

Innovation Applied

Quantitative Analysis of Opiates in Urine using Exactive LC-MS System

Clinical Research Applications Group
Thermo Fisher Scientific
San Jose CA

Forensic Toxicology use Only

Analytes and Internal Standards

No	Analyte	Internal Standard
1	Morphine	Morphine-D6
2	Hydromorphone	Hydromorphone-D6
3	Oxymorphone	Oxymorphone-D3
4	Codeine	Codeine-D6
5	Dihydrocodeine	Dihydrocodeine-D6
6	Oxycodone	Oxycodone-D6
7	Hydrocodone	Hydrocodone-D6
8	Meperidine	Meperidine-D4
9	Normeperidine	Meperidine-D4

Sample preparation

- Sample preparation
 - Acid hydrolysis of 2mL urine
 - SPE cleanup
 - Reconstitution volume 300 uL
 - 5 uL of injected into LC-MS system
- Standards and QC samples
 - Analytes and internal standards Cerilliant.
 - Calibrators - standard spiked in blank urine
 - QC samples contained only some analytes

Analytes

Accurate Masses and Retention Times

No	Analyte	Exact mass	Ret time
1	Morphine	286.1438	2.66
2	Hydromorphone	286.1438	3.04
3	Oxymorphone	302.1387	2.86
4	Codeine	300.1594	3.38
5	Dihydrocodeine	302.1751	3.28
6	Oxycodone	316.1543	3.63
7	Hydrocodone	300.1594	3.78
8	Meperidine	248.1645	4.63
9	Normeperidine	234.1488	4.54

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Internal Standards

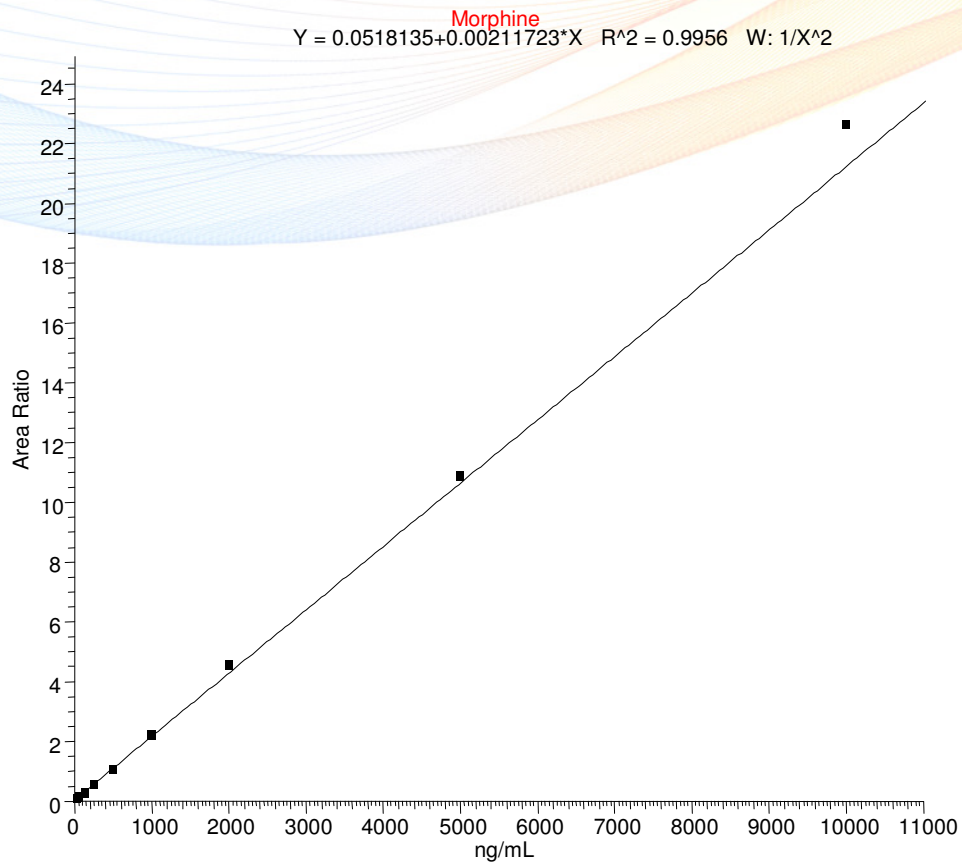
Accurate Masses and Retention Times

No	Analyte	Exact mass	Ret time
1	Morphine-D6	292.1814	2.66
2	Hydromorphone-D6	292.1814	3.04
3	Oxymorphone-D3	305.1575	2.86
4	Codeine-D6	306.1971	3.38
5	Dihydrocodeine-D6	308.2127	3.28
6	Oxycodone-D6	322.1920	3.63
7	Hydrocodone-D6	306.1971	3.78
8	Meperidine-D4	252.1896	4.63

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Morphine

Excellent Linearity and Dynamic Range

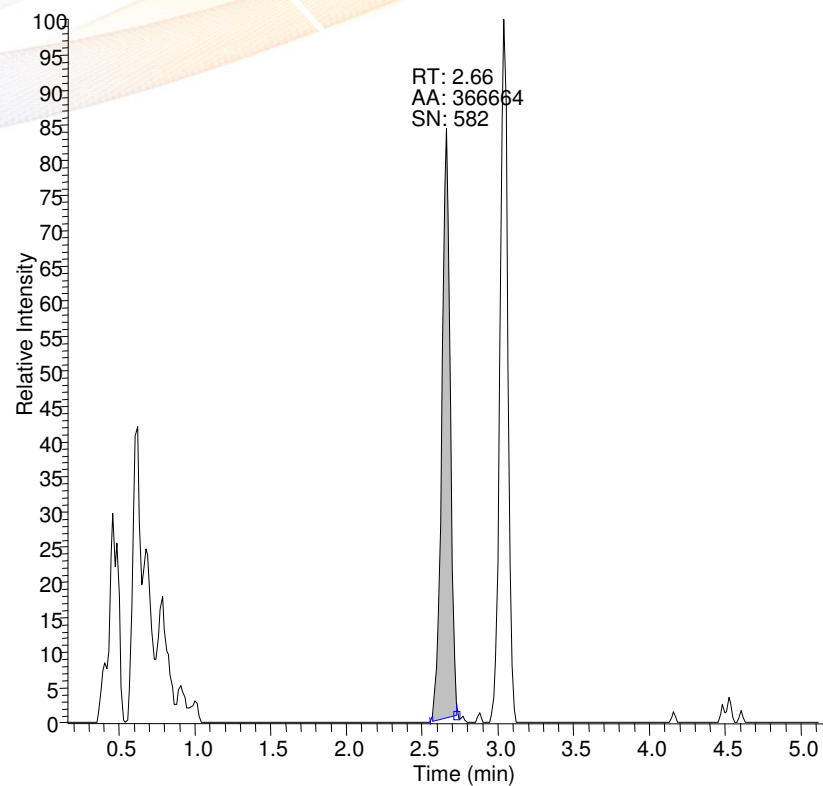


Calibrator ng/mL	% difference
25	2.05
50	1.35
125	-10.21
250	-5.68
500	-5.58
1000	2.00
2000	7.16
5000	2.23
10000	6.69

Morphine Calibrator

S/N = 582 @ 25 ng/mL

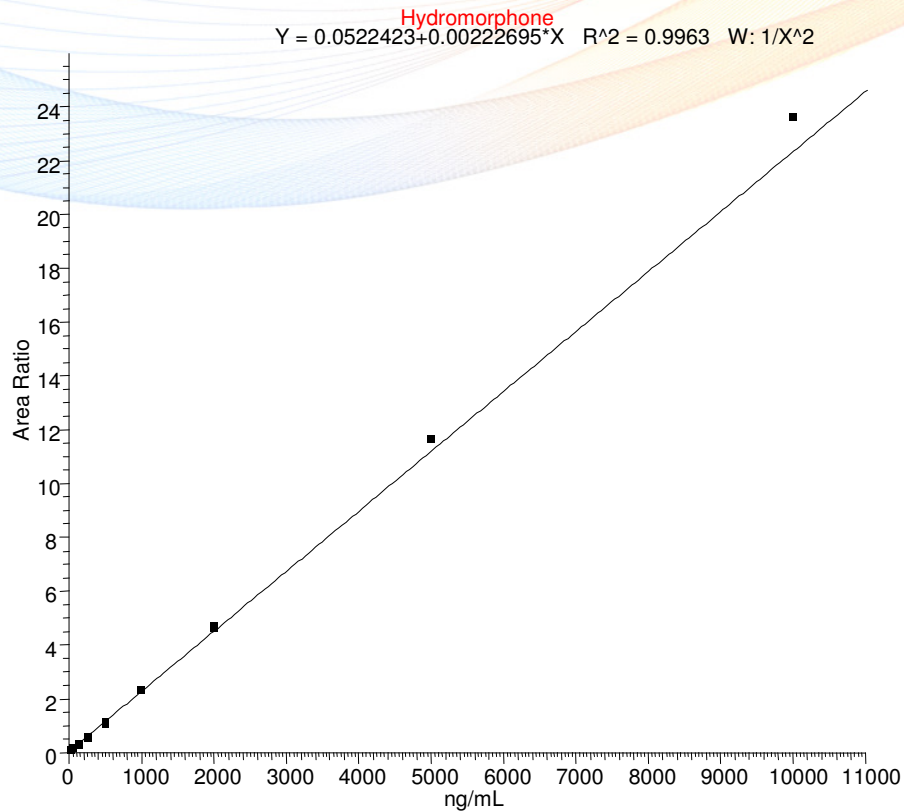
25ng_mL_std - m/z: 286.14286145SM: 5 E5
F: FTMS (0,0) + p Es+ Full ms [232.00-370.00]



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Hydromorphone

Excellent Linearity and Dynamic Range



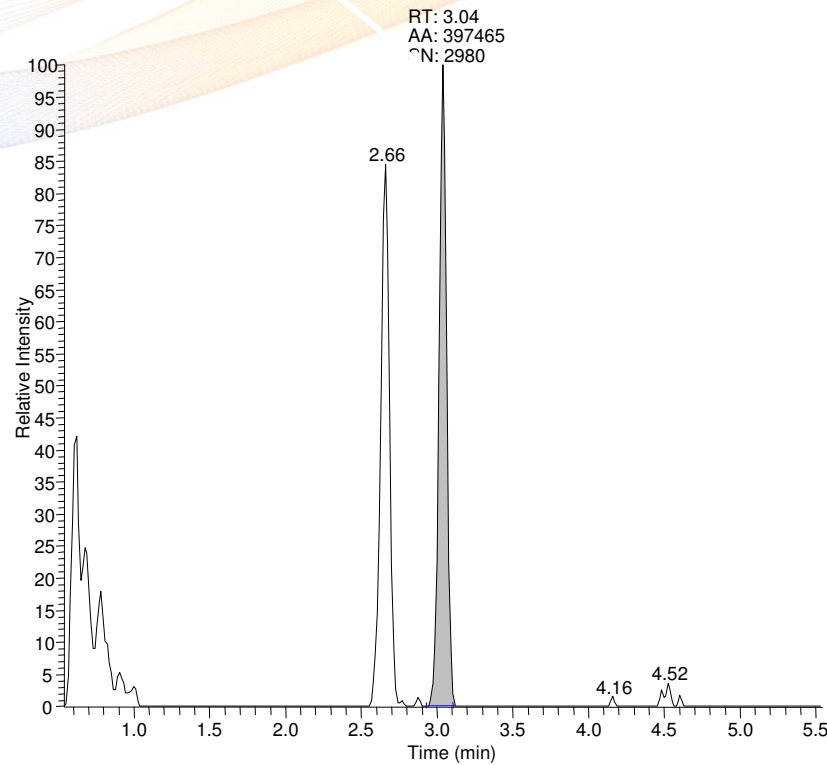
Calibrator ng/mL	% difference
25	-0.03
50	4.42
125	-5.65
250	-9.51
500	-4.63
1000	1.87
2000	3.47
5000	4.26
10000	5.79

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Hydromorphone Calibrator

S/N = 2980 @ 25 ng/mL

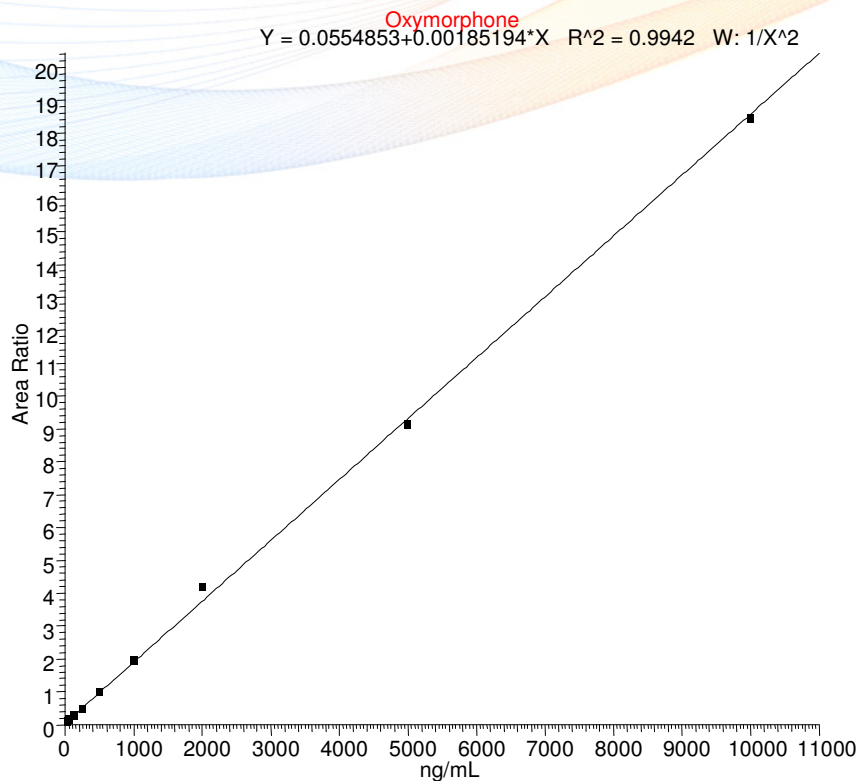
25ng_mL_std - m/z= 286.14-286.14
F: FTMS [0,0] + p ESI Full ms [232.00-370.00]



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Oxymorphone

Excellent Linearity and Dynamic Range



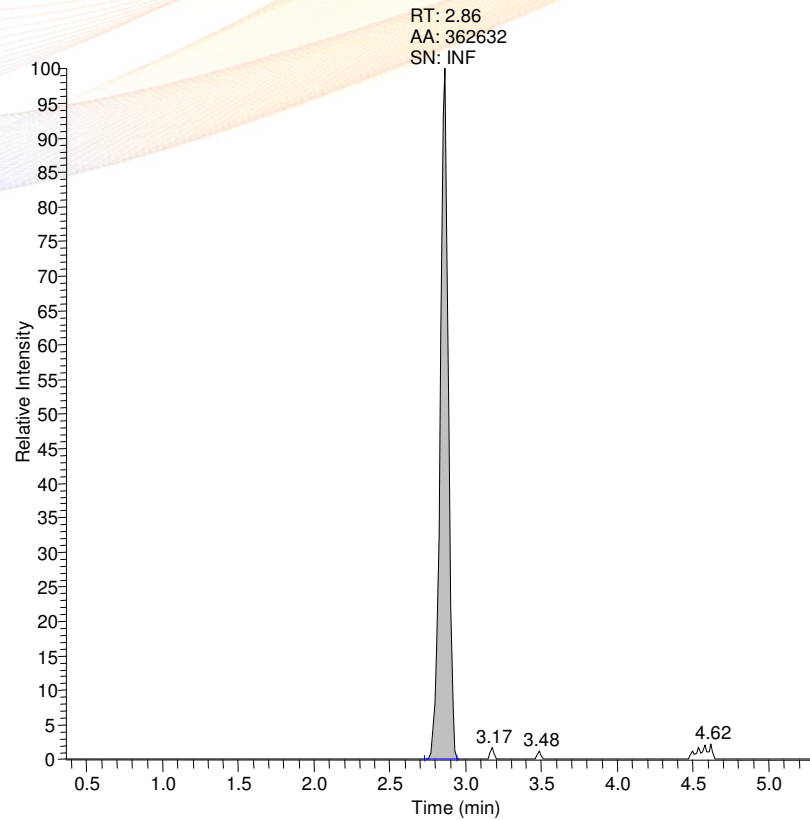
Calibrator ng/mL	% difference
25	-1.37
50	7.92
125	-9.97
250	-7.78
500	-0.33
1000	2.96
2000	11.23
5000	-1.98
10000	-0.67

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Oxymorphone Calibrator

S/N Very High @ 25 ng/mL

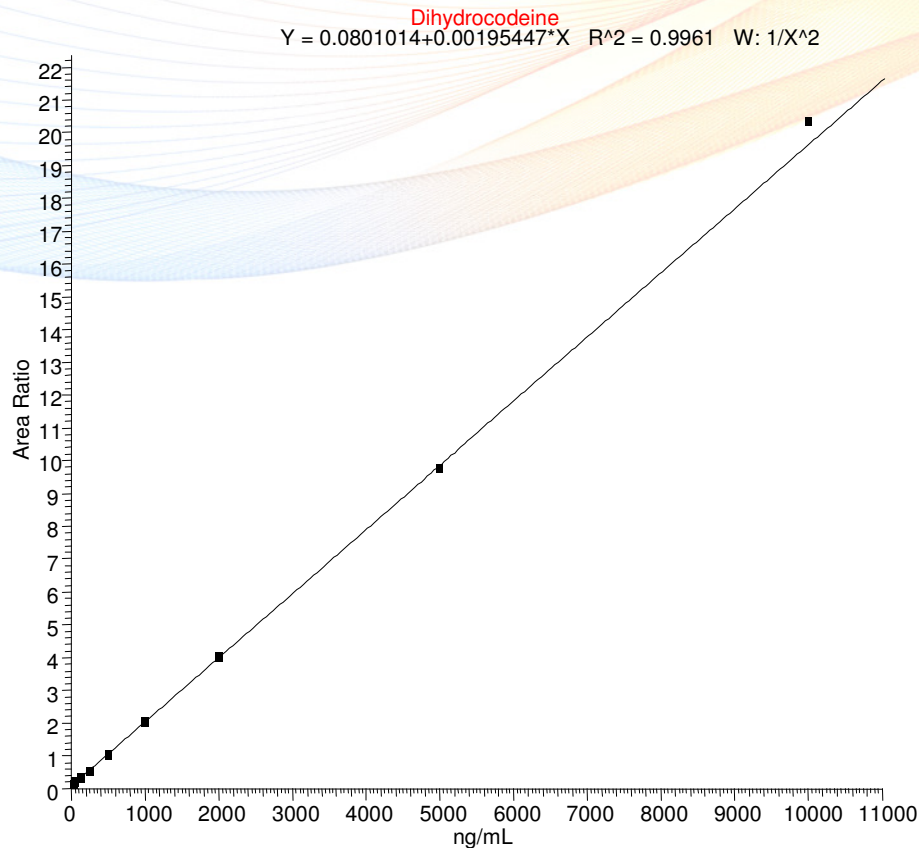
25ng_mL_std - m/z= 302.14-302.1465.549.49E4
F: FTMS {0,0} + p ESI Full ms [232.00-370.00]



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Dihydrocodeine

Excellent Linearity and Dynamic Range



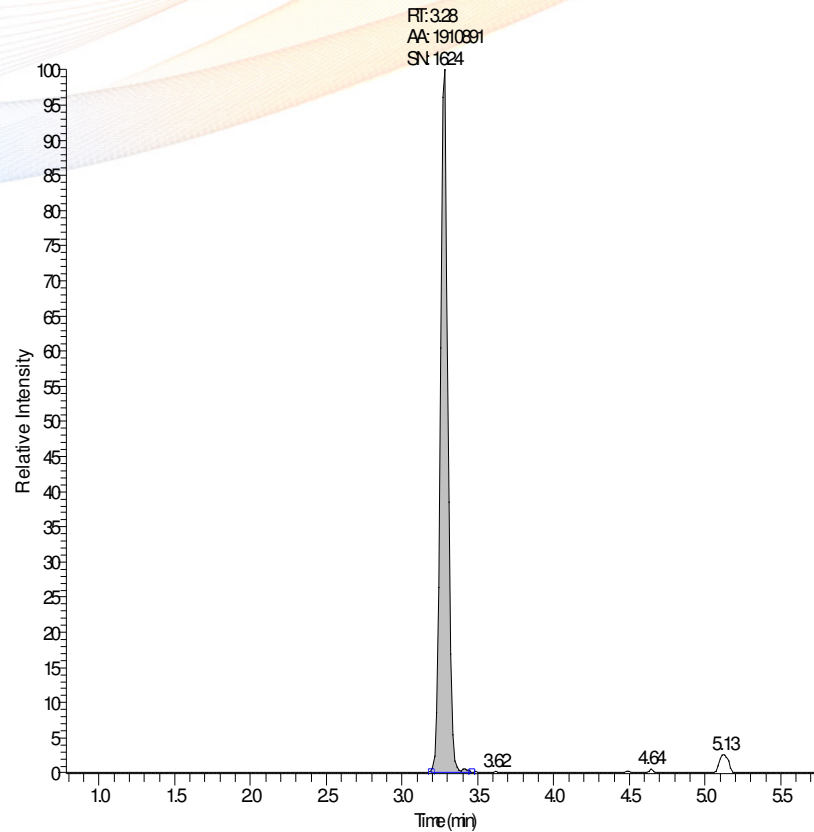
Calibrator ng/mL	% difference
25	-5.07
50	12.30
125	-1.62
250	-6.01
500	-3.61
1000	0.57
2000	0.84
5000	-1.04
10000	3.65

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Dihydrocodeine Calibrator

S/N = 1600 @ 25 ng/mL

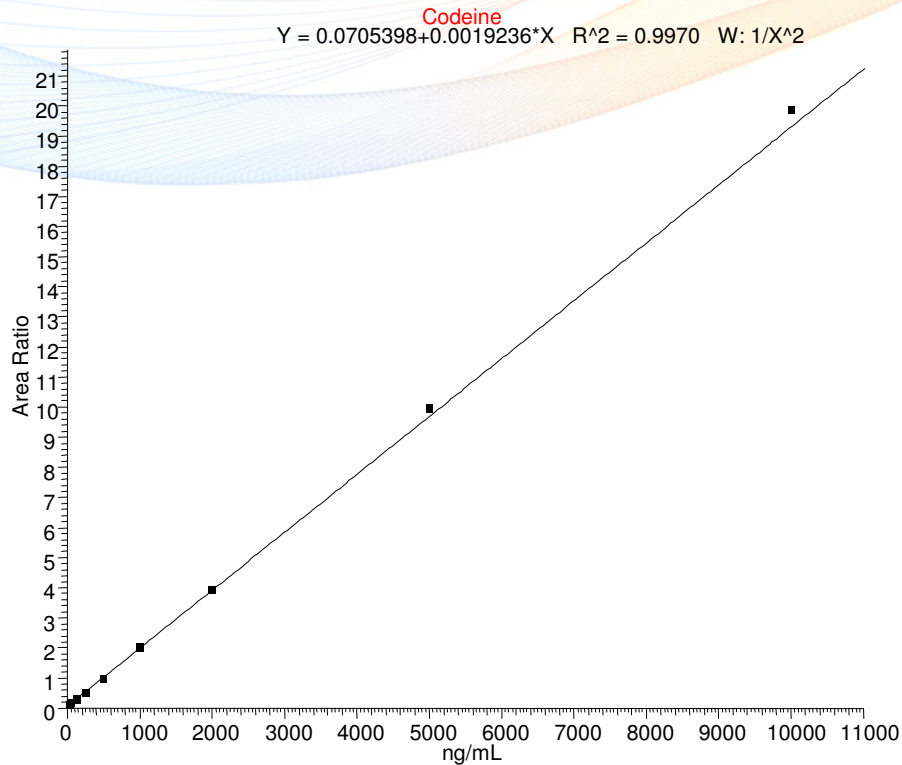
25ng_mL_std-mz=302.18-302.18 SM5 RT: 0.78-5.78 NL: 548E5
F:FTMS(0.0) +pESI Fullms[232.00-370.00]



Forensic Toxicology use Only

Codeine

Excellent Linearity and Dynamic Range



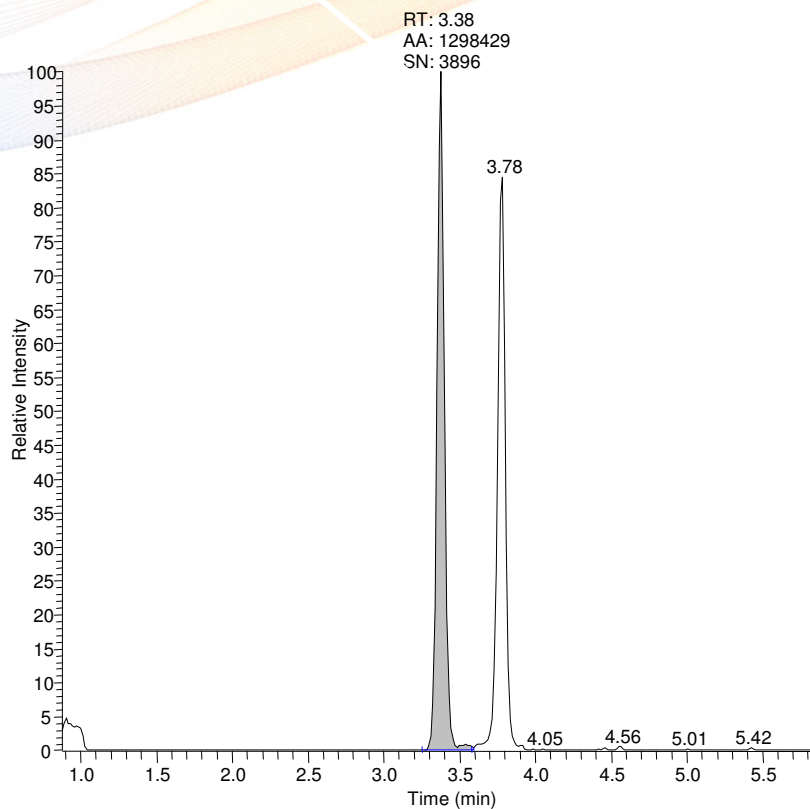
Calibrator ng/mL	% difference
25	-3.18
50	9.62
125	-5.07
250	-4.33
500	-4.88
1000	1.28
2000	0.74
5000	2.81
10000	3.00

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Codeine Calibrator

S/N = 3896 @ 25 ng/mL

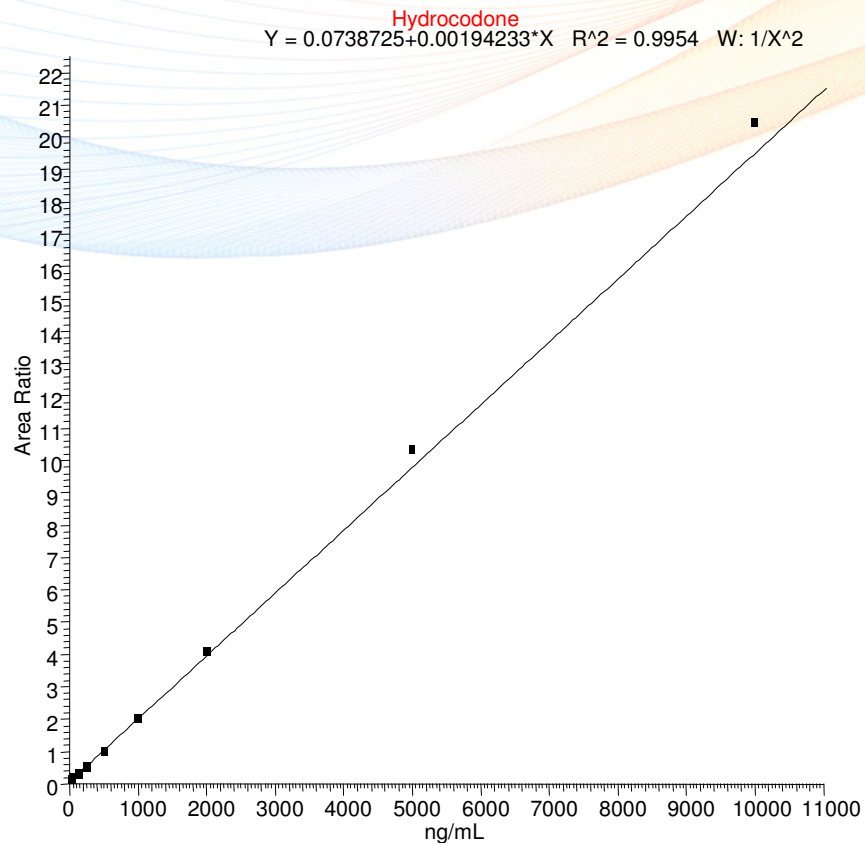
25ng_mL_std - m/z= 300.16-311.06-313.04-315.06
F: FTMS [0,0] + p ESI Full ms [2.00-370.00]



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Hydrocodone

Excellent Linearity and Dynamic Range

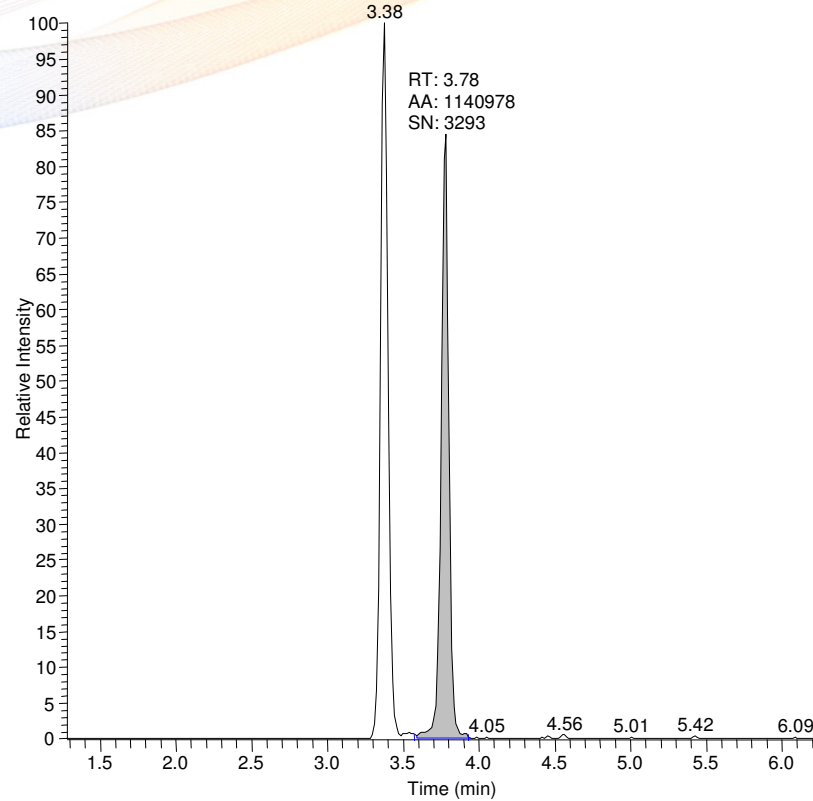


Calibrator ng/mL	% difference
25	-1.59
50	8.18
125	-7.88
250	-7.03
500	-6.19
1000	0.25
2000	3.44
5000	5.87
10000	4.94

Hydrocodone Calibrator

S/N = 3290 @ 25 ng/mL

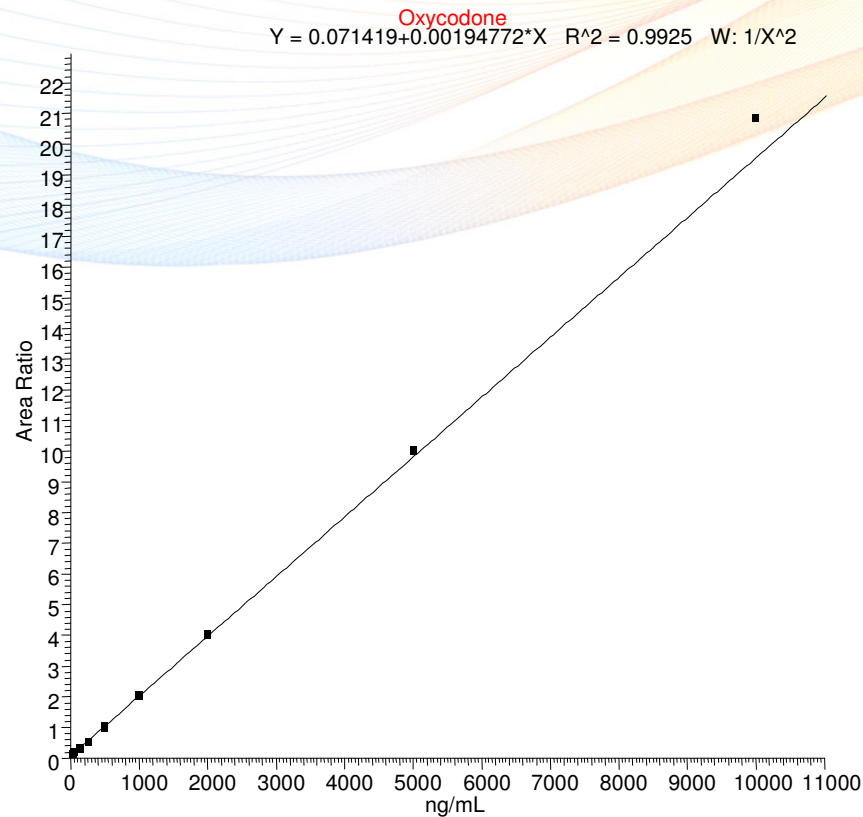
25ng_mL_std - m/z= 300.16-300.16 SM 3.68E5
F: FTMS {0,0} + p ESI Full ms [232.00-370.00]



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Oxycodone

Excellent Linearity and Dynamic Range



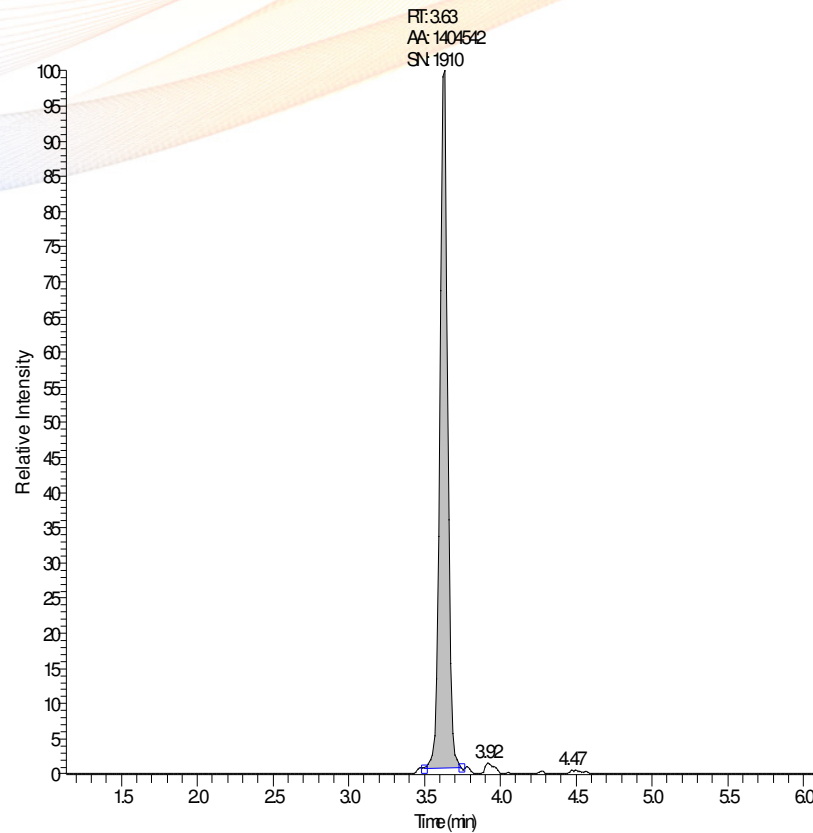
Calibrator ng/mL	% difference
25	-4.72
50	15.04
125	-9.11
250	-8.68
500	-3.29
1000	0.60
2000	1.26
5000	2.20
10000	6.70

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Oxycodone Calibrator

S/N = 1900 @ 25 ng/mL

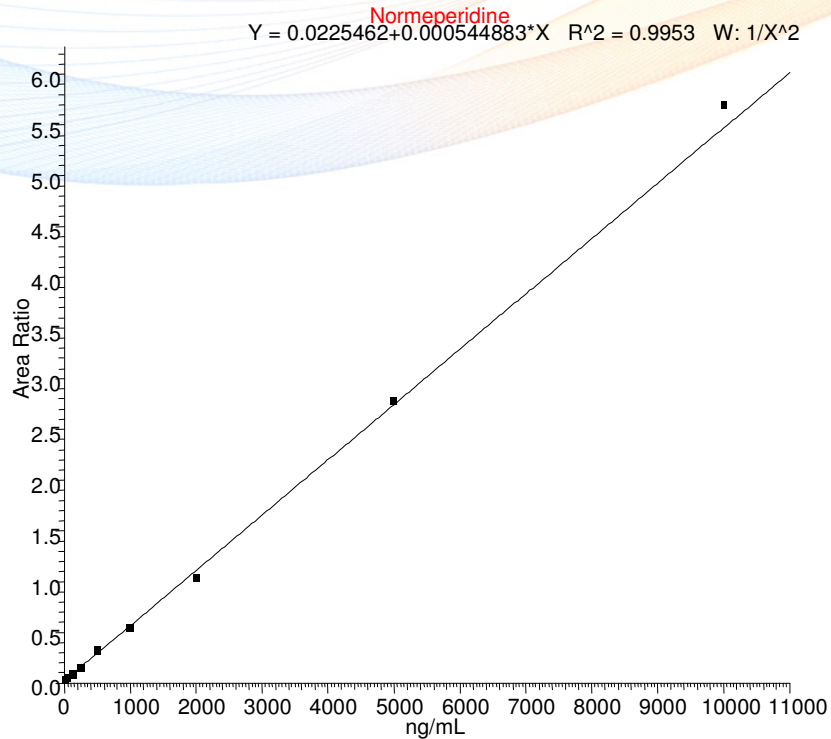
25ng_mL_std-mz=316.1531615 SM5 RT: 1.13-6.13 N: 388E5
F:FTMS(0.0) +pESI Fullms[232.0037000]



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Normeperidine

Excellent Linearity and Dynamic Range

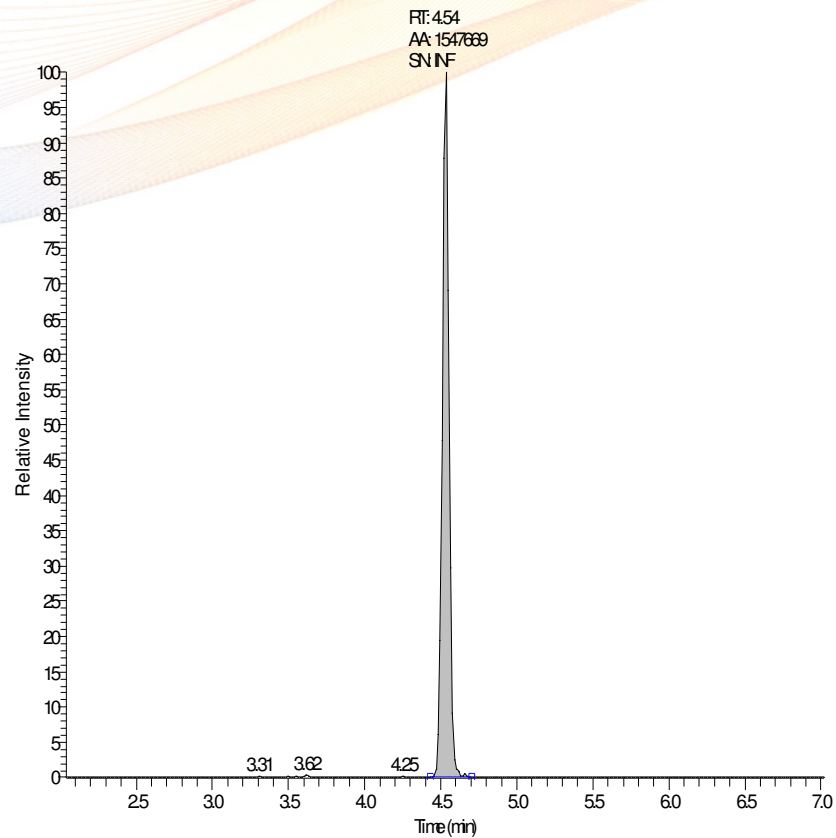


Calibrator ng/mL	% difference
25	-2.05
50	7.05
125	-7.32
250	-3.89
500	10.13
1000	-2.76
2000	-6.57
5000	1.19
10000	4.21

Normeperidine Calibrator

S/N Very High @ 25 ng/mL

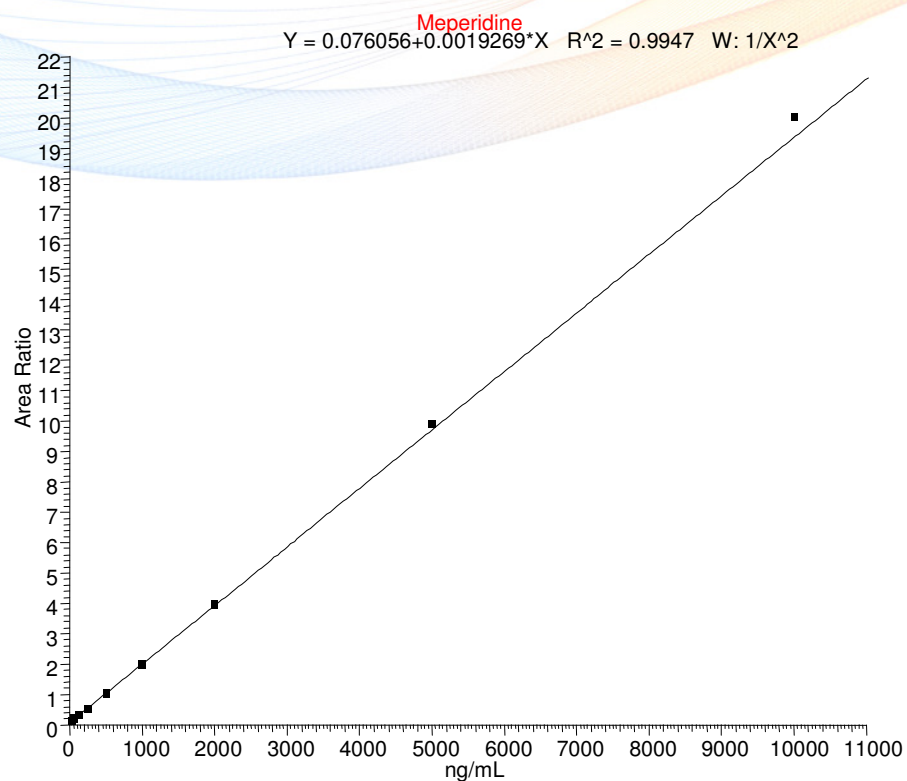
25ng_mL_std-mz=234.15234.15 SM5 RT: 204-702 N: 507E5
F:FTMS(0.0) +pESI Full ms[232.0037000]



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Meperidine

Excellent Linearity and Dynamic Range

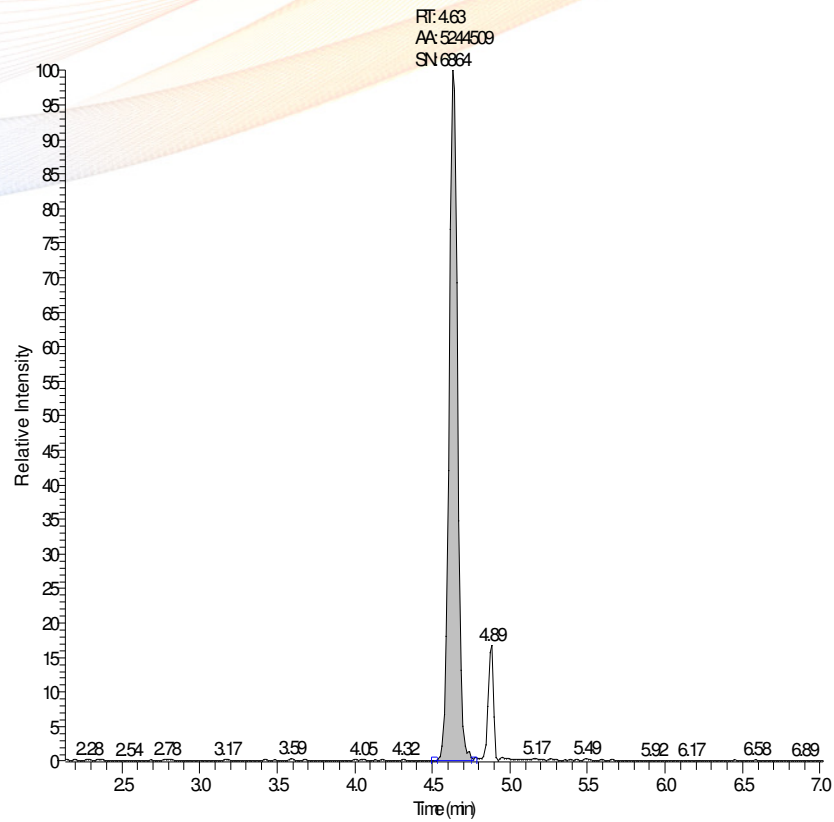


Calibrator ng/mL	% difference
25	-5.32
50	14.42
125	-5.22
250	-5.68
500	-3.79
1000	-0.80
2000	0.77
5000	2.19
10000	3.53

Meperidine Calibrator

S/N = 6800 @ 25 ng/mL

25ng_mL_std-mz=248.16248.16 SM5 RT: 2.13-7.02 N: 1.38E6
F:FTMS(0.0) +pESI Fullms[232.00-370.00]



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Accuracy and Precision on QCs

Low: 300 ng/mL; High: 2500 ng/mL

No	Analyte	%Accuracy		Precision n=5	
		Low QC	High QC	Low QC	High QC
1	Morphine	87.9	114	0.45	2.7
2	Hydromorphone	82.5	-	3.5	-
3	Oxymorphone	89.5	-	0.53	-
4	Codeine	93.0	110	3.7	1.5
5	Dihydrocodeine	-	-	-	-
6	Oxycodone	87.5	-	2.1	-
7	Hydrocodone	84.8	-	1.1	-
8	Meperidine	-	-	-	-
9	Normeperidine	-	-	-	-

Note: QC's didn't have all analytes

LC Method

- Column GOLD PFP, 150 x 2.1 mm, 5 μ m
- Column at room temperature
- Mobile phases
 - A: 20 mM Ammonium Acetate and 0.1% FA in DI water
 - B: 0.1% FA in ACN
- LC gradient

Time	%A	%B	Flow (uL/min)
0	98	2	600
0.5	98	2	600
3.5	60	40	600
3.51	0	100	600
5.0	0	100	600
5.01	98	2	600
8.0	98	2	600

MS Method

- Source parameters
 - HESI in positive ionization
 - Spray voltage: 4000
 - Heater temp: 350
 - Capillary temp: 320
 - Sheet gas: 10
 - Aux gas: 5
- Exactive MS parameters
 - Positive ionization mode
 - Full scan data
 - Mass range: 232-270
 - Resolution: 100000
 - Max Injection time: 10 ms

Data Processing Method

- Extracted ion chromatogram with 5 ppm mass accuracy
- Analyte to Internal standard peaks area ratios were used in quantitative calculations