



Uncertainty of Measurement for Quantitative Methods

| | |
|--|--|
| Analyte | Opioids (morphine, oxymorphone, hydromorphone, O-desmethyltramadol, codeine, 6-acetylmorphine, oxycodone, hydrocodone, tramadol, norbuprenorphine, and buprenorphine) |
| Unit of Measure | ng/mL |
| Analyst Preparing Uncertainty Estimation | Sara Dempsey |
| Responsible Supervisor | Dayong Lee |
| Primary Matrix | Blood |
| Secondary Matrices | N/A |
| Lowest Calibrator Concentration | 0.5 ng/mL for norbuprenorphine and buprenorphine; 5 ng/mL for morphine, oxymorphone, hydromorphone, O-desmethyltramadol, codeine, 6-acetylmorphine, oxycodone, hydrocodone, and tramadol |
| Highest Calibrator Concentration | 100 ng/mL for norbuprenorphine and buprenorphine; 500 ng/mL for morphine, oxymorphone, hydromorphone, O-desmethyltramadol, codeine, 6-acetylmorphine, oxycodone, hydrocodone, and tramadol |
| Equipment/Instrument | LCMS-2 |
| Instrument Serial Number | SG1936G104 |
| Method | OPI1.M |

Uncertainty of Measurement Approval

Analyst: _____ Date

Responsible Supervisor: _____ Date

Quality Director: _____ Date

| Uncertainty of Measurement Budget | | | |
|-----------------------------------|---|---|---|
| Category | Uncertainty Component | Method of Evaluation | Comment |
| Staff | Multiple Analysts | Type A: Measurement Reproducibility | Pooled %RSD of historical QC data. |
| | Training | Type A: Measurement Reproducibility | |
| | Experience | Type A: Measurement Reproducibility | |
| Calibrators | Purity of Certified Reference Material (CRM) | Type B: Certified Reference Material COA | Maximum reported uncertainty from CRM COA. |
| | Dilution of CRM to make working calibrator solution C or F (Pipette) | Type A: External calibration certificates | Maximum %RSD reported on external calibration certificates across all pipettes with relevant volume range. |
| | Dilution of CRM to make working calibrator solution C or F (Volumetric Flask) | Type B: Volumetric flask certificate of calibration | Maximum reported uncertainty on certificates of calibration of NIST traceable flasks as described in SOP version 3.5. |
| | Dilution of working calibrator solution C or F to make working calibrator solution B or E (Pipette) | Type A: External calibration certificates | Maximum %RSD reported on external calibration certificates across all pipettes with relevant volume range. |
| | Dilution of working calibrator solution C or F to make working calibrator solution B or E (Volumetric Flask) | Type B: Volumetric flask certificate of calibration | Maximum reported uncertainty on certificates of calibration of NIST traceable flasks as described in SOP version 3.5. |
| | Dilution of working calibrator solution B or E to make working calibrator solution A or D (Pipette) | Type A: External calibration certificates | Maximum %RSD reported on external calibration certificates across all pipettes with relevant volume range. |
| | Dilution of working calibrator solution B or E to make working calibrator solution A or D (Volumetric Flask) | Type B: Volumetric flask certificate of calibration | Maximum reported uncertainty on certificates of calibration of NIST traceable flasks as described in SOP version 3.5. |
| | Volume of working calibrator solution A, B, C, D, E, or F to make calibrators (Pipette) | Type A: External calibration certificates | Maximum %RSD reported on external calibration certificates across all pipettes with relevant volume range. |
| | Matrix Effects | No influence | Method calibrated and controlled in blank blood. |
| Internal Standard | Purity of CRM | No influence | Same IS lot used throughout batch. |
| | Preparation of Internal Standard | No influence | Same IS lot used throughout batch. |
| Case Sampling | Pipetting of case sample | Type A: External calibration certificates | Maximum %RSD reported on external calibration certificates across all pipettes with relevant volume range. |
| | Homogenization | Type A: Measurement Reproducibility | |
| | Temperature | Type A: Measurement Reproducibility | |
| Sample Preparation | Mixing and homogeneity in vial | Type A: Measurement Reproducibility | All uncertainty generated by the sample preparation procedure is controlled by use of internal standard. |
| | Sources of uncertainty during sample preparation may include but are not limited to: addition of solvents, vortexing, transferring solvent layers. | Type A: Measurement Reproducibility | |
| Instrumental Analysis | Instrument parameter settings (e.g., oven temperatures, injection volume, injection types, incubation time and temperature, column condition, autosampler syringe, autosampler reproducibility, etc). | Type A: Measurement Reproducibility | |
| Analyte Stability | Sample stability during analysis | Type A: Measurement Reproducibility | |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Morphine

Analytical Range: 5-500 ng/mL

Sample volume (in μL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 $\mu\text{g/mL}$): Add 50 μL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 $\mu\text{g/mL}$): Dilute 1 mL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 $\mu\text{g/mL}$): Dilute 200 μL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 μL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 μL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 μL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 μL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 μL of working solution C into 1 mL blood

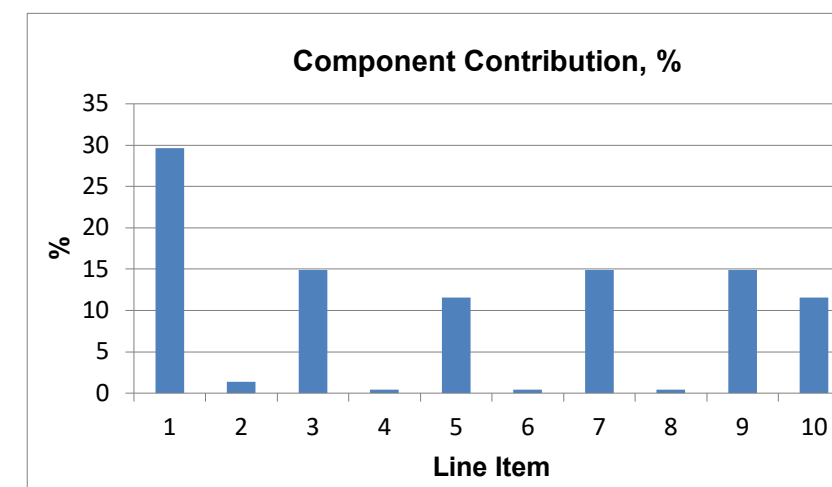
Calibrator 6 (250 ng/mL): 50 μL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 μL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of morphine in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|---|
| 1 | Measurement Reproducibility | 5.308 | % | Normal | A | 1.00 | 57 | 5.308 | 30 | |
| 2 | CRM Uncertainty | 0.500 | % | Normal | B | 2.00 | infinite | 0.250 | 1 | |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | u_c | | | | | | | 7.630 | % |
| Expanded Uncertainty (95.45% CI) | | $UM (k=2)$ | | | | | | | 15.259 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Morphine

Pooled %RSD: **5.308**

k

7

| Control | LQC | LQC | MQC | MQC | HQC | HQC | PM 100 |
|----------------------------------|-------------------------|-------------------------|---------------------------|-------------------------|----------------------------|----------------------------|----------------------------------|
| Lot | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH | C3546 |
| Avg Control Result | 14.46 | 15.01 | 93.28 | 91.35 | 355.17 | 358.77 | 77.18 |
| Control SD | 0.55 | 0.93 | 6.27 | 3.31 | 17.40 | 21.41 | 3.86 |
| %RSD | 3.83 | 6.18 | 6.73 | 3.63 | 4.90 | 5.97 | 5.00 |
| (N-1)x(%RSD)² | 338.14 | 572.15 | 1040.35 | 197.51 | 479.97 | 534.13 | 275.00 |
| C_{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 | 77.18 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 | 61.74 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 | 92.62 |
| N | 24 | 16 | 24 | 16 | 21 | 16 | 12 |
| OPI_20201102B_SD | 14.29 15.36 14.69 | | 94.61 90.17 88.88 | | 366.71 341.97 354.36 | | |
| OPI_20201104B_PK | 14.99 13.45 14.79 | | 91.32 89.68 91.63 | | 361.29 356.34 341.57 | | |
| OPI_20201105B_PK | 14.38 15.01 13.83 | | 90.61 88.45 92.88 | | 334.56 320.59 329.50 | | 75.22 78.23 76.82 78.29 |
| OPI_20201109B_SD | 14.49 15.27 14.21 | | 88.50 92.59 89.42 | | 374.55 347.17 346.01 | | |
| OPI1_20201112B_PK | 13.90 14.28 13.52 | | 90.61 95.46 92.79 | | ND ND ND | | 79.96 85.05 75.54 82.01 |
| OPI1_20201113B_CLR | 15.17 13.87 13.94 | | 110.03 90.38 111.36 | | 351.27 347.23 367.99 | | 75.91 74.95 73.38 70.79 |
| OPI1_20201116B_SD | 14.39 15.16 14.86 | | 98.47 99.06 96.75 | | 379.70 382.23 388.08 | | |
| OPI1_20201119B_SD | 14.36 14.81 13.98 | | 91.20 86.36 87.50 | | 360.43 349.80 357.23 | | |
| OPI_20201130B_PK | | 16.52 16.74 16.46 | | 98.03 94.53 96.92 | | 359.28 386.89 381.40 | |
| OPI1_20201201B_SD | | 14.62 14.68 | | 94.40 91.06 | | 375.29 358.72 | |
| OPI1_20201202B_PK | | 14.83 15.01 14.00 | | 91.18 89.60 87.81 | | 355.94 406.28 358.26 | |
| OPI1_20201203B_PK | | 14.97 16.07 14.31 | | 91.09 90.11 88.71 | | 351.92 340.14 320.26 | |
| OPI1_20201214B_PK | | 14.80 14.52 13.77 | | 86.75 86.80 90.88 | | 333.23 339.33 362.66 | |
| OPI1_20201215B_PK | | 14.31 14.48 | | 90.64 93.01 | | 361.93 348.71 | |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|----------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Morphine | Cerilliant | FE06231704 | 1.000 | 0.005 | 0.500 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Oxymorphone

Analytical Range: 5-500 ng/mL

Sample volume (in µL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 µg/mL): Add 50 µL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 µg/mL): Dilute 1 mL of 5 µg/mL solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 µg/mL): Dilute 200 µL of 5 µg/mL solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 µL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 µL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 µL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 µL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 µL of working solution C into 1 mL blood

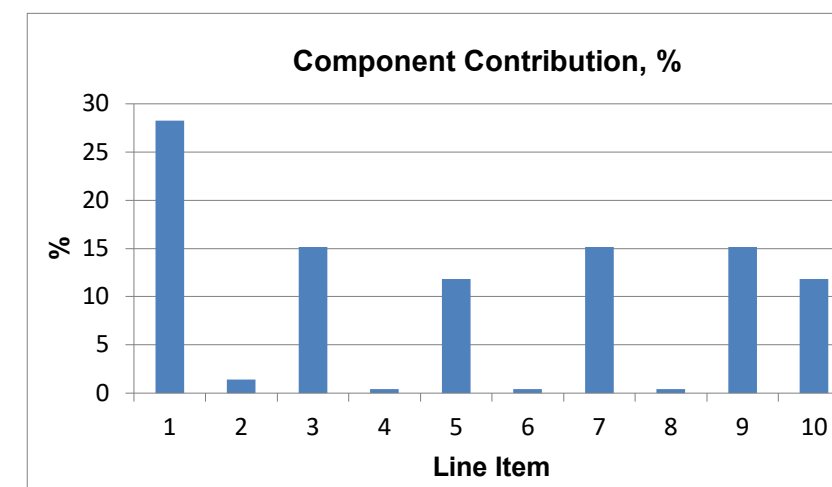
Calibrator 6 (250 ng/mL): 50 µL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 µL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of oxymorphone in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|---|
| 1 | Measurement Reproducibility | 4.969 | % | Normal | A | 1.00 | 57 | 4.969 | 28 | |
| 2 | CRM Uncertainty | 0.500 | % | Normal | B | 2.00 | infinite | 0.250 | 1 | |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | u_c | | | | | | | 7.398 | % |
| Expanded Uncertainty (95.45% CI) | | $UM (k=2)$ | | | | | | | 14.795 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Oxymorphone

Pooled %RSD: **4.969**

k

7

| Control | LQC | LQC | MQC | MQC | HQC | HQC | PM 100 |
|---------------------------|-------------------------|-------------------------|----------------------------|----------------------------|----------------------------|------------------------------|----------------------------------|
| Lot | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH | C3546 |
| Avg Control Result | 14.95 | 14.82 | 99.71 | 94.06 | 358.08 | 346.65 | 76.49 |
| Control SD | 0.51 | 1.01 | 5.66 | 5.98 | 12.08 | 14.20 | 3.57 |
| %RSD | 3.45 | 6.81 | 5.68 | 6.36 | 3.37 | 4.10 | 4.67 |
| (N-1)x(%RSD) ² | 272.97 | 696.50 | 741.65 | 606.27 | 261.77 | 218.07 | 239.44 |
| C _{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 | 76.49 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 | 61.19 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 | 91.79 |
| N | 24 | 16 | 24 | 16 | 24 | 14 | 12 |
| OPI_20201102B_SD | 15.54 15.92 16.13 | | 106.97 101.21 103.08 | | 368.33 346.72 357.16 | | |
| OPI_20201104B_PK | 15.09 14.65 15.54 | | 98.22 96.98 97.83 | | 362.06 347.35 356.77 | | |
| OPI_20201105B_PK | 14.92 15.41 14.65 | | 96.87 98.21 104.08 | | 359.51 345.82 349.44 | | 79.91 79.83 78.44 78.97 |
| OPI_20201109B_SD | 14.70 15.17 15.21 | | 98.09 100.65 99.93 | | 375.14 354.57 361.91 | | |
| OPI1_20201112B_PK | 14.15 14.65 14.37 | | 94.93 100.89 99.34 | | 371.72 375.17 360.93 | | 77.50 82.50 71.53 74.33 |
| OPI1_20201113B_CLR | 15.08 14.55 14.58 | | 118.97 99.62 106.94 | | 367.71 363.63 366.90 | | 75.86 74.55 72.72 71.75 |
| OPI1_20201116B_SD | 15.01 15.20 14.98 | | 98.66 97.64 96.58 | | 350.99 379.59 355.14 | | |
| OPI1_20201119B_SD | 14.49 14.67 14.09 | | 94.37 91.16 91.93 | | 344.25 344.13 328.95 | | |
| OPI_20201130B_PK | | 15.78 15.47 16.45 | | 103.72 100.55 100.43 | | 366.67 370.08 358.01 | |
| OPI1_20201201B_SD | | 14.91 14.23 | | 97.18 95.84 | | 364.38 350.65 | |
| OPI1_20201202B_PK | | 15.80 15.87 15.42 | | 98.90 99.27 95.35 | | 346.63 326.30 349.54 | |
| OPI1_20201203B_PK | | 15.41 15.20 14.58 | | 93.21 95.32 92.67 | | 343.50 335.71 326.12 | |
| OPI1_20201214B_PK | | 13.81 13.20 13.74 | | 86.01 87.15 88.46 | | 318.51* 316.70* 336.16 | |
| OPI1_20201215B_PK | | 13.88 13.33 | | 85.22 85.74 | | 343.58 335.78 | |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|-------------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Oxymorphone | Cerilliant | FE07131606 | 1.000 | 0.005 | 0.500 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Hydromorphone

Analytical Range: 5-500 ng/mL

Sample volume (in μL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 $\mu\text{g/mL}$): Add 50 μL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 $\mu\text{g/mL}$): Dilute 1 mL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 $\mu\text{g/mL}$): Dilute 200 μL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 μL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 μL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 μL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 μL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 μL of working solution C into 1 mL blood

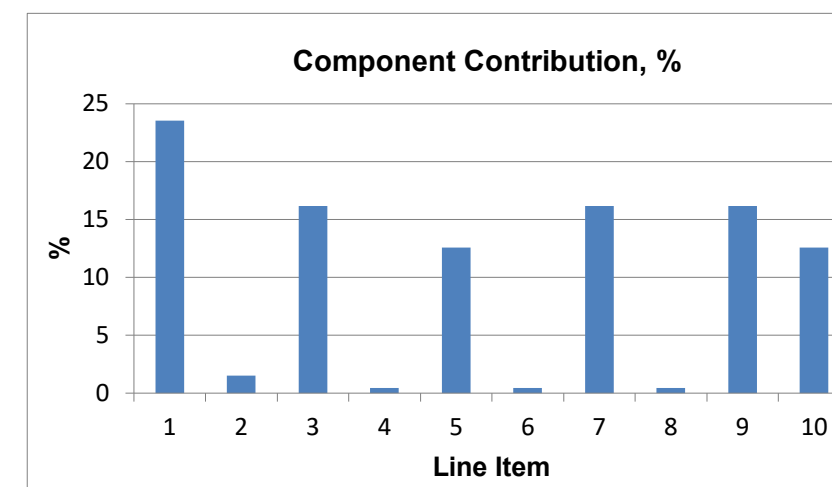
Calibrator 6 (250 ng/mL): 50 μL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 μL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of hydromorphone in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|---|
| 1 | Measurement Reproducibility | 3.880 | % | Normal | A | 1.00 | 57 | 3.880 | 24 | |
| 2 | CRM Uncertainty | 0.500 | % | Normal | B | 2.00 | infinite | 0.250 | 2 | |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 13 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 13 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | u_c | | | | | | | 6.715 | % |
| Expanded Uncertainty (95.45% CI) | | $UM (k=2)$ | | | | | | | 13.429 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Hydromorphone

Pooled %RSD: **3.880**

k

7

| Control | LQC | LQC | MQC | MQC | HQC | HQC | PM 100 |
|----------------------------------|-------------------------|-------------------------|--------------------------|---------------------------|----------------------------|----------------------------|----------------------------------|
| Lot | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH | C3546 |
| Avg Control Result | 14.46 | 15.06 | 94.80 | 94.95 | 359.93 | 356.72 | 77.31 |
| Control SD | 0.49 | 0.91 | 2.82 | 3.79 | 11.33 | 13.11 | 3.27 |
| %RSD | 3.42 | 6.02 | 2.97 | 4.00 | 3.15 | 3.68 | 4.23 |
| (N-1)x(%RSD)² | 269.43 | 542.80 | 202.85 | 239.47 | 227.84 | 202.70 | 196.39 |
| C_{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 | 77.31 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 | 61.85 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 | 92.77 |
| N | 24 | 16 | 24 | 16 | 24 | 16 | 12 |
| OPI_20201102B_SD | 14.81 14.32 14.51 | | 98.44 93.77 90.01 | | 376.02 334.08 338.02 | | |
| OPI_20201104B_PK | 15.31 14.01 15.31 | | 93.69 95.84 97.33 | | 367.56 363.01 357.28 | | |
| OPI_20201105B_PK | 14.84 15.21 13.80 | | 95.68 96.08 98.39 | | 368.26 356.93 355.15 | | 80.06 80.57 79.51 80.26 |
| OPI_20201109B_SD | 14.42 14.39 13.73 | | 91.66 94.75 93.47 | | 370.77 359.29 356.85 | | |
| OPI1_20201112B_PK | 14.23 14.17 13.89 | | 92.49 96.57 96.55 | | 368.12 360.51 361.02 | | 76.11 81.35 70.30 78.22 |
| OPI1_20201113B_CLR | 14.92 14.77 14.16 | | 100.96 98.92 94.18 | | 380.62 374.88 368.98 | | 75.74 76.31 75.19 74.16 |
| OPI1_20201116B_SD | 13.95 15.18 14.36 | | 95.57 93.36 92.00 | | 343.56 352.58 356.28 | | |
| OPI1_20201119B_SD | 14.20 14.69 13.84 | | 93.30 90.08 92.07 | | 357.86 349.98 360.82 | | |
| OPI_20201130B_PK | | 15.94 16.28 15.94 | | 99.85 100.66 100.83 | | 372.49 379.36 379.75 | |
| OPI1_20201201B_SD | | 15.07 14.12 | | 96.38 97.61 | | 363.65 358.43 | |
| OPI1_20201202B_PK | | 15.04 15.22 15.57 | | 96.23 98.31 92.48 | | 349.12 345.56 350.21 | |
| OPI1_20201203B_PK | | 15.44 15.62 14.44 | | 93.01 94.01 90.75 | | 359.87 348.67 332.51 | |
| OPI1_20201214B_PK | | 13.94 13.05 14.10 | | 88.61 90.60 91.18 | | 348.59 343.63 354.15 | |
| OPI1_20201215B_PK | | 16.08 15.16 | | 94.19 94.48 | | 366.88 354.62 | |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|---------------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Hydromorphone | Cerilliant | FE03301705 | 1.000 | 0.005 | 0.500 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: O-desmethyltramadol

Analytical Range: 5-500 ng/mL

Sample volume (in μL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 $\mu\text{g/mL}$): Add 50 μL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 $\mu\text{g/mL}$): Dilute 1 mL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 $\mu\text{g/mL}$): Dilute 200 μL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 μL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 μL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 μL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 μL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 μL of working solution C into 1 mL blood

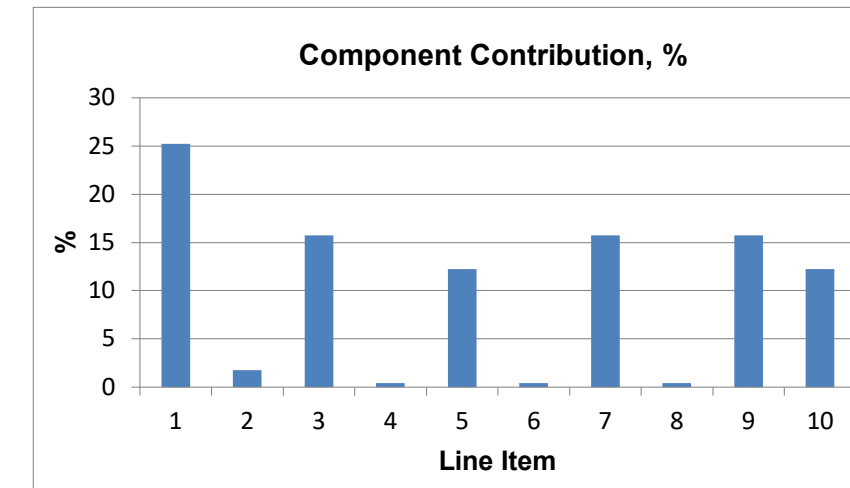
Calibrator 6 (250 ng/mL): 50 μL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 μL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of O-desmethyltramadol in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|---|
| 1 | Measurement Reproducibility | 4.269 | % | Normal | A | 1.00 | 57 | 4.269 | 25 | |
| 2 | CRM Uncertainty | 0.600 | % | Normal | B | 2.00 | infinite | 0.300 | 2 | |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | u_c | | | | | | | 6.949 | % |
| Expanded Uncertainty (95.45% CI) | | $UM (k=2)$ | | | | | | | 13.898 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

O-desmethyltramadol

Pooled %RSD: **4.269**

k

6

| Control Lot | LQC 200916K-Q-MXL | LQC 201130K-Q-MXL | MQC 200916K-Q-MXM | MQC 201130K-Q-MXM | HQC 200916K-Q-MXH | HQC 201130K-Q-MXH |
|----------------------------------|-------------------------|-------------------------|----------------------------|-------------------------|----------------------------|----------------------------|
| Avg Control Result | 15.21 | 15.40 | 96.75 | 93.12 | 386.91 | 365.11 |
| Control SD | 0.47 | 0.59 | 3.47 | 4.29 | 18.81 | 20.53 |
| %RSD | 3.07 | 3.86 | 3.58 | 4.61 | 4.86 | 5.62 |
| (N-1)x(%RSD)² | 216.65 | 223.91 | 282.75 | 318.47 | 543.87 | 474.16 |
| C_{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 |
| N | 24 | 16 | 23 | 16 | 24 | 16 |
| OPI_20201102B_SD | 15.92 15.62 15.69 | | 96.70 98.41 96.91 | | 397.61 366.10 379.57 | |
| OPI_20201104B_PK | 15.68 15.12 16.03 | | 97.97 99.30 98.45 | | 420.53 404.93 401.88 | |
| OPI_20201105B_PK | 15.30 15.67 14.83 | | 98.99 94.53 101.80 | | 371.57 378.48 376.23 | |
| OPI_20201109B_SD | 15.11 15.63 15.12 | | 92.77 96.24 98.22 | | 425.56 402.82 394.52 | |
| OPI1_20201112B_PK | 14.96 15.05 14.71 | | 95.08 102.25 96.88 | | 413.88 403.29 400.85 | |
| OPI1_20201113B_CLR | 15.28 14.62 14.03 | | 104.89 90.48 103.74* | | 374.46 376.22 375.15 | |
| OPI1_20201116B_SD | 14.82 15.39 15.16 | | 91.45 96.39 92.94 | | 364.43 366.57 367.76 | |
| OPI1_20201119B_SD | 14.84 15.62 14.93 | | 96.66 93.32 94.72 | | 365.03 370.40 387.99 | |
| OPI_20201130B_PK | | 15.91 16.18 16.88 | | 99.46 97.12 97.22 | | 385.54 387.84 384.87 |
| OPI1_20201201B_SD | | 14.88 15.36 | | 94.02 100.87 | | 396.91 392.84 |
| OPI1_20201202B_PK | | 15.53 15.75 15.50 | | 96.21 95.65 91.43 | | 368.53 345.28 362.68 |
| OPI1_20201203B_PK | | 14.87 15.38 14.52 | | 88.76 92.44 89.00 | | 361.10 355.81 340.27 |
| OPI1_20201214B_PK | | 15.52 15.26 14.89 | | 91.45 92.55 88.99 | | 364.72 326.40 368.89 |
| OPI1_20201215B_PK | | 14.74 15.20 | | 86.73 88.02 | | 357.66 342.37 |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|---------------------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| O-desmethyltramadol | Cerilliant | FN08261902 | 1.000 | 0.006 | 0.600 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Codeine

Analytical Range: 5-500 ng/mL

Sample volume (in μL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 $\mu\text{g/mL}$): Add 50 μL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 $\mu\text{g/mL}$): Dilute 1 mL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 $\mu\text{g/mL}$): Dilute 200 μL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 μL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 μL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 μL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 μL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 μL of working solution C into 1 mL blood

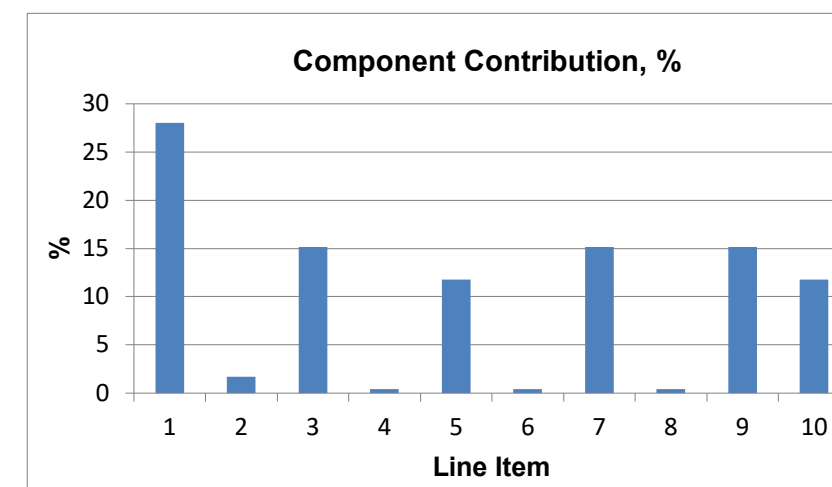
Calibrator 6 (250 ng/mL): 50 μL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 μL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of codeine in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|---|
| 1 | Measurement Reproducibility | 4.935 | % | Normal | A | 1.00 | 56 | 4.935 | 28 | |
| 2 | CRM Uncertainty | 0.600 | % | Normal | B | 2.00 | infinite | 0.300 | 2 | |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | u_c | | | | | | | 7.377 | % |
| Expanded Uncertainty (95.45% CI) | | $UM (k=2)$ | | | | | | | 14.753 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Codeine

Pooled %RSD: **4.935**

k

7

| Control | LQC | LQC | MQC | MQC | HQC | HQC | PM 100 |
|---------------------------|-------------------------|-------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------------|
| Lot | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH | C3546 |
| Avg Control Result | 14.16 | 15.08 | 98.38 | 99.43 | 386.20 | 397.92 | 75.61 |
| Control SD | 0.67 | 0.84 | 5.18 | 4.94 | 19.15 | 9.99 | 4.56 |
| %RSD | 4.75 | 5.54 | 5.27 | 4.97 | 4.96 | 2.51 | 6.03 |
| (N-1)x(%RSD) ² | 518.49 | 460.34 | 610.27 | 370.61 | 565.36 | 94.53 | 399.90 |
| C _{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 | 75.61 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 | 60.49 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 | 90.73 |
| N | 24 | 16 | 23 | 16 | 24 | 16 | 12 |
| OPI_20201102B_SD | 13.51 13.74 13.81 | | 94.80 89.81 87.46 | | 389.72 359.12 341.40 | | |
| OPI_20201104B_PK | 14.70 13.20 14.13 | | 98.60 97.94 99.06 | | 374.81 374.05 348.99 | | |
| OPI_20201105B_PK | 14.62 14.43 13.07 | | 98.34 100.46 105.24 | | 383.61 365.00 375.08 | | 75.35 74.77 77.08 74.81 |
| OPI_20201109B_SD | 14.02 14.31 13.96 | | 100.92 99.21 98.81 | | 414.92 399.94 393.52 | | |
| OPI1_20201112B_PK | 13.63 13.89 13.46 | | 101.49 102.73 100.43 | | 396.73 413.27 382.12 | | 69.37 74.56 66.96 72.55 |
| OPI1_20201113B_CLR | 15.99 14.99 14.19 | | 98.75 111.71 97.40* | | 402.18 414.04 396.87 | | 81.01 82.24 79.63 79.00 |
| OPI1_20201116B_SD | 14.15 14.97 14.37 | | 103.30 95.66 91.53 | | 398.12 397.96 382.05 | | |
| OPI1_20201119B_SD | 13.41 15.03 14.16 | | 93.67 98.31 94.57 | | 393.00 389.49 382.83 | | |
| OPI_20201130B_PK | | 16.06 16.41 16.41 | | 110.03 102.91 109.13 | | 408.52 412.19 405.75 | |
| OPI1_20201201B_SD | | 14.39 13.96 | | 99.38 89.83 | | 392.19 383.98 | |
| OPI1_20201202B_PK | | 15.05 15.26 15.05 | | 100.92 98.26 97.21 | | 390.65 401.60 389.75 | |
| OPI1_20201203B_PK | | 15.35 15.71 13.58 | | 95.61 95.45 98.04 | | 400.20 394.62 382.34 | |
| OPI1_20201214B_PK | | 15.29 15.20 14.51 | | 98.29 99.28 101.19 | | 402.00 387.27 395.82 | |
| OPI1_20201215B_PK | | 15.03 14.02 | | 99.03 96.25 | | 416.16 403.68 | |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|---------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Codeine | Cerilliant | FE05241802 | 1.000 | 0.006 | 0.600 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: 6-acetylmorphine

Analytical Range: 5-500 ng/mL

Sample volume (in µL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 µg/mL): Add 50 µL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 µg/mL): Dilute 1 mL of 5 µg/mL solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 µg/mL): Dilute 200 µL of 5 µg/mL solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 µL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 µL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 µL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 µL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 µL of working solution C into 1 mL blood

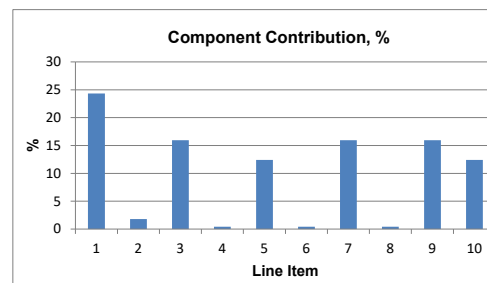
Calibrator 6 (250 ng/mL): 50 µL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 µL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of 6-acetylmorphine in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % |
|-----------|-----------------------------|-------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|
| 1 | Measurement Reproducibility | 4.073 | % | Normal | A | 1.00 | 58 | 4.073 | 24 |
| 2 | CRM Uncertainty | 0.600 | % | Normal | B | 2.00 | infinite | 0.300 | 2 |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 |



100

| | | | |
|----------------------------------|------------|--------|---|
| Combined Standard Uncertainty | u_c | 6.830 | % |
| Expanded Uncertainty (95.45% CI) | $UM (k=2)$ | 13.660 | % |

NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

6-acetylmorphine

Pooled %RSD: **4.073**

| Control Lot | k | | | | | |
|----------------------------------|-------------------------|-------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | LQC | LQC | MQC | MQC | HQC | HQC |
| | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH |
| Avg Control Result | 14.50 | 16.21 | 98.09 | 103.45 | 414.73 | 395.65 |
| Control SD | 0.47 | 0.92 | 4.19 | 4.42 | 15.39 | 13.03 |
| %RSD | 3.23 | 5.65 | 4.27 | 4.27 | 3.71 | 3.29 |
| (N-1)x(%RSD)² | 240.07 | 478.78 | 418.68 | 273.75 | 316.83 | 162.78 |
| C_{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 |
| N | 24 | 16 | 24 | 16 | 24 | 16 |
| OPI_20201102B_SD | 14.05 14.29 14.97 | | 96.74 98.83 99.13 | | 406.85 397.54 407.31 | |
| OPI_20201104B_PK | 14.42 13.78 14.94 | | 96.12 97.25 98.01 | | 421.17 399.64 398.29 | |
| OPI_20201105B_PK | 14.15 14.73 13.60 | | 96.57 96.79 102.01 | | 407.25 390.27 403.21 | |
| OPI_20201109B_SD | 14.59 15.00 14.46 | | 101.16 102.48 101.08 | | 433.46 431.41 423.12 | |
| OPI1_20201112B_PK | 14.44 14.81 13.92 | | 99.01 104.21 99.70 | | 427.44 443.71 430.26 | |
| OPI1_20201113B_CLR | 15.31 14.55 13.80 | | 84.31 104.44 91.24 | | 433.86 434.63 428.71 | |
| OPI1_20201116B_SD | 14.10 14.98 14.52 | | 99.77 97.92 97.30 | | 405.12 401.83 406.55 | |
| OPI1_20201119B_SD | 14.50 15.25 14.76 | | 94.32 98.97 96.88 | | 398.83 401.21 421.77 | |
| OPI_20201130B_PK | | 17.29 16.98 17.73 | | 109.65 105.32 108.85 | | 408.66 418.13 410.45 |
| OPI1_20201201B_SD | | 14.41 14.56 | | 100.35 93.41 | | 412.33 386.14 |
| OPI1_20201202B_PK | | 16.41 16.96 15.96 | | 107.87 105.21 103.78 | | 382.64 387.04 395.79 |
| OPI1_20201203B_PK | | 16.53 16.71 15.60 | | 100.22 103.88 101.71 | | 386.15 391.59 380.18 |
| OPI1_20201214B_PK | | 16.93 16.15 15.79 | | 105.51 107.92 104.51 | | 409.92 391.44 405.37 |
| OPI1_20201215B_PK | | 15.71 15.71 | | 99.00 97.98 | | 385.75 378.83 |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|------------------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| 6-acetylmorphine | Cerilliant | FE07161901 | 1.000 | 0.006 | 0.600 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Oxycodone

Analytical Range: 5-500 ng/mL

Sample volume (in μL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 $\mu\text{g/mL}$): Add 50 μL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 $\mu\text{g/mL}$): Dilute 1 mL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 $\mu\text{g/mL}$): Dilute 200 μL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 μL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 μL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 μL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 μL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 μL of working solution C into 1 mL blood

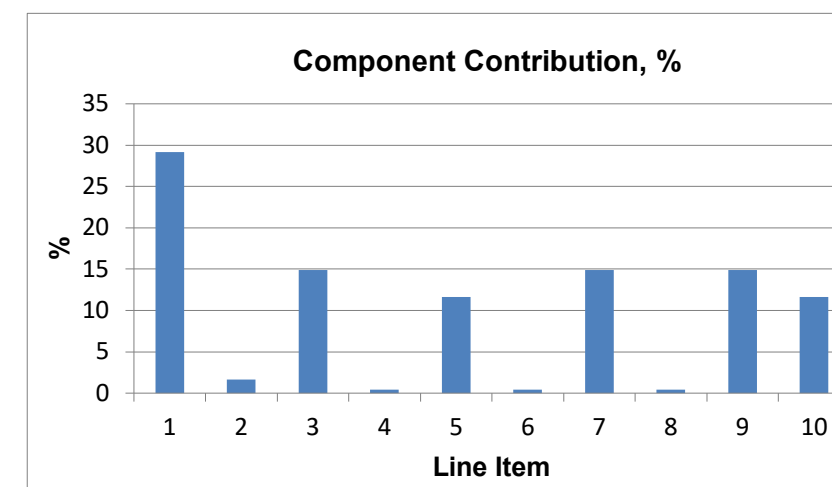
Calibrator 6 (250 ng/mL): 50 μL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 μL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of oxycodone in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|----------------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|----------|
| 1 | Measurement Reproducibility | 5.214 | % | Normal | A | 1.00 | 57 | 5.214 | 29 | |
| 2 | CRM Uncertainty | 0.600 | % | Normal | B | 2.00 | infinite | 0.300 | 2 | |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | <i>u_c</i> | | | | | | | 7.566 | % |
| Expanded Uncertainty (95.45% CI) | | UM (k=2) | | | | | | | 15.133 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Oxycodone

Pooled %RSD: **5.214**

k

7

| Control | LQC | LQC | MQC | MQC | HQC | HQC | PM 100 |
|----------------------------------|-------------------------|-------------------------|--------------------------|-------------------------|----------------------------|----------------------------|----------------------------------|
| Lot | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH | C3546 |
| Avg Control Result | 14.09 | 14.18 | 92.56 | 88.72 | 365.37 | 360.04 | 78.66 |
| Control SD | 0.71 | 0.67 | 4.69 | 2.97 | 14.03 | 29.37 | 4.67 |
| %RSD | 5.04 | 4.69 | 5.07 | 3.35 | 3.84 | 8.16 | 5.93 |
| (N-1)x(%RSD)² | 584.69 | 329.86 | 591.25 | 168.25 | 339.24 | 998.13 | 386.94 |
| C_{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 | 78.66 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 | 62.93 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 | 94.39 |
| N | 24 | 16 | 24 | 16 | 24 | 16 | 12 |
| OPI_20201102B_SD | 15.22 14.35 14.59 | | 100.24 94.09 94.22 | | 408.21 349.57 359.29 | | |
| OPI_20201104B_PK | 15.07 14.23 15.19 | | 94.34 95.58 98.09 | | 393.03 377.99 365.00 | | |
| OPI_20201105B_PK | 15.09 15.05 14.31 | | 94.63 96.22 101.41 | | 384.00 369.98 371.12 | | 83.42 83.89 84.59 82.33 |
| OPI_20201109B_SD | 13.87 13.37 13.35 | | 85.87 88.97 87.47 | | 358.48 354.06 350.26 | | |
| OPI1_20201112B_PK | 13.90 13.46 13.20 | | 89.93 87.71 90.66 | | 376.18 364.92 358.67 | | 76.66 83.18 71.87 79.07 |
| OPI1_20201113B_CLR | 14.40 13.57 12.84 | | 97.17 91.02 100.81 | | 363.26 362.58 361.28 | | 76.17 76.05 74.01 72.71 |
| OPI1_20201116B_SD | 13.66 14.58 14.05 | | 90.86 90.30 87.27 | | 360.02 353.27 360.36 | | |
| OPI1_20201119B_SD | 13.45 14.10 13.24 | | 88.17 87.90 88.40 | | 355.80 353.26 358.39 | | |
| OPI_20201130B_PK | | 15.10 15.12 15.19 | | 94.12 90.36 93.32 | | 364.02 367.93 459.88 | |
| OPI1_20201201B_SD | | 13.76 13.65 | | 92.70 91.51 | | 385.50 356.78 | |
| OPI1_20201202B_PK | | 14.33 14.60 14.51 | | 89.35 89.20 85.10 | | 355.82 346.52 351.13 | |
| OPI1_20201203B_PK | | 14.07 14.91 13.63 | | 87.57 88.55 86.59 | | 349.33 341.82 336.55 | |
| OPI1_20201214B_PK | | 14.10 13.10 13.65 | | 86.49 85.61 88.11 | | 356.02 351.19 358.85 | |
| OPI1_20201215B_PK | | 13.88 13.33 | | 85.22 85.74 | | 343.58 335.78 | |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|-----------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Oxycodone | Cerilliant | FE08241701 | 1.000 | 0.006 | 0.600 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Hydrocodone

Analytical Range: 5-500 ng/mL

Sample volume (in µL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 µg/mL): Add 50 µL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 µg/mL): Dilute 1 mL of 5 µg/mL solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 µg/mL): Dilute 200 µL of 5 µg/mL solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 µL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 µL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 µL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 µL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 µL of working solution C into 1 mL blood

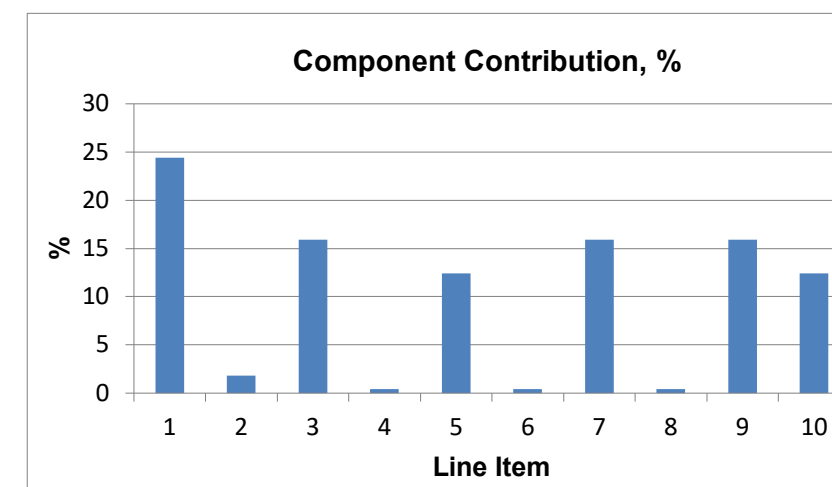
Calibrator 6 (250 ng/mL): 50 µL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 µL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of hydrocodone in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|---|
| 1 | Measurement Reproducibility | 4.092 | % | Normal | A | 1.00 | 57 | 4.092 | 24 | |
| 2 | CRM Uncertainty | 0.600 | % | Normal | B | 2.00 | infinite | 0.300 | 2 | |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | u_c | | | | | | | 6.842 | % |
| Expanded Uncertainty (95.45% CI) | | $UM (k=2)$ | | | | | | | 13.683 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Hydrocodone

Pooled %RSD: **4.092**
k
7

| Control | LQC | LQC | MQC | MQC | HQC | HQC | PM 100 |
|----------------------------------|-------------------------|-------------------------|--------------------------|--------------------------|----------------------------|----------------------------|----------------------------------|
| Lot | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH | C3546 |
| Avg Control Result | 14.04 | 14.70 | 93.22 | 92.53 | 369.47 | 369.55 | 86.87 |
| Control SD | 0.56 | 0.64 | 3.55 | 4.05 | 15.21 | 12.41 | 4.23 |
| %RSD | 4.02 | 4.33 | 3.81 | 4.38 | 4.12 | 3.36 | 4.87 |
| (N-1)x(%RSD)² | 371.59 | 281.34 | 333.44 | 287.18 | 389.82 | 169.17 | 260.67 |
| C_{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 | 86.87 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 | 69.50 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 | 104.24 |
| N | 24 | 16 | 24 | 16 | 24 | 16 | 12 |
| OPI_20201102B_SD | 14.33 13.80 13.13 | | 97.12 87.78 86.69 | | 372.58 332.90 330.96 | | |
| OPI_20201104B_PK | 14.15 13.30 14.72 | | 92.18 94.17 91.42 | | 374.10 369.42 357.99 | | |
| OPI_20201105B_PK | 14.27 14.67 13.40 | | 93.49 93.05 99.06 | | 378.01 359.77 359.72 | | 92.27 84.98 84.01 89.25 |
| OPI_20201109B_SD | 13.91 13.85 13.01 | | 90.90 92.45 91.77 | | 375.29 360.68 356.73 | | |
| OPI1_20201112B_PK | 13.83 14.38 13.40 | | 94.38 88.51 91.73 | | 385.59 357.70 374.60 | | 91.73 93.51 81.79 90.43 |
| OPI1_20201113B_CLR | 14.79 14.41 13.39 | | 101.30 94.64 91.24 | | 384.28 379.71 378.55 | | 82.62 83.83 84.64 83.44 |
| OPI1_20201116B_SD | 14.19 14.89 14.26 | | 97.51 98.39 93.94 | | 380.37 373.29 375.59 | | |
| OPI1_20201119B_SD | 14.07 14.96 13.92 | | 92.67 92.99 89.80 | | 390.00 370.59 388.89 | | |
| OPI_20201130B_PK | | 15.71 15.64 15.84 | | 102.36 96.02 96.59 | | 379.83 394.95 394.52 | |
| OPI1_20201201B_SD | | 13.95 14.41 | | 97.13 93.48 | | 376.86 362.66 | |
| OPI1_20201202B_PK | | 14.80 15.22 14.48 | | 94.62 94.00 90.39 | | 361.46 369.68 370.96 | |
| OPI1_20201203B_PK | | 14.42 14.62 13.90 | | 92.21 88.65 89.04 | | 365.47 371.32 360.12 | |
| OPI1_20201214B_PK | | 14.98 14.25 13.78 | | 88.32 88.35 91.06 | | 354.56 352.68 367.05 | |
| OPI1_20201215B_PK | | 14.46 14.77 | | 89.38 88.95 | | 372.65 358.00 | |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|-------------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Hydrocodone | Cerilliant | FE0421902 | 1.000 | 0.006 | 0.600 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Tramadol

Analytical Range: 5-500 ng/mL

Sample volume (in μL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution C (5 $\mu\text{g/mL}$): Add 50 μL of 1 mg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution B (0.5 $\mu\text{g/mL}$): Dilute 1 mL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Working Calibrator Solution A (0.1 $\mu\text{g/mL}$): Dilute 200 μL of 5 $\mu\text{g/mL}$ solution into 10 mL volumetric flask.

Calibrator 1 (5 ng/mL): 50 μL of working solution A into 1 mL blood

Calibrator 2 (10 ng/mL): 100 μL of working solution A into 1 mL blood

Calibrator 3 (25 ng/mL): 50 μL of working solution B into 1 mL blood

Calibrator 4 (50 ng/mL): 100 μL of working solution B into 1 mL blood

Calibrator 5 (125 ng/mL): 25 μL of working solution C into 1 mL blood

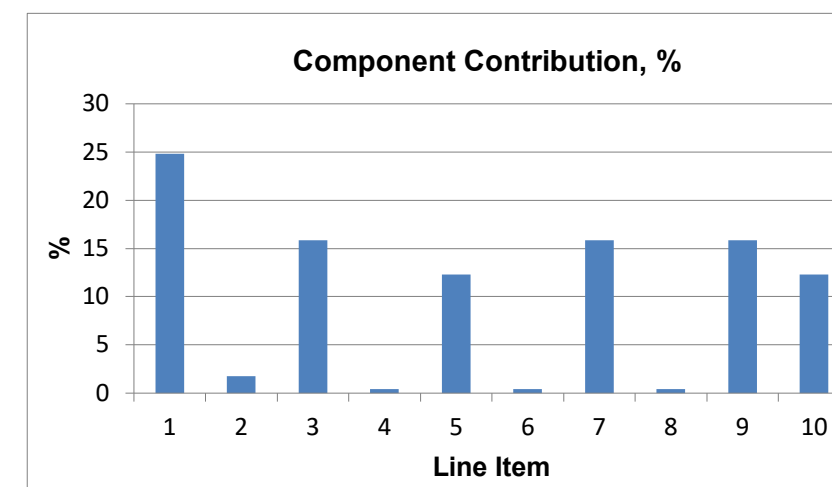
Calibrator 6 (250 ng/mL): 50 μL of working solution C into 1 mL blood

Calibrator 7 (500 ng/mL): 100 μL of working solution C into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of tramadol in blood
Analytical Range: 5-500 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|---|
| 1 | Measurement Reproducibility | 4.177 | % | Normal | A | 1.00 | 57 | 4.177 | 25 | |
| 2 | CRM Uncertainty | 0.600 | % | Normal | B | 2.00 | infinite | 0.300 | 2 | |
| 3 | 50 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 200 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | u_c | | | | | | | 6.893 | % |
| Expanded Uncertainty (95.45% CI) | | $UM (k=2)$ | | | | | | | 13.785 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution C using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution C in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution B using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution B in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution C to working calibrator solution A using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution C to working calibrator solution A in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution A, B, or C to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Tramadol

Pooled %RSD: **4.177**

k

7

| Control | LQC | LQC | MQC | MQC | HQC | HQC | PM 100 |
|----------------------------------|-------------------------|-------------------------|---------------------------|-------------------------|----------------------------|----------------------------|----------------------------------|
| Lot | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH | C3546 |
| Avg Control Result | 14.98 | 15.61 | 96.70 | 93.71 | 378.19 | 382.49 | 82.74 |
| Control SD | 0.48 | 0.82 | 4.83 | 3.25 | 13.56 | 13.99 | 4.12 |
| %RSD | 3.17 | 5.24 | 4.99 | 3.47 | 3.58 | 3.66 | 4.98 |
| (N-1)x(%RSD)² | 231.80 | 412.13 | 572.73 | 180.83 | 257.04 | 200.75 | 273.25 |
| C_{Target} | 15.00 | 15.00 | 100.00 | 100.00 | 400.00 | 400.00 | 82.74 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 12.00 | 12.00 | 80.00 | 80.00 | 320.00 | 320.00 | 66.19 |
| Acceptance Range High | 18.00 | 18.00 | 120.00 | 120.00 | 480.00 | 480.00 | 99.29 |
| N | 24 | 16 | 24 | 16 | 21 | 16 | 12 |
| OPI_20201102B_SD | 15.11 15.24 15.49 | | 95.84 96.73 97.52 | | 372.78 363.41 375.02 | | |
| OPI_20201104B_PK | 14.96 13.88 14.86 | | 109.11 95.63 97.25 | | N/A N/A N/A | | |
| OPI_20201105B_PK | 15.15 15.66 14.56 | | 96.37 95.67 101.61 | | 371.31 358.13 366.40 | | 84.87 86.13 86.27 84.06 |
| OPI_20201109B_SD | 15.13 15.40 14.77 | | 92.95 94.30 94.37 | | 398.30 389.29 385.31 | | |
| OPI1_20201112B_PK | 14.59 15.12 14.16 | | 97.09 99.44 97.39 | | 397.59 407.93 395.87 | | 83.34 90.08 78.87 85.58 |
| OPI1_20201113B_CLR | 15.69 14.72 14.44 | | 104.15 93.56 108.00 | | 379.79 383.52 378.59 | | 79.60 79.32 77.50 77.22 |
| OPI1_20201116B_SD | 14.78 15.61 15.27 | | 92.88 93.81 92.22 | | 370.10 359.08 372.59 | | |
| OPI1_20201119B_SD | 14.54 15.50 14.77 | | 93.69 90.27 90.91 | | 369.70 367.65 379.53 | | |
| OPI_20201130B_PK | | 16.50 16.53 16.85 | | 97.76 94.83 96.04 | | 396.62 406.78 411.86 | |
| OPI1_20201201B_SD | | 14.61 14.96 | | 96.44 97.35 | | 379.05 367.73 | |
| OPI1_20201202B_PK | | 16.30 16.06 16.04 | | 97.76 96.29 94.38 | | 378.79 366.71 379.52 | |
| OPI1_20201203B_PK | | 15.50 15.50 14.13 | | 87.90 90.55 88.29 | | 368.16 386.51 368.67 | |
| OPI1_20201214B_PK | | 16.48 15.46 15.15 | | 92.85 92.14 89.94 | | 389.87 371.38 387.96 | |
| OPI1_20201215B_PK | | 14.59 15.03 | | 92.75 94.06 | | 388.84 371.40 | |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|----------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Tramadol | Cerilliant | FE05241803 | 1.000 | 0.006 | 0.600 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Norbuprenorphine

Analytical Range: 0.5-100 ng/mL

Sample volume (in µL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution F (1 µg/mL): Add 100 µL of 100 µg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution E (0.1 µg/mL): Dilute 1 mL of 1 µg/mL solution into 10 mL volumetric flask.

Working Calibrator Solution D (0.01 µg/mL): Dilute 1 mL of 0.1 µg/mL solution into 10 mL volumetric flask.

Calibrator 1 (0.5 ng/mL): 50 µL of working solution D into 1 mL blood

Calibrator 2 (1 ng/mL): 100 µL of working solution D into 1 mL blood

Calibrator 3 (5 ng/mL): 50 µL of working solution E into 1 mL blood

Calibrator 4 (10 ng/mL): 100 µL of working solution E into 1 mL blood

Calibrator 5 (25 ng/mL): 25 µL of working solution F into 1 mL blood

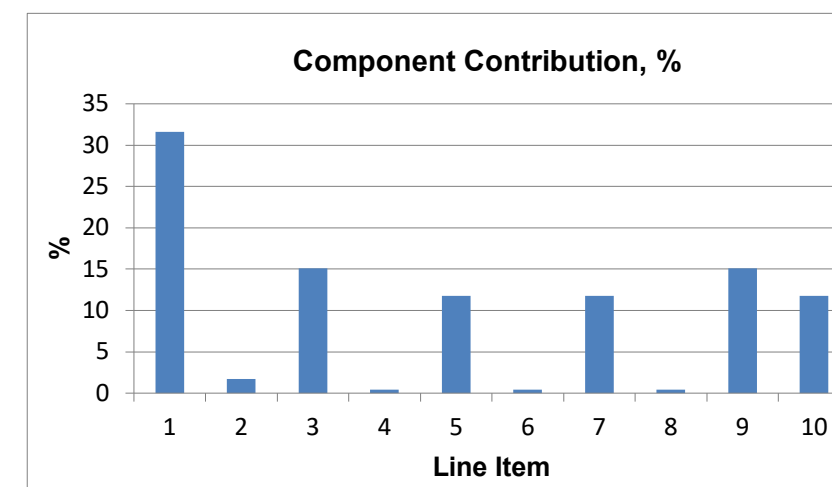
Calibrator 6 (50 ng/mL): 50 µL of working solution F into 1 mL blood

Calibrator 7 (100 ng/mL): 100 µL of working solution F into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of norbuprenorphine in blood
Analytical Range: 0.5-100 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % | |
|---|-----------------------------|----------------------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|----------|
| 1 | Measurement Reproducibility | 5.576 | % | Normal | A | 1.00 | 55 | 5.576 | 32 | |
| 2 | CRM Uncertainty | 0.600 | % | Normal | B | 2.00 | infinite | 0.300 | 2 | |
| 3 | 100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 7 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 | |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 15 | |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 | |
| | | | | | | | | | 100 | |
| Combined Standard Uncertainty | | <i>u_c</i> | | | | | | | 7.639 | % |
| Expanded Uncertainty (95.45% CI) | | UM (k=2) | | | | | | | 15.278 | % |



NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution F using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution F in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution F to working calibrator solution E using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution F to working calibrator solution E in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution E to working calibrator solution D using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution E to working calibrator solution D in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution D, E, F to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Norbuprenorphine

Pooled %RSD: **5.576**

k

6

| Control Lot | LQC | LQC | MQC | MQC | HQC | HQC |
|----------------------------------|-----------------------|----------------------|--------------------------|----------------------|-------------------------|-------------------------|
| | 200916K-Q-MXL | 201130K-Q-MXL | 200916K-Q-MXM | 201130K-Q-MXM | 200916K-Q-MXH | 201130K-Q-MXH |
| Avg Control Result | 1.52 | 1.46 | 9.65 | 9.23 | 73.12 | 74.84 |
| Control SD | 0.12 | 0.12 | 0.32 | 0.27 | 2.90 | 2.41 |
| %RSD | 8.22 | 8.50 | 3.32 | 2.98 | 3.97 | 3.21 |
| (N-1)x(%RSD)² | 1486.00 | 1083.93 | 231.72 | 133.04 | 361.94 | 154.97 |
| C_{Target} | 1.50 | 1.50 | 10.00 | 10.00 | 80.00 | 80.00 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 1.20 | 1.20 | 8.00 | 8.00 | 64.00 | 64.00 |
| Acceptance Range High | 1.80 | 1.80 | 12.00 | 12.00 | 96.00 | 96.00 |
| N | 23 | 16 | 22 | 16 | 24 | 16 |
| OPI_20201102B_SD | 1.47 1.47 1.72 | | 10.16 9.96 10.05 | | 78.39 73.67 76.70 | |
| OPI_20201104B_PK | 1.77 1.37* 1.79 | | 9.44 9.17 9.17 | | 72.05 70.23 70.40 | |
| OPI_20201105B_PK | 1.52 1.44 1.39 | | 9.54 9.51 9.84 | | 76.19 71.03 71.60 | |
| OPI_20201109B_SD | 1.47 1.59 1.54 | | 9.53 9.88 9.56 | | 74.53 74.16 73.14 | |
| OPI1_20201112B_PK | 1.39 1.38 1.54 | | 9.18 9.98 9.28 | | 72.35 76.80 73.18 | |
| OPI1_20201113B_CLR | 1.56 1.37 1.40 | | 13.78* 10.16 4.51* | | 77.64 74.37 76.27 | |
| OPI1_20201116B_SD | 1.38 1.45 1.50 | | 9.28 9.86 9.51 | | 70.54 69.33 73.17 | |
| OPI1_20201119B_SD | 1.61 1.67 1.49 | | 9.90 9.60 9.66 | | 66.61 71.04 71.44 | |
| OPI_20201130B_PK | | 1.31 1.40 1.41 | | 9.70 9.19 9.15 | | 74.49 77.00 78.83 |
| OPI1_20201201B_SD | | 1.44 1.66 | | 9.78 8.96 | | 76.63 76.16 |
| OPI1_20201202B_PK | | 1.36 1.55 1.79 | | 9.53 9.46 9.30 | | 73.35 72.96 77.46 |
| OPI1_20201203B_PK | | 1.46 1.51 1.41 | | 9.18 9.32 8.97 | | 74.53 77.43 75.25 |
| OPI1_20201214B_PK | | 1.42 1.43 1.32 | | 8.88 9.05 9.18 | | 73.91 73.58 74.81 |
| OPI1_20201215B_PK | | 1.46 1.39 | | 9.03 8.92 | | 71.44 69.60 |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|------------------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Norbuprenorphine | Cerilliant | FE05041901 | 0.100 | 0.001 | 0.600 | 2.00 |

Uncertainty of Measurement Specification

Method Information

Method Name: OPI1.M

Traceability of Calibrators: Certificate of Analysis

Reporting of Uncertainty: Uncertainty of measurement for the analyte quantified in this assay is reported at a 95.45% Confidence Interval (k=2).

Measurement Information

Analyte: Buprenorphine

Analytical Range: 0.5-100 ng/mL

Sample volume (in µL): 1000

$$C_{\text{Measurand}} = C_{\text{Calibrators}} \times \frac{RR_{\text{measurand}}}{RR_{\text{Calibrators}}}$$

Measurement Process: where:

C = Concentration

$$RR = \text{Response Ratio} = \frac{\text{Area}_{\text{Analyte}}}{\text{Area}_{\text{Internal Standard}}}$$

Calibration Preparation Information

Working Calibrator Solution F (1 µg/mL): Add 100 µL of 100 µg/mL CRM into 10 mL volumetric flask.

Working Calibrator Solution E (0.1 µg/mL): Dilute 1 mL of 1 µg/mL solution into 10 mL volumetric flask.

Working Calibrator Solution D (0.01 µg/mL): Dilute 1 mL of 0.1 µg/mL solution into 10 mL volumetric flask.

Calibrator 1 (0.5 ng/mL): 50 µL of working solution D into 1 mL blood

Calibrator 2 (1 ng/mL): 100 µL of working solution D into 1 mL blood

Calibrator 3 (5 ng/mL): 50 µL of working solution E into 1 mL blood

Calibrator 4 (10 ng/mL): 100 µL of working solution E into 1 mL blood

Calibrator 5 (25 ng/mL): 25 µL of working solution F into 1 mL blood

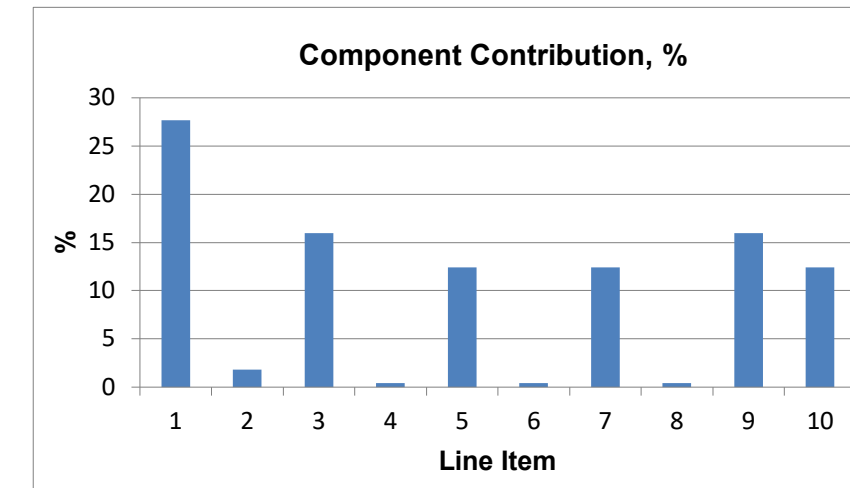
Calibrator 6 (50 ng/mL): 50 µL of working solution F into 1 mL blood

Calibrator 7 (100 ng/mL): 100 µL of working solution F into 1 mL blood

Uncertainty of Measurement Estimation Form

Measurement: Quantitation of buprenorphine in blood
Analytical Range: 0.5-100 ng/mL
Method: OPI1.M

| Line Item | Uncertainty Component | Value | Units | Distribution | Type | Divisor | Degrees Freedom (n-k) | Standard Uncertainty | Component Contribution % |
|-----------|-----------------------------|-------|-------|--------------|------|---------|-----------------------|----------------------|--------------------------|
| 1 | Measurement Reproducibility | 4.617 | % | Normal | A | 1.00 | 55 | 4.617 | 28 |
| 2 | CRM Uncertainty | 0.600 | % | Normal | B | 2.00 | infinite | 0.300 | 2 |
| 3 | 100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 |
| 4 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 |
| 5 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 |
| 6 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 |
| 7 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 |
| 8 | 10 mL Volumetric flask | 0.140 | % | Normal | B | 2.00 | infinite | 0.070 | 0 |
| 9 | 25-100 µL with Pipette | 2.667 | % | Normal | A | 1.00 | 4 | 2.667 | 16 |
| 10 | 1 mL with Pipette | 2.076 | % | Normal | A | 1.00 | 4 | 2.076 | 12 |



100

| | | | |
|---|-----------------|---------------|----------|
| Combined Standard Uncertainty | u_c | 6.970 | % |
| Expanded Uncertainty (95.45% CI) | UM (k=2) | 13.940 | % |

NOTE: Regardless of the number of digits that are showing in a cell, Excel carries the maximum number of significant figures in the background and will use the entire number for further calculations.

The basis for the data above:

| | | |
|----|-----------------------------|--|
| 1 | Measurement Reproducibility | Pooled %RSD from historical control data; normal distribution; divisor = 1. |
| 2 | CRM Uncertainty | Maximum uncertainty from certificates of analysis; COAs report expanded uncertainty at k = 2. |
| 3 | 20-200 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of CRM to working calibrator solution F using 20-200 µL pipette. |
| 4 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of CRM to working calibrator solution F in 10 mL volumetric flask. |
| 5 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution F to working calibrator solution E using 100-1000 µL pipette. |
| 6 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution F to working calibrator solution E in 10 mL volumetric flask. |
| 7 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of working calibrator solution E to working calibrator solution D using 100-1000 µL pipette. |
| 8 | 10 mL Volumetric flask | Maximum uncertainty from certificates of calibration; COCs report expanded uncertainty at k = 2; dilution of working calibrator solution E to working calibrator solution D in 10 mL volumetric flask. |
| 9 | 20-200 µL Pipette | Largest maximum %RSD from data on calibration certificates; transfer of working calibrator solution D, E, F to calibrator (1-7) using 20-200 µL pipette. |
| 10 | 100-1000 µL Pipette | Maximum %RSD from data on calibration certificates; transfer of blood sample using 100-1000 µL pipette. |

Measurement Reproducibility

Buprenorphine

Pooled %RSD: **4.617**

k
6

| Control Lot | LQC 200916K-Q-MXL | LQC 201130K-Q-MXL | MQC 200916K-Q-MXM | MQC 201130K-Q-MXM | HQC 200916K-Q-MXH | HQC 201130K-Q-MXH |
|----------------------------------|-----------------------|----------------------|---------------------------|-----------------------|-------------------------|-------------------------|
| Avg Control Result | 1.50 | 1.52 | 9.84 | 9.43 | 72.69 | 78.80 |
| Control SD | 0.09 | 0.09 | 0.29 | 0.35 | 2.38 | 4.52 |
| %RSD | 5.70 | 5.88 | 2.96 | 3.75 | 3.27 | 5.74 |
| (N-1)x(%RSD)² | 713.68 | 518.52 | 183.44 | 210.98 | 245.60 | 494.06 |
| C_{Target} | 1.50 | 1.50 | 10.00 | 10.00 | 80.00 | 80.00 |
| Control Acceptability (±) | 20% | 20% | 20% | 20% | 20% | 20% |
| Acceptance Range Low | 1.20 | 1.20 | 8.00 | 8.00 | 64.00 | 64.00 |
| Acceptance Range High | 1.80 | 1.80 | 12.00 | 12.00 | 96.00 | 96.00 |
| N | 23 | 16 | 22 | 16 | 24 | 16 |
| OPI_20201102B_SD | 1.43 1.58 1.50 | | 9.60 10.23 10.18 | | 74.24 71.34 70.84 | |
| OPI_20201104B_PK | 1.54 1.84* 1.71 | | 10.05 9.88 9.76 | | 72.85 69.01 68.61 | |
| OPI_20201105B_PK | 1.46 1.41 1.47 | | 10.17 9.82 10.22 | | 72.38 71.50 70.74 | |
| OPI_20201109B_SD | 1.51 1.49 1.65 | | 9.62 10.16 9.81 | | 76.86 73.86 73.39 | |
| OPI1_20201112B_PK | 1.36 1.48 1.44 | | 9.54 9.80 9.43 | | 72.46 75.01 71.95 | |
| OPI1_20201113B_CLR | 1.54 1.49 1.34 | | 17.09* 10.19 10.38* | | 77.65 76.90 75.53 | |
| OPI1_20201116B_SD | 1.57 1.54 1.54 | | 10.11 9.64 9.66 | | 71.13 71.07 71.11 | |
| OPI1_20201119B_SD | 1.53 1.57 1.45 | | 9.75 9.20 9.64 | | 72.72 70.85 72.57 | |
| OPI_20201130B_PK | | 1.52 1.56 1.67 | | 10.10 9.33 9.33 | | 84.11 85.46 85.16 |
| OPI1_20201201B_SD | | 1.61 1.61 | | 9.98 9.24 | | 72.69 71.61 |
| OPI1_20201202B_PK | | 1.44 1.40 1.64 | | 9.15 9.61 9.05 | | 75.88 77.62 79.26 |
| OPI1_20201203B_PK | | 1.46 1.51 1.41 | | 9.18 9.32 8.97 | | 74.53 77.43 75.25 |
| OPI1_20201214B_PK | | 1.63 1.55 1.47 | | 9.65 10.05 9.43 | | 77.76 79.17 86.58 |
| OPI1_20201215B_PK | | 1.41 1.50 | | 9.20 9.24 | | 79.24 79.07 |

CRM Information

| Analyte | CRM Source | Lot Number | CRM Concentration (mg/mL) | CRM Expanded Uncertainty (mg/mL) | CRM % Uncertainty | k |
|---------------|------------|------------|---------------------------|----------------------------------|-------------------|------|
| Buprenorphine | Cerilliant | FE08031802 | 0.100 | 0.0006 | 0.600 | 2.00 |

Calibration Data from External Calibration Certificates (20-200 µL Pipettes)

| |
|-----------------|
| MAX %RSD |
| 2.667 |

| Date of Calibration | Target Volume (µL) | (% Actual Imprecision) | | | | |
|---------------------|--------------------|------------------------|---------|---------|---------|---------|
| | | PU05581 | PU08808 | PU08932 | PU08933 | PU08937 |
| 4/3/2018 | 20.0 | 0.378 | | | | |
| | 100.0 | 0.292 | | | | |
| | 200 | 0.208 | | | | |
| 7/3/2018 | 20.0 | 0.106 | 0.651 | 0.224 | 0.734 | 1.042 |
| | 100.0 | 0.358 | 0.210 | 0.215 | 0.078 | 0.501 |
| | 200 | 0.141 | 0.140 | 0.131 | 0.349 | 0.258 |
| 10/2/2018 | 20.0 | 0.254 | 0.578 | 0.491 | 1.004 | 0.444 |
| | 100.0 | 0.123 | 0.337 | 0.344 | 0.114 | 0.265 |
| | 200 | 0.623 | 0.511 | 0.456 | 0.173 | 0.554 |
| 1/14/2019 | 20.0 | 0.319 | 0.136 | 0.556 | 0.461 | 0.394 |
| | 100.0 | 0.173 | 2.667 | 0.173 | 0.098 | 0.157 |
| | 200 | 0.143 | 0.196 | 0.103 | 0.117 | 0.206 |
| 4/3/2019 | 20.0 | 0.418 | 0.307 | 0.215 | 0.408 | 0.249 |
| | 100.0 | 0.134 | 2.306 | 0.135 | 0.162 | 0.378 |
| | 200 | 0.149 | 0.115 | 0.163 | 0.121 | 0.121 |
| 7/8/2019 | 20.0 | 0.401 | 0.737 | 1.942 | 0.625 | 2.603 |
| | 100.0 | 0.103 | 0.481 | 0.341 | 0.433 | 0.223 |
| | 200 | 0.035 | 0.167 | 0.407 | 0.345 | 0.187 |
| 11/12/2019 | 20.0 | 0.323 | 0.318 | 1.288 | 0.162 | 0.512 |
| | 100.0 | 0.271 | 1.451 | 0.184 | 0.224 | 0.662 |
| | 200 | 0.464 | 0.400 | 0.224 | 0.504 | 0.089 |
| 1/13/2020 | 20.0 | 0.338 | 0.400 | 0.283 | 0.693 | 0.305 |
| | 100.0 | 0.076 | 0.212 | 0.140 | 0.059 | 0.059 |
| | 200 | 0.075 | 0.071 | 0.077 | 0.063 | 0.087 |
| 4/10/2020 | 20.0 | 0.670 | 1.750 | 0.880 | 0.540 | 2.240 |
| | 100.0 | 0.200 | 0.260 | 0.240 | 0.170 | 0.080 |
| | 200 | 0.160 | 0.130 | 0.080 | 0.210 | 0.110 |
| 7/2/2020 | 20.0 | 1.270 | 1.680 | 1.330 | 1.610 | 1.430 |
| | 100.0 | 0.190 | 0.090 | 0.320 | 0.160 | 0.240 |
| | 200 | 0.120 | 0.490 | 0.690 | 0.500 | 0.300 |
| 10/9/2020 | 20.0 | 0.560 | 1.880 | 0.980 | 0.410 | 0.650 |
| | 100.0 | 0.170 | 0.220 | 0.140 | 0.270 | 0.150 |
| | 200 | 0.180 | 0.250 | 0.120 | 0.140 | 0.130 |

Calibration Data from External Calibration Certificates (100-1000 µL Pipettes)

| |
|-----------------|
| MAX %RSD |
| 2.076 |

| Date of Calibration | Target Volume (µL) | (% Actual Imprecision) | | | | |
|---------------------|--------------------|------------------------|---------|---------|---------|---------|
| | | PU10398 | PU10409 | PU10413 | PU05621 | PU08823 |
| 4/3/2018 | 100.0 | | | | 0.398 | |
| | 500.0 | | | | 0.149 | |
| | 1000 | | | | 0.256 | |
| 7/3/2018 | 100.0 | 0.296 | 0.126 | 0.555 | 0.257 | 0.312 |
| | 500.0 | 0.311 | 0.275 | 0.206 | 0.104 | 0.080 |
| | 1000 | 0.133 | 0.113 | 0.207 | 0.030 | 0.140 |
| 10/2/2018 | 100.0 | 0.371 | 1.953 | 0.330 | 0.499 | 0.199 |
| | 500.0 | 0.281 | 0.260 | 0.489 | 0.373 | 0.210 |
| | 1000 | 0.327 | 0.373 | 0.307 | 0.372 | 0.258 |
| 1/14/2019 | 100.0 | 0.328 | 0.243 | 0.305 | 0.030 | 0.172 |
| | 500.0 | 0.219 | 0.211 | 0.387 | 0.222 | 0.175 |
| | 1000 | 0.375 | 0.345 | 0.293 | 0.325 | 0.104 |
| 4/3/2019 | 100.0 | 1.315 | 0.380 | 2.076 | 0.381 | 0.202 |
| | 500.0 | 0.207 | 0.155 | 0.272 | 0.266 | 0.055 |
| | 1000 | 0.241 | 0.330 | 0.257 | 0.201 | 0.192 |
| 7/8/2019 | 100.0 | 0.277 | 0.190 | 0.136 | 0.212 | 0.361 |
| | 500.0 | 0.235 | 0.193 | 0.160 | 0.102 | 0.206 |
| | 1000 | 0.222 | 0.195 | 0.231 | 0.185 | 0.235 |
| 11/12/2019 | 100.0 | 0.341 | 0.172 | 0.421 | | 0.506 |
| | 500.0 | 1.112 | 0.165 | 0.235 | | 0.428 |
| | 1000 | 0.144 | 0.447 | 0.101 | | 0.240 |
| 1/13/2020 | 100.0 | 0.744 | 0.172 | 0.263 | 0.531 | 0.144 |
| | 500.0 | 0.042 | 0.310 | 0.357 | 0.139 | 0.214 |
| | 1000 | 0.065 | 0.148 | 0.056 | 0.160 | 0.132 |
| 4/10/2020 | 100.0 | 0.240 | 0.210 | 0.340 | 0.390 | 0.270 |
| | 500.0 | 0.160 | 0.610 | 0.140 | 0.070 | 0.120 |
| | 1000 | 0.080 | 0.070 | 0.110 | 0.100 | 0.090 |
| 7/2/2020 | 100.0 | 0.820 | 0.290 | 0.460 | 1.130 | 0.540 |
| | 500.0 | 0.190 | 0.190 | 0.200 | 0.430 | 0.100 |
| | 1000 | 0.150 | 0.110 | 0.160 | 0.370 | 0.070 |
| 10/9/2020 | 100.0 | 0.570 | 0.450 | 0.370 | 0.190 | 0.740 |
| | 500.0 | 0.060 | 0.140 | 0.470 | 0.080 | 0.120 |
| | 1000 | 0.080 | 0.060 | 0.210 | 0.160 | 0.110 |

10 mL NIST Traceable Volumetric Flask UM Data

MAX UM Value (%) **0.140**

| Serial Number | Actual Capacity (mL) | UM Value (%) | k | Certificate Date | End Date |
|---------------|----------------------|--------------|------|------------------|------------|
| CH24986 | 10.0025 | 0.12 | 2.00 | 8/15/2016 | 8/15/2026 |
| CH45140 | 10.0061 | 0.12 | 2.00 | 10/26/2016 | 10/26/2026 |
| CH45225 | 10.0028 | 0.11 | 2.00 | 10/25/2016 | 10/25/2026 |
| CH45373 | 10.0076 | 0.12 | 2.00 | 10/26/2016 | 10/26/2026 |
| CH49431 | 10.0074 | 0.12 | 2.00 | 10/25/2016 | 10/25/2026 |
| CH45384 | 9.9971 | 0.11 | 2.00 | 10/26/2016 | 10/26/2026 |
| CH98159 | 10.0025 | 0.11 | 2.00 | 8/3/2017 | 8/3/2027 |
| CH98283 | 10.00 | 0.11 | 2.00 | 8/3/2017 | 8/3/2027 |
| CH98391 | 10.00 | 0.12 | 2.00 | 8/3/2017 | 8/3/2027 |
| CI22375 | 10.00 | 0.14 | 2.00 | 8/10/2017 | 8/10/2027 |
| CI22463 | 10.0018 | 0.11 | 2.00 | 8/10/2017 | 8/10/2027 |
| CI23370 | 10.00 | 0.12 | 2.00 | 8/3/2017 | 8/3/2027 |