

**REVIEWED**  
By Tamara Salazar at 1:42 pm, May 11, 2021

 5/7/2021

**Worklist: 4958**

<u>LAB CASE</u>	<u>ITEM</u>	<u>ITEM TYPE</u>	<u>DESCRIPTION</u>	
P2021-1379	12	URINE	AM 28 Urine Multi-Drug Confirmation Panel 2 by LC-QQ	
P2021-1379	8	URINE	AM 28 Urine Multi-Drug Confirmation Panel 2 by LC-QQ	

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

Extraction Date: 5/06/2021

Analyst: Amber Gerheart

Plate lot#: 200514

Plate Expiration: 11/14/2020

**Mobile phase A:** 5mM Amm Form + 0.01% FA    **Mobile phase B:** 0.01% Formic Acid in MeOH

**Blank Blood Lot:** 20L20723

**Blank Urine Lot:** POC031319

**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. Urine hydrolysis pipette 250 ul urine in blank well, add 40 ul BG Turbo, add 100 ul 500 mM sodium phosphate buffer, mix for at least 5 minutes at ambient temperature. Pipette **250µL blood (calibrated pipette) or 250µL hydrolyzed urine** in wells of analytical (standards) plate. **Pipette ID: 42**
- 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/urine+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. **If run contains urine, add 50µL 1% HCl in MeOH to wells and place plate cover on plate before drying (optional)**. Place on SPE Dry and evaporate to dryness at approx. 35°C.
- 16. Reconstitute in **100µL 20% MeOH** and heat seal plate with foil.

### Post-Analytic

- 1. Create batch and process data.
- 2. Make necessary changes to integration limits
- 3. Integration linear and R<sup>2</sup> 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? Yes, see comments Add Control data to QC tracking spreadsheet.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports.

COMMENTS: *Calibration Curve Range:* flurazepam 5-250 ng/mL, norketamine 5-250 ng/mL

Compounds Evaluated: 7-aminoflunitrazepam, acetyl fentanyl, acetyl norfentanyl, amitriptyline, flurazepam, norketamine

**Idaho State Police  
Forensic Services**

**Request for Departure from an Analytical Method or Quality Standard**

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Date of Request

5/07/21

Requestor

Amber Gerheart

Analytical Method/Quality Standard

Toxicology Analytical Method 28

4.1.9 Add 900 $\mu$ L of ethyl acetate and allow to flow through for approximately 5 minutes under gravity.

4.1.10 Apply positive pressure for approximately 10-15 seconds. (*Positive pressure manifold setting for this step is between 12-15 PSI*).

4.1.11 Add 900 $\mu$ L of ethyl acetate and allow to flow through for approximately 5 minutes under gravity.

Request

On 5/6/21 I skipped 4.1.10 and did not apply pressure between additions of ethyl acetate. After the second addition of ethyl acetate I waited 5 minutes and then applied pressure (4.1.12). I then continued with the extraction as written. I am requesting to use the data from this run as all calibrators, QCs (internal and external), and negative controls met parameters and there did not appear to be any impact on the analysis.

**Review**

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Departure approved

Comments:

Departure Not Approved

Comments:

Celena Shrum

Date: 05/07/2021

Title: Toxicology Discipline Lead

	1	2	3	4	5	6	7	8	9	10	11	12
A	IS + Cal. 1	IS + QC_1	P2021-1379-8	IS + Cal. 1	IS + QC_1	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 8	IS + Sample	IS + Sample	IS + Cal. 8
B	IS + Cal. 2	IS + QC_2	P2021-1379-12	IS + Cal. 2	IS + QC_2	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 7	IS + Sample	IS + Sample	IS + Cal. 7
C	IS + Cal. 3	IS + QC_3	IS + Sample	IS + Cal. 3	IS + QC_3	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 6	IS + Sample	IS + Sample	IS + Cal. 6
D	IS + Cal. 4	IS + QC_4	IS + Sample	IS + Cal. 4	IS + QC_4	IS + Sample	IS + Sample	IS + Sample	IS + Cal. 5	IS + Sample	IS + Sample	IS + Cal. 5
E	IS + Cal. 5	Negative Blood	IS + Sample	IS + Cal. 5	IS + Sample	IS + Sample	IS + Sample	IS + QC_4	IS + Cal. 4	IS + Sample	IS + QC_4	IS + Cal. 4
F	IS + Cal. 6	Negative Urine	IS + Sample	IS + Cal. 6	IS + Sample	IS + Sample	IS + Sample	IS + QC_3	IS + Cal. 3	IS + Sample	IS + QC_3	IS + Cal. 3
G	IS + Cal. 7	Urine External	IS + Sample	IS + Cal. 7	IS + Sample	IS + Sample	IS + Sample	IS + QC_2	IS + Cal. 2	IS + Sample	IS + QC_2	IS + Cal. 2
H	IS + Cal. 8	Blood External	IS + Sample	IS + Cal. 8	IS + Sample	IS + Sample	IS + Sample	IS + QC_1	IS + Cal. 1	IS + Sample	IS + QC_1	IS + Cal. 1

All wells to contain 60 µl of Trapping Solution



	1	2	3	4	5	6	7	8	9	10	11	12
A				IS + Cal. 1	IS + QC_1	P2021-1379-8						
B				IS + Cal. 2	IS + QC_2	P2021-1379-12						
C				IS + Cal. 3	IS + QC_3							
D				IS + Cal. 4	IS + QC_4							
E				IS + Cal. 5	Negative Blood							
F				IS + Cal. 6	Negative Urine							
G				IS + Cal. 7	Urine External							
H				IS + Cal. 8	Blood External							

All wells to contain 60 µl of Trapping Solution

AS

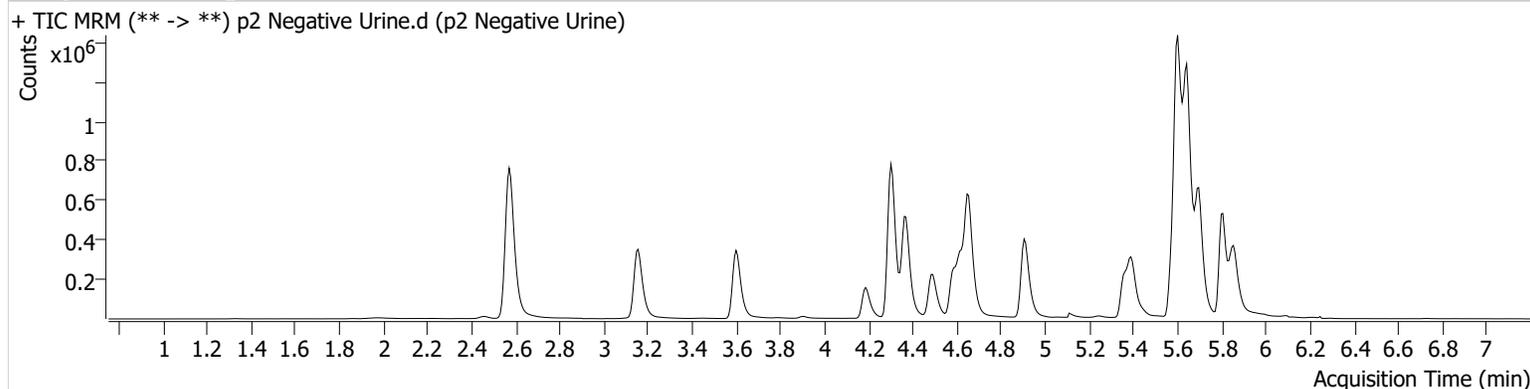


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Negative Urine.d
<b>Type</b>	Sample	<b>Sample</b>	p2 Negative Urine
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-F5	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 8:23:59 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



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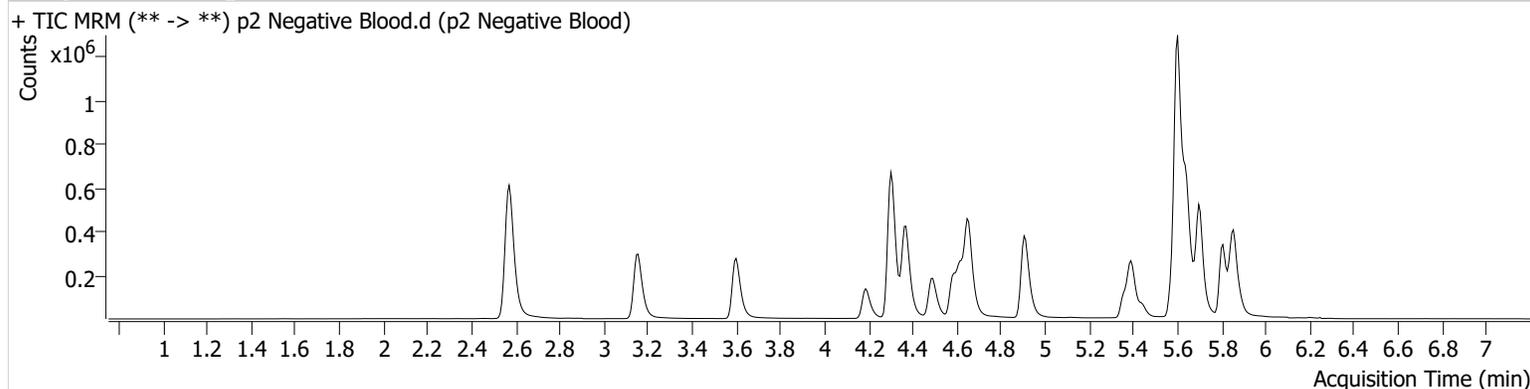


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**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Negative Blood.d
<b>Type</b>	Sample	<b>Sample</b>	p2 Negative Blood
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-E5	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 8:02:48 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



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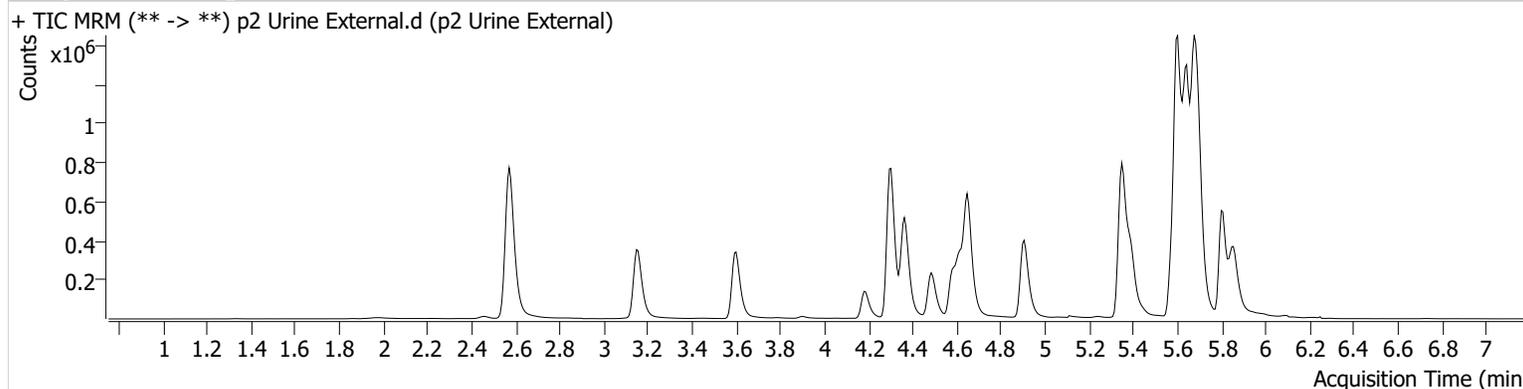


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**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
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<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Urine External.d
<b>Type</b>	Sample	<b>Sample</b>	p2 Urine External
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-G5	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 8:45:09 PM		
<b>Sample Info.</b>			

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Amitriptyline	5.678	519344	3021.91	88.0	2254.37	413914	46.6596 ng/ml
Flurazepam	5.345	1444151	2895.36	11.1	9381.10	226949	63.3559 ng/ml

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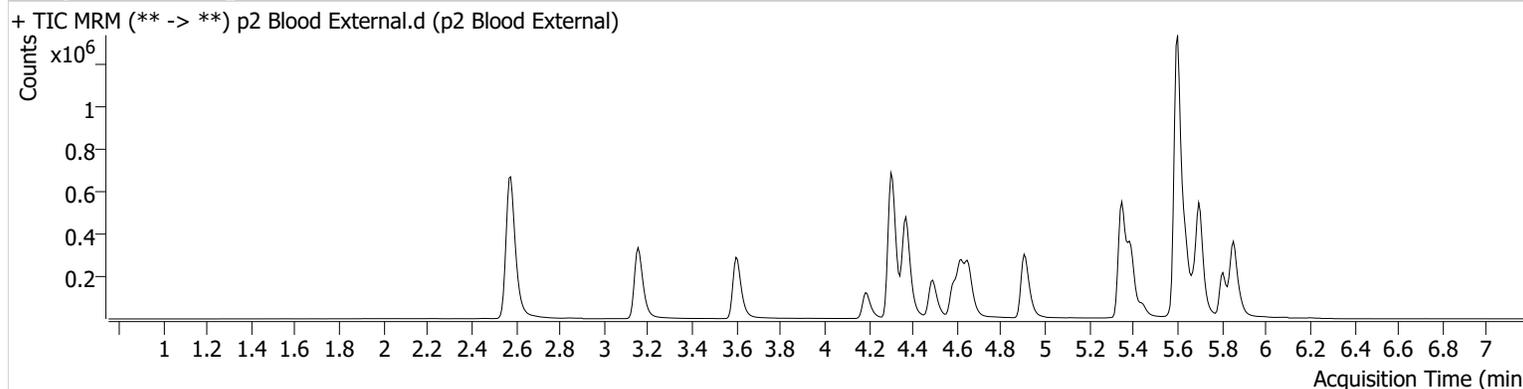


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Blood External.d
<b>Type</b>	Sample	<b>Sample</b>	p2 Blood External
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-H5	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 9:06:20 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Amitriptyline	5.678	69804	1623.83	89.0	425.17	37416	69.6391 ng/ml
Flurazepam	5.345	1203115	5980.10	11.2	179603.08	330756	37.1166 ng/ml



# Idaho State Police Forensic Services

## AM #28 Blood/Urine Multi-Drug Confirmatory Analysis by LCMS-QQQ---Panel 2

### Methanol External Control Solution (Lot: 121720)

100 ul each 1 mg/mL stock solution in 9800 ul MeOH

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>	<i>Expiration Date</i>
Methanol (LCMS)	Fisher	197468	
Amitriptyline	Cerilliant	FN02202004	03/31/2025
Flurazepam	Cerilliant	FE08231902	11/30/2024
Prepared:	12/17/2020		
Prepared By:	Celena Shrum		
Expires:	12/17/2021		

### Blood External Control Solution (Lot: WS121720)

100 ul of methanol external control solution was added to 9900ul of blood.

Approximately 100ng/mL of each compound.

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>
Negative Blood	Lampire	20L20725
Methanol External Control Solution	-	121720
Prepared:	12/17/2020	
Prepared by:	Celena Shrum	
Expires:	12/17/2021	

### Urine External Control Solution (Lot: WS121720)

100 ul of methanol external control solution was added to 9900ul of urine.

Approximately 100ng/mL of each compound.

<i>Component</i>	<i>Source</i>	<i>Source Lot Number</i>
Negative Urine		POC031319
Methanol External Control Solution	-	121720
Prepared:	12/17/2020	
Prepared by:	Celena Shrum	
Expires:	12/17/2021	

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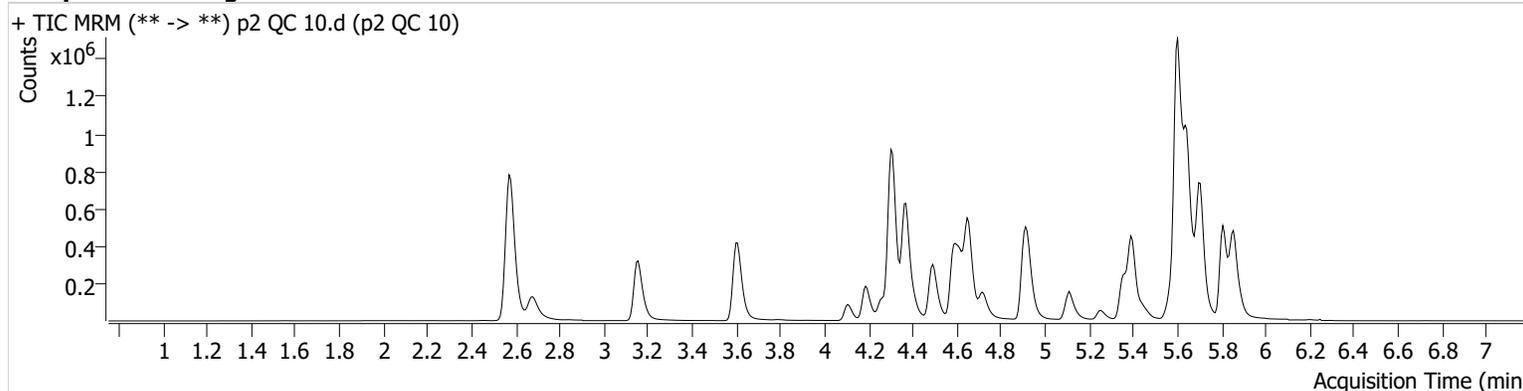


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 QC 10.d
<b>Type</b>	QC	<b>Sample</b>	p2 QC 10
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-A5	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 6:48:38 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	114043	3467.38	24.6	579.57	592515	10.7042 ng/ml
acetyl-fentanyl	4.666	20646	1010.82	64.2	11484.83	1087879	1.0717 ng/ml
acetyl-norfentanyl	3.183	22603	1311.72	33.1	152.30	882024	1.0022 ng/ml
Amitriptyline	5.678	55720	603.32	70.1	253.36	189130	10.5456 ng/ml
Flurazepam	5.345	220788	3947.43	11.2	4673.46	291053	9.4042 ng/ml
Norketamine	4.108	38420	285.35	485.6	2409.08	882024	10.6178 ng/ml

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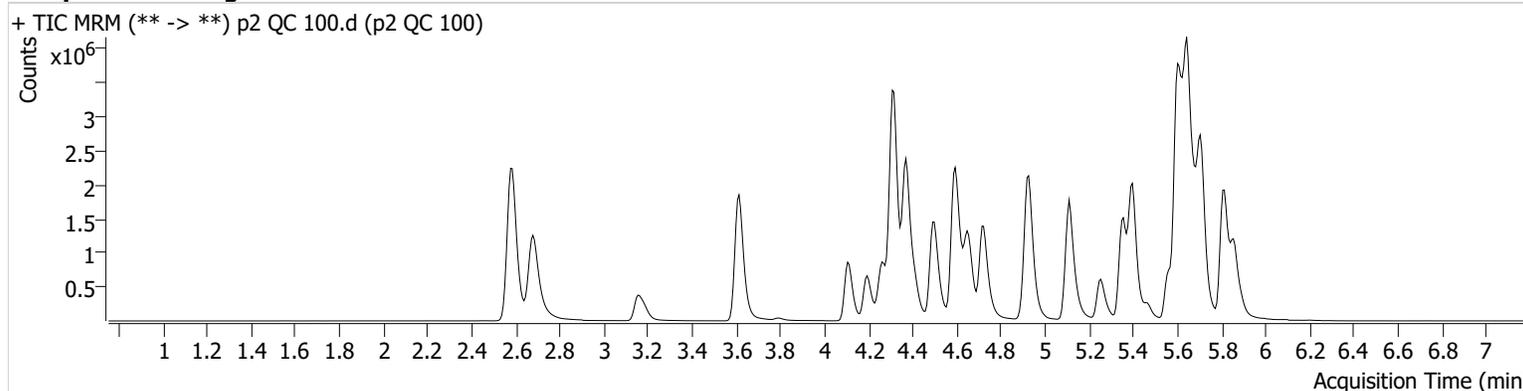


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 QC 100.d
<b>Type</b>	QC	<b>Sample</b>	p2 QC 100
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-B5	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 10:09:52 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	1207778	1674.15	26.3	8444.83	673078	105.3388 ng/ml
acetyl-fentanyl	4.666	236093	416804.75	62.3	5841.86	1242215	10.1339 ng/ml
acetyl-norfentanyl	3.183	246358	6409.58	32.9	965.38	1003030	9.8878 ng/ml
Amitriptyline	5.678	609697	555.28	76.8	4193.38	232396	98.1473 ng/ml
Flurazepam	5.345	2411171	1121.36	11.4	338580.46	254464	93.3135 ng/ml
Norketamine	4.108	392431	3306.72	491.1	4412.86	1003030	102.4342 ng/ml

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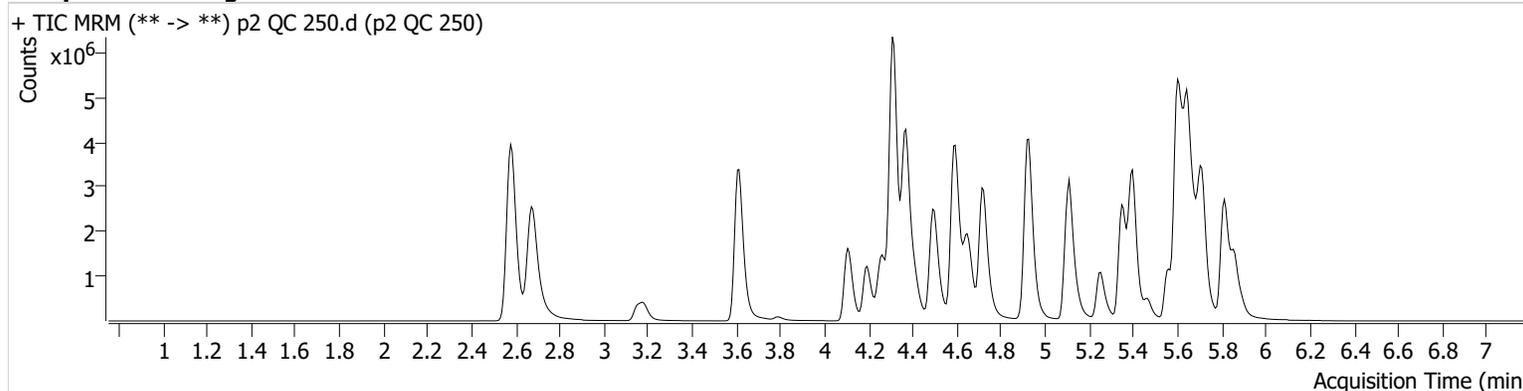


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 QC 250.d
<b>Type</b>	QC	<b>Sample</b>	p2 QC 250
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-C5	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 7:09:49 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	2549943	1374.84	26.1	26466.69	595184	252.4287 ng/ml
acetyl-fentanyl	4.666	426356	3015.94	63.5	655542.46	876596	25.8301 ng/ml
acetyl-norfentanyl	3.183	580382	81221.91	32.6	15822.85	902987	25.9280 ng/ml
Amitriptyline	5.678	746648	13497.74	74.9	904.07	114754	244.2048 ng/ml
Flurazepam	5.345	4787785	899.48	11.2	13403.90	187674	247.6746 ng/ml
Norketamine	4.108	753217	3200.81	503.2	81568.51	902987	219.3930 ng/ml

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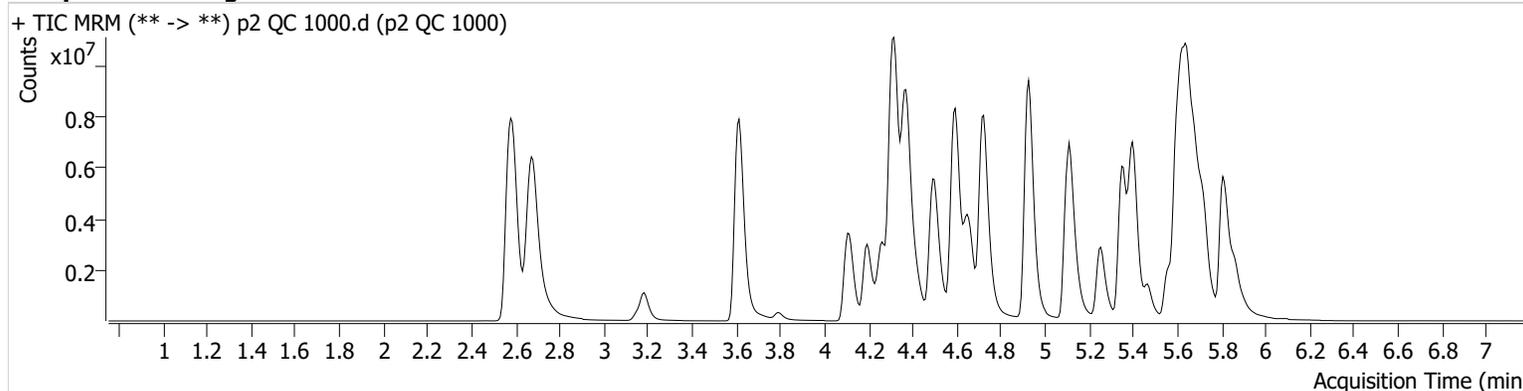


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 QC 1000.d
<b>Type</b>	QC	<b>Sample</b>	p2 QC 1000
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-D5	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 7:31:00 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	7337440	2386.66	25.7	28406.87	498294	869.2200 ng/ml
acetyl-fentanyl	4.666	1084026	105740.26	67.3	1059489.14	530612	108.2841 ng/ml
acetyl-norfentanyl	3.183	2128821	963322.03	32.7	1784.72	866678	99.1799 ng/ml
Amitriptyline	5.678	2435232	6017.33	76.0	3633.52	92633	988.3193 ng/ml
* <del>Flurazepam</del>	<del>5.345</del>	<del>12622402</del>	<del>122952.45</del>	<del>11.3</del>	<del>728.49</del>	<del>65187</del>	<del>1866.0253 ng/ml</del>
* <del>Norketamine</del>	<del>4.108</del>	<del>1823360</del>	<del>6586.52</del>	<del>508.6</del>	<del>384472.01</del>	<del>866678</del>	<del>554.6963 ng/ml</del>

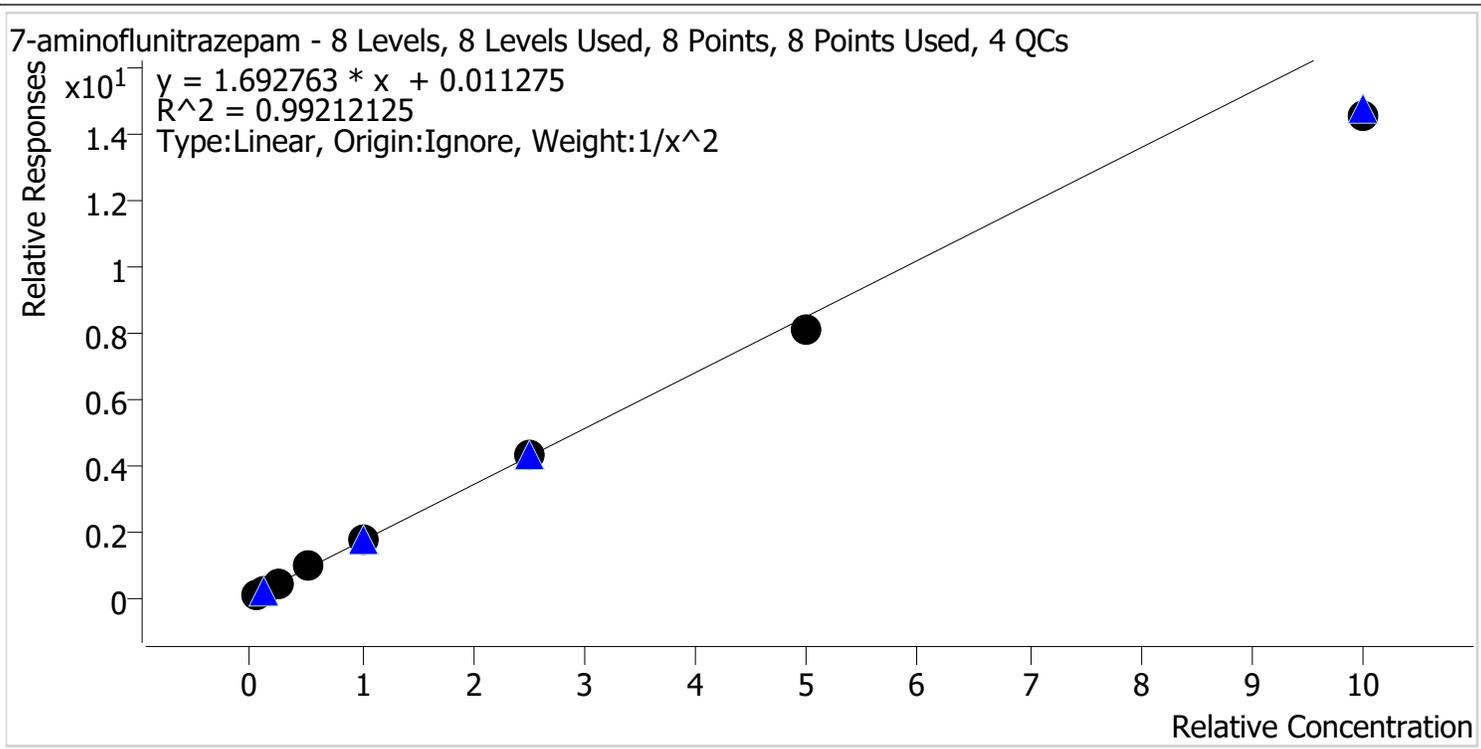
\*Outside curve range

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# AM #28 Multi-Drug Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Last Cal. Update** 5/10/2021 10:27 AM  
**Analyst Name** ISP\datastor  
**Analyte** 7-aminoflunitrazepam **Internal Standard** 7-aminoflunitrazepam-D7



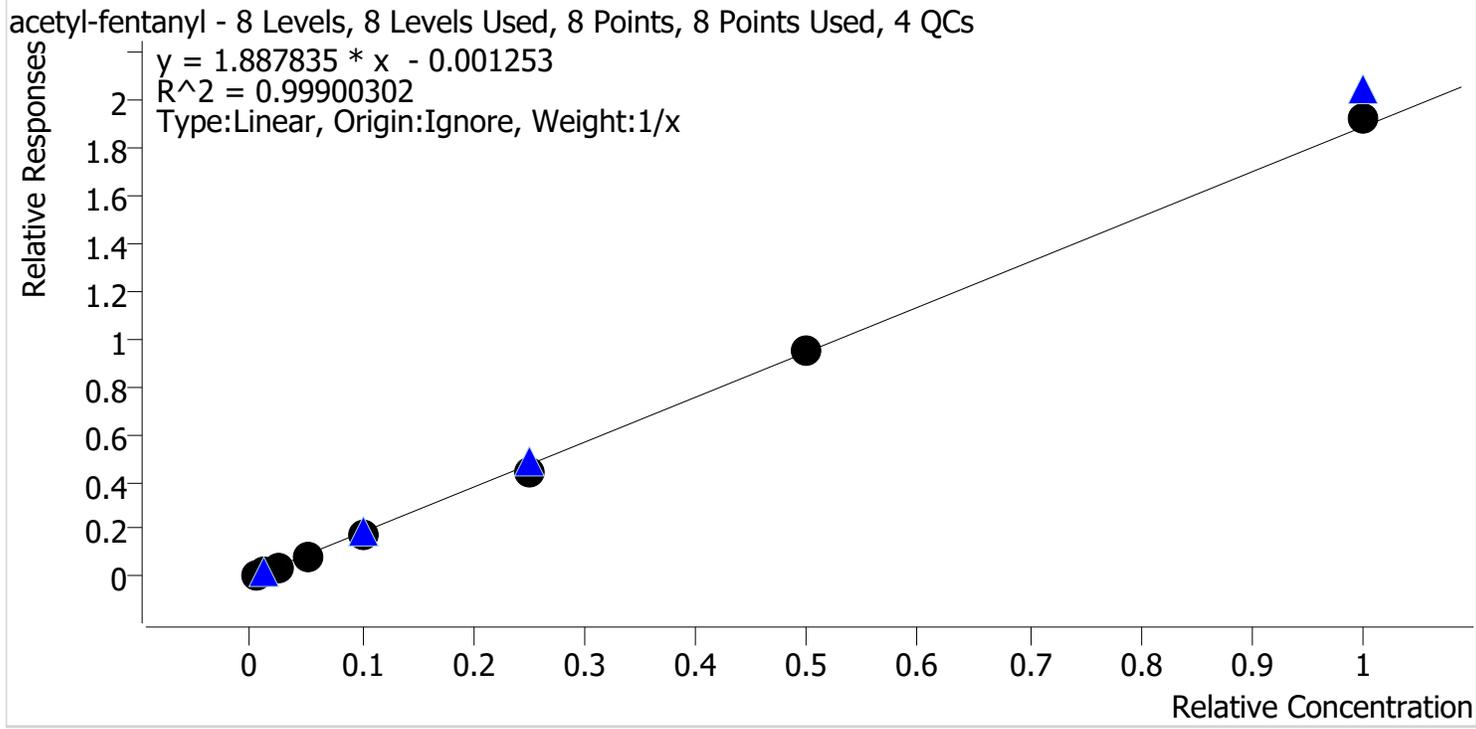
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	4.7	94.0
p2 Cal 2- 10ng	2	✓	10.0	10.8	108.1
p2 Cal 3 -25ng	3	✓	25.0	26.5	106.2
p2 Cal 4-50ng	4	✓	50.0	53.3	106.6
p2 Cal 5-100ng	5	✓	100.0	103.3	103.3
p2 Cal 6-250ng	6	✓	250.0	250.7	100.3
p2 Cal 7-500ng	7	✓	500.0	479.6	95.9
p2 Cal 8-1000ng	8	✓	1000.0	856.0	85.6

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**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Last Cal. Update** 5/10/2021 10:27 AM  
**Analyst Name** ISP\datastor  
**Analyte** acetyl-fentanyl **Internal Standard** acetyl-fentanyl-d5



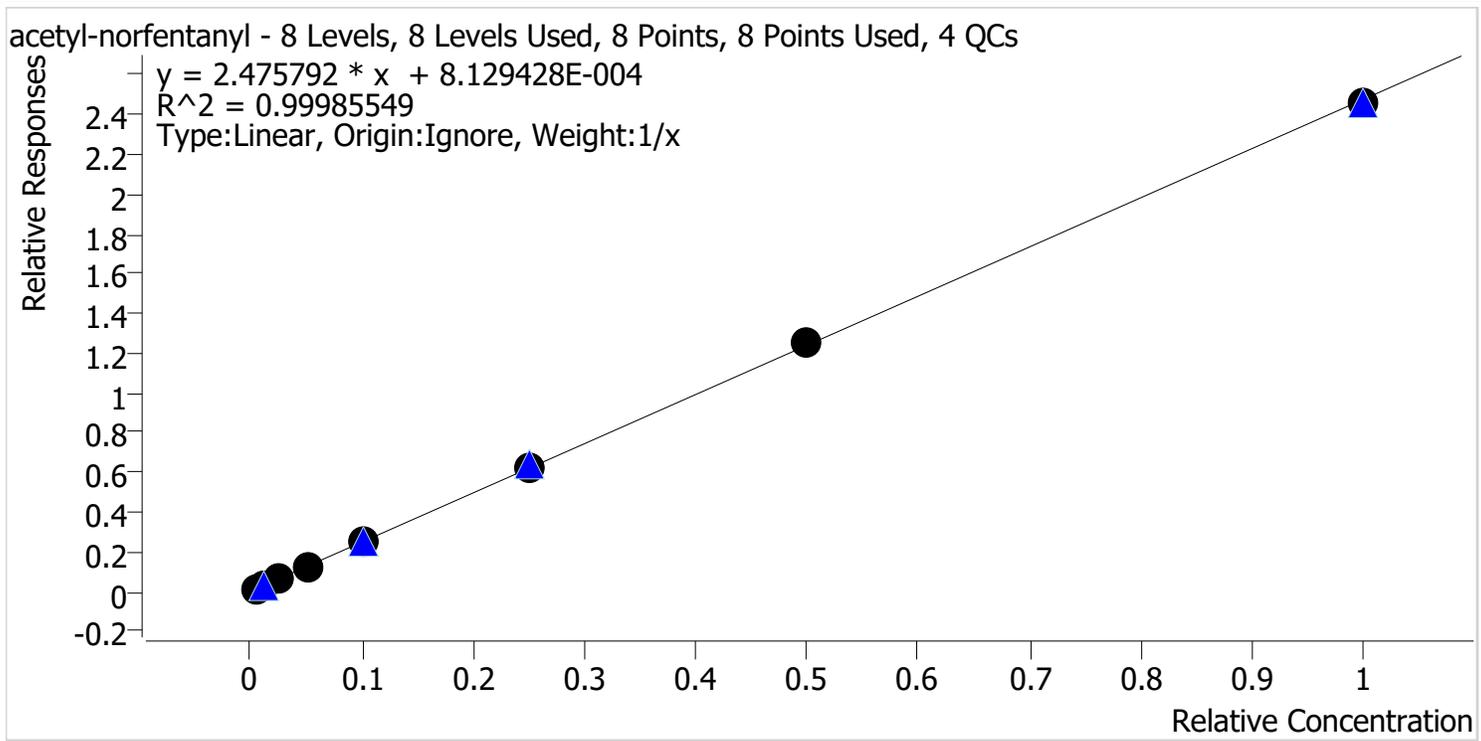
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	0.5	0.6	110.9
p2 Cal 2- 10ng	2	✓	1.0	1.1	107.5
p2 Cal 3 -25ng	3	✓	2.5	2.4	94.9
p2 Cal 4-50ng	4	✓	5.0	4.8	96.1
p2 Cal 5-100ng	5	✓	10.0	9.4	93.5
p2 Cal 6-250ng	6	✓	25.0	23.7	94.9
p2 Cal 7-500ng	7	✓	50.0	50.0	100.0
p2 Cal 8-1000ng	8	✓	100.0	102.1	102.1

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# AM #28 Multi-Drug Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Last Cal. Update** 5/10/2021 10:27 AM  
**Analyst Name** ISP\datastor  
**Analyte** acetyl-norfentanyl **Internal Standard** acetyl-norfentanyl-d5



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	0.5	0.5	94.4
p2 Cal 2- 10ng	2	✓	1.0	1.0	102.7
p2 Cal 3 -25ng	3	✓	2.5	2.5	98.9
p2 Cal 4-50ng	4	✓	5.0	5.1	102.2
p2 Cal 5-100ng	5	✓	10.0	10.1	101.4
p2 Cal 6-250ng	6	✓	25.0	25.0	100.1
p2 Cal 7-500ng	7	✓	50.0	50.7	101.4
p2 Cal 8-1000ng	8	✓	100.0	99.1	99.1

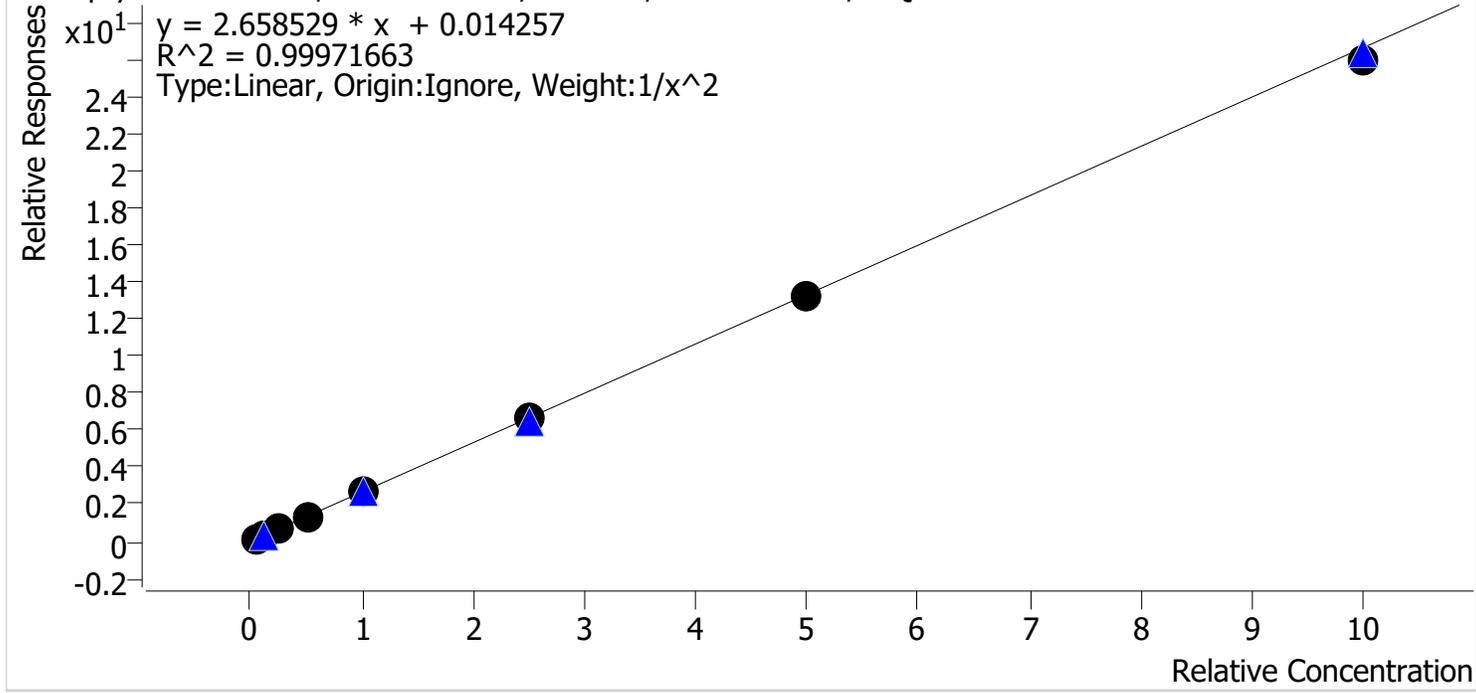
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# AM #28 Multi-Drug Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Last Cal. Update** 5/10/2021 10:27 AM  
**Analyst Name** ISP\datastor  
**Analyte** Amitriptyline **Internal Standard** Amitriptyline-D3

Amitriptyline - 8 Levels, 8 Levels Used, 8 Points, 8 Points Used, 4 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	5.0	99.7
p2 Cal 2- 10ng	2	✓	10.0	9.9	99.4
p2 Cal 3 -25ng	3	✓	25.0	25.6	102.5
p2 Cal 4-50ng	4	✓	50.0	50.4	100.7
p2 Cal 5-100ng	5	✓	100.0	100.1	100.1
p2 Cal 6-250ng	6	✓	250.0	251.0	100.4
p2 Cal 7-500ng	7	✓	500.0	499.6	99.9
p2 Cal 8-1000ng	8	✓	1000.0	972.2	97.2

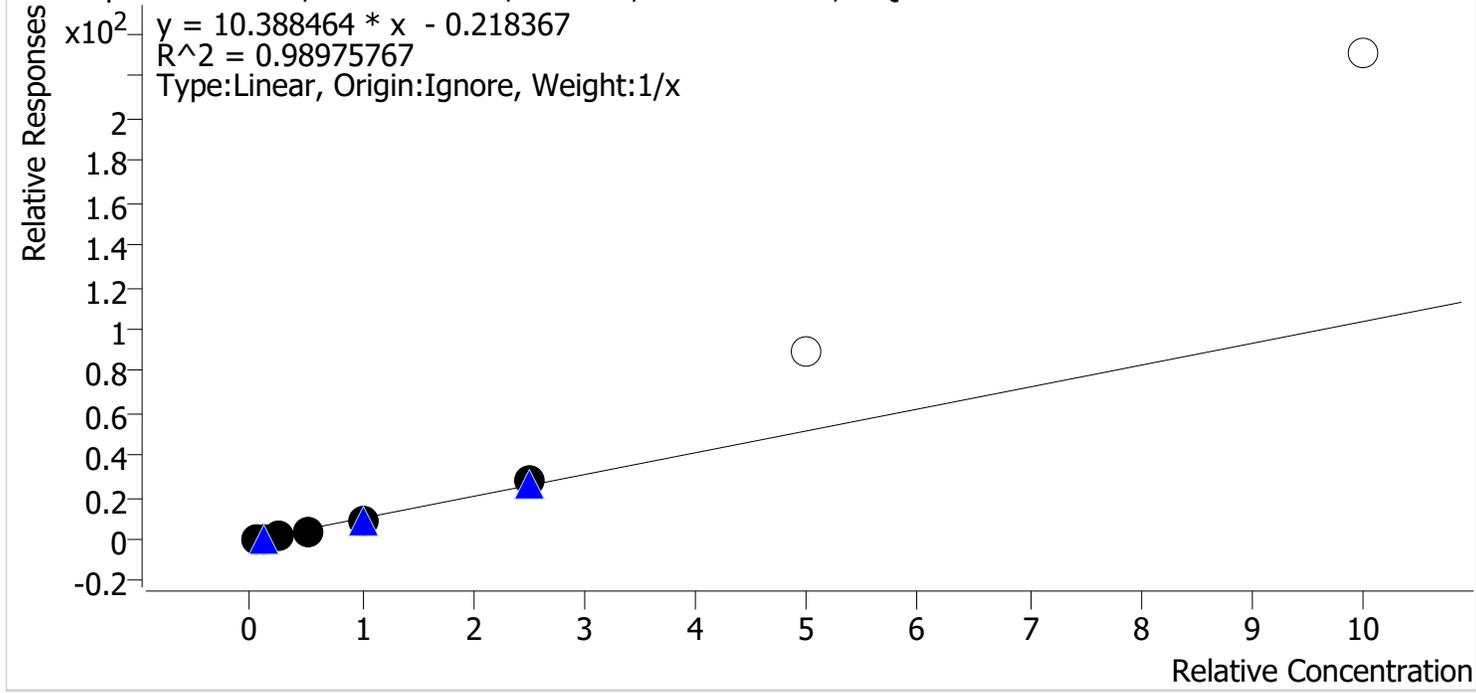
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# AM #28 Multi-Drug Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Last Cal. Update** 5/10/2021 10:27 AM  
**Analyst Name** ISP\datastor  
**Analyte** Flurazepam **Internal Standard** Flunitrazepam-D7

Flurazepam - 8 Levels, 6 Levels Used, 8 Points, 6 Points Used, 4 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	5.8	117.0
p2 Cal 2- 10ng	2	✓	10.0	10.1	101.5
p2 Cal 3 -25ng	3	✓	25.0	25.3	101.4
p2 Cal 4-50ng	4	✓	50.0	41.4	82.8
p2 Cal 5-100ng	5	✓	100.0	90.8	90.8
p2 Cal 6-250ng	6	✓	250.0	266.5	106.6
* p2 Cal 7-500ng	7	✗	500.0	855.1	171.0
* p2 Cal 8-1000ng	8	✗	1000.0	2226.4	222.6

\*Cal 7 and 8 due to accuracy

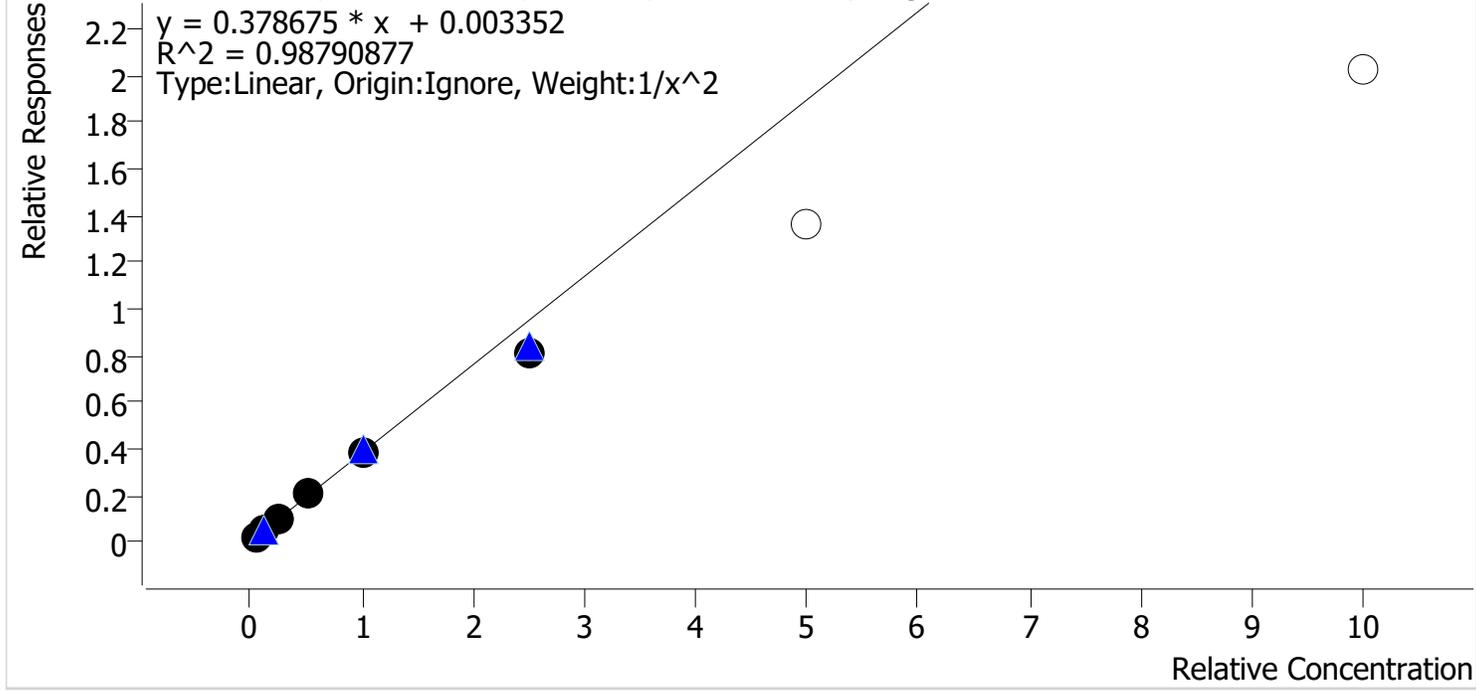
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# AM #28 Multi-Drug Quant. Calibration Curve Report

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Last Cal. Update** 5/10/2021 10:27 AM  
**Analyst Name** ISP\datastor  
**Analyte** Norketamine **Internal Standard** acetyl-norfentanyl-d5

Norketamine - 8 Levels, 6 Levels Used, 8 Points, 6 Points Used, 4 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	4.7	94.4
p2 Cal 2- 10ng	2	✓	10.0	10.8	107.8
p2 Cal 3 -25ng	3	✓	25.0	26.9	107.5
p2 Cal 4-50ng	4	✓	50.0	52.1	104.3
p2 Cal 5-100ng	5	✓	100.0	101.5	101.5
p2 Cal 6-250ng	6	✓	250.0	211.4	84.6
* p2 Cal 7-500ng	7	✗	500.0	360.5	72.1
* p2 Cal 8-1000ng	8	✗	1000.0	534.3	53.4

\*Cal 7 and 8 due to accuracy

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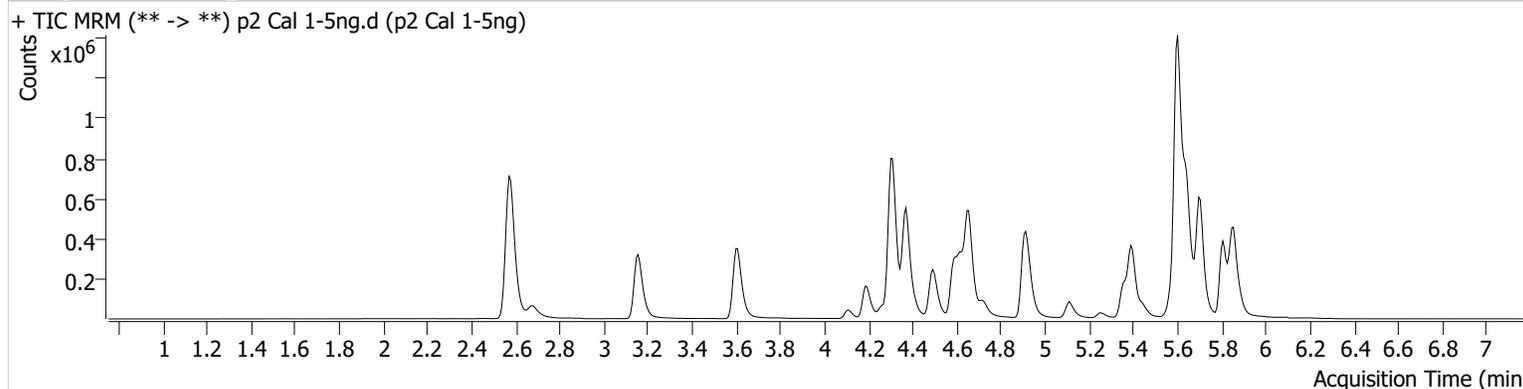


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Cal 1-5ng.d
<b>Type</b>	Cal	<b>Sample</b>	p2 Cal 1-5ng
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-A4	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 5:02:36 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	52664	2401.97	26.6	2500.83	579802	4.6998 ng/ml
acetyl-fentanyl	4.666	10829	298.86	61.0	4276.81	1174854	0.5546 ng/ml
acetyl-norfentanyl	3.183	10991	108.87	35.3	317.44	879725	0.4718 ng/ml
Amitriptyline	5.678	20099	243.72	68.3	178.81	136874	4.9871 ng/ml
Flurazepam	5.345	117075	126094.97	11.2	21811.49	300862	5.8478 ng/ml
Norketamine	4.108	18678	175.24	492.4	949.48	879725	4.7215 ng/ml

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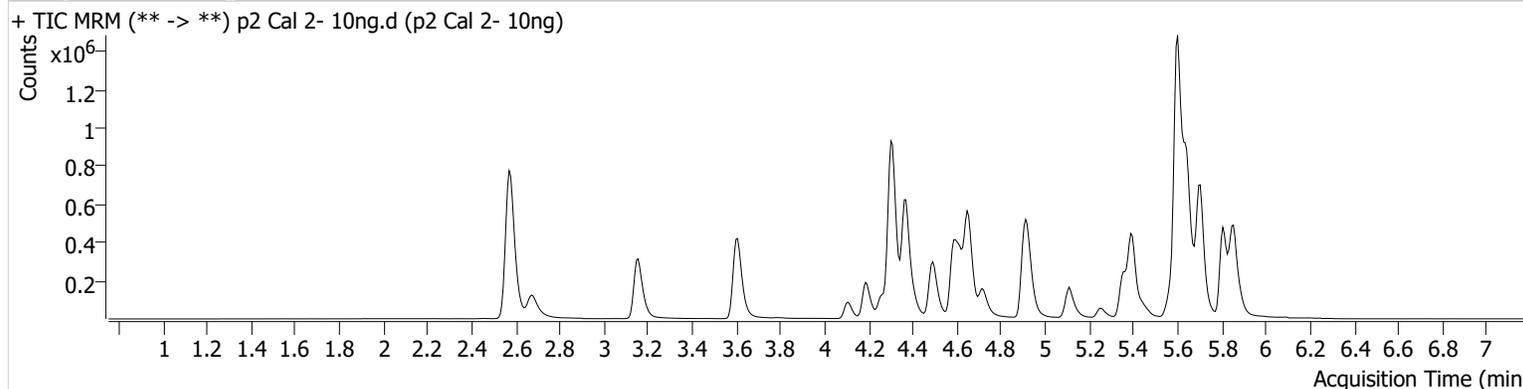


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Cal 2- 10ng.d
<b>Type</b>	Cal	<b>Sample</b>	p2 Cal 2- 10ng
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-B4	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 5:13:21 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	109043	299.79	25.1	19278.04	561368	10.8090 ng/ml
acetyl-fentanyl	4.666	21568	101.90	60.6	116.09	1132328	1.0753 ng/ml
acetyl-norfentanyl	3.183	22711	1759.03	34.8	584.08	865716	1.0268 ng/ml
Amitriptyline	5.678	39900	328.01	77.8	224.46	143281	9.9384 ng/ml
Flurazepam	5.345	250233	410893.12	11.2	46784.22	299444	10.1461 ng/ml
Norketamine	4.108	38229	291.46	486.5	1818.98	865716	10.7763 ng/ml

AS

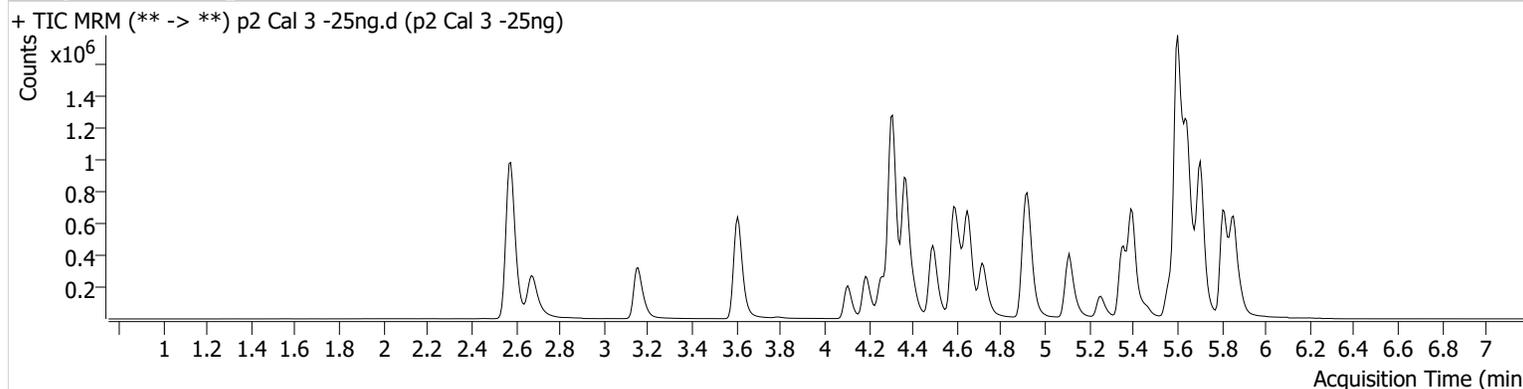


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Cal 3 -25ng.d
<b>Type</b>	Cal	<b>Sample</b>	p2 Cal 3 -25ng
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-C4	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 5:23:56 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	226959	550.67	26.0	382.81	492660	26.5487 ng/ml
acetyl-fentanyl	4.666	53712	598.72	62.8	41601.58	1233387	2.3732 ng/ml
acetyl-norfentanyl	3.183	54235	510.83	32.6	301.52	874798	2.4713 ng/ml
Amitriptyline	5.678	95820	567.00	74.0	441.09	137826	25.6143 ng/ml
Flurazepam	5.345	671754	975.91	10.7	83570.43	278200	25.3455 ng/ml
Norketamine	4.108	91931	720.34	478.4	2726.21	874798	26.8662 ng/ml

AS

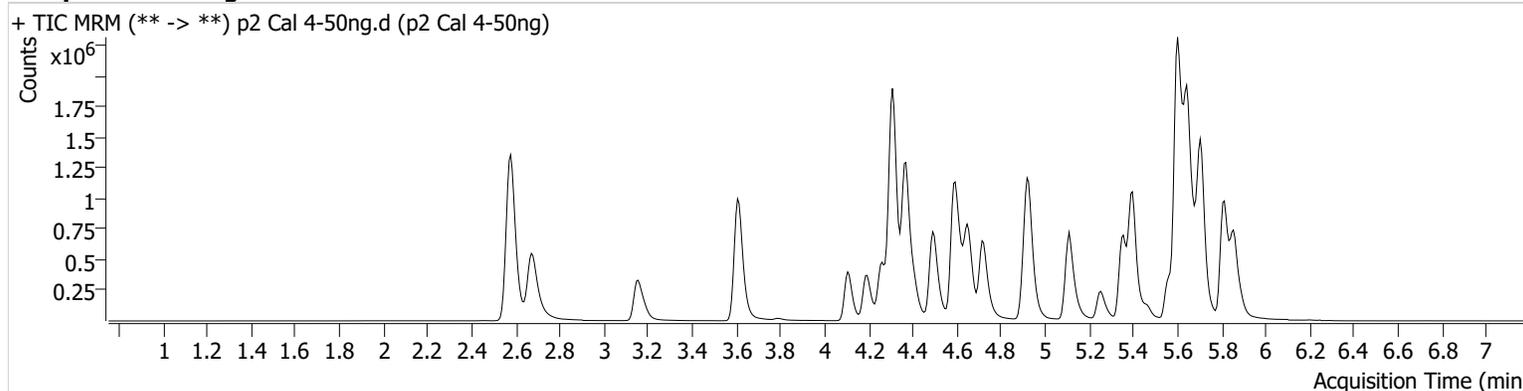


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Cal 4-50ng.d
<b>Type</b>	Cal	<b>Sample</b>	p2 Cal 4-50ng
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-D4	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 5:34:30 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	526540	9073.74	26.0	1509.87	576270	53.3110 ng/ml
acetyl-fentanyl	4.666	91942	3862.04	62.6	43981.17	1028198	4.8031 ng/ml
acetyl-norfentanyl	3.183	114175	486.61	32.8	2762.31	896784	5.1096 ng/ml
Amitriptyline	5.678	206690	831.24	71.5	663.28	152711	50.3743 ng/ml
Flurazepam	5.345	1092819	1052884.45	11.3	47713.04	267585	41.4150 ng/ml
Norketamine	4.108	180091	1279.18	483.9	44712.10	896784	52.1467 ng/ml

AS

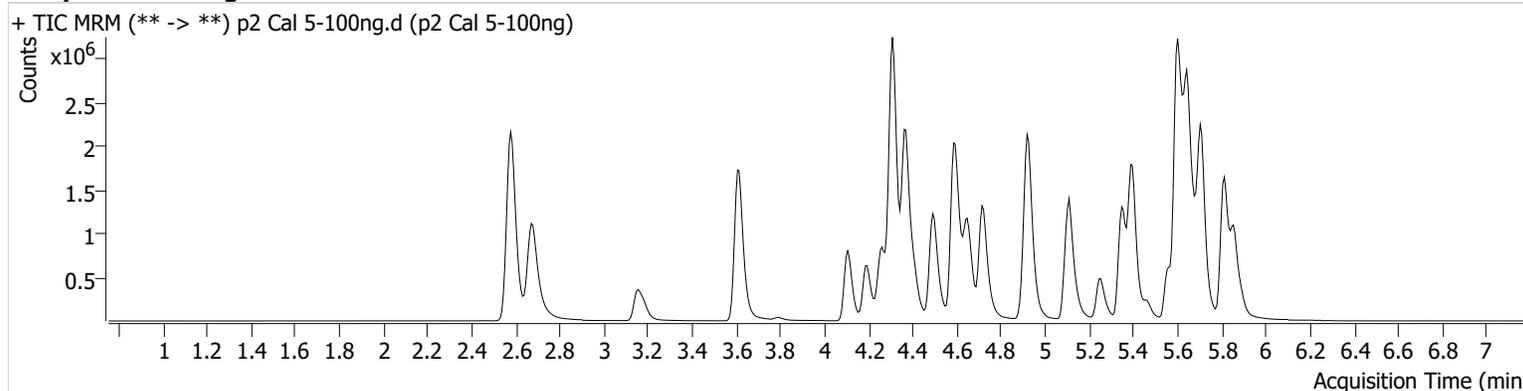


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Cal 5-100ng.d
<b>Type</b>	Cal	<b>Sample</b>	p2 Cal 5-100ng
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-E4	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 5:45:05 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	1091301	1121.20	26.5	2193.38	620121	103.2954 ng/ml
acetyl-fentanyl	4.666	191797	3677.58	63.0	153138.51	1094213	9.3513 ng/ml
acetyl-norfentanyl	3.183	236162	20703.65	33.1	1056.77	937985	10.1367 ng/ml
Amitriptyline	5.678	335902	9011.49	75.7	727.86	125554	100.0967 ng/ml
Flurazepam	5.345	2339225	2856821.05	11.1	753.30	253906	90.7866 ng/ml
Norketamine	4.108	363602	1886.40	487.4	17497.10	937985	101.4827 ng/ml

AS

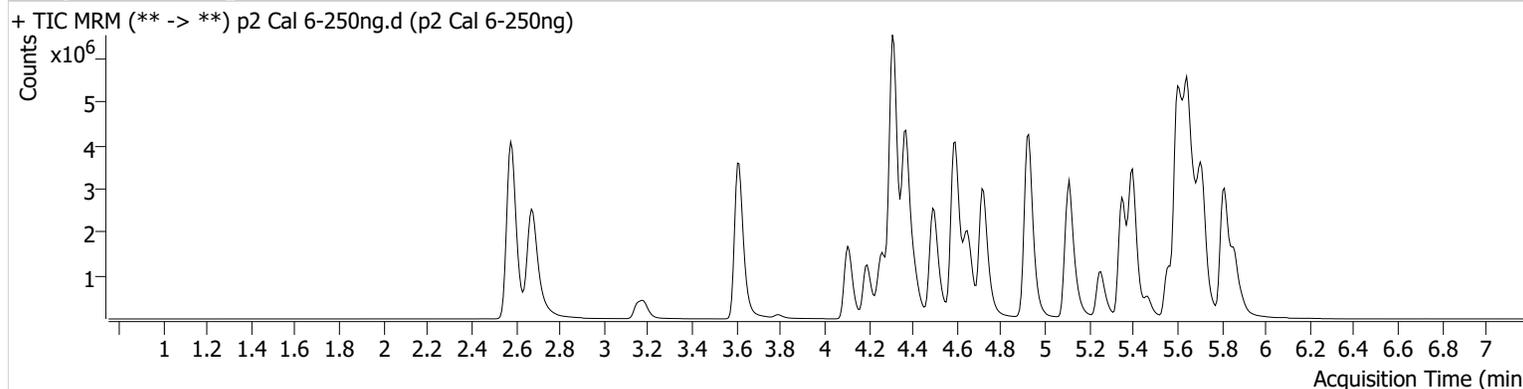


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Cal 6-250ng.d
<b>Type</b>	Cal	<b>Sample</b>	p2 Cal 6-250ng
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-F4	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 5:55:39 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	2664314	1176.39	26.2	2681.83	626128	250.7112 ng/ml
acetyl-fentanyl	4.666	420567	7885.32	63.7	274249.35	941291	23.7336 ng/ml
acetyl-norfentanyl	3.183	591302	2325.07	33.3	695.67	953233	25.0223 ng/ml
Amitriptyline	5.678	903478	18323.69	75.6	1152.22	135082	251.0458 ng/ml
Flurazepam	5.345	5148404	123673.30	11.1	746342.63	187470	266.4589 ng/ml
Norketamine	4.108	766326	6044.93	509.1	3066.47	953233	211.4135 ng/ml

AS

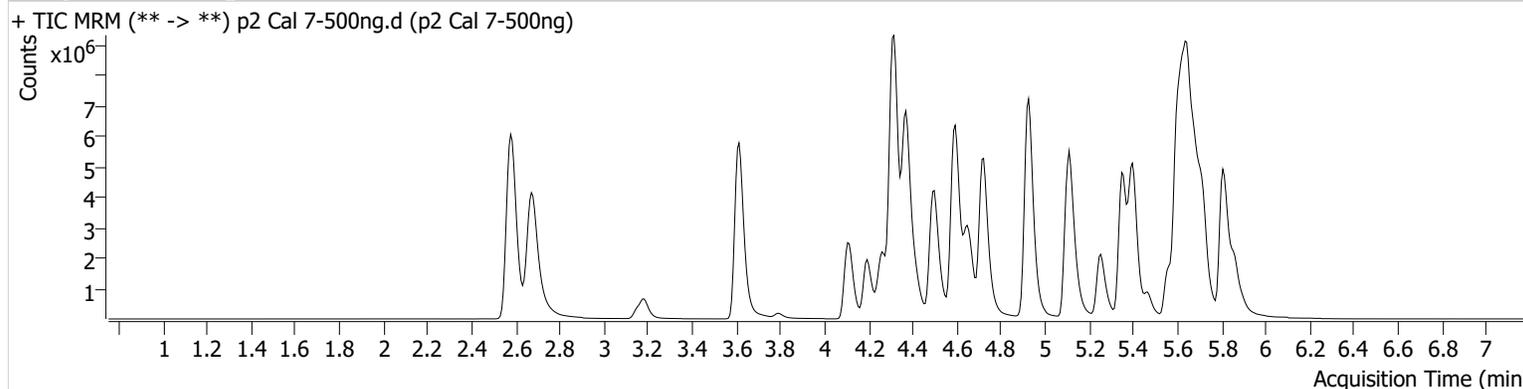


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Cal 7-500ng.d
<b>Type</b>	Cal	<b>Sample</b>	p2 Cal 7-500ng
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-G4	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 6:06:15 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	4532743	2614.87	26.2	30193.30	557545	479.6037 ng/ml
acetyl-fentanyl	4.666	828129	39851.23	63.8	1629314.99	878548	49.9972 ng/ml
acetyl-norfentanyl	3.183	1132012	66519.44	33.4	34728.98	901235	50.7011 ng/ml
Amitriptyline	5.678	1978190	583.98	78.0	2125.98	148764	499.6458 ng/ml
Flurazepam	5.345	9232962	1300.93	11.1	4144.93	104189	855.1384 ng/ml
Norketamine	4.108	1233458	10331.69	508.4	392285.33	901235	360.5406 ng/ml

AG

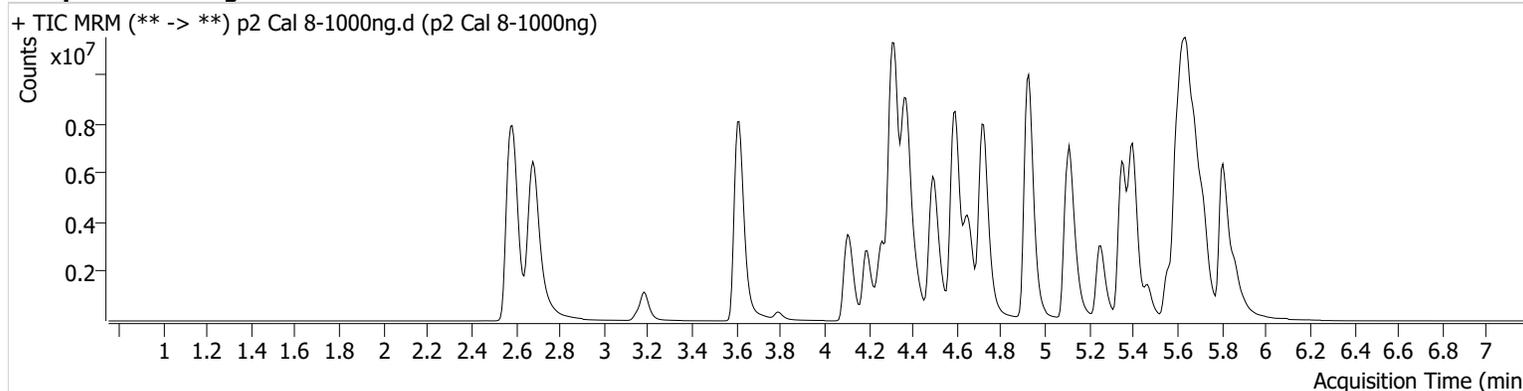


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2021\AM 27-28\050621 AM 28 P2 AG\QuantResults\Correct AM 28 P2 evaluated.batch.bin  
**Calibration Last Update** 5/10/2021 10:27:01 AM

<b>Instrument</b>	Falco (069901)	<b>Data File</b>	p2 Cal 8-1000ng.d
<b>Type</b>	Cal	<b>Sample</b>	p2 Cal 8-1000ng
<b>Acq. Method</b>	AM 28 MDQ P2.m	<b>Operator</b>	Amber Gerheart
<b>Sample Position</b>	P6-H4	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	5/6/2021 6:16:51 PM		

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-aminoflunitrazepam	4.647	7500274	1037.07	26.2	4949.01	517234	855.9658 ng/ml
acetyl-fentanyl	4.666	1139185	205260.88	67.5	340417.03	591340	102.1117 ng/ml
acetyl-norfentanyl	3.183	2226511	84644.60	32.6	29852.63	907541	99.0605 ng/ml
Amitriptyline	5.678	2770560	24660.66	77.9	1093.06	107132	972.2271 ng/ml
Flurazepam	5.345	13597661	864.01	11.3	509.18	58847	2226.3807 ng/ml
Norketamine	4.108	1839364	14049.17	516.3	148000.38	907541	534.3372 ng/ml