

REVIEWED
By Sarah Pickle at 10:20 am, Jul 24, 2020

TS

7/23/2020

Worklist: 4383

| <u>LAB CASE</u> | <u>ITEM</u> | <u>ITEM TYPE</u> | <u>DESCRIPTION</u> |
|-----------------|-------------|------------------|--|
| C2020-1110 | 1 | BCK | AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ |
| P2020-1623 | 1 | BCK | AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ |
| P2020-1624 | 1 | BCK | AM 28 Blood Multi-Drug Quant Panel 2 by LC-QQQ |



AM# 28: Multi-Drug Quantitation in Blood by LC-MS/MS

TS

Extraction Date: 07/23/2020
Plate lot#: Item #: IDP-112-2 Lot:200514

Analyst: Tamara Salazar
Plate Expiration: 11/14/20

Mobile phase A: 5mM Amm Form + 0.01% FA
0.5M Ammonium Hydroxide

Mobile phase B: 0.01% Formic Acid in MeOH
Ethyl Acetate 20% Methanol in Water

Blank Blood Lot: 445283-4

Column: Agilent 120 EC-C18 (2.1x 100-2.7um)

LCMS-QQQ ID: 069901

Pre-Analytic:

- 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.
- 3. Create worklist.

Analytic:

- 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- 2. Pipette **250µL blood (calibrated pipette) Pipette ID: 42** in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 4. Pipette **250µL 0.5M ammonium hydroxide** in wells of analytical plate.
- 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- 6. Transfer **300µL of blood+base** mixture to corresponding wells of SLE+ plate.
- 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent).
(Load at 85-100 PSI- Selector to the right)
- 8. Wait 5 minutes.
- 9. Add **900uL ethyl acetate.**
- 10. Wait 5 minutes.
- 11. Apply positive pressure for approx. 15 seconds. *(10-15 PSI- Selector to the left).*
- 12. Add **900uL ethyl acetate.**
- 13. Wait 5 minutes.
- 14. Apply positive pressure for approx. 15 seconds. *(10-15 PSI- Selector to the left).*
- 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.
- 16. Reconstitute in **100µL 20% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- 1. Create batch and process data.
- 2. Make necessary changes to integration limits
- 3. Integration linear and R² values ≥0.98 for each analyte.
- 4. For unknown samples and controls: response ratio within 20% of average of controls and standards, RT within +/- 5% (tramadol RT +/-2%), S/N for primary transition >10 and secondary transitions >5.
- 5. Did all QCs pass for each analyte? Y / N _____ Add Control data to QC tracking spreadsheet.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports.

COMMENTS: *Compounds evaluated: Ephedrine/Pseudoephedrine 5-1000, Etizolam 5-250*

TS

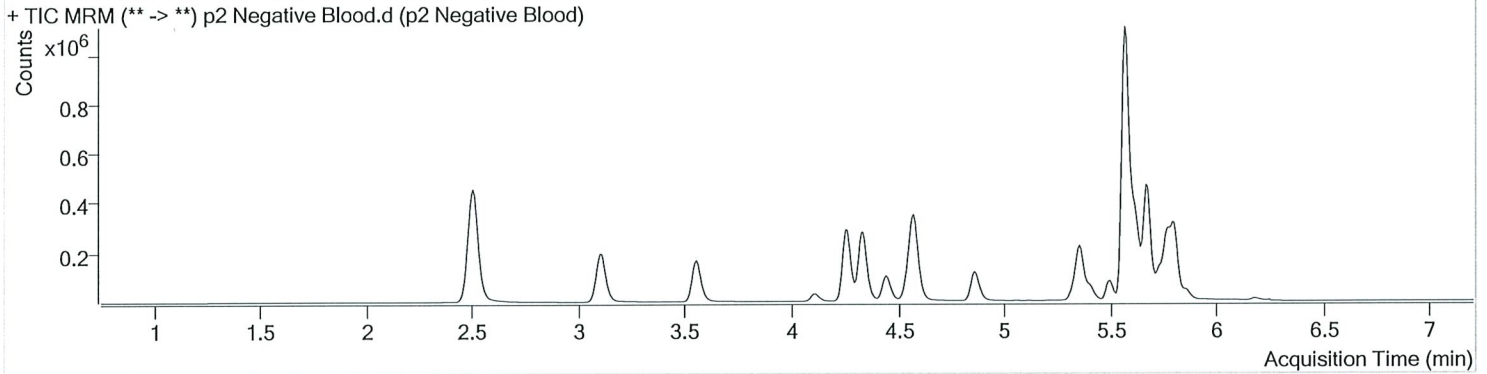


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|-----------------------|------------------|---------------------|
| Instrument | Falco | Data File | p2 Negative Blood.d |
| Type | Sample | Sample | p2 Negative Blood |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-E2 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/24/2020 12:22:49 AM | | |
| Sample Info. | | | |

Sample Chromatogram



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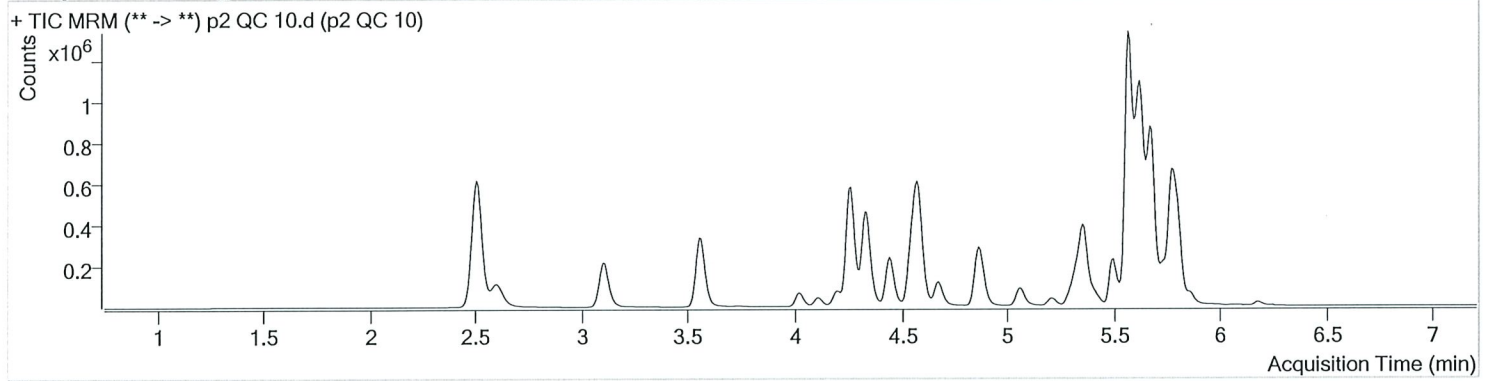
AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
 P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|-----------------------|------------------|----------------|
| Instrument | Falco | Data File | p2 QC 10.d |
| Type | QC | Sample | p2 QC 10 |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-A2 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 10:37:43 PM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|--------|---------|-------|--------|------------|---------------|
| Etizolam | 5.791 | 60272 | ∞ | 25.3 | ∞ | 864630 | 9.7912 ng/ml |
| Pseudoephedrine | 2.514 | 377004 | 1416.36 | 15.5 | 735.65 | 1730386 | 10.2502 ng/ml |

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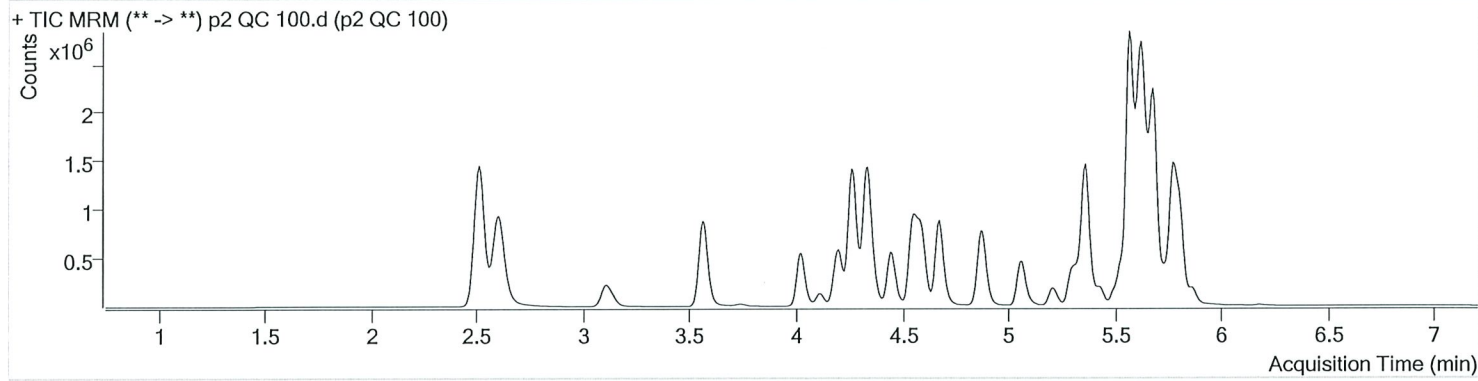
AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
 P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|-----------------------|------------------|----------------|
| Instrument | Falco | Data File | p2 QC 100.d |
| Type | QC | Sample | p2 QC 100 |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-B2 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 10:58:45 PM | | |

Sample Info.

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|---------|----------|-------|----------|------------|---------------|
| Etizolam | 5.791 | 529993 | 4426.66 | 26.3 | ∞ | 699086 | 80.3668 ng/ml |
| Pseudoephedrine | 2.520 | 3205155 | 57315.69 | 15.3 | 70009.63 | 1516668 | 96.1739 ng/ml |

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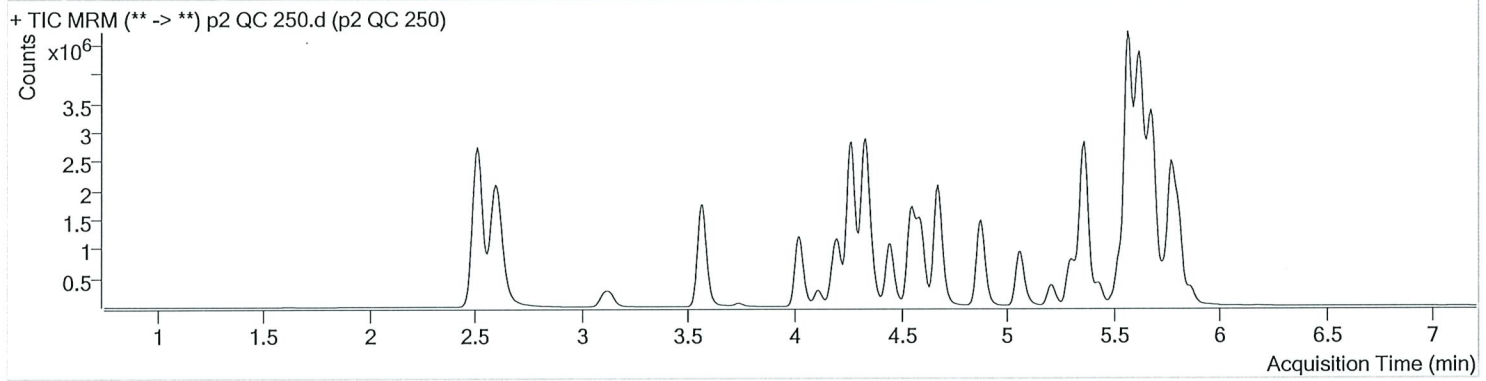


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|-----------------------|------------------|----------------|
| Instrument | Falco | Data File | p2 QC 250.d |
| Type | QC | Sample | p2 QC 250 |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-C2 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 11:19:44 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|---------|---------|-------|---------|------------|----------------|
| Etizolam | 5.791 | 1120070 | 4716.30 | 26.6 | 5203.52 | 514870 | 225.6692 ng/ml |
| Pseudoephedrine | 2.514 | 7451215 | 5102.58 | 15.0 | 4811.93 | 1384035 | 244.4293 ng/ml |

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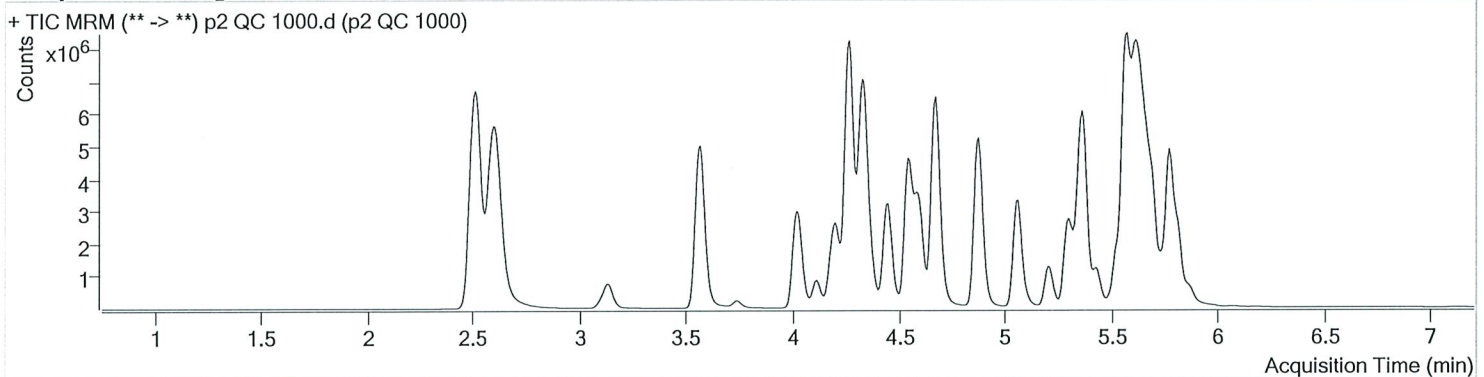
AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin

Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|-----------------------|------------------|----------------|
| Instrument | Falco | Data File | p2 QC 1000.d |
| Type | QC | Sample | p2 QC 1000 |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-D2 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 11:40:46 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|---------------------|------------------|--------------------|--------------------|-----------------|--------------------|-------------------|----------------------------|
| Etizolam | 5.797 | 2521539 | 3277.59 | 26.3 | 3428.34 | 182584 | 1418.4613 ng/ml |
| Pseudoephedrine | 2.514 | 22870205 | 207971.44 | 14.6 | 423878.20 | 1033757 | 1003.2803 ng/ml |

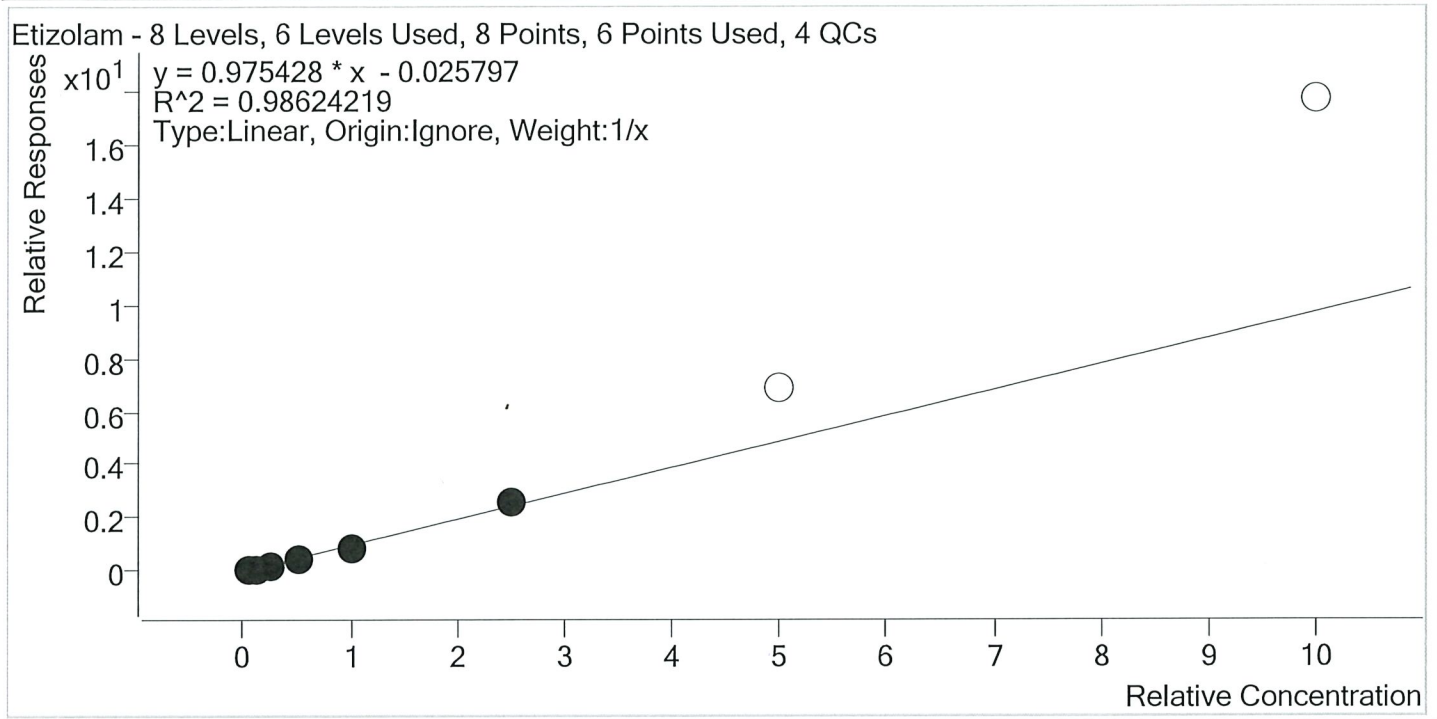
* outside of curve range. - 7/24/2020 TS

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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Last Cal. Update 7/24/2020 9:18 AM
Analyst Name ISP\datastor
Analyte Etizolam Internal Standard Estazolam-D5



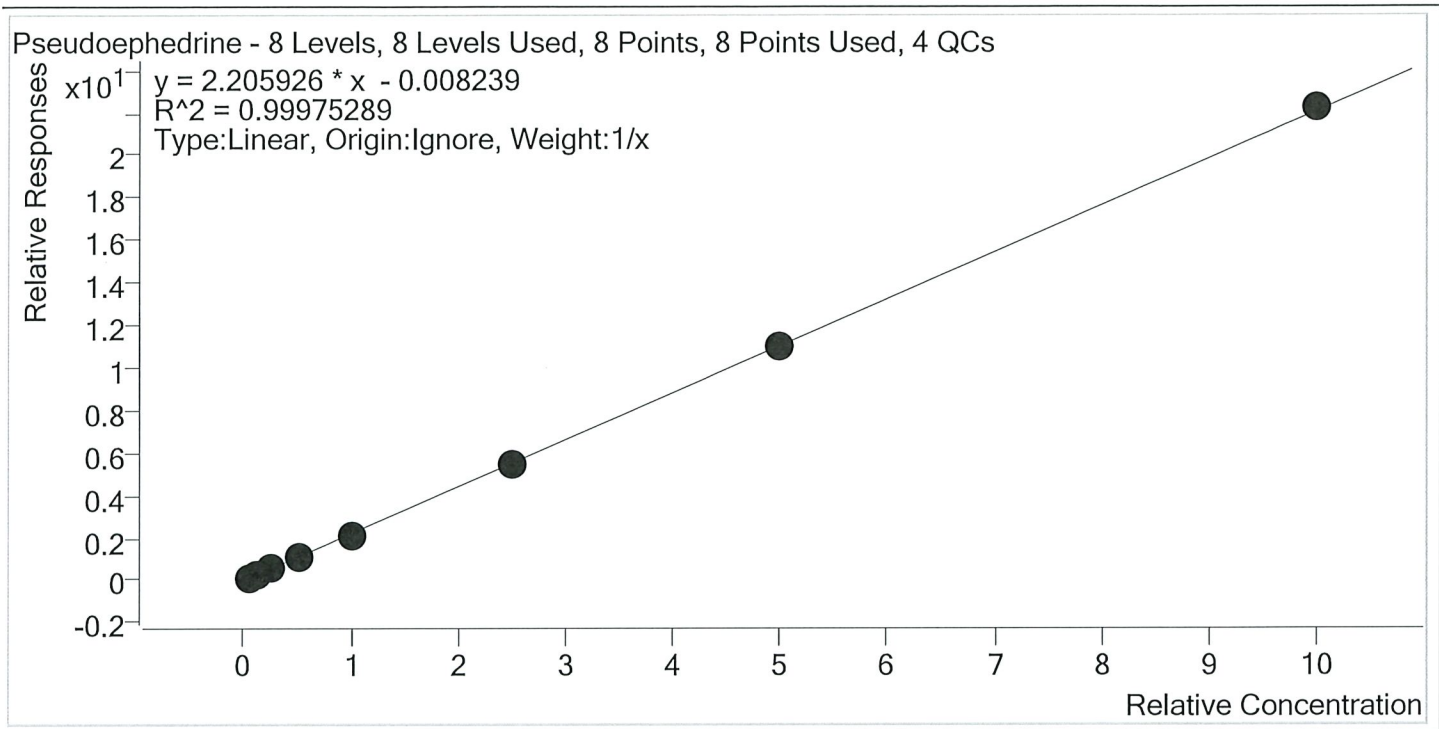
| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|-----------------|-------|---------|------------------------|---------------------|----------|
| p2 Cal 1-5ng | 1 | ✓ | 5.0 | 6.2 | 124.4 |
| p2 Cal 2- 10ng | 2 | ✓ | 10.0 | 10.7 | 107.0 |
| p2 Cal 3 -25ng | 3 | ✓ | 25.0 | 21.9 | 87.5 |
| p2 Cal 4-50ng | 4 | ✓ | 50.0 | 41.9 | 83.8 |
| p2 Cal 5-100ng | 5 | ✓ | 100.0 | 89.3 | 89.3 |
| p2 Cal 6-250ng | 6 | ✓ | 250.0 | 270.0 | 108.0 |
| p2 Cal 7-500ng | 7 | × | 500.0 | 701.2 | 140.2 |
| p2 Cal 8-1000ng | 8 | × | 1000.0 | 1823.5 | 182.4 |

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AM #28 Multi-Drug Quant. Calibration Curve Report

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
 P2_casework.batch.bin
Last Cal. Update 7/24/2020 9:18 AM
Analyst Name ISP\datastor
Analyte Pseudoephedrine **Internal Standard** Pseudoephedrine-D3



| Sample | Level | Enabled | Expected Concentration | Final Concentration | Accuracy |
|-----------------|-------|---------|------------------------|---------------------|----------|
| p2 Cal 1-5ng | 1 | ✓ | 5.0 | 5.2 | 104.2 |
| p2 Cal 2- 10ng | 2 | ✓ | 10.0 | 10.6 | 106.5 |
| p2 Cal 3 -25ng | 3 | ✓ | 25.0 | 24.2 | 96.8 |
| p2 Cal 4-50ng | 4 | ✓ | 50.0 | 48.2 | 96.4 |
| p2 Cal 5-100ng | 5 | ✓ | 100.0 | 97.1 | 97.1 |
| p2 Cal 6-250ng | 6 | ✓ | 250.0 | 244.1 | 97.6 |
| p2 Cal 7-500ng | 7 | ✓ | 500.0 | 502.6 | 100.5 |
| p2 Cal 8-1000ng | 8 | ✓ | 1000.0 | 1007.9 | 100.8 |

AM #28 Multi-Drug Quant. Results

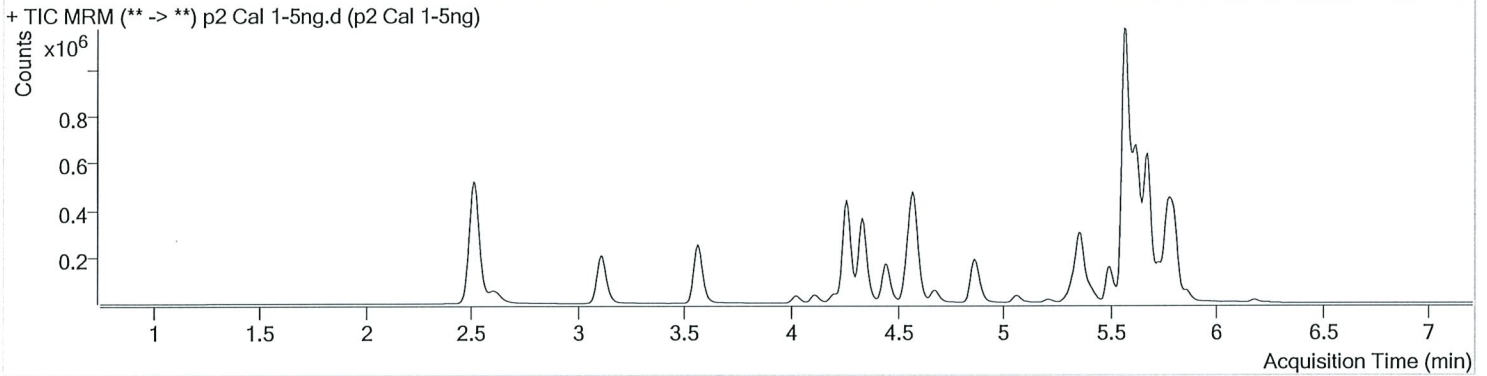
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Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|----------------------|------------------|----------------|
| Instrument | Falco | Data File | p2 Cal 1-5ng.d |
| Type | Cal | Sample | p2 Cal 1-5ng |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-A1 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 8:41:57 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|--------|----------|-------|---------|------------|--------------|
| Etizolam | 5.797 | 31017 | 37693.20 | 26.5 | 7283.56 | 889404 | 6.2199 ng/ml |
| Pseudoephedrine | 2.527 | 172831 | 2263.12 | 14.8 | 69.53 | 1619620 | 5.2110 ng/ml |

AM #28 Multi-Drug Quant. Results

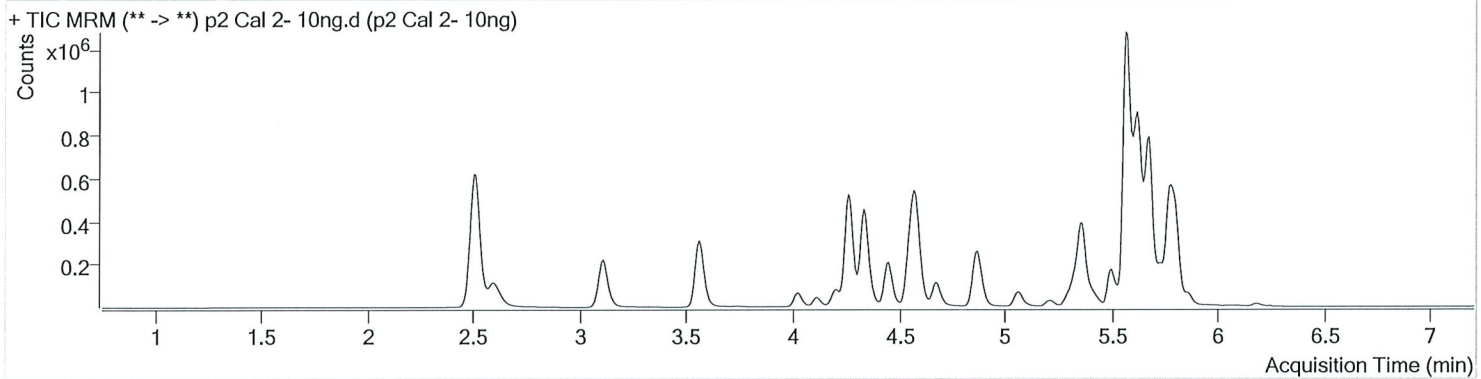
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Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|----------------------|------------------|------------------|
| Instrument | Falco | Data File | p2 Cal 2- 10ng.d |
| Type | Cal | Sample | p2 Cal 2- 10ng |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-B1 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 8:52:36 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|--------|---------|-------|----------|------------|---------------|
| Etizolam | 5.797 | 68023 | ∞ | 26.8 | 24526.87 | 865487 | 10.7022 ng/ml |
| Pseudoephedrine | 2.520 | 385205 | 5053.72 | 15.1 | 3896.56 | 1699797 | 10.6466 ng/ml |

TS

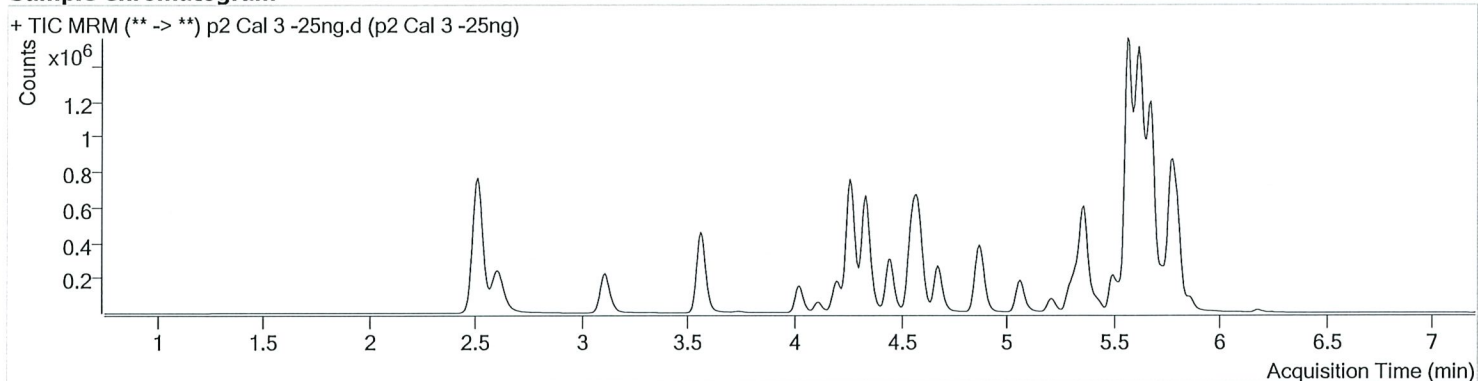


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

Instrument Falco **Data File** p2 Cal 3 -25ng.d
Type Cal **Sample** p2 Cal 3 -25ng
Acq. Method MDQ P2 061720.m **Operator** Tamara Salazar
Sample Position P1-C1 **Comment**
Injection Volume 2
Acq. Date-Time 7/23/2020 9:03:06 PM
Sample Info.

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|--------|-----------|-------|---------|------------|---------------|
| Etizolam | 5.797 | 154535 | 279139.82 | 25.1 | 1390.20 | 824297 | 21.8645 ng/ml |
| Pseudoephedrine | 2.520 | 887748 | 1312.37 | 15.4 | 7629.18 | 1688935 | 24.2014 ng/ml |

AM #28 Multi-Drug Quant. Results

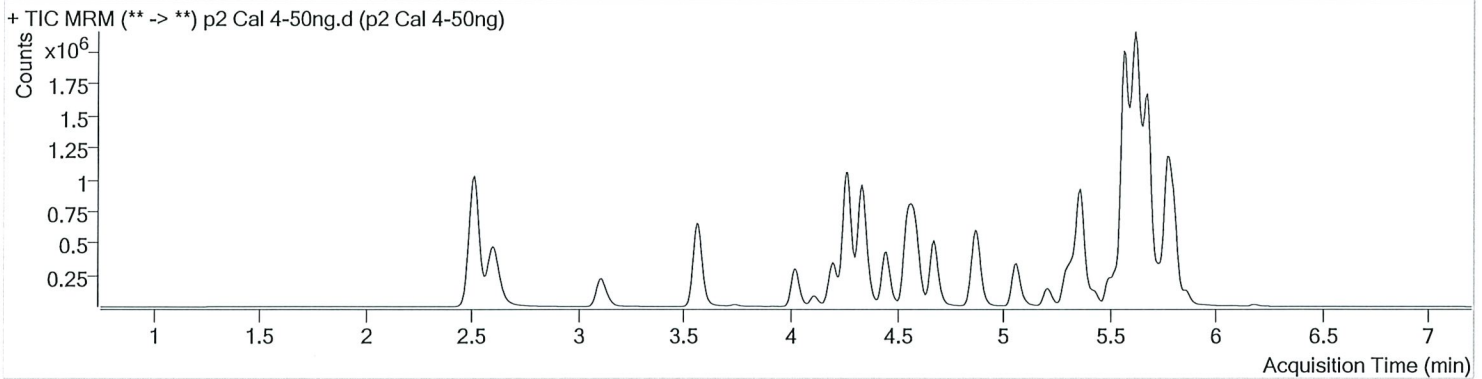
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Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|----------------------|------------------|-----------------|
| Instrument | Falco | Data File | p2 Cal 4-50ng.d |
| Type | Cal | Sample | p2 Cal 4-50ng |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-D1 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 9:13:37 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|---------|----------|-------|---------|------------|---------------|
| Etizolam | 5.791 | 298277 | 8690.17 | 26.8 | ∞ | 778766 | 41.9107 ng/ml |
| Pseudoephedrine | 2.520 | 1725526 | 61007.86 | 15.4 | 3188.34 | 1634897 | 48.2189 ng/ml |

TS

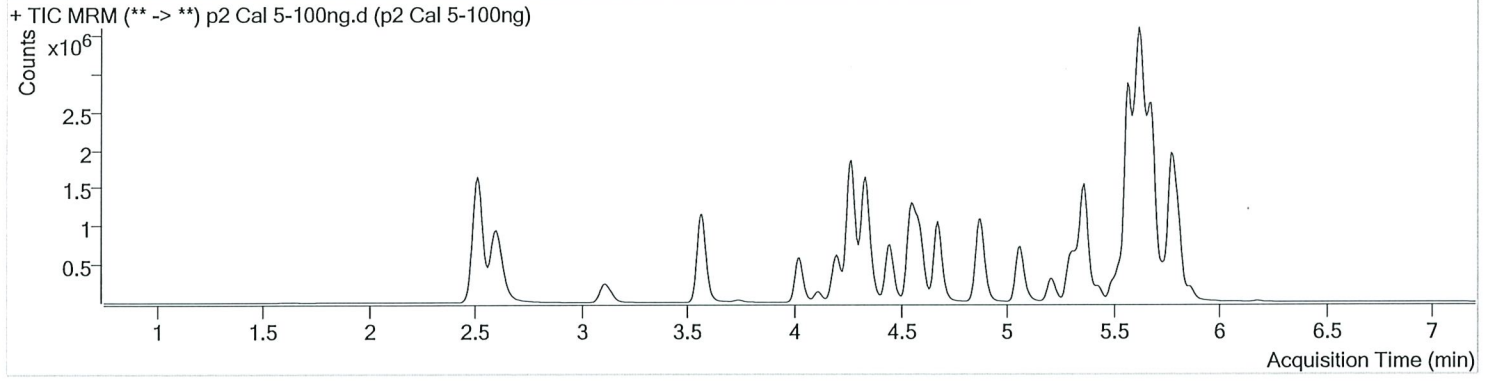


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
 P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|----------------------|------------------|------------------|
| Instrument | Falco | Data File | p2 Cal 5-100ng.d |
| Type | Cal | Sample | p2 Cal 5-100ng |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-E1 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 9:24:07 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|---------|----------|-------|---------|------------|---------------|
| Etizolam | 5.791 | 561090 | ∞ | 25.9 | 1682.56 | 663826 | 89.2977 ng/ml |
| Pseudoephedrine | 2.514 | 3575898 | 98375.96 | 15.3 | 1748.80 | 1675649 | 97.1146 ng/ml |

AM #28 Multi-Drug Quant. Results

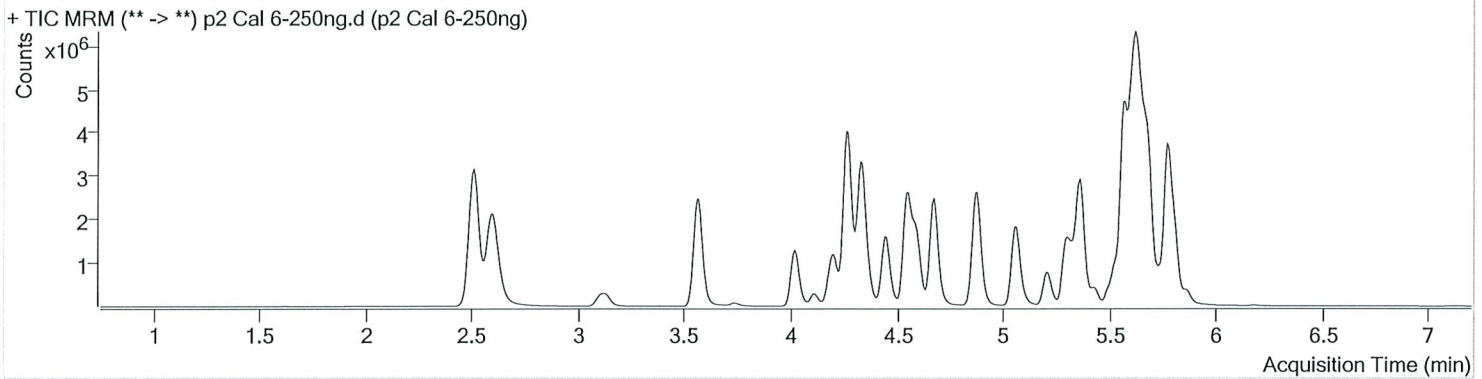
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Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|----------------------|------------------|------------------|
| Instrument | Falco | Data File | p2 Cal 6-250ng.d |
| Type | Cal | Sample | p2 Cal 6-250ng |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-F1 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 9:34:36 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|---------|-----------|-------|----------|------------|----------------|
| Etizolam | 5.797 | 1109572 | 5939.31 | 26.2 | 3429.00 | 425465 | 270.0049 ng/ml |
| Pseudoephedrine | 2.514 | 8405909 | 646394.28 | 15.0 | 52538.20 | 1563423 | 244.1080 ng/ml |

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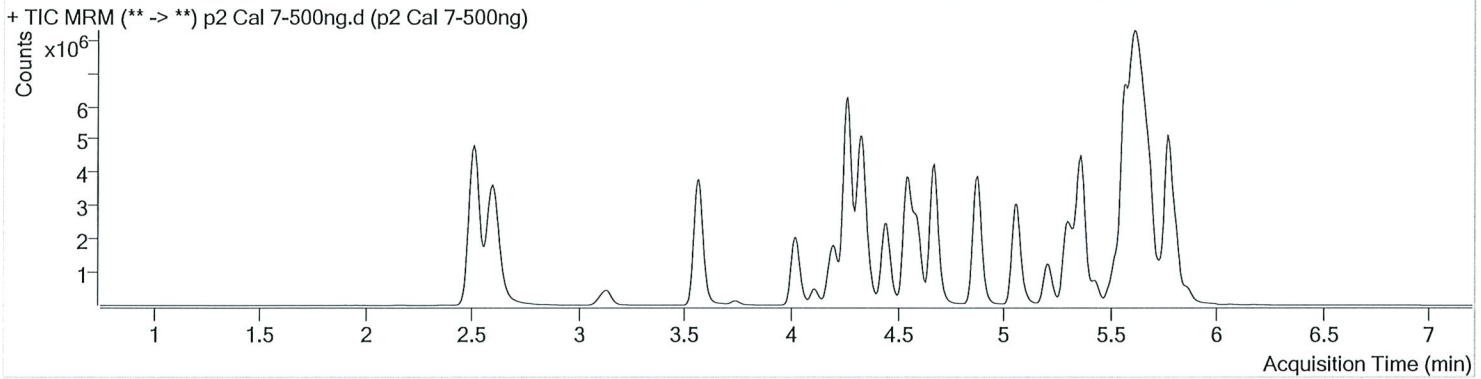
AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin

Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|----------------------|------------------|------------------|
| Instrument | Falco | Data File | p2 Cal 7-500ng.d |
| Type | Cal | Sample | p2 Cal 7-500ng |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-G1 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 9:45:06 PM | | |
| Sample Info. | | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|----------|----------|-------|----------|------------|----------------|
| Etizolam | 5.797 | 1705733 | 4444.68 | 26.4 | 8995.30 | 250345 | 701.1613 ng/ml |
| Pseudoephedrine | 2.514 | 14580444 | 15754.53 | 14.5 | 30847.99 | 1315991 | 502.6314 ng/ml |

TS

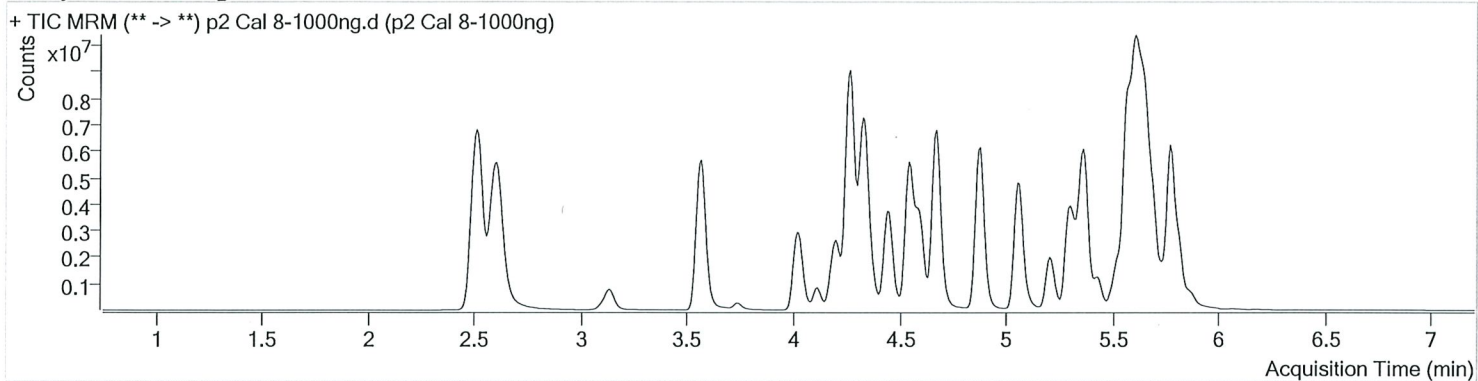


AM #28 Multi-Drug Quant. Results

Batch results D:\MassHunter\Data\2020\AM 27-28\072320 AM 27 AM 28 P1 and P2 TS\QuantResults\AM 28
P2_casework.batch.bin
Calibration Last Update 7/24/2020 9:18:17 AM

| | | | |
|-------------------------|----------------------|------------------|-------------------|
| Instrument | Falco | Data File | p2 Cal 8-1000ng.d |
| Type | Cal | Sample | p2 Cal 8-1000ng |
| Acq. Method | MDQ P2 061720.m | Operator | Tamara Salazar |
| Sample Position | P1-H1 | Comment | |
| Injection Volume | 2 | | |
| Acq. Date-Time | 7/23/2020 9:55:37 PM | | |

Sample Chromatogram



| Name | RT | Resp. | S/N | Ratio | S/N | ISTD Resp. | Final Conc. |
|-----------------|-------|----------|-----|-------|----------|------------|-----------------|
| Etizolam | 5.797 | 2315388 | ∞ | 26.7 | 3121.81 | 130362 | 1823.5042 ng/ml |
| Pseudoephedrine | 2.514 | 23182386 | ∞ | 14.5 | 53120.10 | 1043096 | 1007.8681 ng/ml |