Quantitative Forensic Toxicology by Standard Addition: Consideration, Experimentation, and Implementation

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Disclosure

- I have no conflicts of interest to disclose.

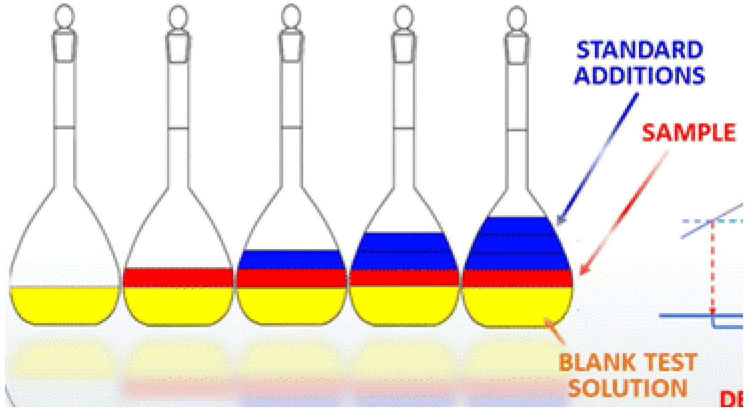
- I am a scientist and employee of FRFF / CFSRE, a 501(c)(3) non-profit research and educational facility.

Standard Addition

- **Definition:** A type of quantitative analysis approach whereby the standard is added directly to the aliquots of analyzed sample
  - Internal calibration model (as opposed to an external calibration model)

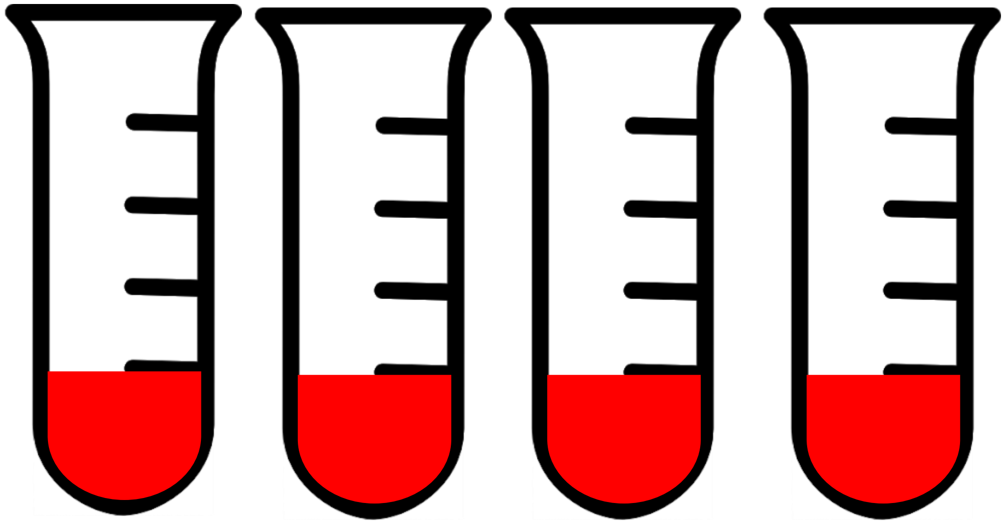
- Various scientific areas use standard addition

- Mechanism to provide accurate and reliable quantitative results in the absence of a traditionally validated assay
  - Rarely encountered substances
  - Novel Psychoactive Substances (NPS)



Standard Addition

- Briefly… how standard addition works:



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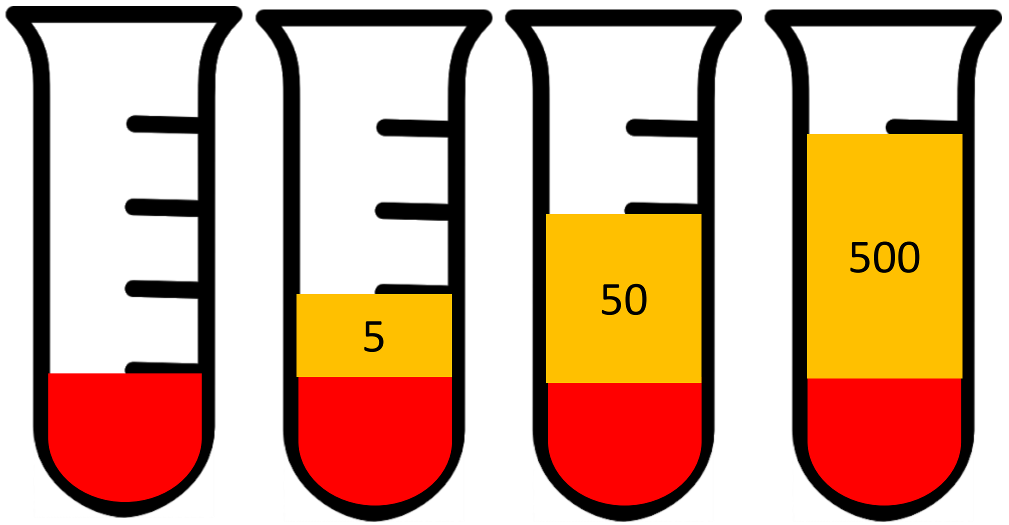

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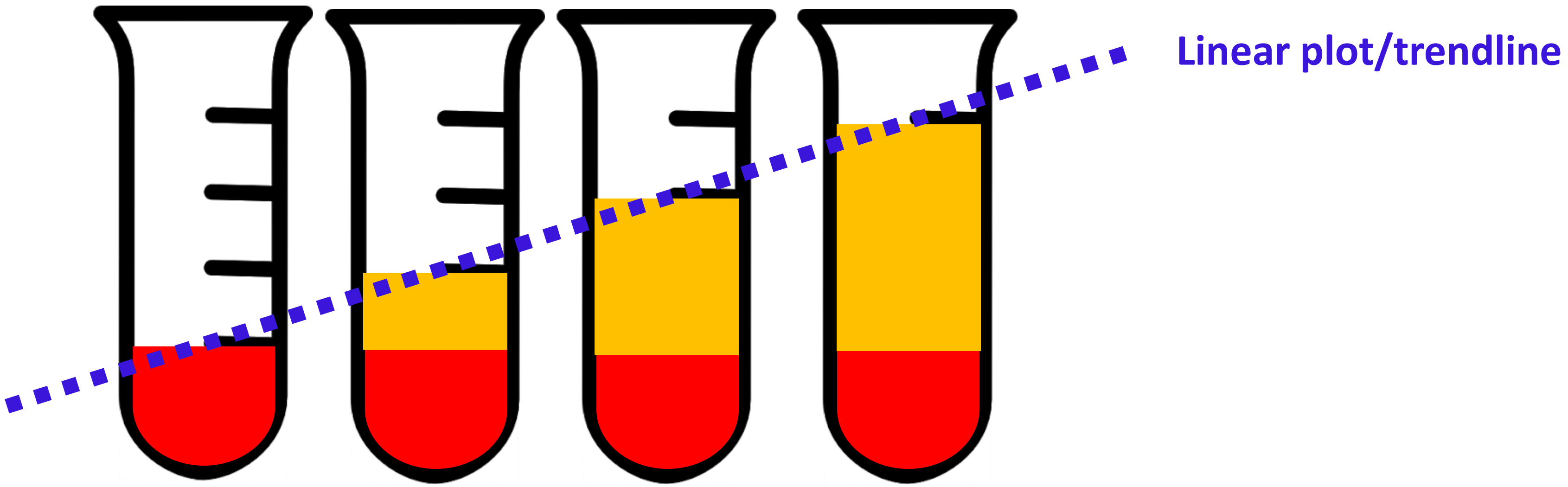

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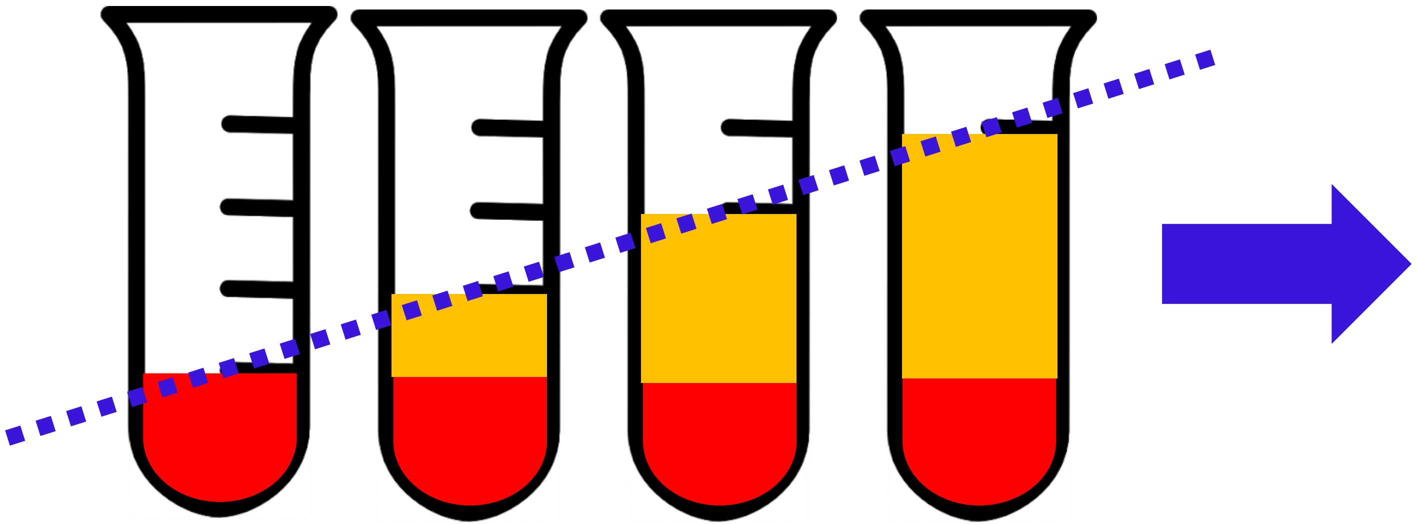

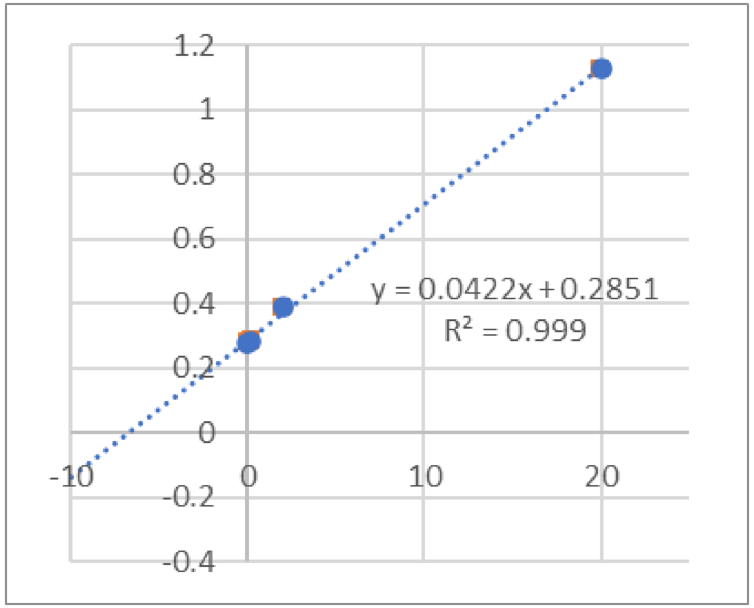

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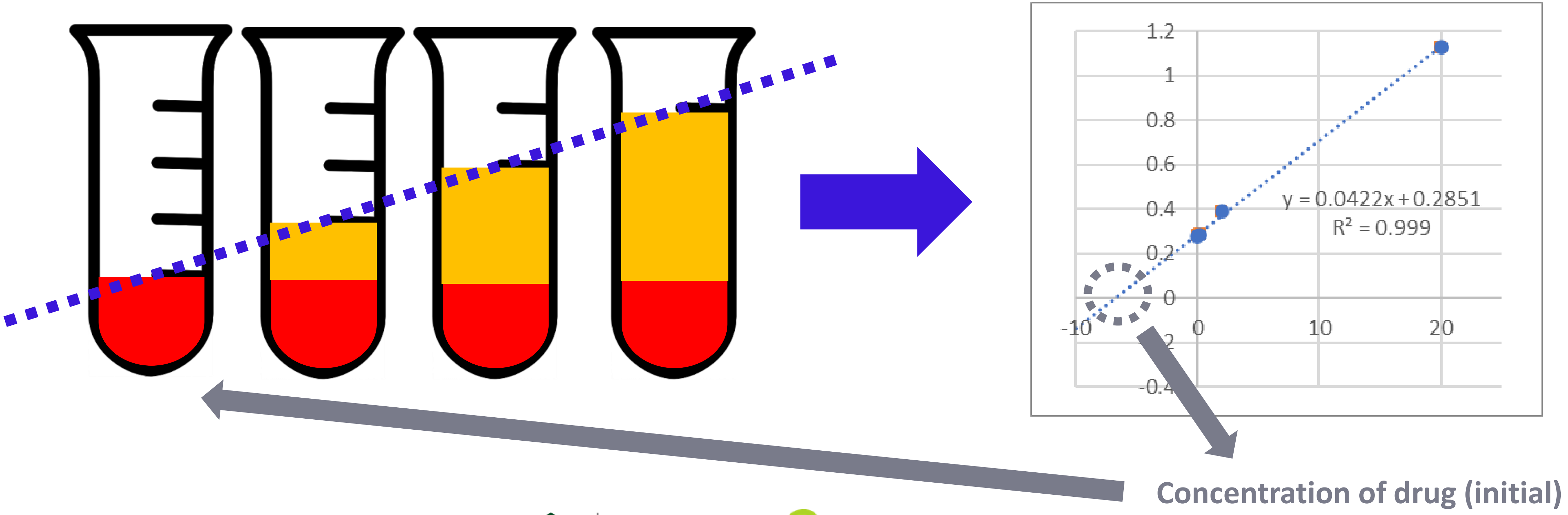

CONSIDERATIONS

Considerations

- **The Good and The Bad:**

- Pros:
  - “Self-validating” approach
  - Different requirements for *validation*
  - Resource savings on small scale
  - When executed properly, more accurate

- Cons:
  - Consumed sample volume (2+ mL per assay)
  - Need to know a ballpark quantitative value
  - For 10+ samples, can become time consuming and resource consuming

- **Additional Considerations:**
  - Desired quantitative range
  - Instrumentation
    - GC-MS vs. LC-MS/MS
  - Matrix type
    - Tissue, vitreous, bile, etc.
  - Matrix effects
    - Can you replicate postmortem blood?
  - Internal standard
  - Etc.

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Considerations

- Standard addition **can not** be applied without assessment of the analytical technique

Standard additions: myth and reality†

Stephen L. R. Ellison[a] and Michael Thompson[b]

DOI: 10.1039/b717660k

Standard additions is a calibration technique devised to eliminate rotational matrix effects in analytical measurement. Although the technique is presented in almost every textbook of analytical chemistry, its behaviour in practice is not well documented and is prone to attract misleading accounts. The most important limitation is that the method cannot deal with translational matrix effects, which need to be handled separately. In addition, because the method involves extrapolation from known data, the method is often regarded as less precise than external calibration (interpolation) techniques. Here, using a generalised model of an analytical system, we look at the behaviour of the method of standard additions under a range of conditions, and find that, if executed optimally, there is no noteworthy loss of precision.

Recommendations for standard additions

1. Make sure that the analytical method is effectively linear over the whole of the required working range.
2. Make sure that any translational interference is eliminated separately.
3. Only one level of added analyte is necessary, with repeated measurements if better precision is required.
4. Let the concentration of the added analyte be as high as is consistent with linearity, and ideally at least five times the original concentration of analyte.

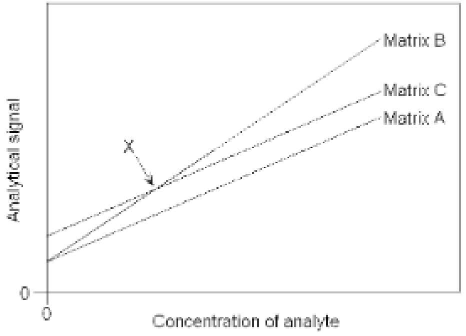

Figure 1. Different types of matrix effect on the analytical signal. Matrix A is the calibration matrix. With Matrix B a rotational effect changes the size of the signal derived from the analyte, but not the intercept. With Matrix C the intercept has been shifted by a translational effect, but the slope is unaffected. At point X the two matrix effects fortuitously have the same outcome.

https://doi.org/10.1039/b717660k

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EXPERIMENTATION

Experimentation

- **Method development and workflow** *(same a traditionally validated assay)*
  - Analytical method
  - Spiking mixes / internal standard
  - Sample preparation protocol / extraction

- **Example:**
  - Waters Xevo TQ-S micro (LC-MS/MS)
  - Column, mobile phase, and gradient
  - MRM transitions
  - Isotonitazene and fentanyl-D5 / 0.1 and 1 ng/µL
  - Liquid-liquid extraction

Table I. LC Gradient Conditions

Time (min)	%A	%B	Flow (mL/min)
Initial	50	50	0.4
1.0	50	50	0.4
4.0	5	95	0.4
5.0	5	95	0.4
5.1	50	50	0.4
6.0	50	50	0.4

Table II. MRM Parameters

Analyte	Cone (V)	Precursor (m/z)	Collision (V)	Product (m/z)	Dwell (s)
Isotonitazene	50	411.2	46	106.9	0.053
			22	100.0	0.053
			44	72.0	0.053
Fentanyl-d_5	56	342.2	24	188.0	0.053
			40	105.0	0.053

Experimentation

- **Method verification** (or "validation")
  - Modeled after ASB Standard 036: *Standard Practices for Method Validation in Forensic Toxicology*
  - These are our proposed experiments to assess the use of standard addition

Required:
- Linearity (target range)
- Limit of detection
- Carryover
- Interferences

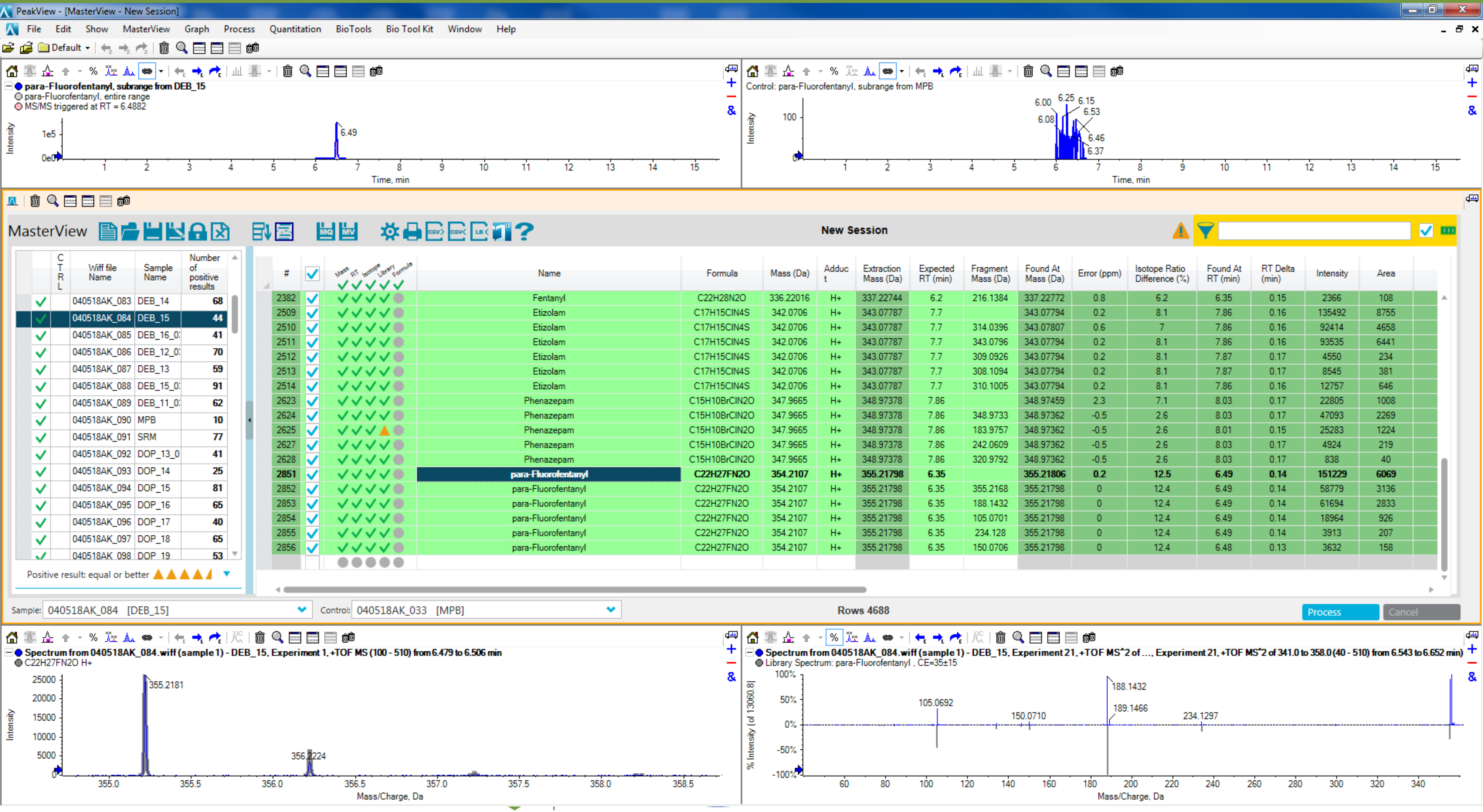
May be required:
- "Controls" (accuracy and precision)
- Stability studies
- Recovery

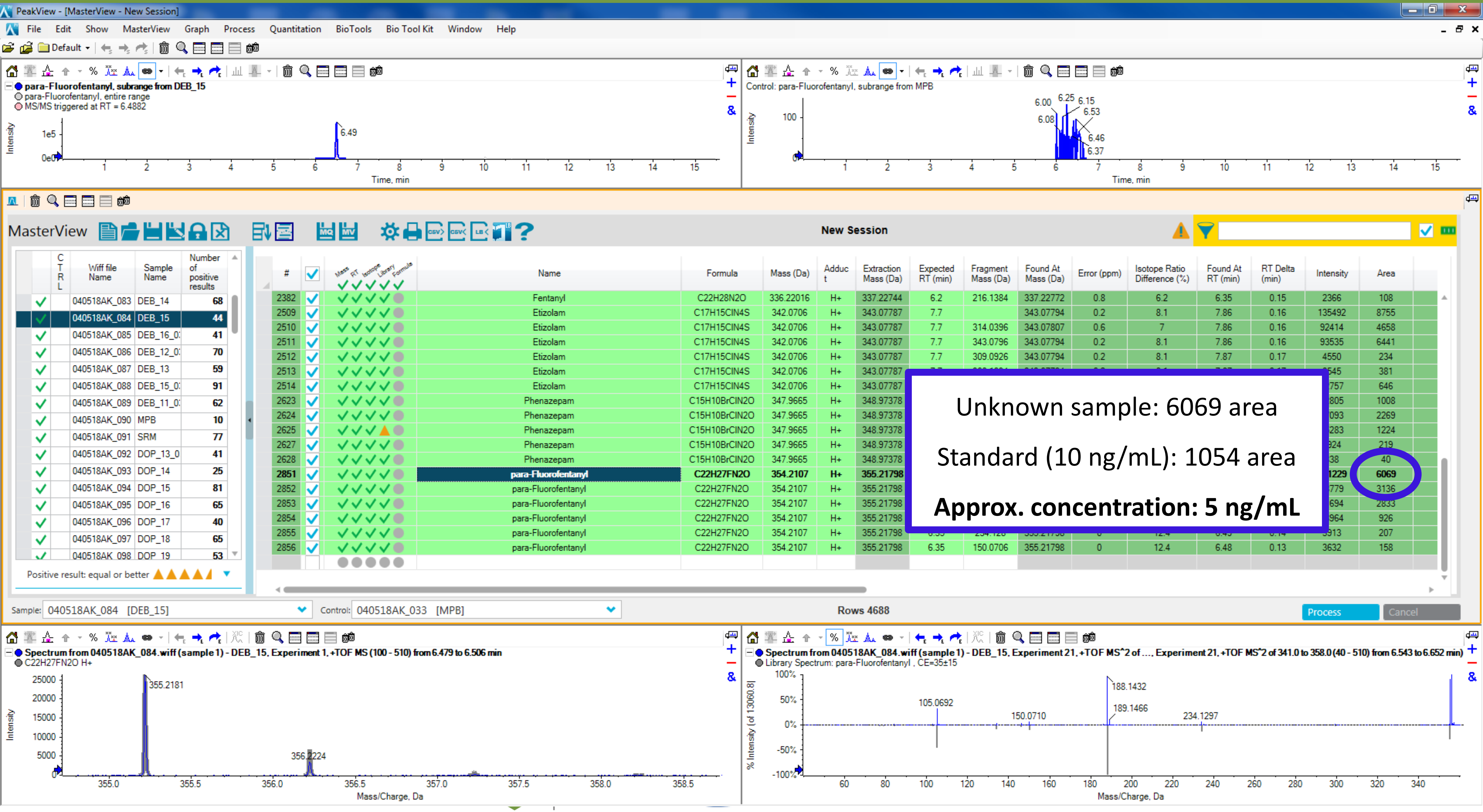
Not required:
- Ion suppression / enhancement
- Dilution integrity

IMPLEMENTATION

Implementation

- [Screening] – Analysis of sample(s) by LC-QTOF-MS
- Method development and verification
- [Confirmation] – Application to authentic sample(s)
- Calculation of concentration (x-intercept)


PeakView - [MasterView - New Session]
File Edit Show MasterView Graph Process Quantitation BioTools Bio Tool Kit Window Help
para-Fluorofentanyl, subrange from DEB_15
para-Fluorofentanyl, entire range
MS/MS triggered at RT = 6.4882
6.49
Time, min
Intensity
Control: para-Fluorofentanyl, subrange from MPB
6.00 6.25 6.15 6.53 6.08 6.46 6.37
MasterView
New Session
Wiff file Name | Sample Name | Number of positive results
040518AK_083 DEB_14 68
040518AK_084 DEB_15 44
040518AK_085 DEB_16_0 41
040518AK_086 DEB_12_0 70
040518AK_087 DEB_13 59
040518AK_088 DEB_15_0 91
040518AK_089 DEB_11_0 62
040518AK_090 MPB 10
040518AK_091 SRM 77
040518AK_092 DOP_13_0 41
040518AK_093 DOP_14 25
040518AK_094 DOP_15 81
040518AK_095 DOP_16 65
040518AK_096 DOP_17 40
040518AK_097 DOP_18 65
040518AK_098 DOP_19 53
Positive result: equal or better
| Name | Formula | Mass (Da) | Adduct | Extraction Mass (Da) | Expected RT (min) | Fragment Mass (Da) | Found At Mass (Da) | Error (ppm) | Isotope Ratio Difference (%) | Found At RT (min) | RT Delta (min) | Intensity | Area
2382 Fentanyl C22H28N2O 336.2016 H+ 337.22744 6.2 216.1384 337.22772 0.8 6.2 6.35 0.15 2366 108
2509 Etizolam C17H15ClN4S 342.0706 H+ 343.07787 7.7 343.07794 0.2 8.1 7.86 0.16 135492 8755
2510 Etizolam C17H15ClN4S 342.0706 H+ 343.07787 7.7 314.0396 343.07807 0.6 7 7.86 0.16 92414 4658
2511 Etizolam C17H15ClN4S 342.0706 H+ 343.07787 7.7 343.0796 343.07794 0.2 8.1 7.86 0.16 93535 6441
2512 Etizolam C17H15ClN4S 342.0706 H+ 343.07787 7.7 309.0926 343.07794 0.2 8.1 7.87 0.17 4550 234
2513 Etizolam C17H15ClN4S 342.0706 H+ 343.07787 7.7 308.1094 343.07794 0.2 8.1 7.87 0.17 8545 381
2514 Etizolam C17H15ClN4S 342.0706 H+ 343.07787 7.7 310.1005 343.07794 0.2 8.1 7.86 0.16 12757 646
2623 Phenazepam C15H10BrClN2O 347.9665 H+ 348.97378 7.86 348.97459 2.3 7.1 8.03 0.17 22805 1008
2624 Phenazepam C15H10BrClN2O 347.9665 H+ 348.97378 7.86 348.9733 348.97362 -0.5 2.6 8.03 0.17 47093 2269
2625 Phenazepam C15H10BrClN2O 347.9665 H+ 348.97378 7.86 183.9757 348.97362 -0.5 2.6 8.01 0.15 25283 1224
2627 Phenazepam C15H10BrClN2O 347.9665 H+ 348.97378 7.86 242.0609 348.97362 -0.5 2.6 8.03 0.17 4924 219
2628 Phenazepam C15H10BrClN2O 347.9665 H+ 348.97378 7.86 320.9792 348.97362 -0.5 2.6 8.03 0.17 838 40
2851 para-Fluorofentanyl C22H27FN2O 354.2107 H+ 355.21798 6.35 355.21806 0.2 12.5 6.49 0.14 151229 6069
2852 para-Fluorofentanyl C22H27FN2O 354.2107 H+ 355.21798 6.35 355.2168 355.21798 0 12.4 6.49 0.14 58779 3136
2853 para-Fluorofentanyl C22H27FN2O 354.2107 H+ 355.21798 6.35 188.1432 355.21798 0 12.4 6.49 0.14 61694 2833
2854 para-Fluorofentanyl C22H27FN2O 354.2107 H+ 355.21798 6.35 105.0701 355.21798 0 12.4 6.49 0.14 18964 926
2855 para-Fluorofentanyl C22H27FN2O 354.2107 H+ 355.21798 6.35 234.128 355.21798 0 12.4 6.49 0.14 3913 207
2856 para-Fluorofentanyl C22H27FN2O 354.2107 H+ 355.21798 6.35 150.0706 355.21798 0 12.4 6.48 0.13 3632 158
Sample: 040518AK_084 [DEB_15]
Control: 040518AK_033 [MPB]
Rows 4688
Process
Cancel
Spectrum from 040518AK_084.wiff (sample 1) - DEB_15, Experiment 1, +TOF MS (100 - 510) from 6.479 to 6.506 min
C22H27FN2O H+
355.2181
356.2224
Mass/Charge, Da
Spectrum from 040518AK_084.wiff (sample 1) - DEB_15, Experiment 21, +TOF MS^2 of ..., Experiment 21, +TOF MS^2 of 341.0 to 358.0 (40 - 510) from 6.543 to 6.652 min
Library Spectrum: para-Fluorofentanyl , CE=35±15
188.1432
105.0692
189.1466
150.0710
234.1297
% Intensity (of 13060.8)
Mass/Charge, Da


PeakView - [MasterView - New Session]
para-Fluorofentanyl, subrange from DEB_15
para-Fluorofentanyl, entire range
MS/MS triggered at RT = 6.4882
Control: para-Fluorofentanyl, subrange from MPB
Time, min
MasterView
New Session
Unknown sample: 6069 area
Standard (10 ng/mL): 1054 area
Approx. concentration: 5 ng/mL
Positive result: equal or better
Sample: 040518AK_084 [DEB_15]
Control: 040518AK_033 [MPB]
Rows 4688
Process
Cancel
Spectrum from 040518AK_084.wiff (sample 1) - DEB_15, Experiment 1, +TOF MS (100 - 510) from 6.479 to 6.506 min
C22H27FN2O H+
Mass/Charge, Da
Library Spectrum: para-Fluorofentanyl , CE=35±15

Sample #	Notes	Sample ID and Comments
1	Calibration Model	Reagent blank (DI water)
2		Standard 1: 100 ng/mL
3		Blank Blood -- No ISTD added
4		Standard 2: 50 ng/mL
5		Standard 3: 20 ng/mL
6		Standard 4: 10 ng/mL
7		Standard 5: 5 ng/mL
8		Standard 6: 1 ng/mL
9		Standard 7: 0.5 ng/mL
10		Standard 8: 0.25 ng/mL
11		Standard 9: 0.1 ng/mL
12		Blank Blood – with ISTD added
13	Standard Addition Assessment (5 ng/mL)	Std Add-1 (No up-spike)
14		Std Add-2 (0.2 ng/mL up-spike)
15		Std Add-3 (2 ng/mL up-spike)
16		Std Add-4 (20 ng/mL up-spike)
17	Standard Addition Assessment (10 ng/mL)	Std Add-1 (No up-spike)
18		Std Add-2 (0.2 ng/mL up-spike)
19		Std Add-3 (2 ng/mL up-spike)
20		Std Add-4 (20 ng/mL up-spike)
21	Recovery (20 ng/mL)	Pre-spike-1
22		Pre-spike-2
23		Pre-spike-3
24		Post-spike-1
25		Post-spike-2
26		Post-spike-3
27	Matrix Interferences (10 different sources)	Matrix Blood 1
28		Matrix Blood 2
29		Matrix Blood 3
30		Matrix Blood 4
31		Matrix Blood 5
32		Matrix Blood 6
33		Matrix Blood 7
34		Matrix Blood 8
35		Matrix Blood 9
36		Matrix Blood 10
37	Analyte/Internal Standard Interferences (Highest Calibrator and Normal ISTD Conc.)	Highest Cal. 1 – 100 ng/mL
38		Highest Cal. 2 – 100 ng/mL
39		Highest Cal. 3 – 100 ng/mL
40		Highest Cal. 4 – 100 ng/mL
41		Highest Cal. 5 – 100 ng/mL
42		Internal Standard 1 – 20 ng/mL
43		Internal Standard 2 – 20 ng/mL
44		Internal Standard 3 – 20 ng/mL
45		Internal Standard 4 – 20 ng/mL
46		Internal Standard 5 – 20 ng/mL

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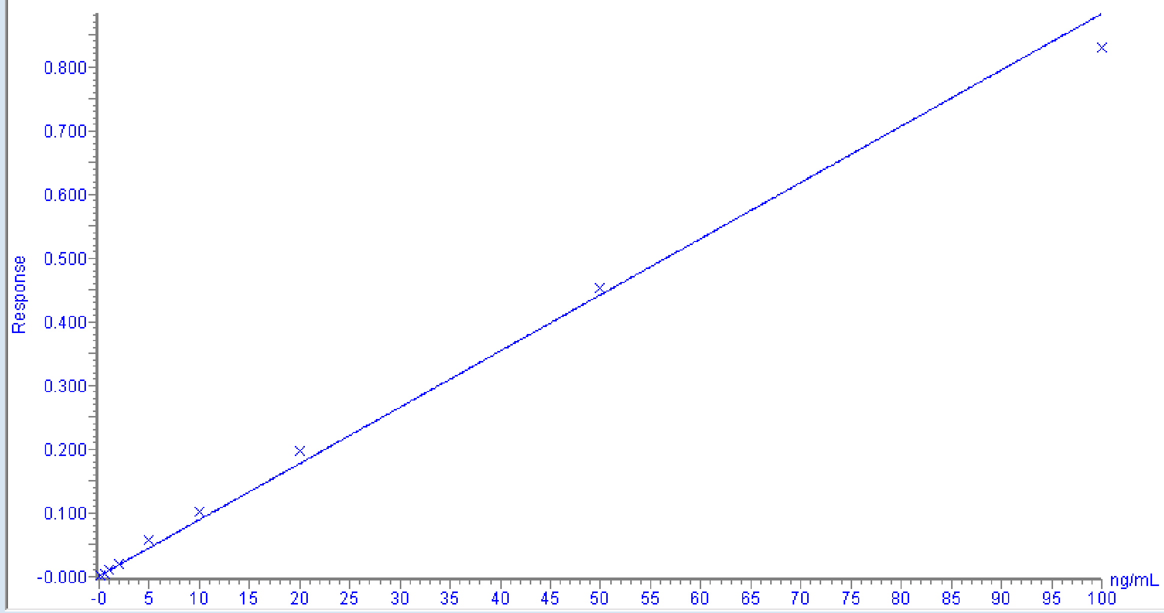
Brorphine

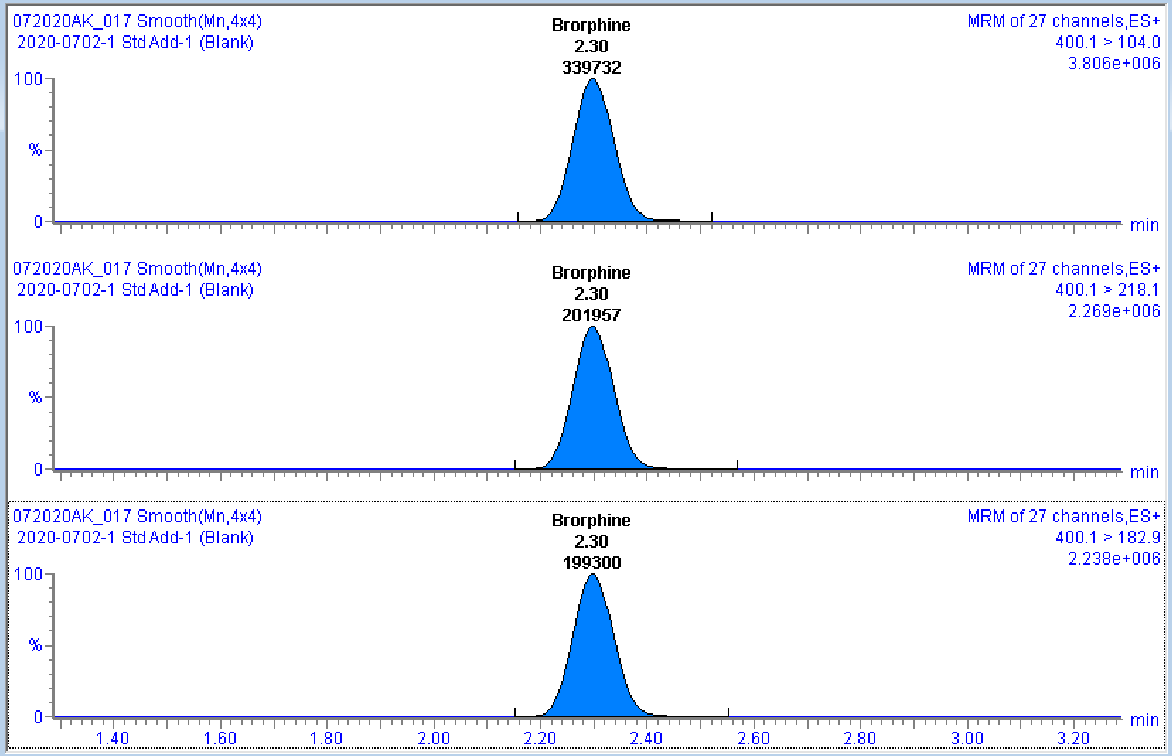
	Vial	#	Name	ID	Type	Std. Conc	ng/mL	%Dev	Area	RT	IS Area	Response	S/N	1° Ratio (Actual)	1° Ratio Flag	RRT	2° Ratio (...	2° Ratio Flag
1	2:A,1	1	072020AK_004	MPB														
2	2:A,3	2	072020AK_005	Reagent Blank														
3	2:A,4	3	072020AK_006	Standard 1: 100 ng/mL	Standard	100.000	93.84	-6.2	2903842	2.30	3503228	0.829	33524	1.738	YES	1.247	1.764	YES
4	2:A,5	4	072020AK_007	Matrix Blank - No ISTD														
5	2:A,6	5	072020AK_008	Standard 2: 50 ng/mL	Standard	50.000	51.23	2.5	1574149	2.30	3473626	0.453	39840	1.689	YES	1.247	1.703	YES
6	2:A,7	6	072020AK_009	Standard 3: 20 ng/mL	Standard	20.000	22.14	10.7	661915	2.30	3366567	0.197	19248	1.667	YES	1.247	1.701	YES
7	2:A,8	7	072020AK_010	Standard 4: 10 ng/mL	Standard	10.000	11.39	13.9	350035	2.30	3440013	0.102	10497	1.678	YES	1.247	1.706	YES
8	2:B,1	8	072020AK_011	Standard 5: 5 ng/mL	Standard	5.000	6.39	27.9	197671	2.30	3425253	0.058	4471	1.713	YES	1.247	1.718	YES
9	2:B,2	9	072020AK_012	Standard 6: 2 ng/mL	Standard	2.000	2.13	6.7	69786	2.30	3465318	0.020	1374	1.692	YES	1.247	1.710	YES
10	2:B,3	10	072020AK_013	Standard 7: 1 ng/mL	Standard	1.000	1.01	1.3	34492	2.30	3363015	0.010	863	1.643	YES	1.247	1.692	YES
11	2:B,4	11	072020AK_014	Standard 8: 0.5 ng/mL	Standard	0.500	0.45	-10.9	18856	2.30	3590315	0.005	489	1.765	YES	1.247	1.763	YES
12	2:B,5	12	072020AK_015	Standard 9: 0.2 ng/mL	Standard	0.200	0.11	-45.9	7956	2.30	3495998	0.002	229	1.720	YES	1.247	1.890	YES
13	2:B,6	13	072020AK_016	Matrix Blank							3362659							
14	2:B,7	14	072020AK_017	2020-0702-1 Std Add-1 (Blank)			11.53		339732	2.30	3297772	0.103	8074	1.682	YES	1.247	1.705	YES
15	2:B,8	15	072020AK_018	2020-0702-1 Std Add-2 (0.2 ng/mL)			11.24		353171	2.30	3516877	0.100	8432	1.650	YES	1.247	1.666	YES
16	2:C,1	16	072020AK_019	2020-0702-1 Std Add-3 (2 ng/mL)			12.55		414139	2.30	3696472	0.112	9029	1.682	YES	1.247	1.684	YES
17	2:C,2	17	072020AK_020	2020-0702-1 Std Add-4 (20 ng/mL)			32.22		989681	2.30	3466838	0.285	32074	1.703	YES	1.247	1.686	YES
18	2:A,1	18	072020AK_021	MPB														

Calibration: 18 Jan 2021 15:24:11

Compound name: Brorphine
Correlation coefficient: r = 0.996460, r^2 = 0.992932
Calibration curve: 0.0088192 * x + 0.00132165
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Chromatogram



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Brorphine

	Vial	#	Name	ID	Type	Std. Conc	ng/mL	%Dev	Area	RT	IS Area	Response	S/N	1° Ratio (Actual)	1° Ratio Flag	RRT	2° Ratio (...	2° Ratio Flag
1	2:A,1	1	072020AK_004	[illegible]PB														
2	2:A,3	2	072020AK_005	Reagent Blank														
3	2:A,4	3	072020AK_0[illegible]	Standard 1: 100 ng/mL	Standard	100.000	93.84	-6.2	2903842	2.30	3503228	0.829	33524	1.738	YES	1.247	1.764	YES
4	2:A,5	4	072020AK_[illegible]7	Matrix Blank - No ISTD														
5	2:A,6	5	072020AK_[illegible]08	Standard 2: 50 ng/mL	Standard	50.000	51.23	2.5	1574149	2.30	3473626	0.453	39840	1.689	YES	1.247	1.703	YES
6	2:A,7	6	072020AK[illegible]09	Standard 3: 20 ng/mL	Standard	20.000	22.14	10.7	661915	2.30	3366567	0.197	19248	1.667	YES	1.247	1.701	YES
7	2:A,8	7	072020AK[illegible]10	Standard 4: 10 ng/mL	Standard	10.000	11.39	13.9	350035	2.30	3440013	0.102	10497	1.678	YES	1.247	1.706	YES
8	2:B,1	8	072020AK[illegible]11	Standard 5: 5 ng/mL	Standard	5.000	6.39	27.9	197671	2.30	3425253	0.058	4471	1.713	YES	1.247	1.718	YES
9	2:B,2	9	072020AK_[illegible]12	Standard 6: 2 ng/mL	Standard	2.000	2.13	6.7	69786	2.30	3465318	0.020	1374	1.692	YES	1.247	1.710	YES
10	2:B,3	10	072020AK_[illegible]3	Standard 7: 1 ng/mL	Standard	1.000	1.01	1.3	34492	2.30	3363015	0.010	863	1.643	YES	1.247	1.692	YES
11	2:B,4	11	072020AK_0[illegible]	Standard 8: 0.5 ng/mL	Standard	0.500	0.45	-10.9	18856	2.30	3590315	0.005	489	1.765	YES	1.247	1.763	YES
12	2:B,5	12	072020AK_01[illegible]	Standard 9: 0.2 ng/mL	Standard	0.200	0.11	-45.9	7956	2.30	3495998	0.002	229	1.720	YES	1.247	1.890	YES
13	2:B,6	13	072020AK_016	[illegible]atrix Blank							3362659							
14	2:B,7	14	072020AK_017	20[illegible]0702-1 Std A[illegible]1 (Blank)			11.53		339732	2.30	3297772	0.103	8074	1.682	YES	1.247	1.705	YES
15	2:B,8	15	072020AK_018	2020-0[illegible] Std Add-2 (0.2 ng/mL)			11.24		353171	2.30	3516877	0.100	8432	1.650	YES	1.247	1.666	YES
16	2:C,1	16	072020AK_019	2020-0702-1 Std Add-3 (2 ng/mL)			12.55		414139	2.30	3696472	0.112	9029	1.682	YES	1.247	1.684	YES
17	2:C,2	17	072020AK_020	2020-0702-1 Std Add-4 (20 ng/mL)			32.22		989681	2.30	3466838	0.285	32074	1.703	YES	1.247	1.686	YES
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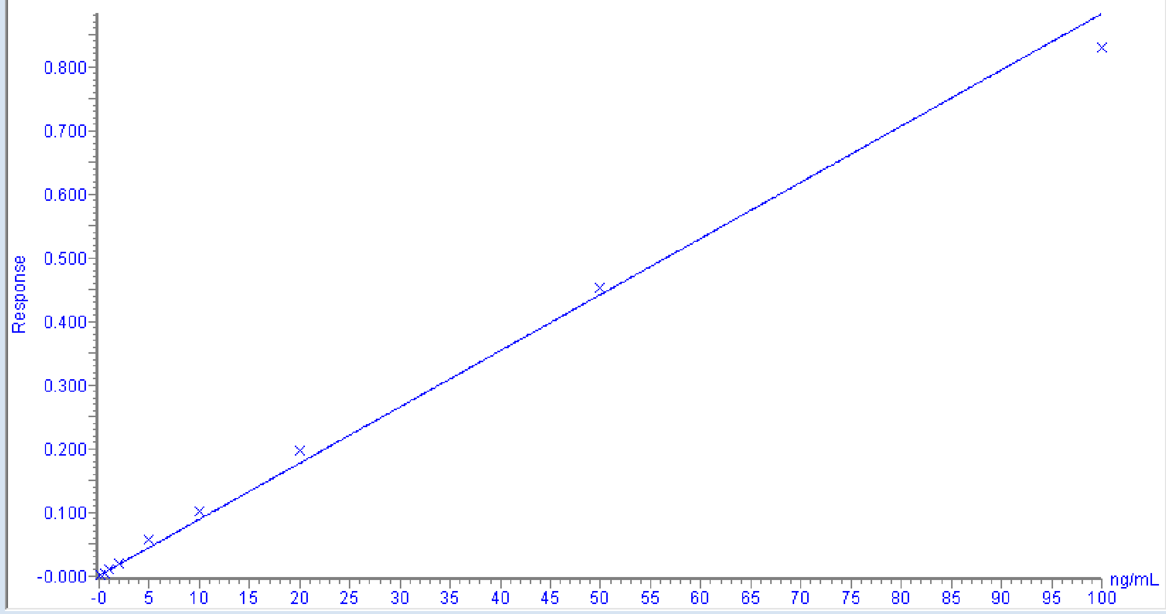
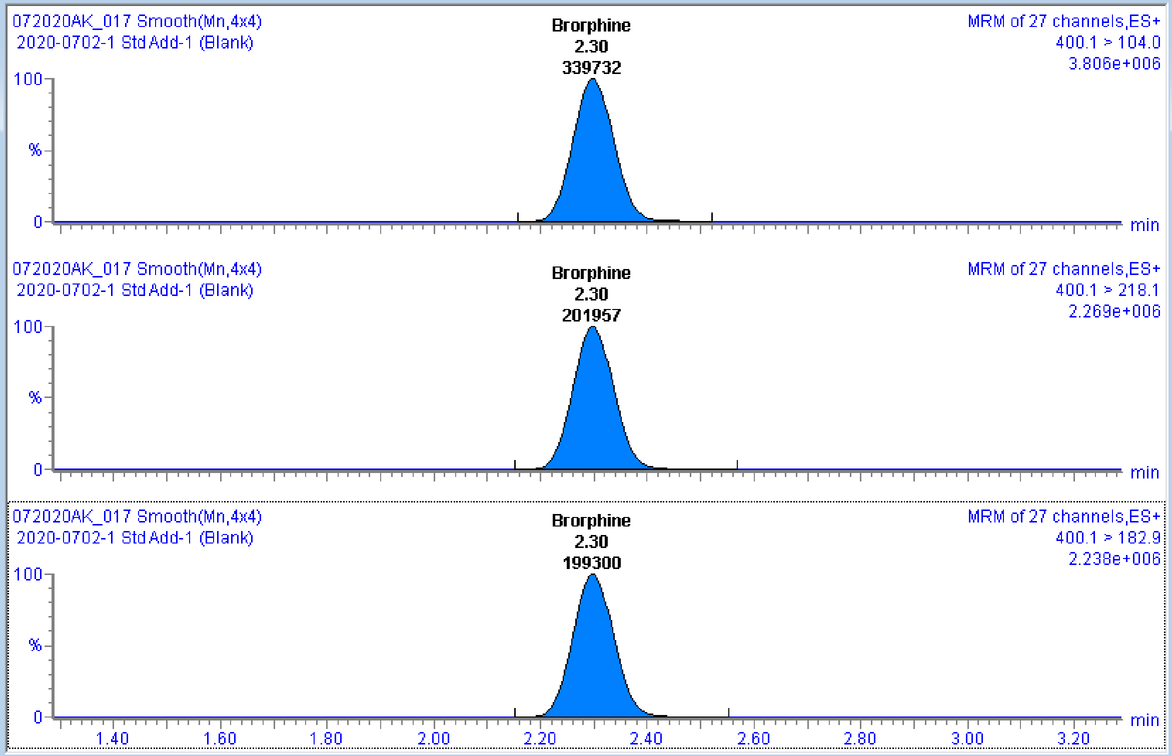
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



Chromatogram



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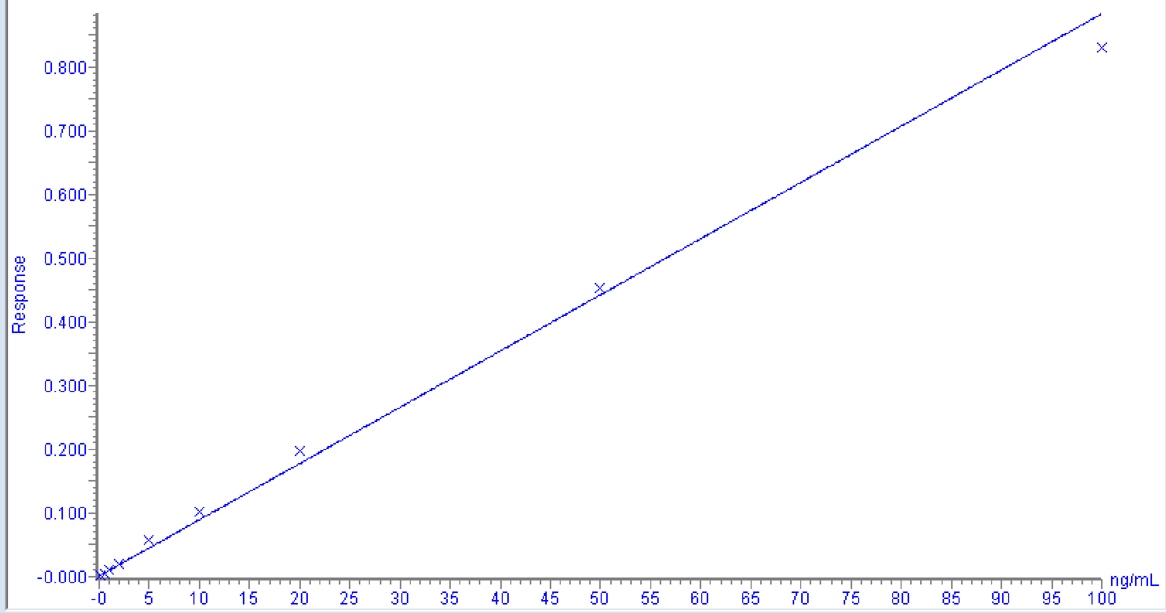
File Edit View Display Processing Window Help

Brorphine

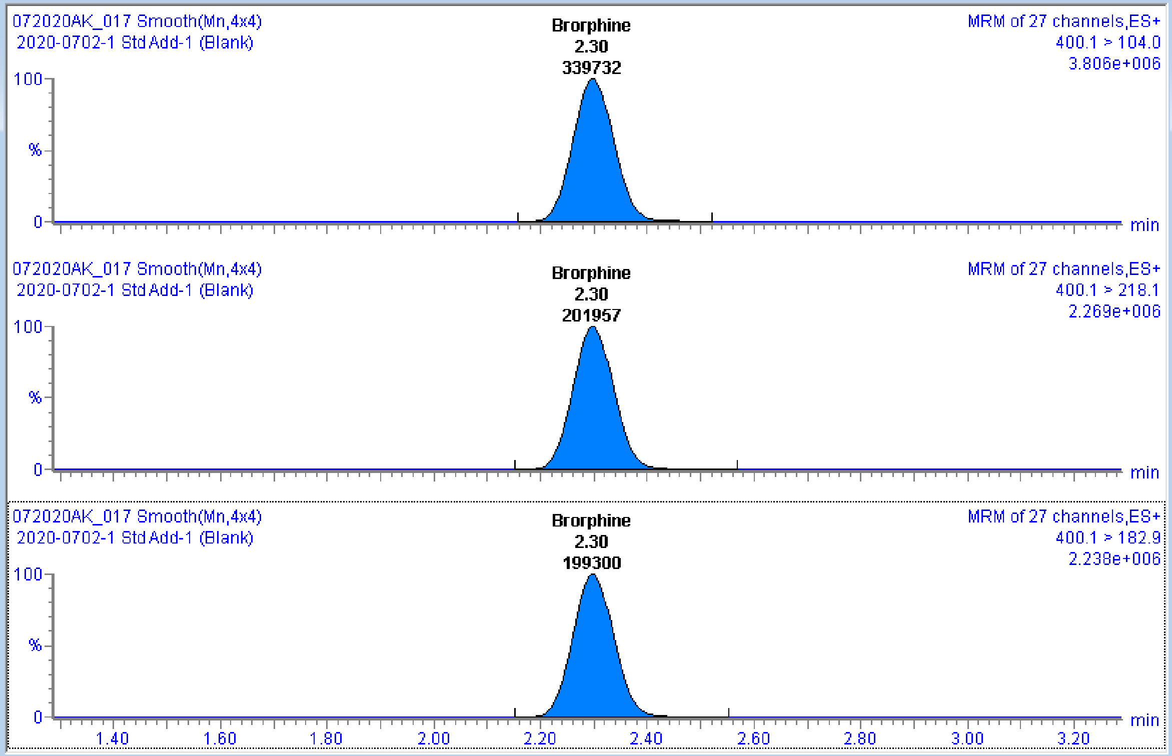
	Vial	#	Name	ID	Type	Std. Conc	ng/mL	%Dev	Area	RT	IS Area	Response	S/N	1° Ratio (Actual)	1° Ratio Flag	RRT	2° Ratio (...	2° Ratio Flag
1	2:A,1	1	072020AK_004	MPB														
2	2:A,3	2	072020AK_005	Reagent Blank														
3	2:A,4	3	072020AK_006	Standard 1: 100 ng/mL	Standard	100.000	93.84	-6.2	2903842	2.30	3503228	0.829	33524	1.738	YES	1.247	1.764	YES
4	2:A,5	4	072020AK_007	Matrix Blank - No ISTD														
5	2:A,6	5	072020AK_008	Standard 2: 50 ng/mL	Standard	50.000	51.23	2.5	1574149	2.30	3473626	0.453	39840	1.689	YES	1.247	1.703	YES
6	2:A,7	6	072020AK_009	Standard 3: 20 ng/mL	Standard	20.000	22.14	10.7	661915	2.30	3366567	0.197	19248	1.667	YES	1.247	1.701	YES
7	2:A,8	7	072020AK_010	Standard 4: 10 ng/mL	Standard	10.000	11.39	13.9	350035	2.30	3440013	0.102	10497	1.678	YES	1.247	1.706	YES
8	2:B,1	8	072020AK_011	Standard 5: 5 ng/mL	Standard	5.000	6.39	27.9	197671	2.30	3425253	0.058	4471	1.713	YES	1.247	1.718	YES
9	2:B,2	9	072020AK_012	Standard 6: 2 ng/mL	Standard	2.000	2.13	6.7	69786	2.30	3465318	0.020	1374	1.692	YES	1.247	1.710	YES
10	2:B,3	10	072020AK_013	Standard 7: 1 ng/mL	Standard	1.000	1.01	1.3	34492	2.30	3363015	0.010	863	1.643	YES	1.247	1.692	YES
11	2:B,4	11	072020AK_014	Standard 8: 0.5 ng/mL	Standard	0.500	0.45	-10.9	18856	2.30	3590315	0.005	489	1.765	YES	1.247	1.763	YES
12	2:B,5	12	072020AK_015	Standard 9: 0.2 ng/mL	Standard	0.200	0.11	-45.9	7956	2.30	3495998	0.002	229	1.720	YES	1.247	1.890	YES
13	2:B,6	13	072020AK_016	Matrix Blank							3362659							
14	2:B,7	14	072020AK_017	2020-0702-1 Std Add-1 (Blank)			11.53		339732	2.30	3297772	0.103	8074	1.682	YES	1.247	1.705	YES
15	2:B,8	15	072020AK_018	2020-0702-1 Std Add-2 (0.2 ng/mL)					353171	2.30	3516877	0.100	8432	1.650	YES	1.247	1.666	YES
16	2:C,1	16	072020AK_019	2020-0702-1 Std Add-3 (2 ng/mL)			12.55		414139	2.30	3696472	0.112	9029	1.682	YES	1.247	1.684	YES
17	2:C,2	17	072020AK_020	2020-0702-1 Std Add-4 (20 ng/mL)			32.22		989681	2.30	3466838	0.285	32074	1.703	YES	1.247	1.686	YES
18	2:A,1	18	072020AK_021	MPB														

Calibration: 18 Jan 2021 15:24:11

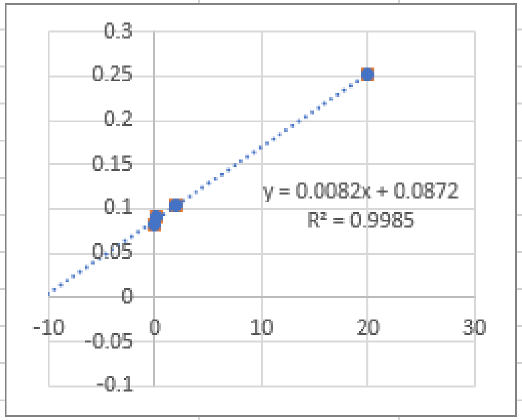
Compound name: Brorphine
Correlation coefficient: r = 0.996460, r^2 = 0.992932
Calibration curve: 0.0088192 * x + 0.00132165
Response type: Internal Std (Ref 1), Area * (IS Conc. / IS Area)
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None



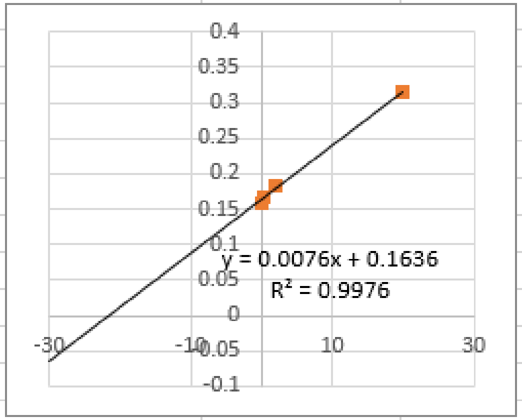
Chromatogram



Concentration: 10 ng/mL		
Sample Name	Conc. (ng/mL)	PAR
Target Sample	0	0.083
Up-spike #1	0.2	0.092
Up-spike #2	2	0.105
Up-spike #3	20	0.252
Y-Intercept	-	**0.0872**
X-Intercept	**10.6**	-



Concentration: 20 ng/mL		
Sample Name	Conc. (ng/mL)	PAR
Target Sample	0	0.159
Up-spike #1	0.2	0.166
Up-spike #2	2	0.183
Up-spike #3	20	0.316
Y-Intercept	-	**0.1636**
X-Intercept	**21.4**	-



Recovery	
Pre-1	0.157
Pre-2	0.158
Pre-3	0.169
Pre-Avg	0.161
Post-1	0.175
Post-2	0.149
Post-3	0.176
Post-Avg	0.167
Recovery	**96.8**

Matrix	No issues
Standard	No issues
ISTD	No issues
Carryover	Mininal (<1000 area)

Verification Data ↑

Authentic Sample →

WO# XXXXXXXX CFSRE# XXXX-XXXX-X		
Sample Name	**Conc. (ng/mL)**	**PAR**
Target Sample	0	0.103
Up-spike #1	0.2	0.1
Up-spike #2	2	0.112
Up-spike #3	20	0.285
Y-Intercept	-	**0.0983**
R2 Correl	-	**0.998**
X-Intercept	**10.6**	-
Cal. Curve	**11.5**	**-9%**

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Conclusions / Take Aways

- Standard addition is a scientifically valid approach for quantitation in forensic toxicology
  - Toxicologists should consider its utility in their labs
- Sample volume consumed may be an issue
- Method assessment is still **required**
- Allows for quantitation when other methods or approaches are not available
  - Particularly useful for new and emerging NPS
- *There are other ways to perform standard addition*

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PAPER
Toxicology

Brorphine—Investigation and quantitation of a new potent synthetic opioid in forensic toxicology casework using liquid chromatography-mass spectrometry

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Abstract
New synthetic opioids continue to appear as novel psychoactive substances (NPS) on illicit drug markets. Isotonitazene emerged in mid-2019, becoming the most prevalent NPS opioid in the United States within a few months. Notification by the Drug Enforcement Administration of its intent to schedule isotonitazene in mid-2020 led to its decline in popularity and replacement with a new NPS opioid: brorphine. Brorphine is a potent synthetic opioid, but little information was previously available regarding its toxicity or involvement in impairment and death. Our laboratory developed an assay for the identification and quantitative confirmation of brorphine using standard addition. Quantitative analysis was performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). *In vitro* and *in vivo* metabolism st

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  - Joseph Homan (co-author)
  - Donna Papsun
- **Forensic partners**

- **Provide Feedback or Insights**
  - We would love to hear from you
  - Fellow colleagues are looking for different perspectives and innovative approached
  - Email: alex.krotulski@cfsre.org

  - *Does your lab use standard addition?*
  - *Do you have an assessment process?*
  - *Do you use standard addition in a high-volume, high-throughput environment?*
  - *Do you have other ideas about implementation?*

NMS LABS

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Thank You!

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