

Innovation Applied

Quantitative Analysis of Synthetic Opioids
in Urine using Exactive LC-MS System

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Forensic Toxicology use Only

Overview

- Customer workflow
- Analytes and Internal Standards used
- Sample Preparation
- Analytes and Internal Standards M/Z and retention times
- Linearity and Dynamic Range data
- Results on QC's
- Conclusions
- Appendix

Customer Workflow

- Quick Semi Quantitative Screening using Exactive
- Positive Samples Run On Immunoassay
- Near future plans to use only Exactive LC-MS
- No Immunoassay

The Workflow Primarily Driven by Cost Effectiveness

Analytes and Internal Standards

No	Analyte	Internal Standard
1	Ketamine	Ketamine-D4
2	Norketamine	Norketamine-D4
3	Nalbuphine	Fentanyl-D5
4	Fentanyl	Fentanyl-D5
5	Norfentanyl	Norfentanyl-D5
6	Alfentanil	Fentanyl-D5
7	Sufentanil	Sufentanil-D5
8	Butorphanol	Fentanyl-D5

Sample Preparation

- Dilute n Shoot
 - 10 X dilution with DI water
- Standards and QC samples
 - Analytes and internal standards Cerilliant.
 - Calibrators - standard spiked in blank urine
 - Commercial QC samples

Analytes and Internal standards

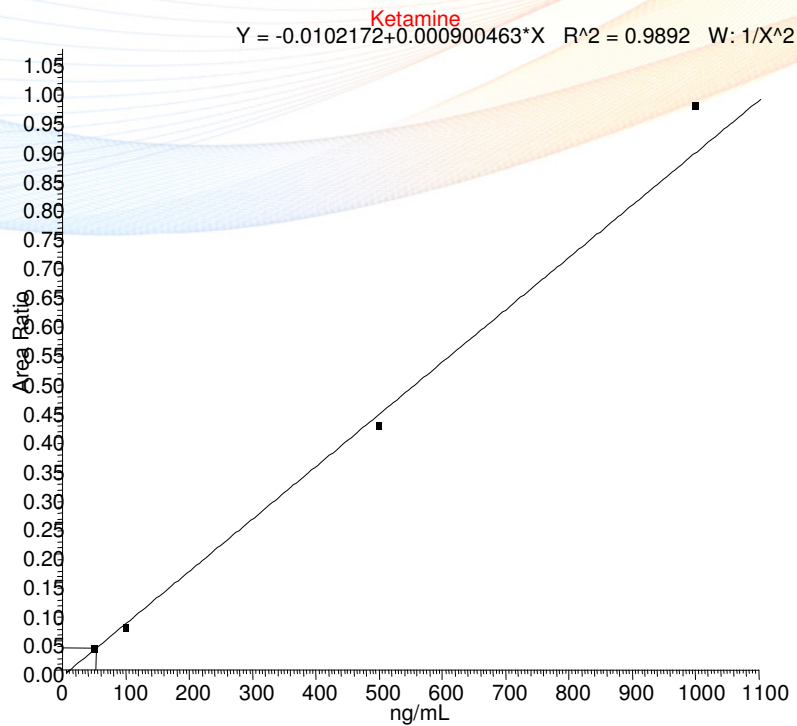
Accurate Masses and Retention Times

No	Analyte	Exact mass	Ret time
1	Ketamine	238.0993	3.29
2	Norketamine	224.0837	3.15
3	Nalbuphine	358.2013	3.18
4	Fentanyl	337.2274	4.51
5	Norfentanyl	233.1648	3.31
6	Alfentanil	417.2609	4.35
7	Sufentanil	387.2100	4.75
8	Butorphanol	328.2271	4.17
9	Ketamine	238.0993	3.29

No	Analyte	Exact mass	Ret time
1	Ketamine-D4	242.1244	3.28
2	Norketamine-D4	228.1088	3.13
3	Fentanyl-D5	342.2588	4.51
4	Norfentanyl-D5	238.1962	3.29
5	Sufentanil-D5	392.2414	4.74

Ketamine

Good Linearity in the Desired Range



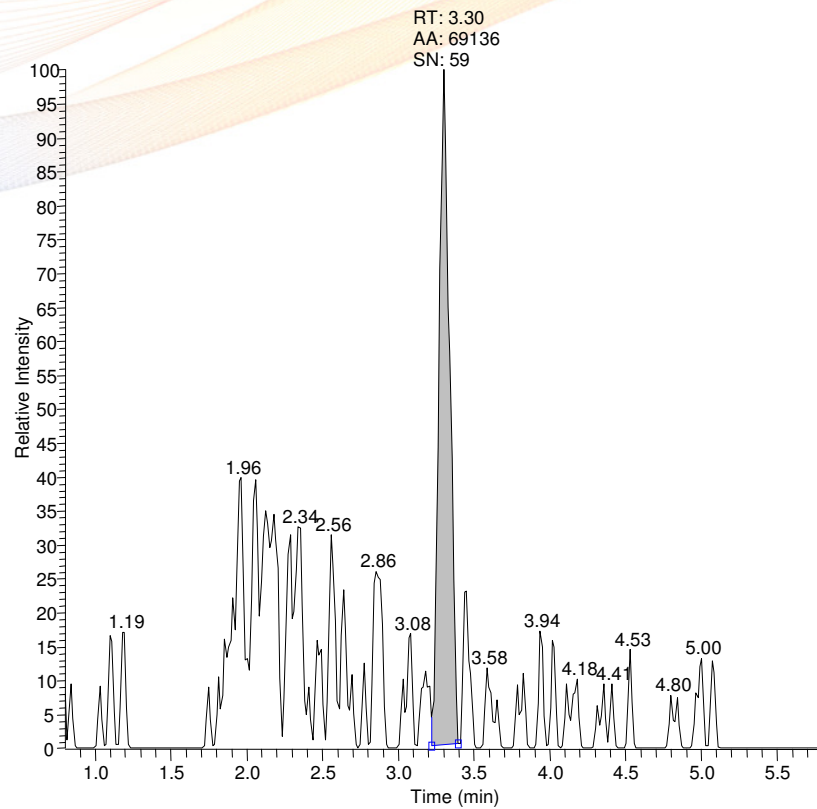
Calibrator ng/mL	% Difference
50	4.51
100	-9.03
500	-4.42
1000	8.94

Note: 4 point cal curve. Objective was semi quantitative analysis

Ketamine Calibrator

S/N = 60 @ 50 ng/mL

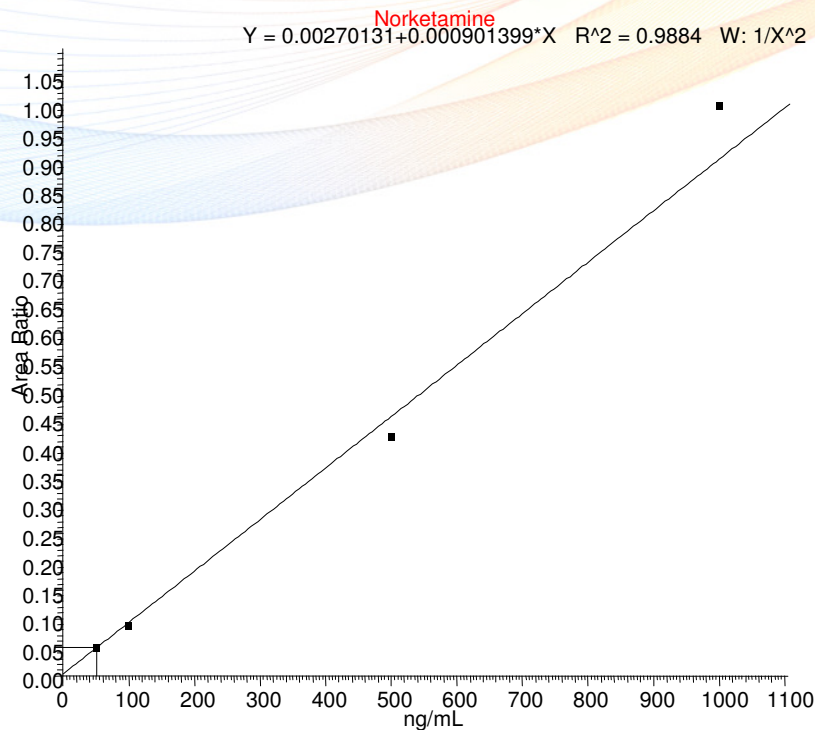
std50 - m/z= 238.10-238.12 RT: 3.30
F: FTMS (0,0) + p ESI Full ms [223.00-418.00]



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Norketamine

Good Linearity in the Desired Range



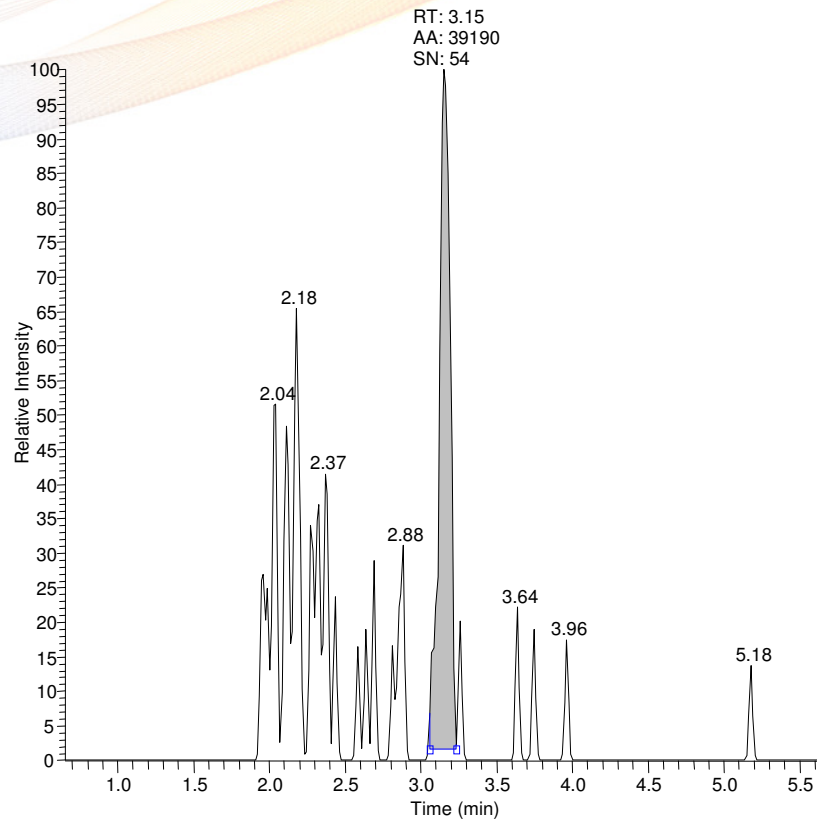
Calibrator ng/mL	% Difference
50	3.38
10	-6.29
500	-7.50
1000	10.41

Note: 4 point cal curve. Objective was semi quantitative analysis

Norketamine Calibrator

S/N = 54 @ 50 ng/mL

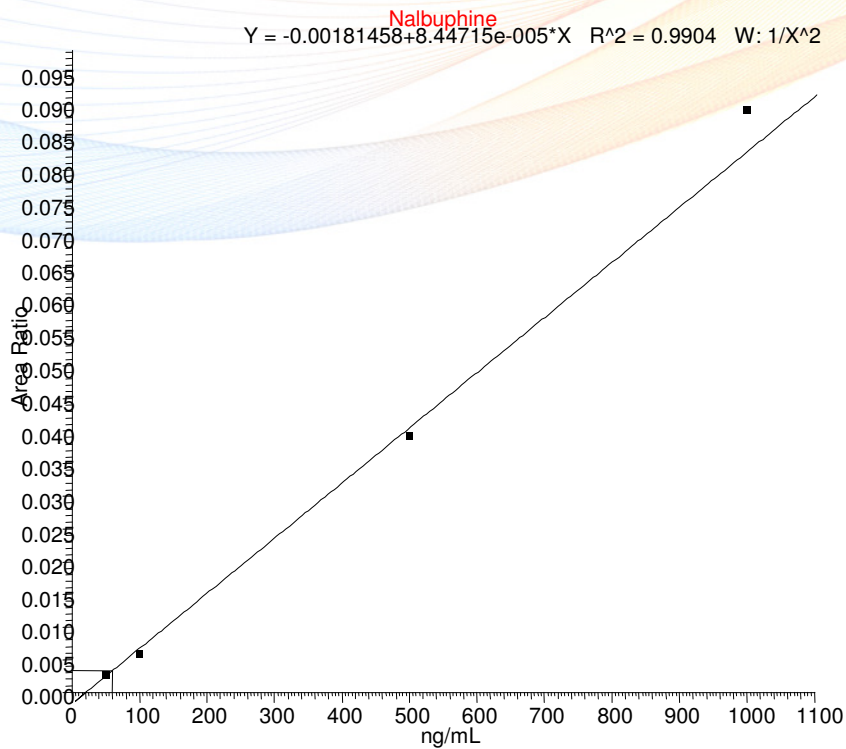
std50 - m/z= 224.08-224.08
F: FTMS {0,0} + p ESI Full ms [223.00-418.00]



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Nalbuphine

Good Linearity in the Desired Range



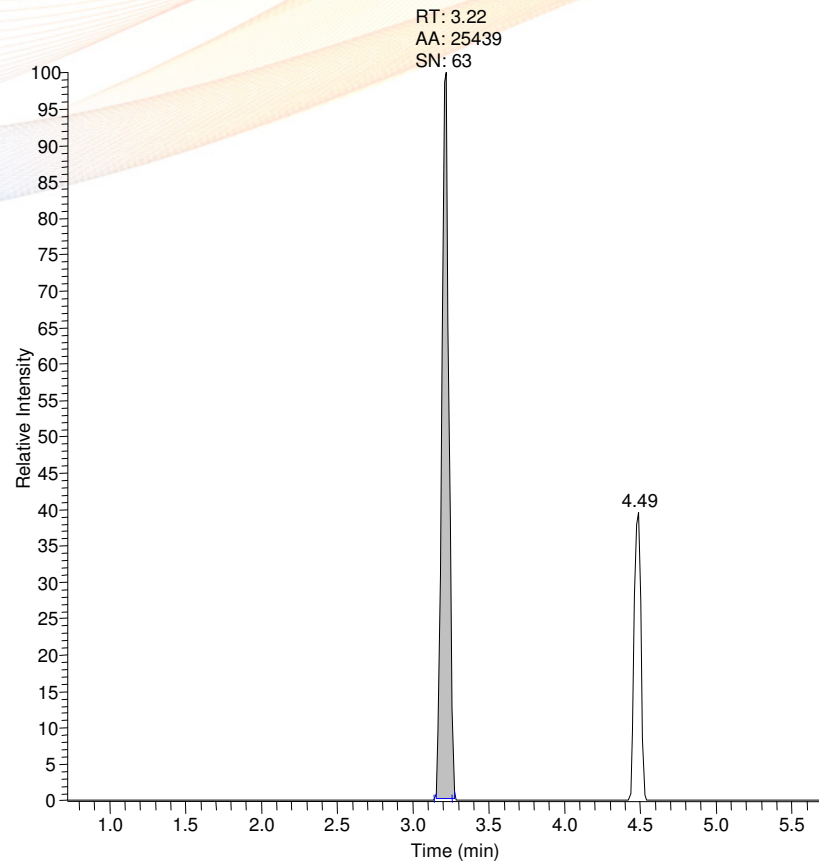
Calibrator ng/mL	% Difference
50	4.64
100	-9.47
500	-2.86
1000	7.69

Note: 4 point cal curve. Objective was semi quantitative analysis

Nalbuphine

S/N = 63 @ 50 ng/mL

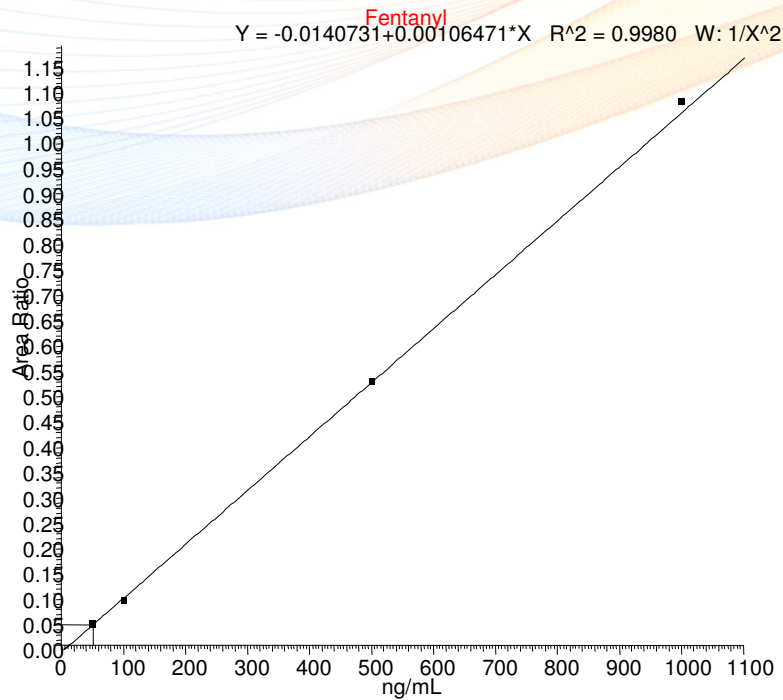
std50 - m/z= 358.20-358.20
F: FTMS {0,0} + p ESI Full ms [223.00-418.00]



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Fentanyl

Good Linearity in the Desired Range



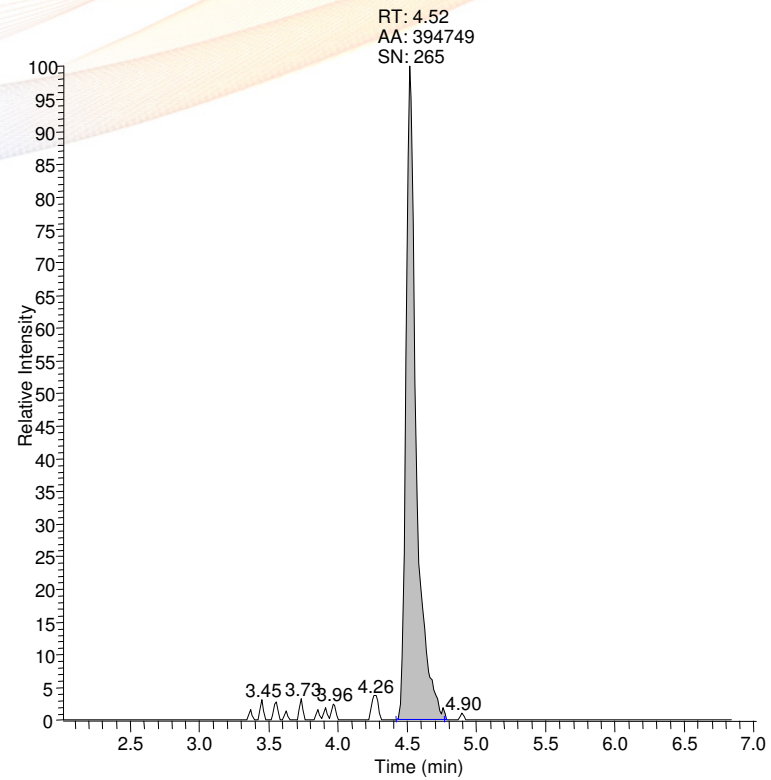
Calibrator ng/mL	% Difference
50	2.36
100	-5.04
500	0.46
1000	2.22

Note: 4 point cal curve. Objective was semi quantitative analysis

Fentanyl Calibrator

S/N = 295 @ 50 ng/mL

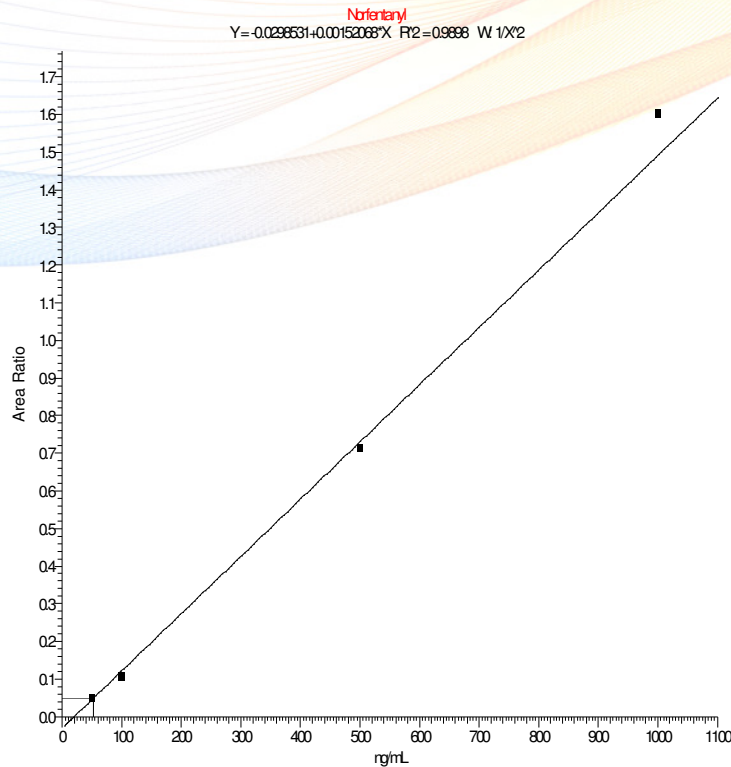
std50 - m/z= 337.23-337.23 SM: 1027.41E4
F: FTMS (0,0) + p ESI Full ms [223.00-418.00]



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Norfentanyl Calibrator

Good Linearity in the Desired Range

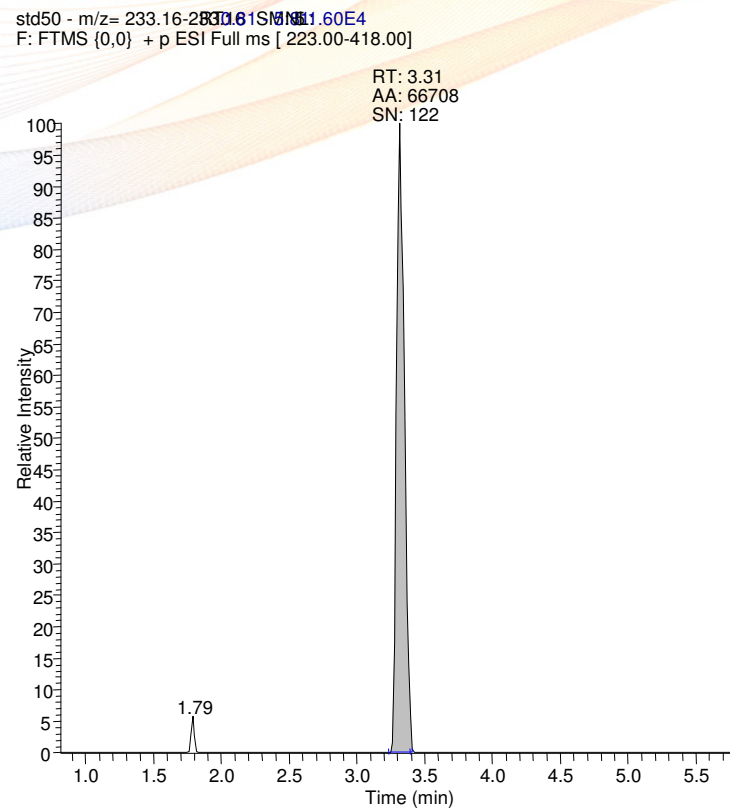


Calibrator ng/mL	% Difference
50	4.96
100	-10.23
500	-2.22
1000	7.49

Note: 4 point cal curve. Objective was semi quantitative analysis

Norfentanyl Calibrator

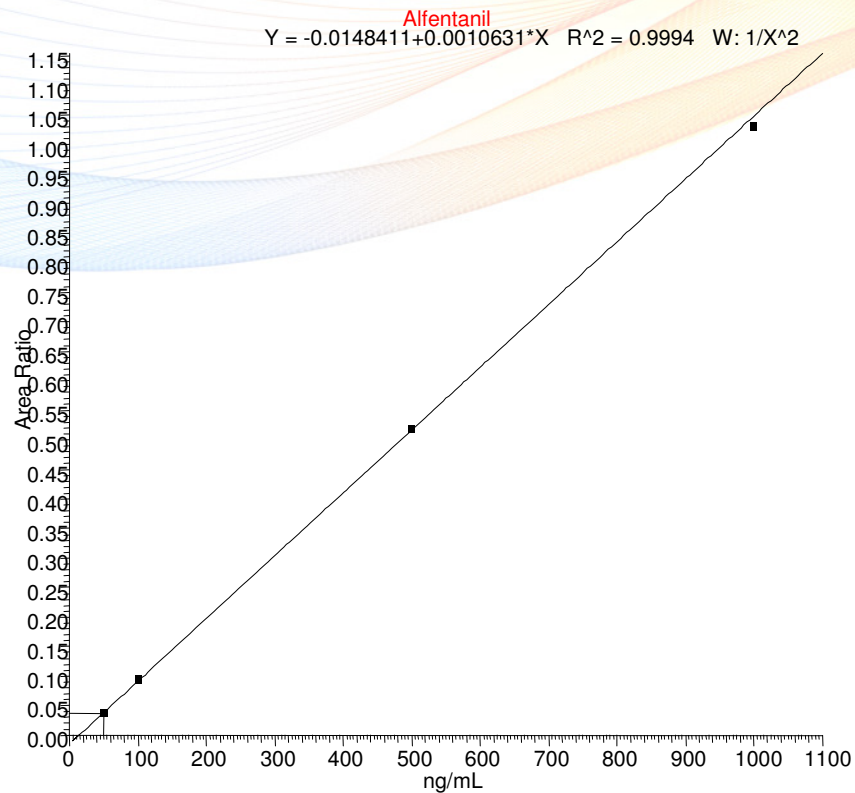
S/N = 122 @ 50 ng/mL



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Alfentanil

Good Linearity in the Desired Range



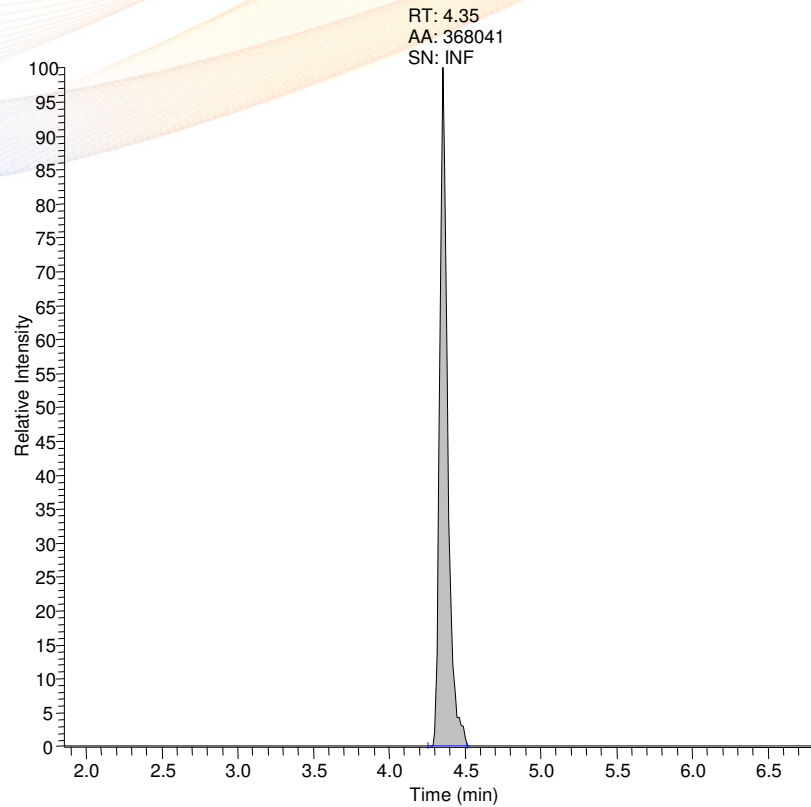
Calibrator ng/mL	% Difference
50	-1.18
100	2.46
500	0.35
1000	-1.62

Note: 4 point cal curve. Objective was semi quantitative analysis

Alfentanil Calibrator

S/N Very High @ 50 ng/mL

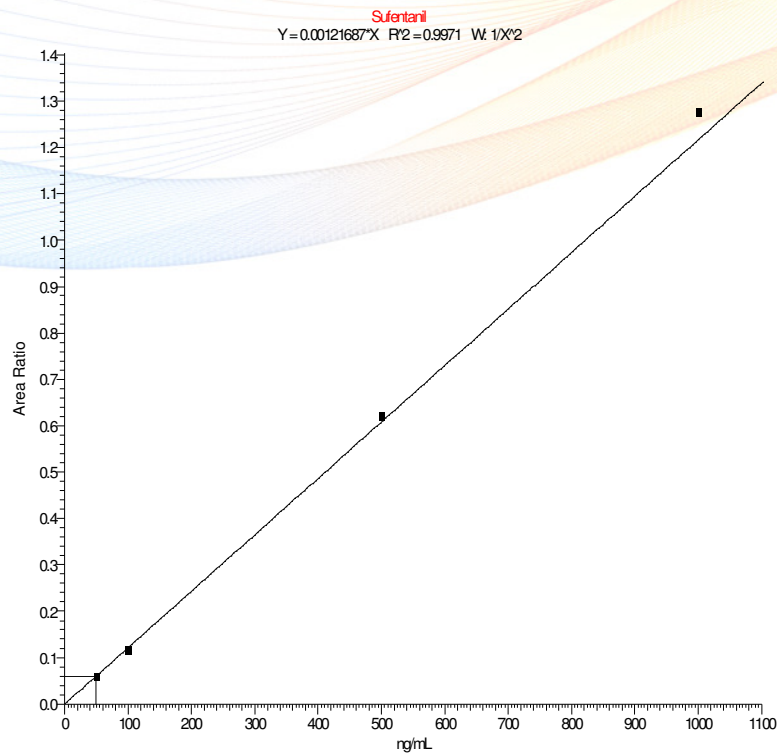
std50 - m/z= 417.26-417.26
F: FTMS {0,0} + p ESI Full ms [223.00-418.00]



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Sulfentanil

Good Linearity in the Desired Range



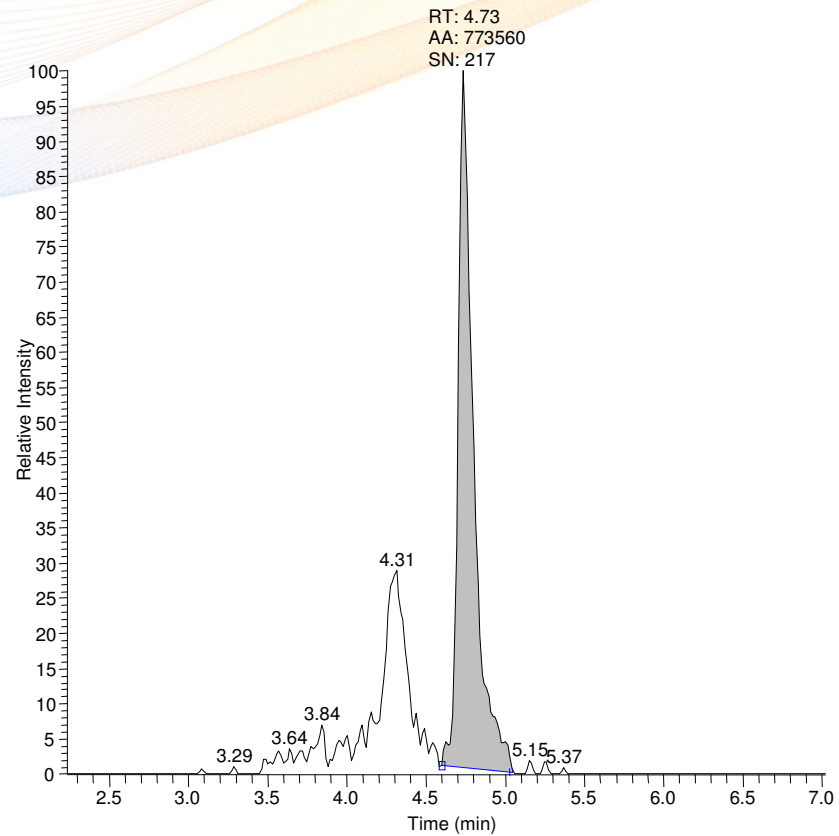
Calibrator ng/mL	% Difference
50	-2.10
100	-4.86
500	2.13
1000	4.83

Note: 4 point cal curve. Objective was semi quantitative analysis

Sulfentanil Calibrator

S/N = 217 @ 50 ng/mL

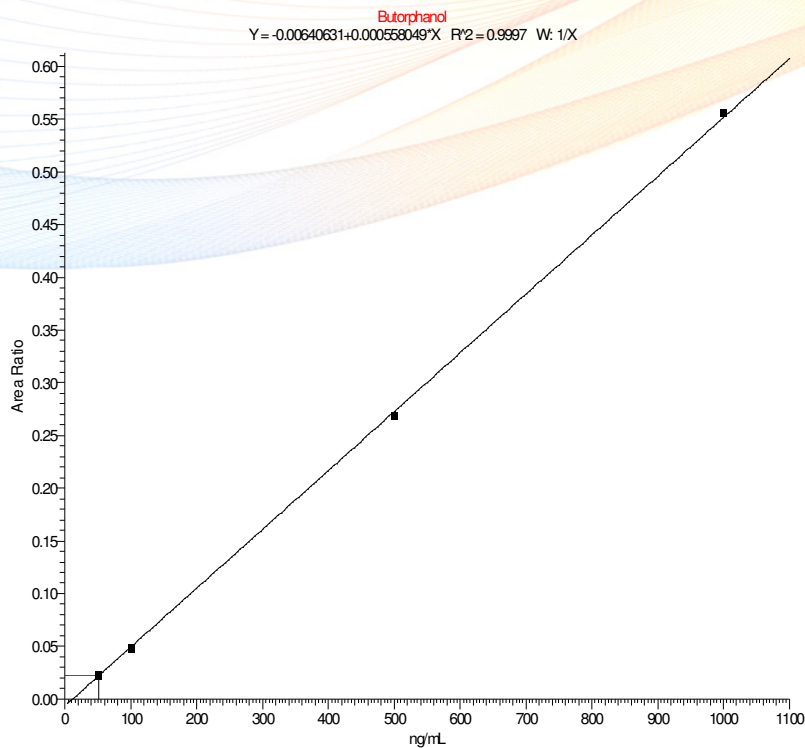
std50 - m/z= 387.21-387.21 RT: 4.73
F: FTMS {0,0} + p ESI Full ms [223.00-418.00]



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Butorphanol

Good Linearity in the Desired Range



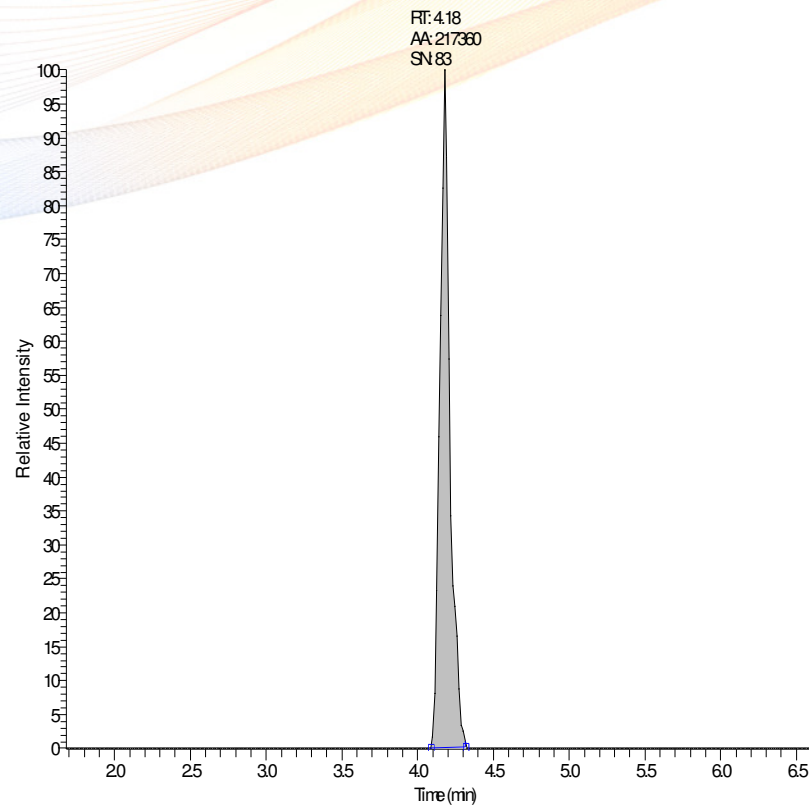
Calibrator ng/mL	% Difference
50	2.72
100	-1.95
500	-1.66
1000	0.89

Note: 4 point cal curve. Objective was semi quantitative analysis

Butorphanol Calibrator

S/N = 83 @ 50 ng/mL

str50-mz=328.23-328.23 SM5 RT: 1.68-6.68 NL: 4.62E4
F: FTMS(0.0) +pESI Full ms[223.00-418.00]



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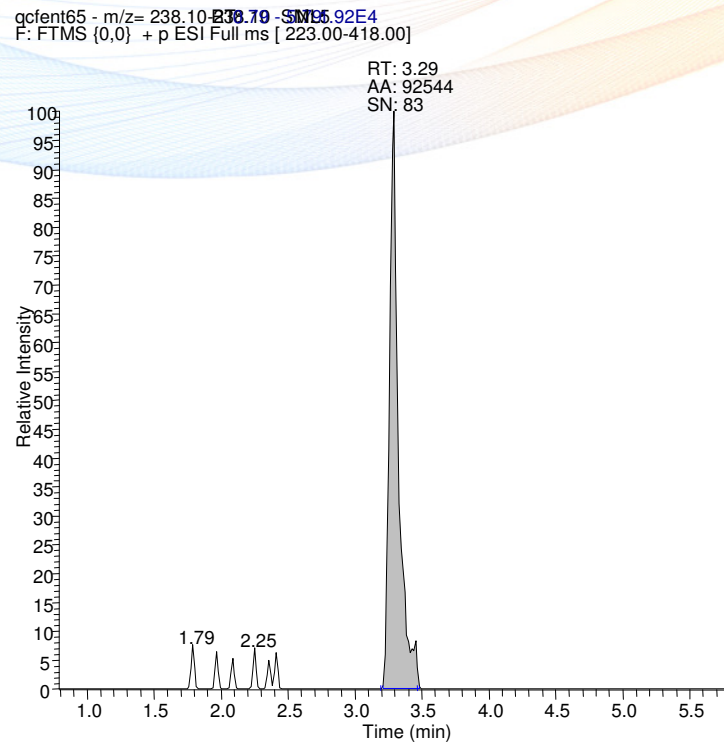
Quality Control Samples

- QC's @ lowest calibrator concn. (50 ng/mL)
- Acceptance Criteria – Values \leq 20% of the target value

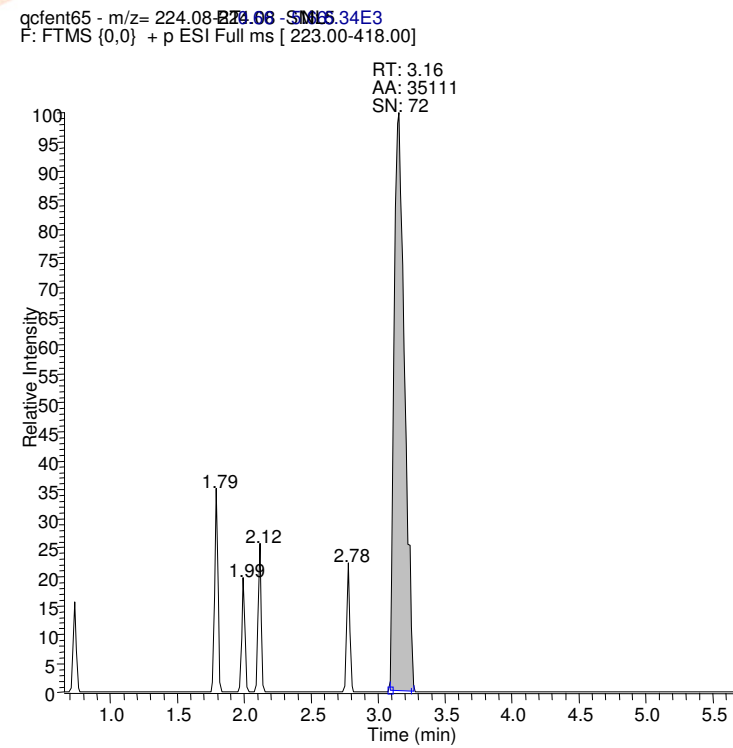
Target Value from the supplier 50ng/mL

QC Results

Ketamine
57.8 ng/mL



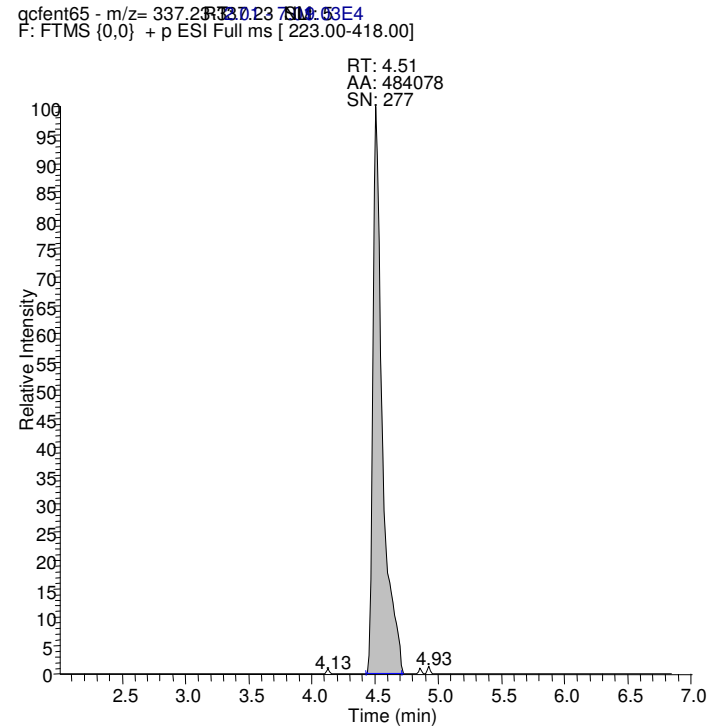
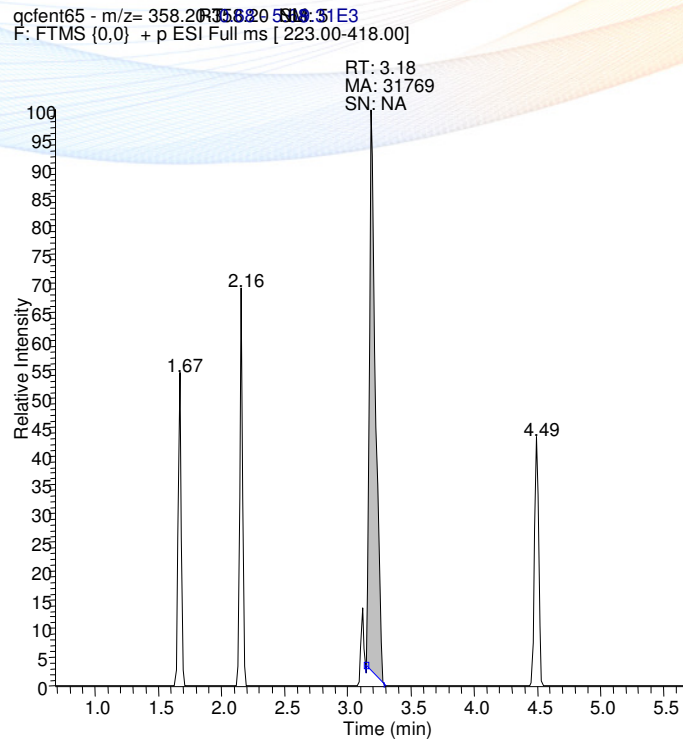
Norketamine
46.2 ng/mL



QC Results

Nalbuphine
59.0 ng/mL

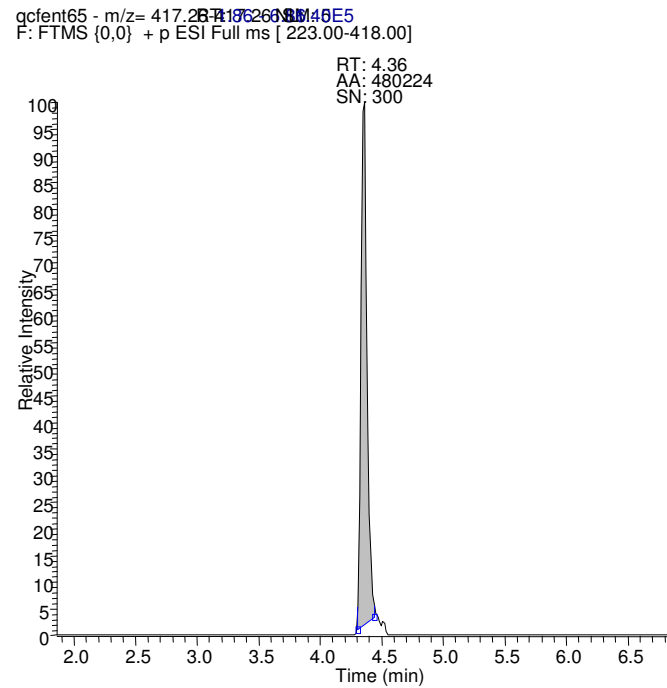
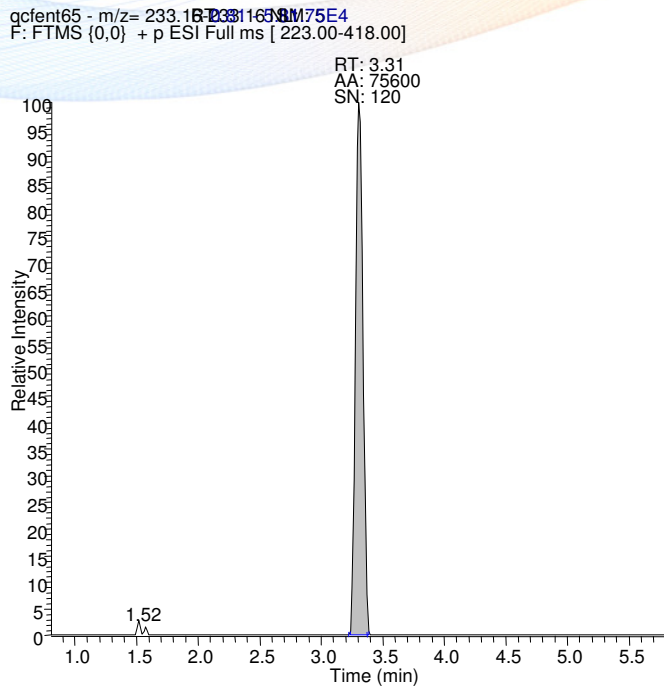
Fentanyl
58.5 ng/mL



QC Results

Norfentanyl
54.3 ng/mL

Alfentanil
59.0 ng/mL

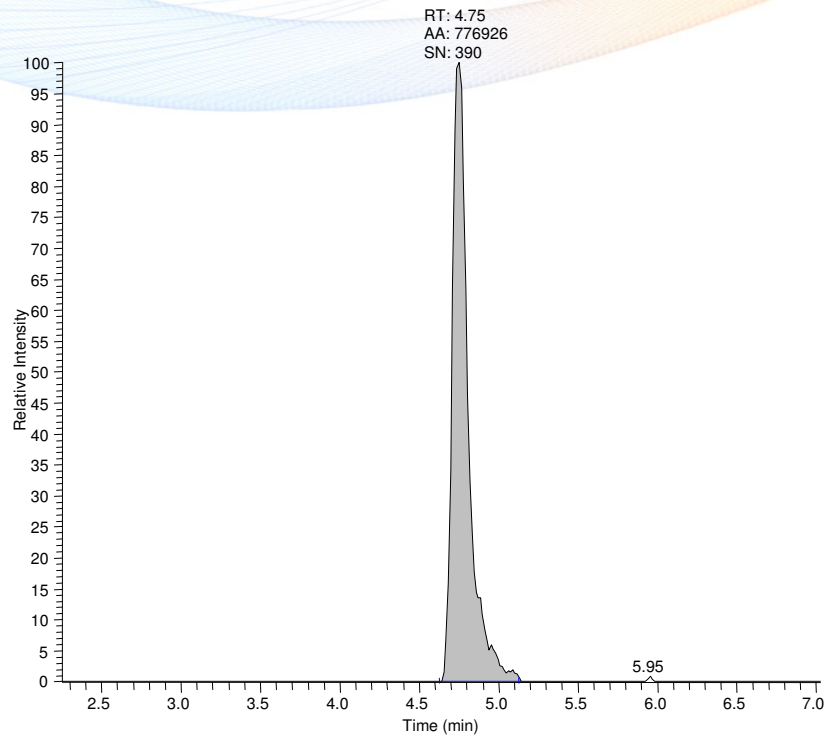


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QC Results

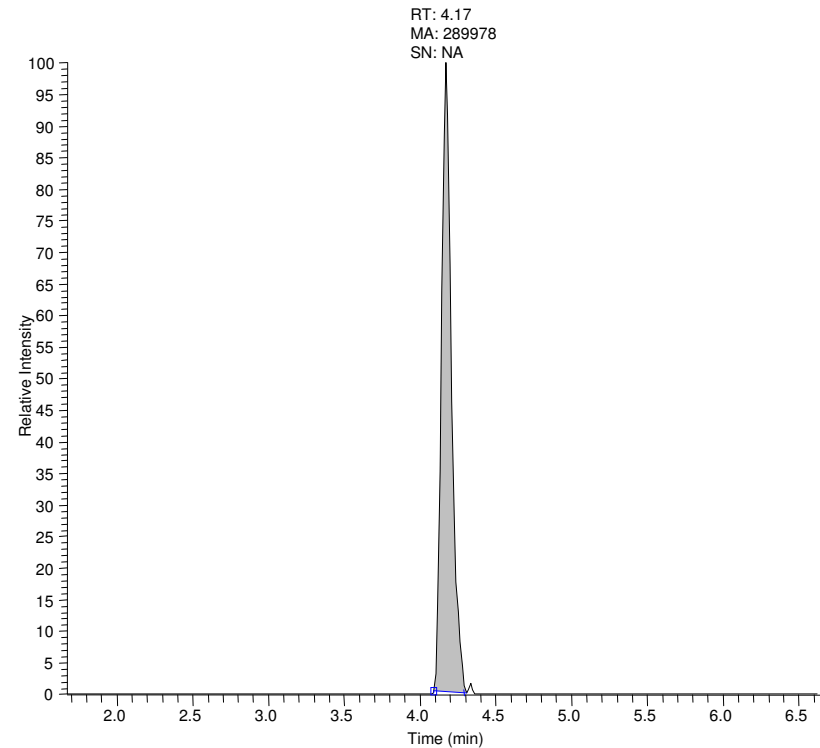
Sulfentanil
49.4 ng/mL

qcfcnt65 - m/z= 387.21-387.21 F: FTMS (0,0) + p ESI Full ms [223.00-418.00]



Butorphanol
60.0 ng/mL

qcfcnt65 - m/z= 328.23-328.23 F: FTMS (0,0) + p ESI Full ms [223.00-418.00]



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Conclusion

- Method met the requirements for a semi quantitative assay
- It is very cost efficient
- Full calibration and further validation will replace immunoassay
- The method is expected to be the only method used in the lab

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- Appendix

LC method

- **Analytical Column:** GOLD PFP, 10 x 2.1 mm, 5 μ m
- **Column temp:** 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

- HESI in positive ionization
- Spray voltage: 4000
- Heater temp: 350
- Capillary temp: 320
- Sheet gas: 10
- Aux gas: 5

Data acquisition method

- Positive ionization mode
- Full scan data
- Mass range: 223-418
- Resolution: 100000
- Max Injection time: 100 ms

Data processing method

- Chromatographic peaks were obtained by reconstructing analytes and internal standard masses with accuracy of 5 ppm.
- Analyte to internal standard peaks area ratios were used in quantitative calculations