C
Flow Rate		0.4 mL/min
Injection Volume		5 µL
Gradient Program		
Time (min)	% Mobile Phase A (0.1% FA in Water)	% Mobile Phase B (0.1% FA in Methanol)
0	100	0
0.5	85	15
3.5	70	30
7.5	0	100
8.5	0	100
8.6	100	0
11	100	0



MRM Table:

MRM							
Analyte	RT (min)	Parent Ion	Product Ion 1	CE	Product Ion 2	CE	Internal Standard
6-Acetylmorphine	4.64	328.1	165.1	36	211.1	25	6-Acetylmorphine-D6
Acetyl fentanyl	6.87	323.2	105.1	33	188.1	21	Fentanyl-D5
Buprenorphine	7.16	468.4	396.3	37	414.3	32	Buprenorphine-D4
Codeine	4.53	300.1	152.1	63	165.1	41	Codeine-D6
EDDP	7.40	278.1	234.1	31	249.2	23	Methadone-D9
Fentanyl	7.12	337.2	105.0	34	188.1	22	Fentanyl-D5
Heroin	6.32	370.1	165.0	46	268.1	27	Heroin-D9
Hydrocodone	5.03	300.1	128.1	56	199.1	29	Hydrocodone-D6
Hydromorphone	3.37	286.1	157.1	40	185.1	29	Hydromorphone-D6
Levorphanol	5.98	258.1	157.1	37	199.1	26	Morphine-D3
Meperidine	6.24	248.2	174.1	19	220.2	20	Meperidine-D4
Morphine	2.96	286.1	152.1	64	165.1	43	Morphine-D3
Methadone	7.59	310.0	104.6	28	264.7	13	Methadone-D9
Naloxone	4.25	328.0	212.1	37	310.1	18	Fentanyl-D5
Naltrexone	4.94	342.2	270.1	26	324.2	19	Fentanyl-D5
Norcodeine	3.79	286.1	152.0	56	165.1	43	Morphine-D3
Norbuprenorphine	6.70	414.3	101.1	36	187.0	35	Norbuprenorphine-D3
Norfentanyl	5.67	233.2	84.1	17	150.1	17	Norfentanyl-D5
Norhydrocodone	4.42	286.0	128.1	55	199.1	27	Norhydrocodone-D3
Normeperidine	6.12	234.1	56.2	23	160.1	16	Normeperidine-D4
Noroxycodone	4.23	302.0	227.1	28	284.1	16	Noroxycodone-D3
Noroxymorphone	2.47	287.9	212.9	29	270.0	17	Morphine-D3
Oxycodone	4.83	316.1	298.2	18	241.1	27	Oxycodone-D6
Oxymorphone	3.10	302.1	227.1	28	284.2	19	Morphine-D3
Tapentadol	5.81	222.1	77.1	45	107.1	29	Norfentanyl-D5
Tramadol	6.03	264.1	42.1	79	58.1	16	Fentanyl-D5



Chromatograms:

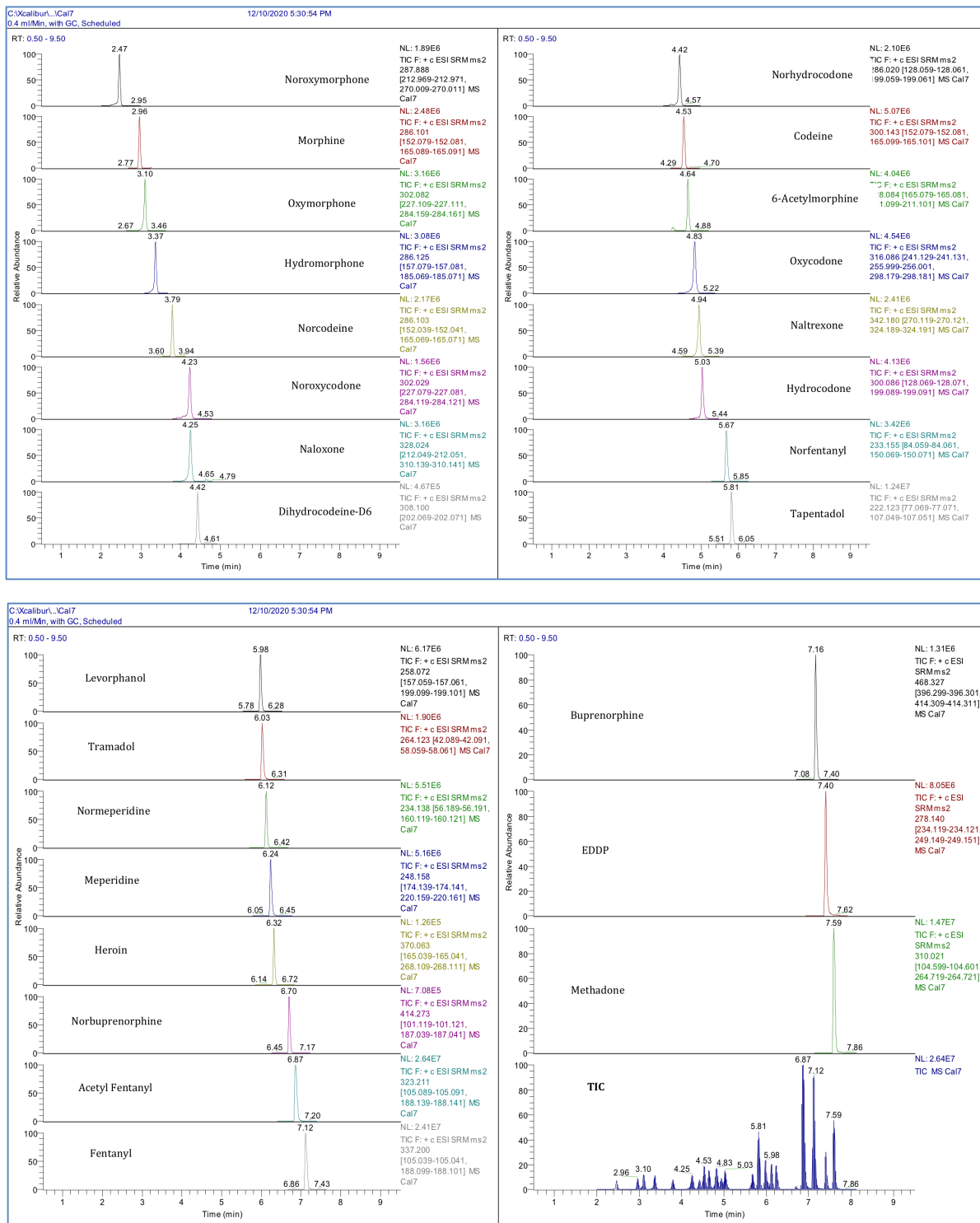


Figure 1: Exemplary chromatogram of the separation obtained using the Selectra® DA UHPLC column. All analytes included in the method eluted in less than 8 minutes. The TIC represents the total ion chromatogram for a single injection.

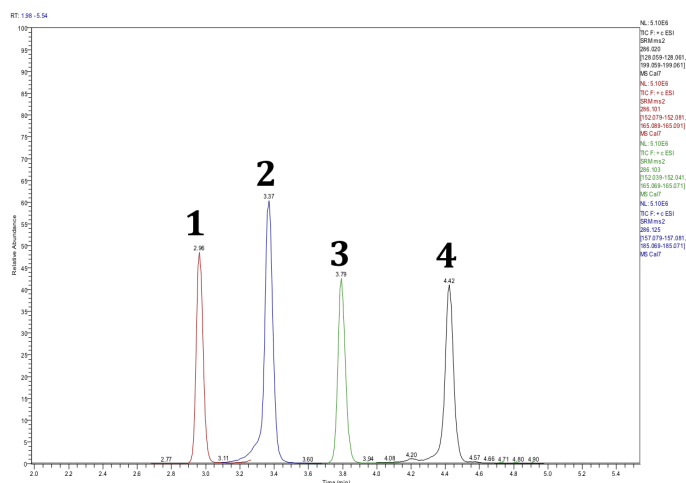


Figure 2a: Complete separation of critical isobaric compounds 1) Morphine, 2) Hydromorphone, 3) Norcodeine, and 4) Norhydrocodone

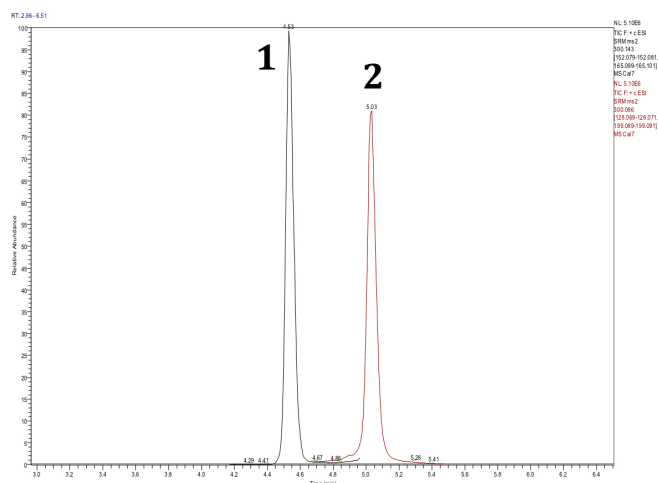


Figure 2b: Complete separation of critical isobaric compounds 1) Codeine and 2) Hydrocodone

Calibration Curves:

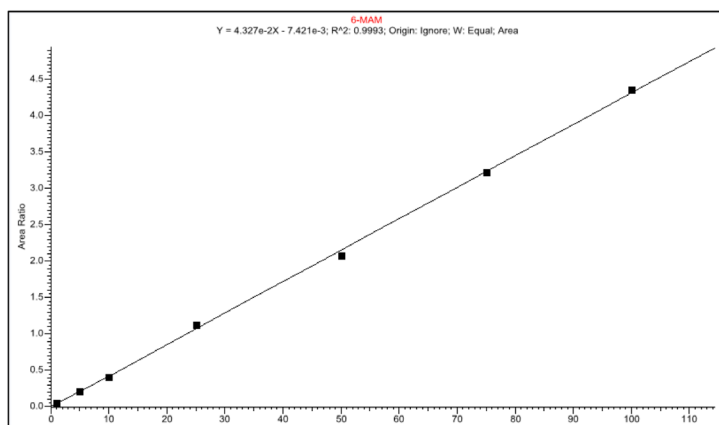


Figure 3a: Example of a 7-point calibration curve for 6-MAM with R^2 of 0.9993 (1, 5, 10, 25, 50 & 100 ng/mL).

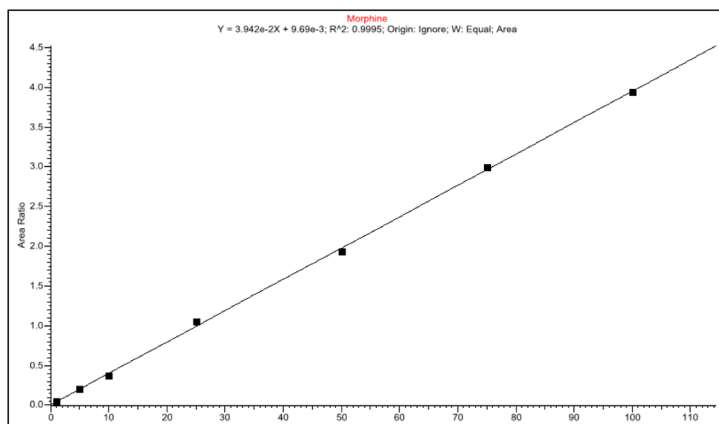


Figure 3b: Example of a 7-point calibration curve for Morphine with R^2 of 0.9995 (1, 5, 10, 25, 50 & 100 ng/mL).



Results:

Urine Recovery (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	75 ng/mL	RSD
6-Acetylmorphine	98%	0.17	103%	0.88	100%	1.06
Acetyl fentanyl	106%	0.28	104%	0.98	99%	3.68
Buprenorphine	98%	0.23	100%	0.67	99%	1.05
Codeine	96%	0.17	104%	0.58	99%	1.99
EDDP	106%	0.13	104%	2.06	95%	2.59
Fentanyl	84%	0.33	102%	1.31	101%	1.17
Heroin	104%	0.14	100%	0.87	99%	1.40
Hydrocodone	103%	0.14	102%	0.64	100%	1.69
Hydromorphone	97%	0.08	103%	0.74	102%	1.03
Levorphanol	106%	0.23	103%	2.58	91%	4.27
Meperidine	97%	0.13	101%	0.59	100%	0.87
Morphine	95%	0.18	102%	0.80	100%	1.81
Methadone	107%	0.17	97%	0.74	97%	1.86
Naloxone	106%	0.31	108%	1.31	94%	5.52
Naltrexone	109%	0.27	108%	1.20	96%	5.85
Norcodeine	109%	0.24	104%	0.97	97%	4.65
Norbuprenorphine	84%	0.43	99%	0.85	98%	1.42
Norfentanyl	92%	0.23	105%	0.81	101%	1.42
Norhydrocodone	102%	0.20	100%	0.95	97%	0.92
Normeperidine	90%	0.17	102%	0.85	102%	0.84
Noroxycodone	103%	0.26	93%	0.87	95%	4.13
Noroxymorphone	102%	0.25	102%	1.27	100%	3.03
Oxycodone	100%	0.19	103%	0.97	103%	1.78
Oxymorphone	110%	0.22	109%	1.30	97%	4.51
Tapentadol	82%	0.26	106%	1.94	99%	2.65
Tramadol	96%	0.35	109%	1.49	99%	2.85



Results:

Blood Recovery (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	75 ng/mL	RSD
6-Acetylmorphine	101%	0.14	104%	0.82	98%	1.74
Acetyl fentanyl	107%	0.31	95%	3.04	100%	1.88
Buprenorphine	101%	0.13	103%	0.57	97%	1.76
Codeine	89%	0.14	106%	0.99	97%	1.51
EDDP	102%	0.56	98%	3.23	100%	3.17
Fentanyl	103%	0.14	108%	0.65	101%	1.30
Heroin	107%	0.30	105%	1.24	95%	2.82
Hydrocodone	97%	0.14	107%	0.44	99%	1.21
Hydromorphone	93%	0.07	106%	0.62	100%	1.18
Levorphanol	100%	0.10	108%	0.99	93%	2.19
Meperidine	94%	0.12	106%	0.78	99%	1.22
Morphine	96%	0.12	104%	0.64	98%	1.44
Methadone	85%	0.15	106%	1.13	101%	1.55
Naloxone	107%	0.38	105%	1.23	102%	2.64
Naltrexone	106%	0.36	108%	1.59	101%	3.51
Norcodeine	94%	0.11	98%	0.63	98%	2.51
Norbuprenorphine	98%	0.15	104%	0.74	98%	1.33
Norfentanyl	88%	0.14	109%	1.09	102%	1.72
Norhydrocodone	98%	0.12	104%	0.77	99%	0.97
Normeperidine	95%	0.15	108%	0.86	101%	1.47
Noroxycodone	95%	0.26	107%	0.77	100%	1.98
Noroxymorphone	95%	0.12	100%	0.92	98%	2.45
Oxycodone	97%	0.13	108%	0.74	99%	1.72
Oxymorphone	103%	0.07	105%	0.55	97%	2.53
Tapentadol	96%	0.17	106%	0.78	98%	2.00
Tramadol	105%	0.53	103%	1.57	104%	3.03

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