

**REVIEWED**  
By Britany Wylie at 10:16 am, Sep 27, 2019

9/23/2019 *BS*

**Worklist: 3704**

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>
P2019-2517	1	164854	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QC
P2019-2594	1	164855	AM 28 Blood Multi-Drug Quant Panel 2 by LC-QC



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

Extraction Date: 09/23/19  
Plate lot#: Item #:IDP-112 Lot:190730

Analyst: Tamara Salazar  
Plate Expiration: 01/30/20

**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:** 445283-2

**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: 3** in wells of analytical (standards) plate.
- 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 067105*
- 4. Pipette **250µL 00.5M 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) Manifold ID: 067104*
- 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.  
*SPE Dry ID: 067103*
- 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.  
Worklist path: *D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS*  
Batch Name: *MDQ P2 wklst 3704\_only evaluated compounds*
- 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? Y / N \_\_\_\_\_ Add Control data to QC tracking spreadsheet.
- 6. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports.

COMMENTS: *Only methylphenidate and pseudoephedrine evaluated.*



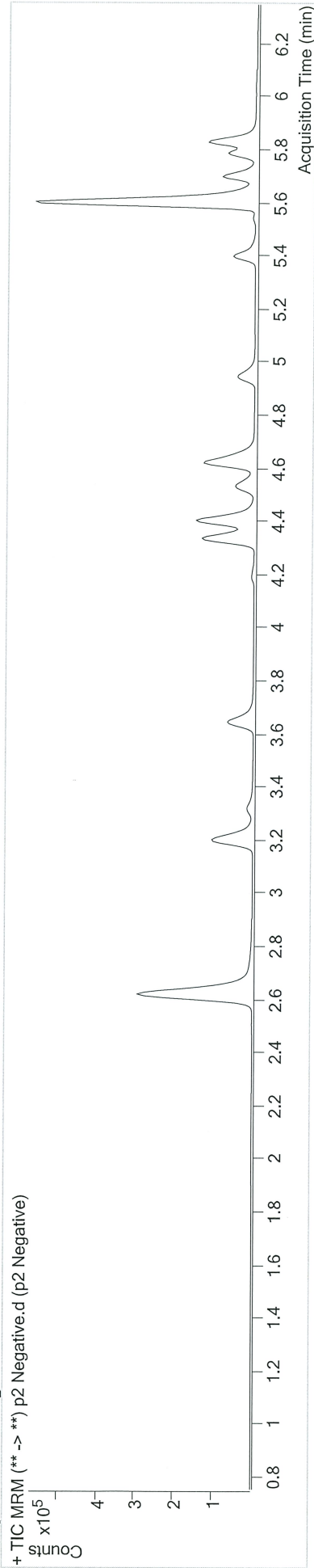
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wklist 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco	<b>Data File</b>	p2 Negative.d
<b>Acq. Method</b>	MDQ P2 Combined 092319.m	<b>Sample Operator</b>	p2 Negative
<b>Sample Position</b>	P1-E6	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 4:06:17 PM		

## Sample Info.

## Sample Chromatogram



TS



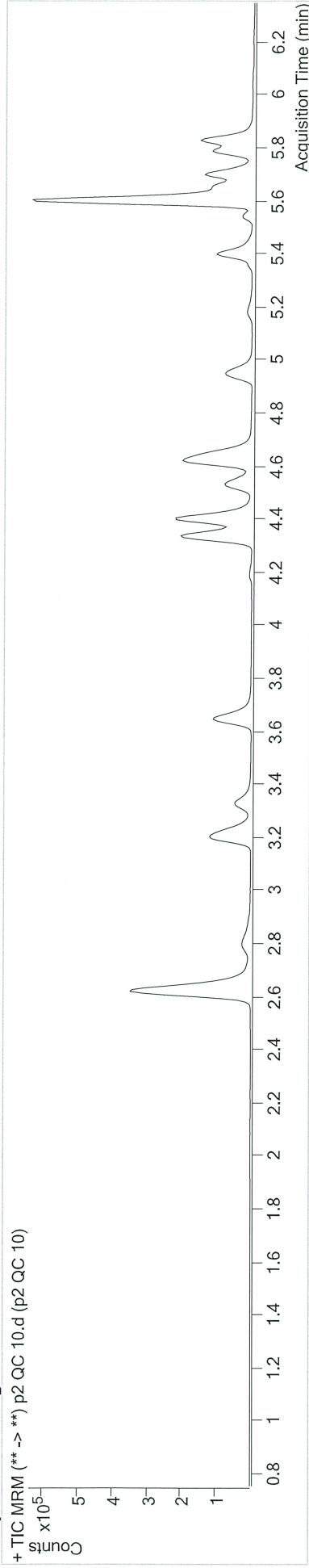
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wk1st 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

**Instrument** Falco  
**Type** QC  
**Acq. Method** MDQ P2 Combined 092319.m  
**Sample Position** P1-A6  
**Injection Volume** 2  
**Acq. Date-Time** 9/23/2019 2:21:15 PM  
**Sample Info.**

**Data File**  
**Sample** p2 QC 10.d  
**Operator** p2 QC 10  
**Comment**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.347	131149	7851.50	24.1	434150	10.3439 ng/ml
Pseudoephedrine	2.637	193485	676.40	14.0	939968	9.9394 ng/ml





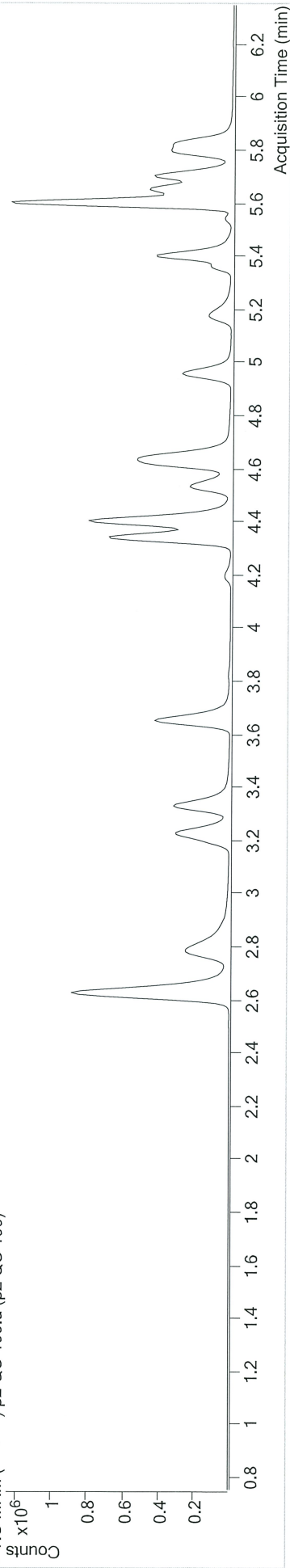
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wklist\_3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco QC	<b>Data File</b>	p2 QC 100.d
<b>Acq. Method</b>	MDQ P2 Combined 092319.m	<b>Sample Operator</b>	p2 QC 100
<b>Sample Position</b>	P1-B6	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 2:42:16 PM		

## Sample Chromatogram

+ TIC MRM (\*\* -> \*\*) p2 QC 100.d (p2 QC 100)



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.347	1249470	43697.94	24.4	425573	103.2134 ng/ml
Pseudoephedrine	2.631	1864519	∞	14.0	916401	100.1930 ng/ml



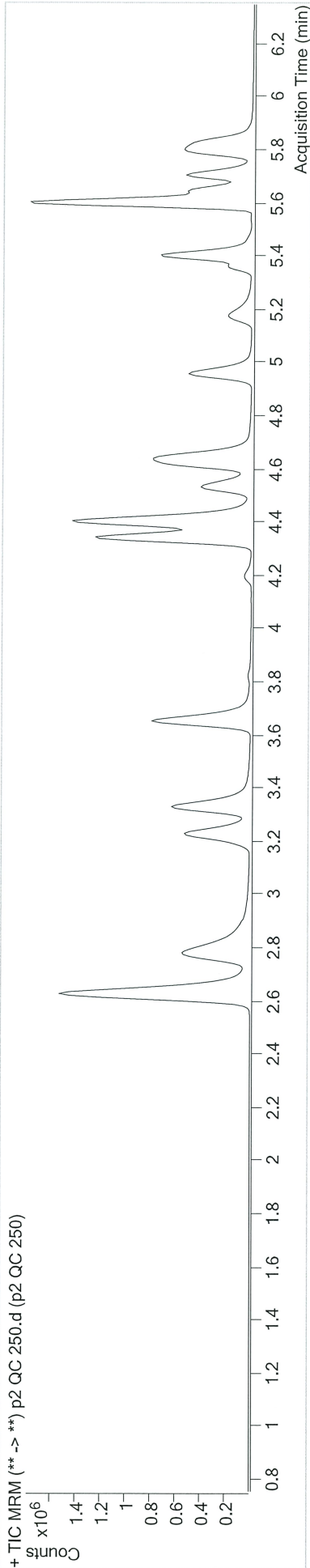


# AM #28 Multi-Drug Quant. Results

Batch results Calibration Last Update D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wklist 3704\_only evaluated compounds.batch.bin  
9/24/2019 9:38:41 AM

Instrument Type Data File  
Falco QC p2 QC 250.d  
Acq. Method MDQ P2 Combined 092319.m  
Sample Position P1-C6  
Injection Volume 2  
Acq. Date-Time 9/23/2019 3:03:15 PM  
Sample Info.

### Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.347	2535442	80651.56	24.5	356011	250.8038 ng/ml
Pseudoephedrine	2.631	3917644	∞	14.0	790062	244.5007 ng/ml



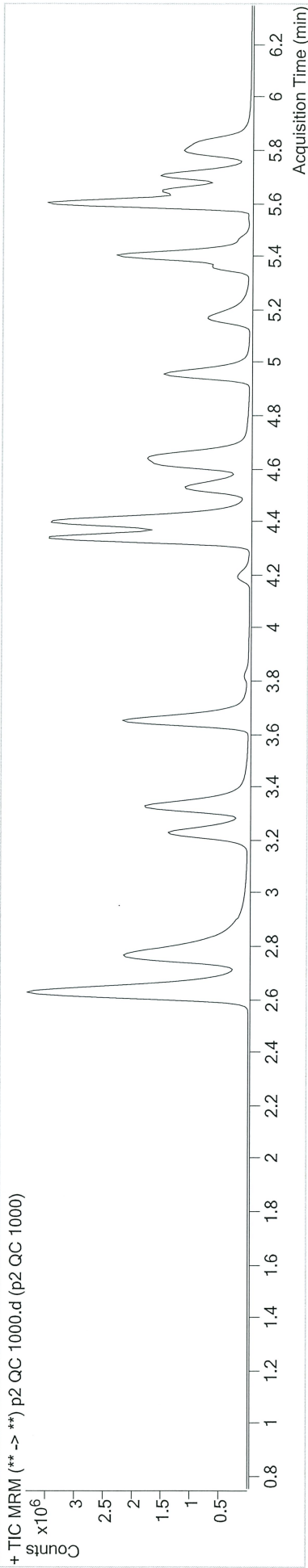


# AM #28 Multi-Drug Quant. Results

Batch results Calibration Last Update D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wk1st 3704\_only evaluated compounds.batch.bin  
9/24/2019 9:38:41 AM

Instrument Type Falco  
QC p2 QC 1000.d  
Acq. Method MDQ P2 Combined 092319.m  
Sample Position P1-D6  
Injection Volume 2  
Acq. Date-Time 9/23/2019 3:24:15 PM  
Sample Info. Comment

### Sample Chromatogram



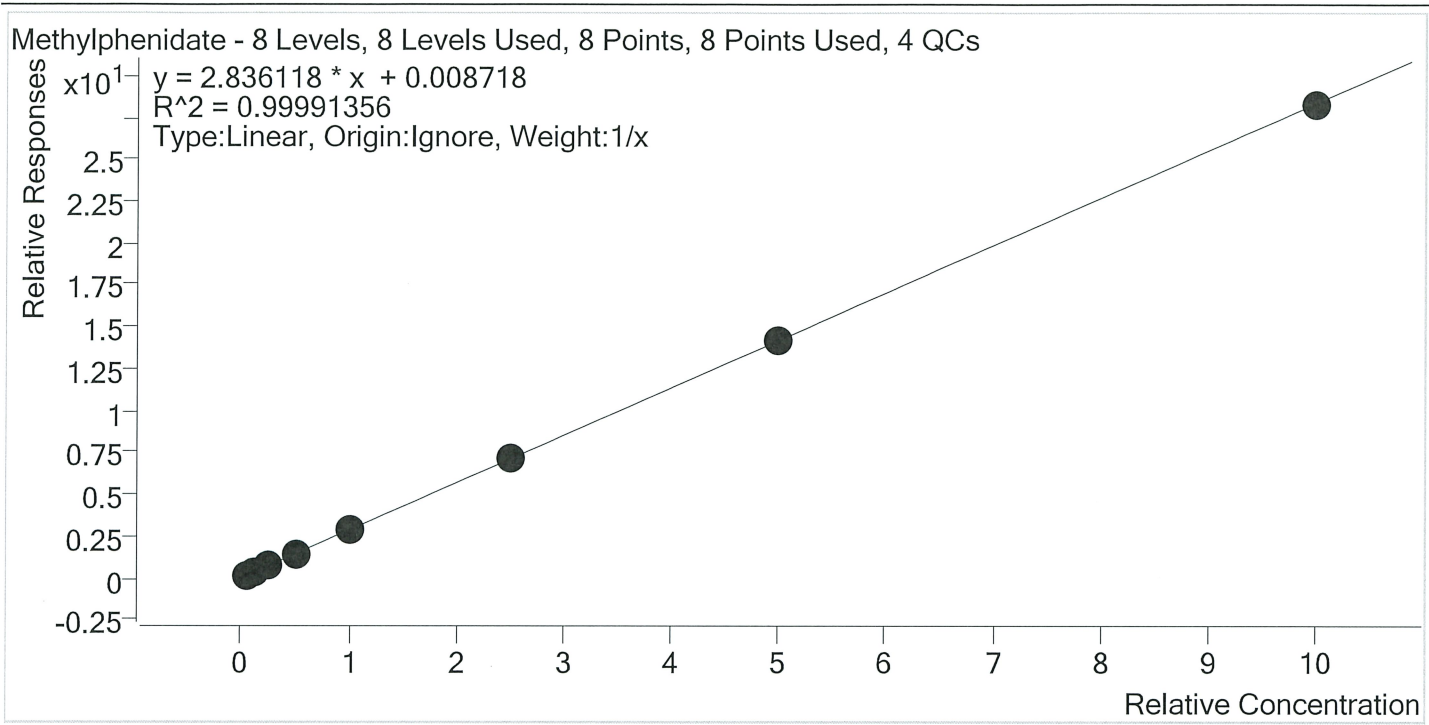
Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methamphetamine	4.347	7769449	566993.22	25.1	272095	1006.4967 ng/ml
Pseudoephedrine	2.631	12376689	∞	13.9	612528	996.9851 ng/ml

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

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wk1st  
 3704\_only evaluated compounds.batch.bin  
**Last Cal. Update** 9/24/2019 9:38 AM  
**Analyst Name** ISP\datastor  
**Analyte** Methylphenidate **Internal Standard** Methylphenidate-D4



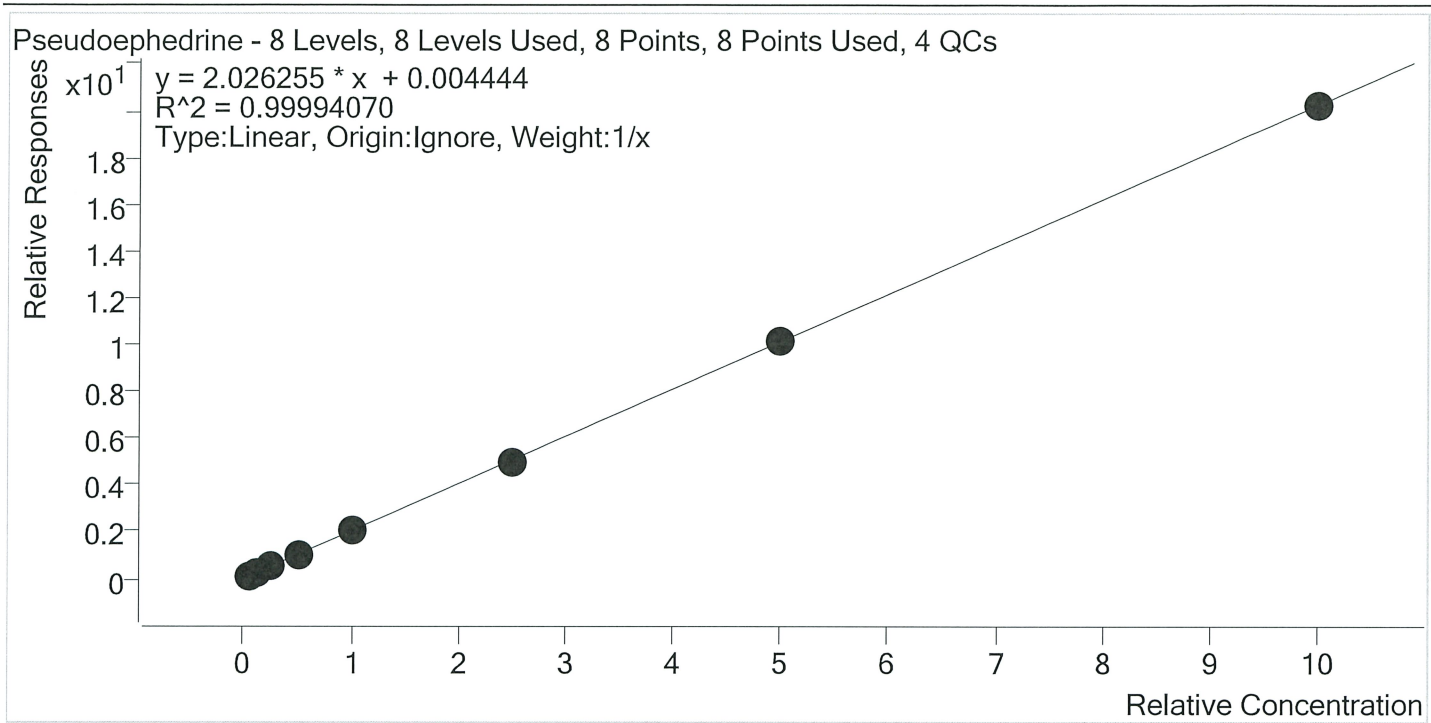
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
p2 Cal 1-5ng	1	✓	5.0	4.9	98.7
p2 Cal 2- 10ng	2	✓	10.0	10.1	101.1
p2 Cal 3 -25ng	3	✓	25.0	25.0	99.8
p2 Cal 4-50ng	4	✓	50.0	48.7	97.4
p2 Cal 5-100ng	5	✓	100.0	102.7	102.7
p2 Cal 6-250ng	6	✓	250.0	250.1	100.1
p2 Cal 7-500ng	7	✓	500.0	503.3	100.7
p2 Cal 8-1000ng	8	✓	1000.0	995.2	99.5





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

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wk1st  
 3704\_only evaluated compounds.batch.bin  
**Last Cal. Update** 9/24/2019 9:38 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	5.0	100.4
p2 Cal 2- 10ng	2	✓	10.0	10.1	100.7
p2 Cal 3 -25ng	3	✓	25.0	24.6	98.3
p2 Cal 4-50ng	4	✓	50.0	49.4	98.8
p2 Cal 5-100ng	5	✓	100.0	102.1	102.1
p2 Cal 6-250ng	6	✓	250.0	248.1	99.3
p2 Cal 7-500ng	7	✓	500.0	503.4	100.7
p2 Cal 8-1000ng	8	✓	1000.0	997.3	99.7

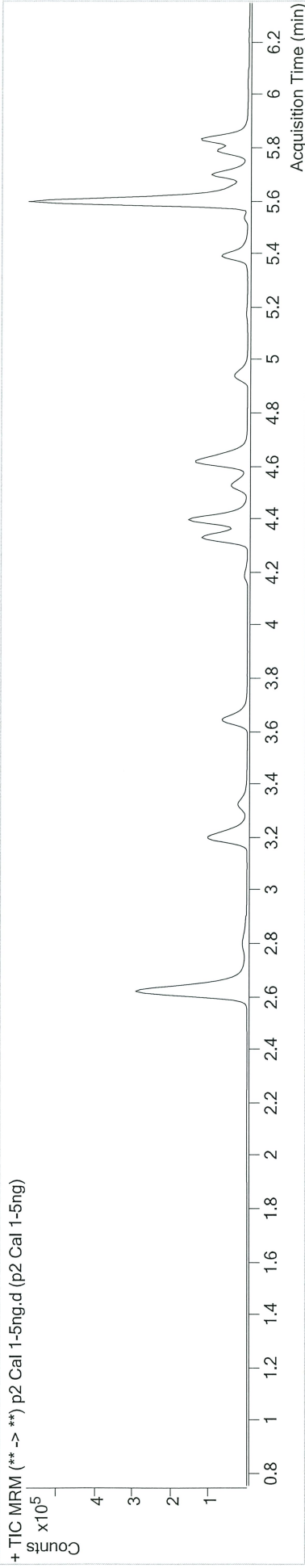


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wk1st 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco Cal	<b>Data File Sample</b>	p2 Cal 1-5ng.d
<b>Acq. Method</b>	MDQ P2 Combined	<b>Operator</b>	p2 Cal 1-5ng
<b>Sample Position</b>	P1-A7	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 12:25:30 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methamphetamine	4.340	43066	2556.92	24.3	289598	4.9361 ng/ml
Pseudoephedrine	2.631	90432	9160.51	13.9	851908	5.0195 ng/ml





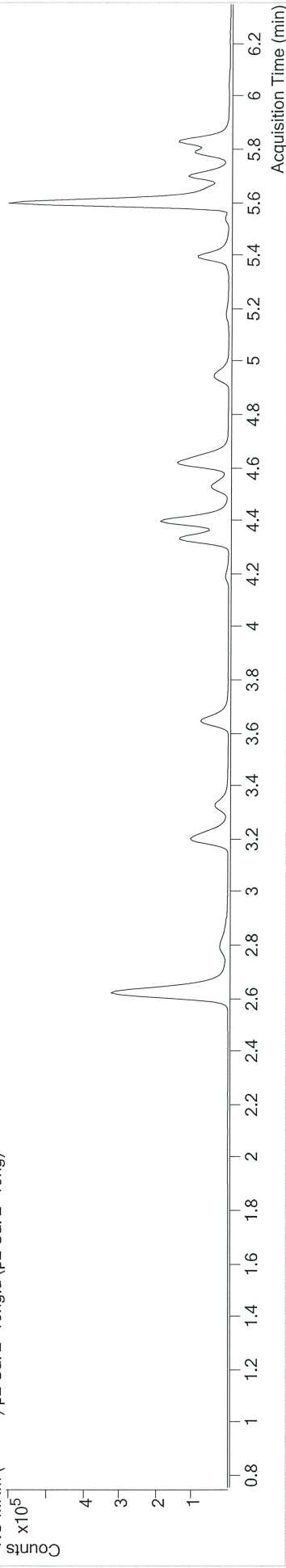
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wk1st 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco Cal	<b>Data File</b>	p2 Cal 2- 10ng.d
<b>Acq. Method</b>	MDQ P2 Combined 092319.m	<b>Sample Operator</b>	p2 Cal 2- 10ng
<b>Sample Position</b>	P1-B7	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 12:36:10 PM		

## Sample Chromatogram

+ TIC MRM (\*\* -> \*\*) p2 Cal 2- 10ng.d (p2 Cal 2- 10ng)



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.340	85474	12438.94	23.6	289308	10.1098 ng/ml
Pseudoephedrine	2.631	180310	9384.44	13.8	864977	10.0685 ng/ml

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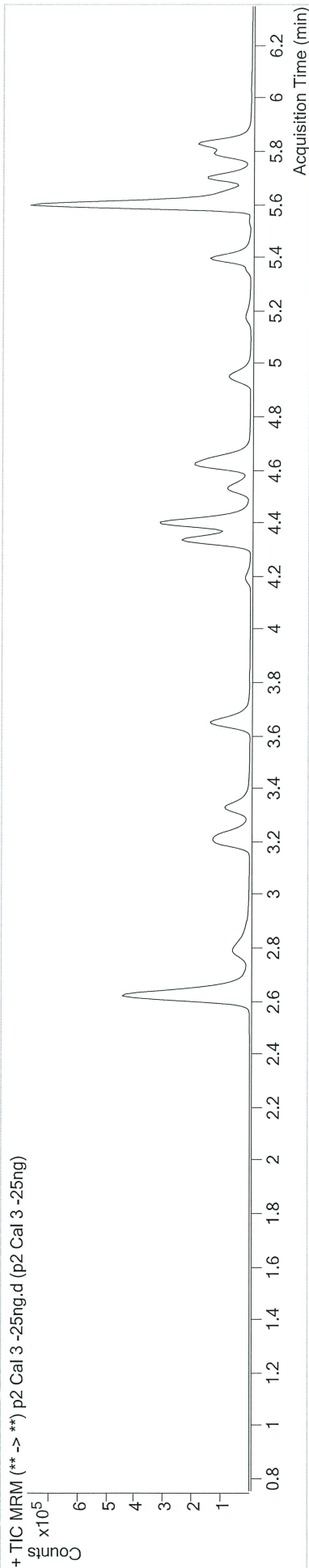


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wkst 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco Cal	<b>Data File</b>	p2 Cal 3 -25ng.d
<b>Acq. Method</b>	MDQ P2 Combined 092319.m	<b>Sample Operator</b>	p2 Cal 3 -25ng
<b>Sample Position</b>	P1-C7	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 12:46:40 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Methylphenidate	4.347	261448	133427.73	24.1	4875.05	364857	24.9587 ng/ml
Pseudoephedrine	2.631	478381	13266.34	14.1	4115.97	951741	24.5869 ng/ml

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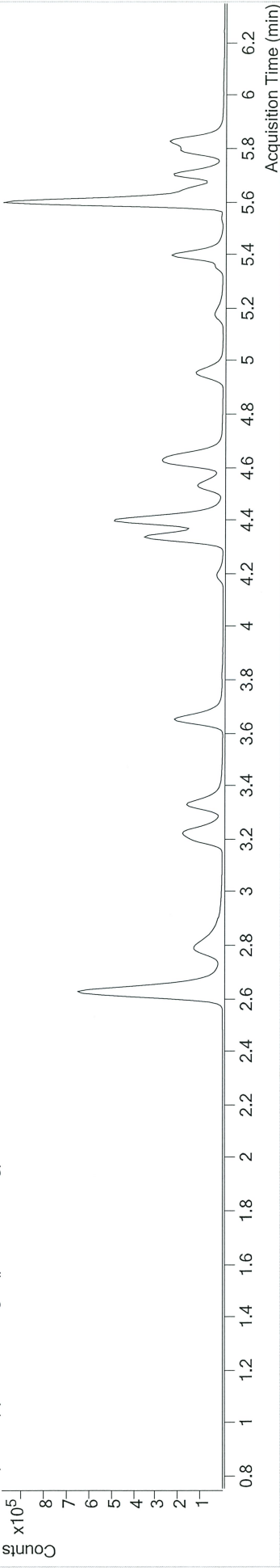
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wklist 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco Cal	<b>Data File</b>	p2 Cal 4-50ng.d
<b>Acq. Method</b>	MDQ P2 Combined 092319.m	<b>Sample Operator</b>	p2 Cal 4-50ng
<b>Sample Position</b>	P1-D7	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 12:57:10 PM		

## Sample Chromatogram

+ TIC MRM (\*\* -> \*\*) p2 Cal 4-50ng.d (p2 Cal 4-50ng)



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.347	511801	10650.81	24.2	368165	48,7083 ng/ml
Pseudoephedrine	2.631	1040623	∞	13.9	1035302	49,3865 ng/ml



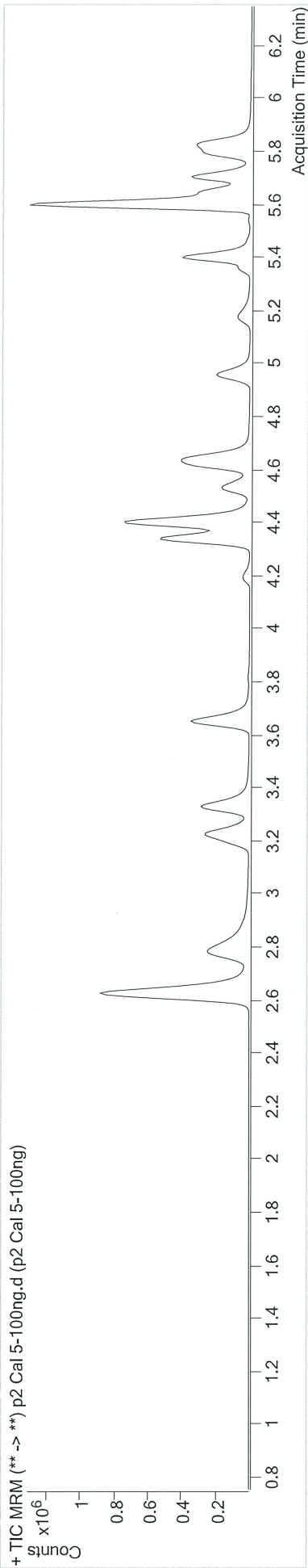


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wk1st 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco Cal	<b>Data File</b>	p2 Cal 5-100ng.d
<b>Acq. Method</b>	MDQ P2 Combined 092319.m	<b>Sample Operator</b>	p2 Cal 5-100ng
<b>Sample Position</b>	P1-E7	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 1:07:40 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.347	951763	24407.51	24.3	325764	102.7078 ng/ml
Pseudoephedrine	2.631	1850567	11663.29	14.1	892202	102.1448 ng/ml

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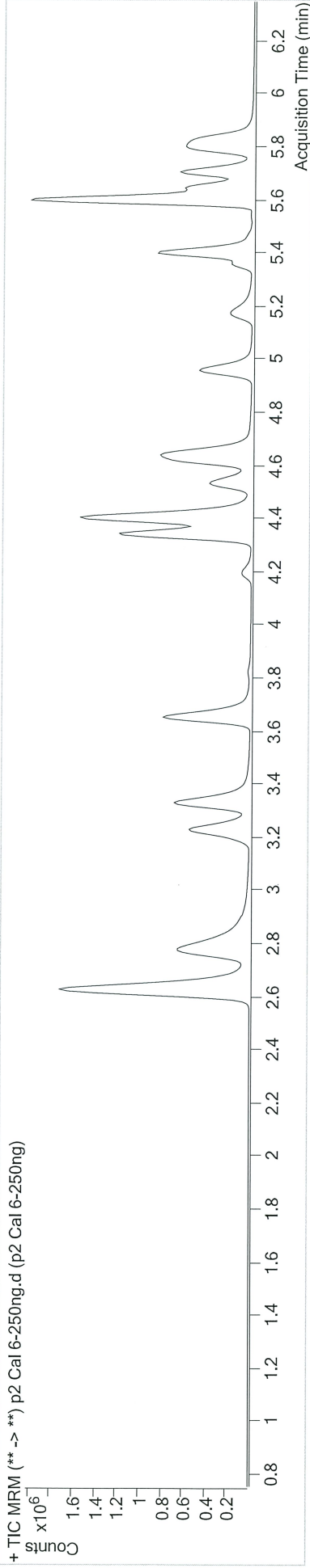


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wk1st 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco Cal	<b>Data File</b>	p2 Cal 6-250ng.d
<b>Acq. Method</b>	MDQ P2 Combined 092319.m	<b>Sample Operator</b>	p2 Cal 6-250ng
<b>Sample Position</b>	P1-F7	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 1:18:10 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.347	2416396	80093.64	24.6	340211	250.1280 ng/ml
Pseudoephedrine	2.631	4471458	∞	14.0	888526	248.1427 ng/ml





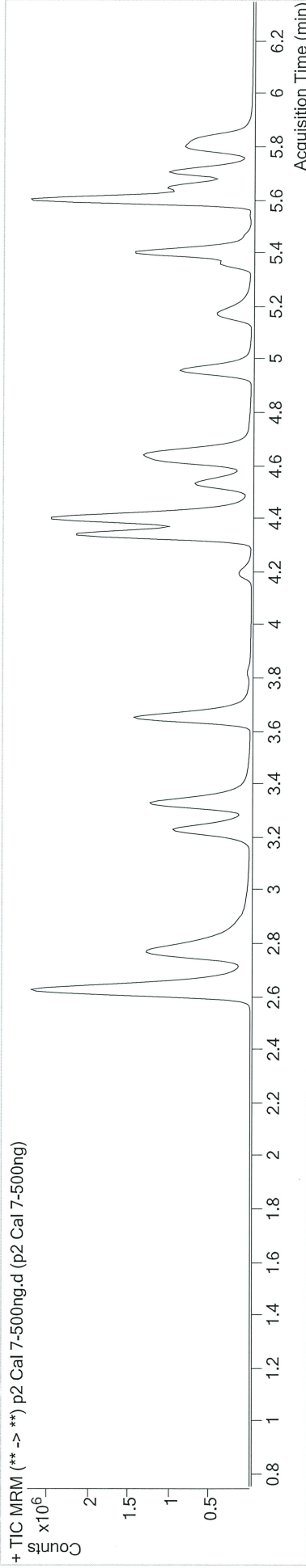
# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wklist 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

**Instrument Type** Falco  
**Acq. Method** Cal  
**Sample Position** MDQ P2 Combined 092319.m  
**Injection Volume** P1-G7  
**Acq. Date-Time** 2  
**Sample Info.** 9/23/2019 1:28:40 PM

**Data File**  
**Sample** p2 Cal 7-500ng.d  
**Operator** p2 Cal 7-500ng  
**Comment**

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.347	4672296	153874.27	24.8	327157	503.2516 ng/ml
Pseudoephedrine	2.631	7892688	359585.09	13.9	773475	503.3791 ng/ml

TS



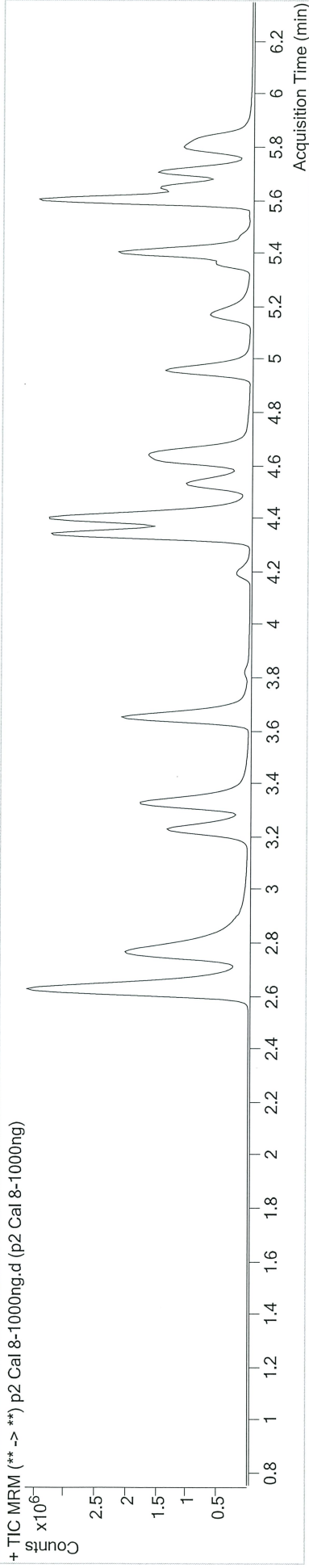


# AM #28 Multi-Drug Quant. Results

**Batch results** D:\MassHunter\Data\2019\AM 28\092319 MDQ P1 and P2 TS\QuantResults\MDQ P2 wklist 3704\_only evaluated compounds.batch.bin  
**Calibration Last Update** 9/24/2019 9:38:41 AM

<b>Instrument Type</b>	Falco Cal	<b>Data File</b>	p2 Cal 8-1000ng.d
<b>Acq. Method</b>	MDQ P2 Combined 092319.m	<b>Sample Operator</b>	p2 Cal 8-1000ng
<b>Sample Position</b>	P1-H7	<b>Comment</b>	
<b>Injection Volume</b>	2		
<b>Acq. Date-Time</b>	9/23/2019 1:39:11 PM		

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	ISTD Resp.	Final Conc.
Methylphenidate	4.347	7242367	∞	24.7	256514	995.1997 ng/ml
Pseudoephedrine	2.631	11599397	213947.44	13.8	573895	997.2721 ng/ml

B