



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

CSDAU206

Clean Screen® DAU
200 mg, 6mL Cartridge

Abalonnase™ Ultra Purified
β-Glucuronidase
UASBETA-GLUC-10

SPHPHO6001-10
Select pH Buffer Pouch
100 mM Phosphate, pH 6.0

SLDA50ID21-18UM
Selectra® DA UHPLC Column
50 X 2.1 mm, 1.8 μm

SLDAGDC20-18UMOPT
Selectra® DA Guard Column
5 X 2.1 mm, 1.8 μm

SLGRDHLDLDR-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 (UHPLC-MS/MS). Clean Screen® DAU is UCT's flagship SPE cartridge 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 included in the method, 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.



CLINICAL



FORENSICS

Sample Pretreatment:

Urine Specimens*:

- To 1 mL of urine add 1 mL of pH 6 phosphate buffer (0.1M) and the 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 the 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 cartridge)

SPE Procedure:

1. Condition Cartridge:

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

2. Apply Sample:

- Load at 1-2 mL/minute

3. Wash Cartridge:

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

4. Elute Analytes:

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

5. Dry Eluate:

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

6. Reconstitute:

- 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)		
Guard Column: Selectra® DA Guard Column (5 X 2.1 mm, 1.8 µm)		
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:

Analyte	RT (min)	MRM					Internal Standard
		Parent Ion	Product Ion 1	CE	Product Ion 2	CE	
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:

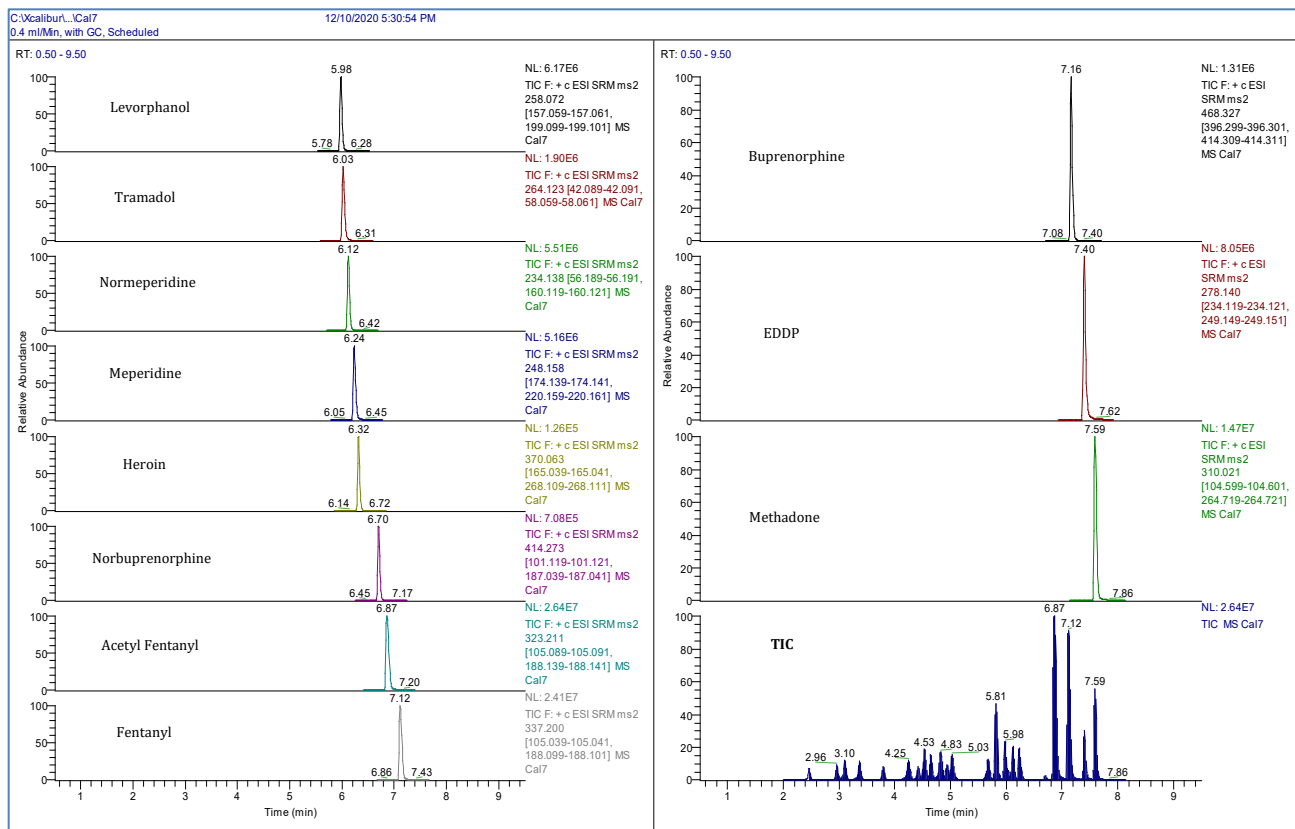
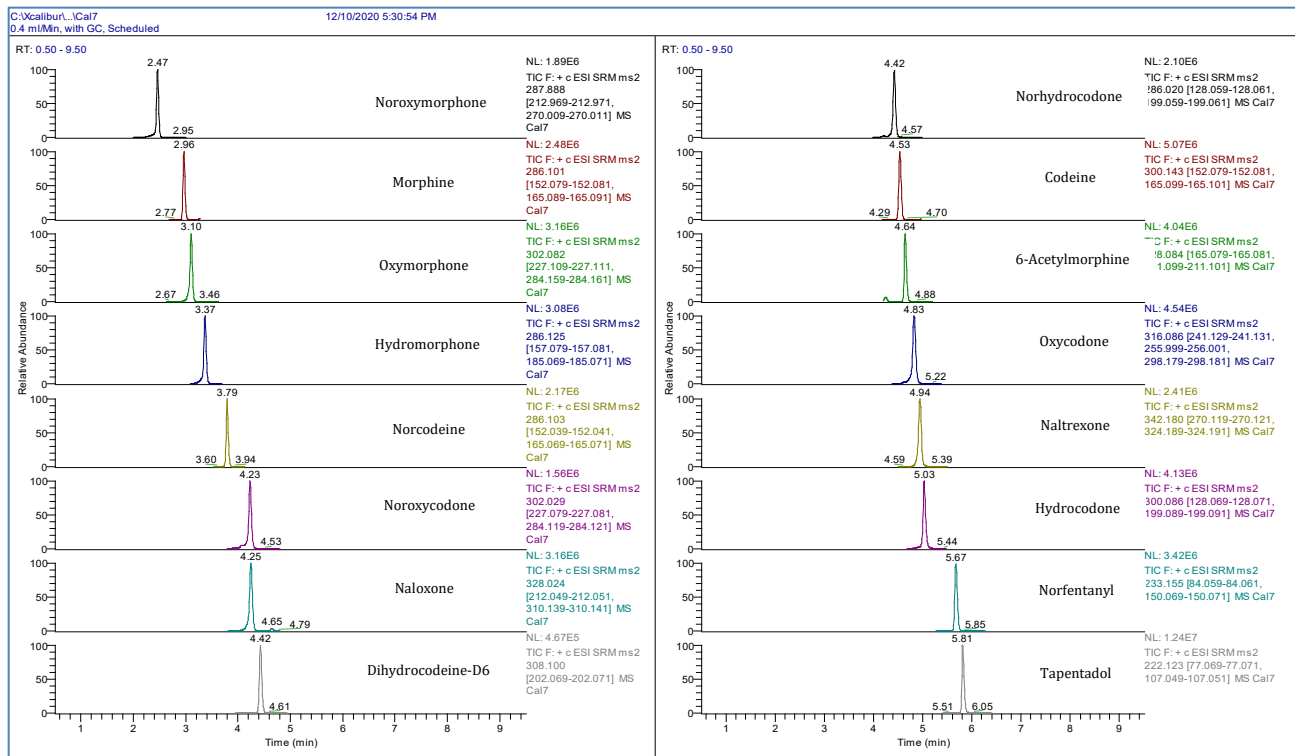


Figure 1: Exemplary chromatogram of the separation obtained using the Selectra® DA UHPLC column. All analytes included in the method eluted in less than 8 minutes. The TIC represents the total ion chromatogram for a single injection.

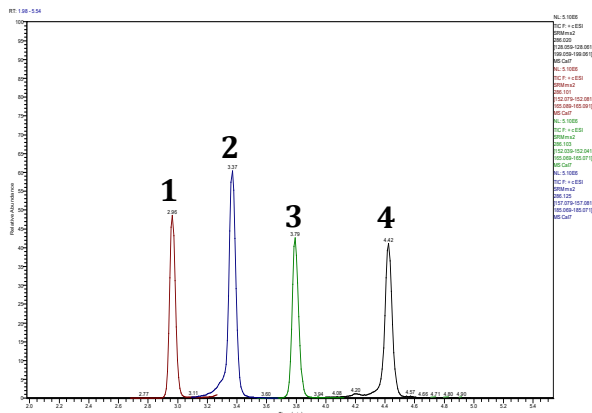


Figure 2a: Complete separation of critical isobaric compounds 1) Morphine, 2) Hydromorphone, 3) Norcodeine, and 4) Norhydrocodone.

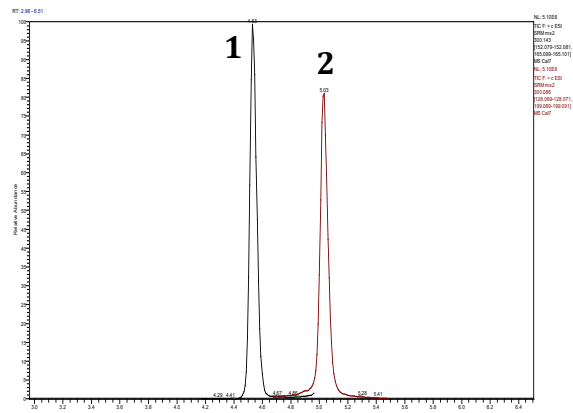


Figure 2b: Complete separation of critical isobaric compounds 1) Codeine and 2) Hydrocodone.

Calibration Curves:

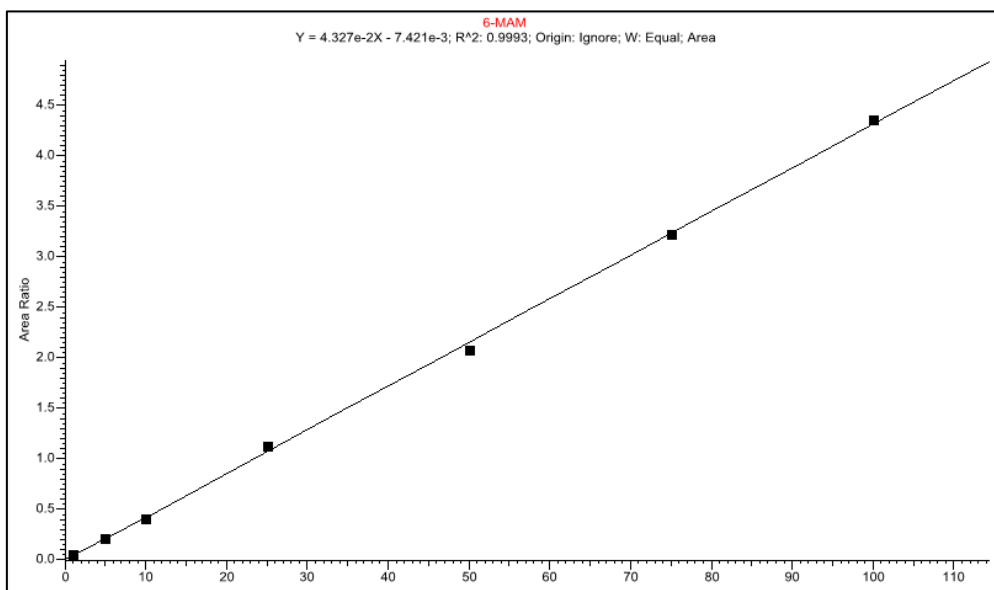


Figure 3a: Example of a 7-point calibration curve for 6-MAM with R^2 of 0.9993 (1, 5, 10, 25, 50 & 100 ng/mL).

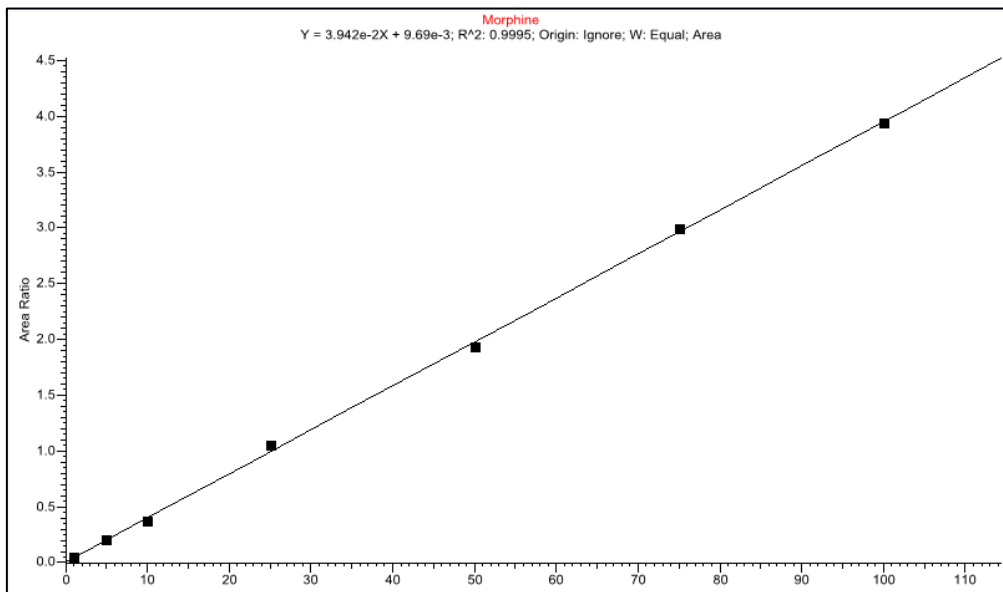


Figure 3b: Example of a 7-point calibration curve for Morphine with R^2 of 0.9995 (1, 5, 10, 25, 50 & 100 ng/mL).



Results:

Urine:

Analyte	Recovery (n=5)					
	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

Blood:

Analyte	Recovery (n=5)					
	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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