

**REVIEWED**

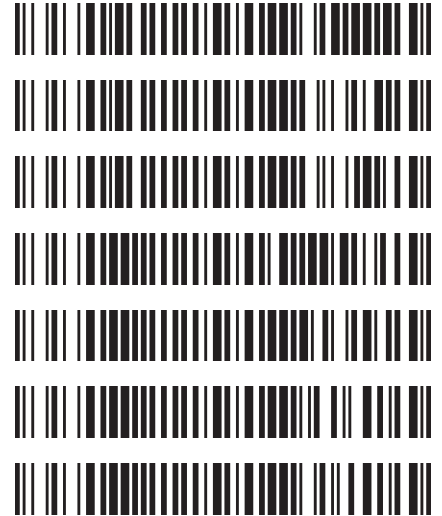
By Anne Nord at 4:45 pm, Jul 19, 2019

7/11/2019

g

**Worklist: 3536**

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>
M2019-2783	3	156968	AM 13 Urine LCMS-QQQ Benzodiazepines and ;
M2019-2908	3	156970	AM 13 Urine LCMS-QQQ Benzodiazepines and ;
M2019-3023	3	156971	AM 13 Urine LCMS-QQQ Benzodiazepines and ;
P2019-1280	1	156990	AM 13 Urine LCMS-QQQ Benzodiazepines and ;
P2019-1895	1	156973	AM 13 Urine LCMS-QQQ Benzodiazepines and ;
P2019-1928	1	156975	AM 13 Urine LCMS-QQQ Benzodiazepines and ;
P2019-1931	1	156978	AM 13 Urine LCMS-QQQ Benzodiazepines and ;



## AM 13: Benzo and Z-Drugs LCMS Urine Extraction

**Extraction Date:** 06/12/2019  
**Negative Blood/Urine Lot:** POC031319  
**Positive Control Working Solution Lot:** WS013119  
**Calibrator Working Solution Lot #'s:** WS021319  
**Oxazepam Glucuronide Lot:** Alltech 715-5035  
**LC Column:** Agilent Poroshell 120 EC-C18 (2.1x75mm; 2.7 Micron)

**Analyst:** Celena Shrum  
**LCMS-QQQ ID:** Abby  
**b-glucuronidase Lot:** Kura 4150

**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. Prep Non-Extracted Calibrators:

Level	ng/mL	Working Solution (ng/μL)	Volume to add (μL)
Blank	-	-	-
1	10	1	10
2	25	1	25
3	50	1	50
4	100	1	100
5	500	10	50
6	1000	10	100
7	3000 (Urine)	10	300

**Analytic:**

- 1. Remove working solutions, external control, negative urine and case samples from cold storage.
- 2. **Blood:** Add 1mL samples, and controls to Extraction tube. **Urine:** To each labeled round bottomed tube add 1mL sample, using negative blood or urine sample for both negative and positive control. Positive control: spike negative blood or urine with 300uL positive control working solution. Urine Required additional glucuronide positive and negative controls.
- 3. To all samples: add 100uL Internal Standard Working Solution.
- 4. For Urine Only: Glucuronide Cleaving:
  - 4.1. Add 20uL of 2M Acetate buffer to all urine samples, Vortex.
  - 4.2. To all but negative glucuronide control: add 76uL Kura b-glucuronidase. Cap and gently rock.
  - 4.3. Place in oven at ~60C for approx. 30 minutes.
- 5. Add 4mL water to all extraction tubes. Urine Samples: Transfer into the extraction tubes.
- 6. Place on tube rocker at ambient temp for ~ 10 minutes.
- 7. Centrifuge for ~ 10 min at ~2500rpm.  
*Optional: To break up emulsions: re-centrifuge at ~3000rpm for ~5 minutes*
- 8. Transfer solvent to tapered bottom tube and evaporate to dryness under nitrogen @ 37C.
- 9. Reconstitute with 100uL of 9:1 mobile phase A:B
- 10. Transfer to labeled ALS vial with insert.
- 11. Run using appropriate LCMS Instrument Acquisition.

**Post-Analytic**

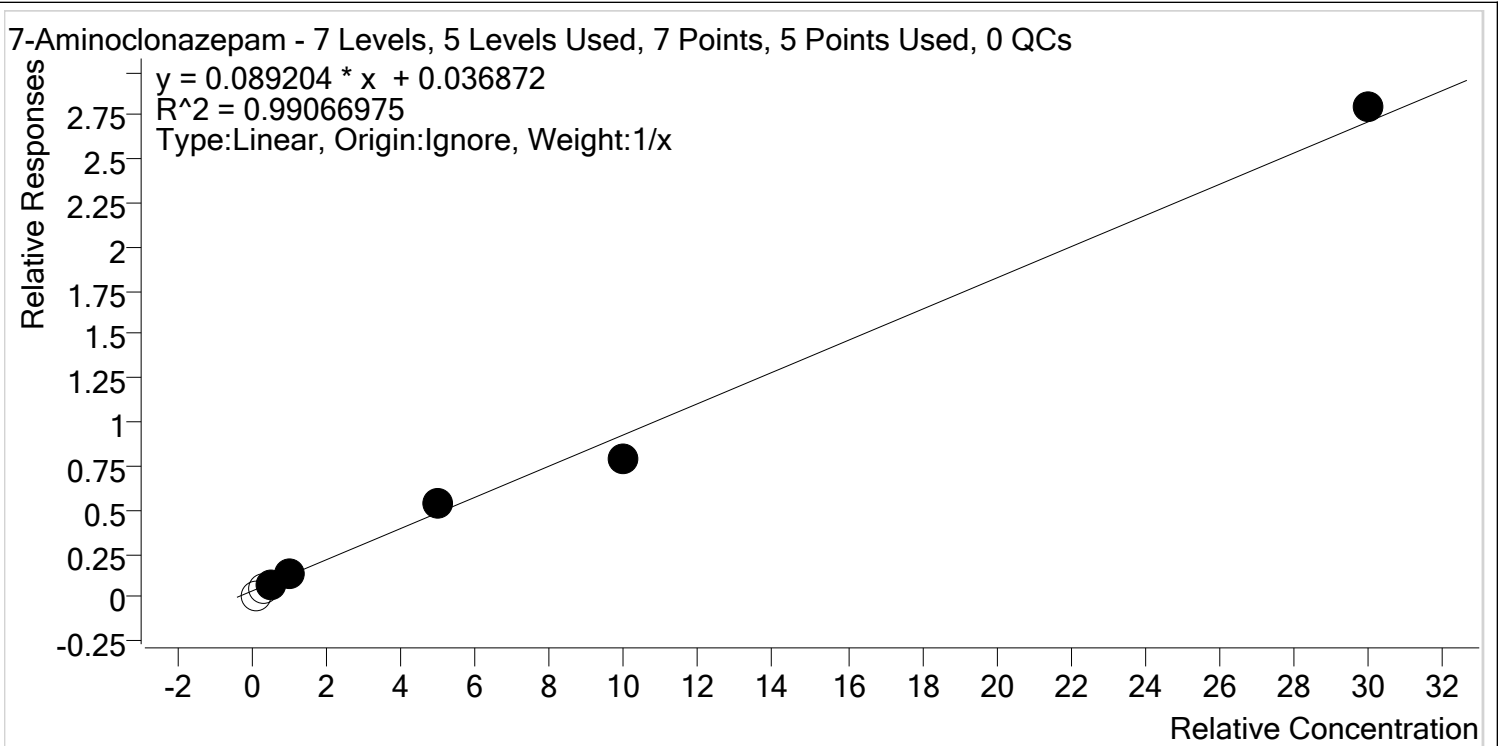
- 1. Complete Data Analysis on all samples and corresponding sample blanks  
 LCMS Data Path: C:\MassHunter\Data\2019\AM 13\AM 13 Worklist 3536\QuantResults\am 13 worklist 3536
- 2. Did positive and negative control samples provide intended response? Y / N
- 3. RT within +/-5% of Calibrators; Ion Ratios within +/- 20% of the calibrator average for the run.
- 4. Central File Packet to include: LIMS Worklist, Method Checklist, Working solution prep sheet, and Control sample LCMS data printouts.

Comments: The samples were run on 7/12/19 but a few of the calibrators and samples failed to inject. New mobile phase needed to be made before the samples could be re injected, so this was done and the samples were re injected. There was a retention time shift between the reinjects and the samples that were run prior. A new data folder was created and all of the samples were re injected. Curve limitations are 7-aminoclonazepam cutoff is 50ng/mL, 7-aminoflunitrazepam cutoff is 100 ng/mL, zopiclone cutoff is 50 ng/mL, alprazolam: 10-1000, clonazepam: 10-1000, diazepam: 10-1000, flunitrazepam: 10-1000, flurazepam: 10-100, midazolam: 10-100, nordiazepam: 10-1000, oxazepam: 10-1000, quetiapine: 10-100, temazepam: 10-1000, zolpidem: 10-100



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** 7-Aminoclonazepam **Internal Standard** 7-Aminoflunitrazepam-D7 ISTD



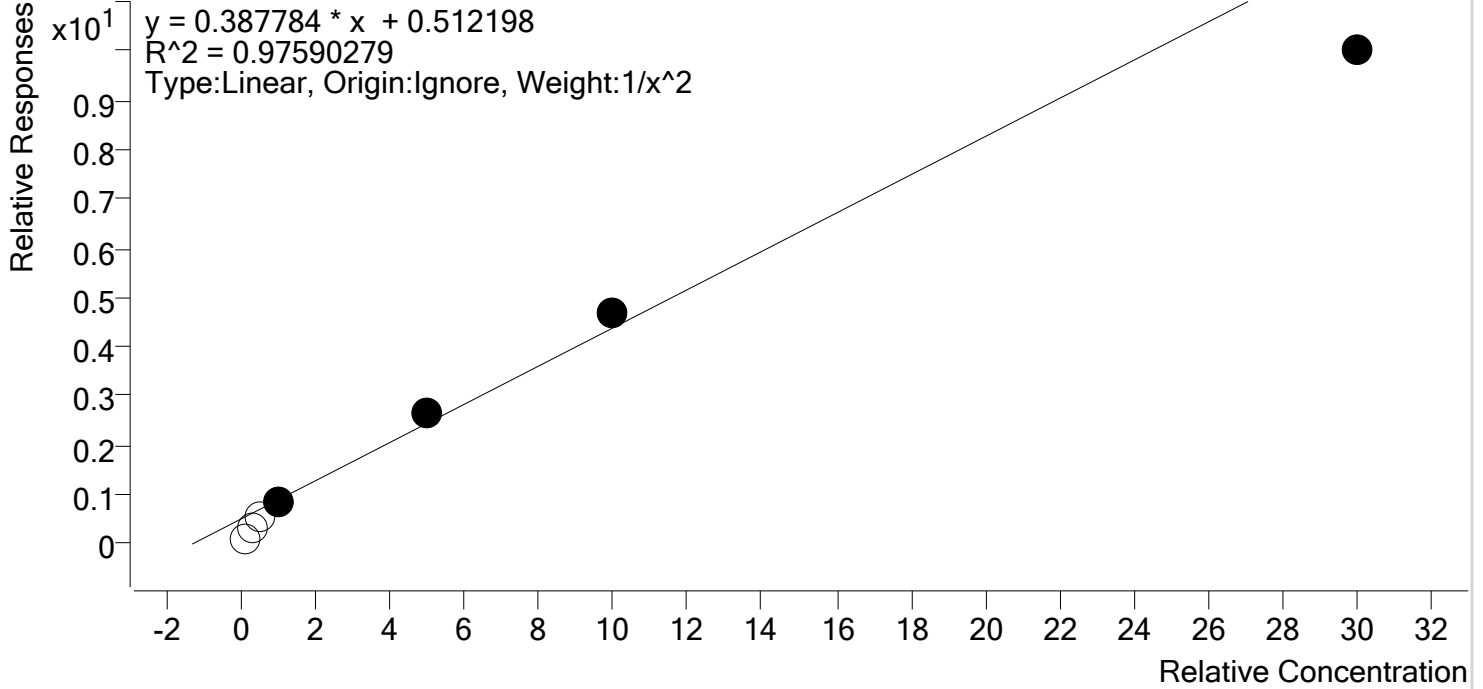
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	x	10.0	0.0	0.0
Cal 2- 25ng	2	x	25.0	4.2	16.9
Cal 3 -50ng	3	✓	50.0	43.1	86.1
Cal 4-100ng-reinject	4	✓	100.0	115.4	115.4
Cal 5-500ng-reinject	5	✓	500.0	554.9	111.0
Cal 6-1000ng-reinject	6	✓	1000.0	843.5	84.4
Cal 7-3000ng	7	✓	3000.0	3093.1	103.1



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** 7-Aminoflunitrazepam **Internal Standard** 7-Aminoflunitrazepam-D7 ISTD

7-Aminoflunitrazepam - 7 Levels, 4 Levels Used, 7 Points, 4 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	x	10.0	0.0	0.0
Cal 2- 25ng	2	x	25.0	0.0	0.0
Cal 3 -50ng	3	x	50.0	8.1	16.1
Cal 4-100ng-reinject	4	✓	100.0	97.3	97.3
Cal 5-500ng-reinject	5	✓	500.0	562.3	112.5
Cal 6-1000ng-reinject	6	✓	1000.0	1085.7	108.6
Cal 7-3000ng	7	✓	3000.0	2451.6	81.7

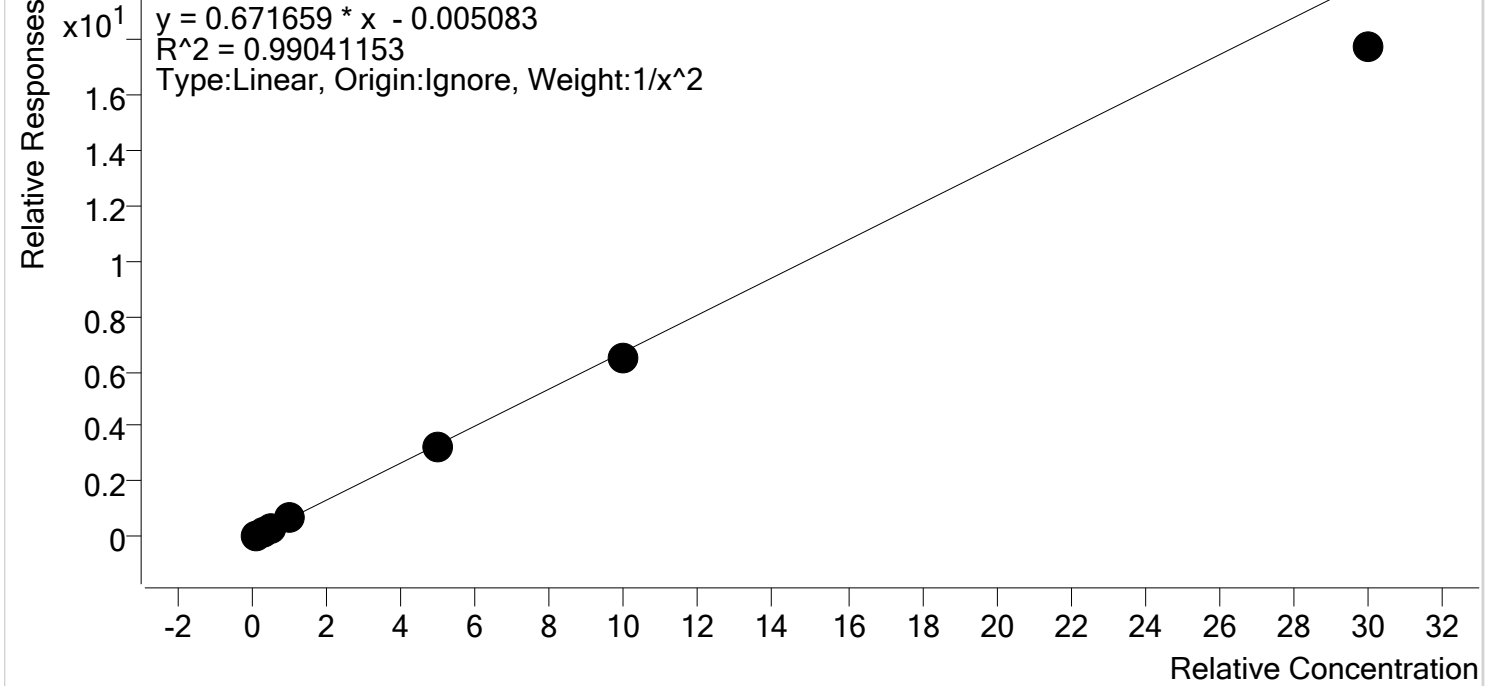




# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** alpha-Hydroxyalprazolam **Internal Standard** alphahydroxyalprazolam -D5 ISTD

alpha-Hydroxyalprazolam - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



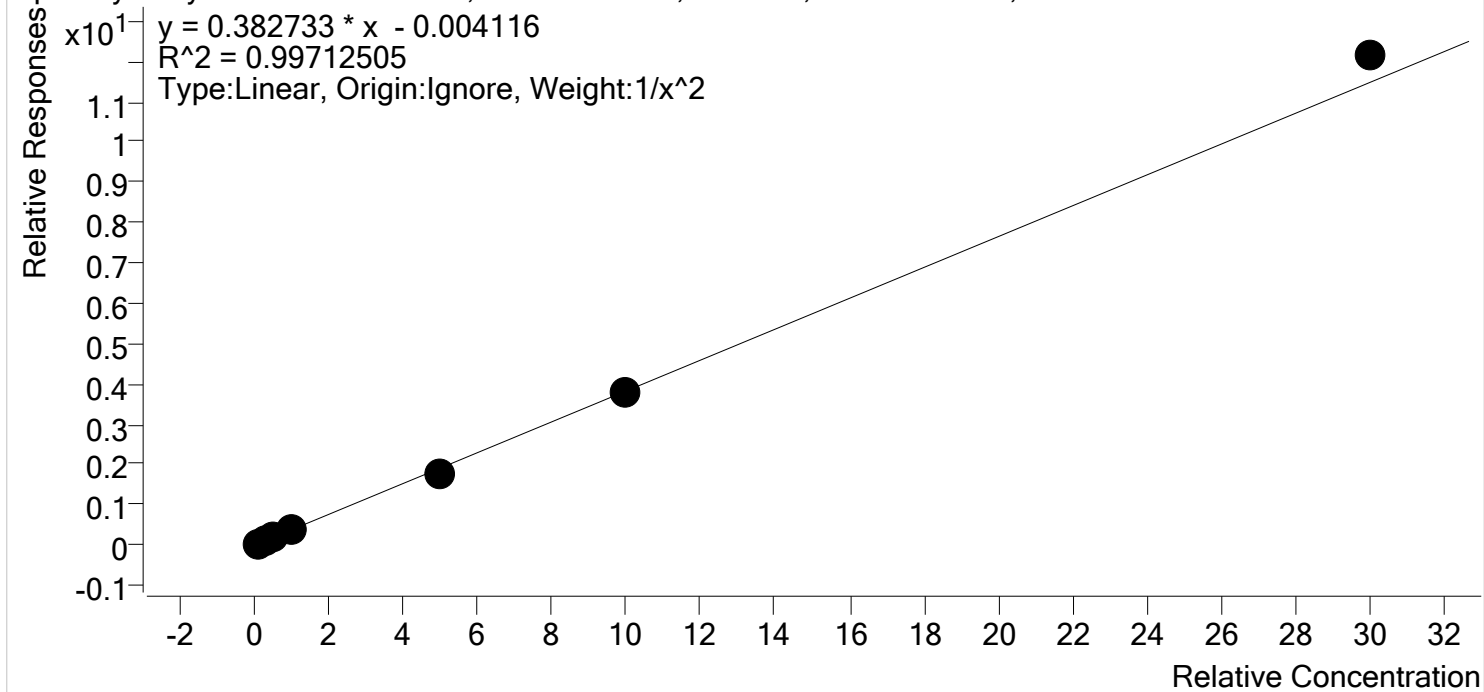
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.3	93.5
Cal 2- 25ng	2	✓	25.0	27.6	110.6
Cal 3 -50ng	3	✓	50.0	55.6	111.1
Cal 4-100ng-reinject	4	✓	100.0	101.4	101.4
Cal 5-500ng-reinject	5	✓	500.0	494.3	98.9
Cal 6-1000ng-reinject	6	✓	1000.0	964.9	96.5
Cal 7-3000ng	7	✓	3000.0	2640.4	88.0



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** alpha-hydroxytriazolam **Internal Standard** Nordiazepam-D5 ISTD

alpha-hydroxytriazolam - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs

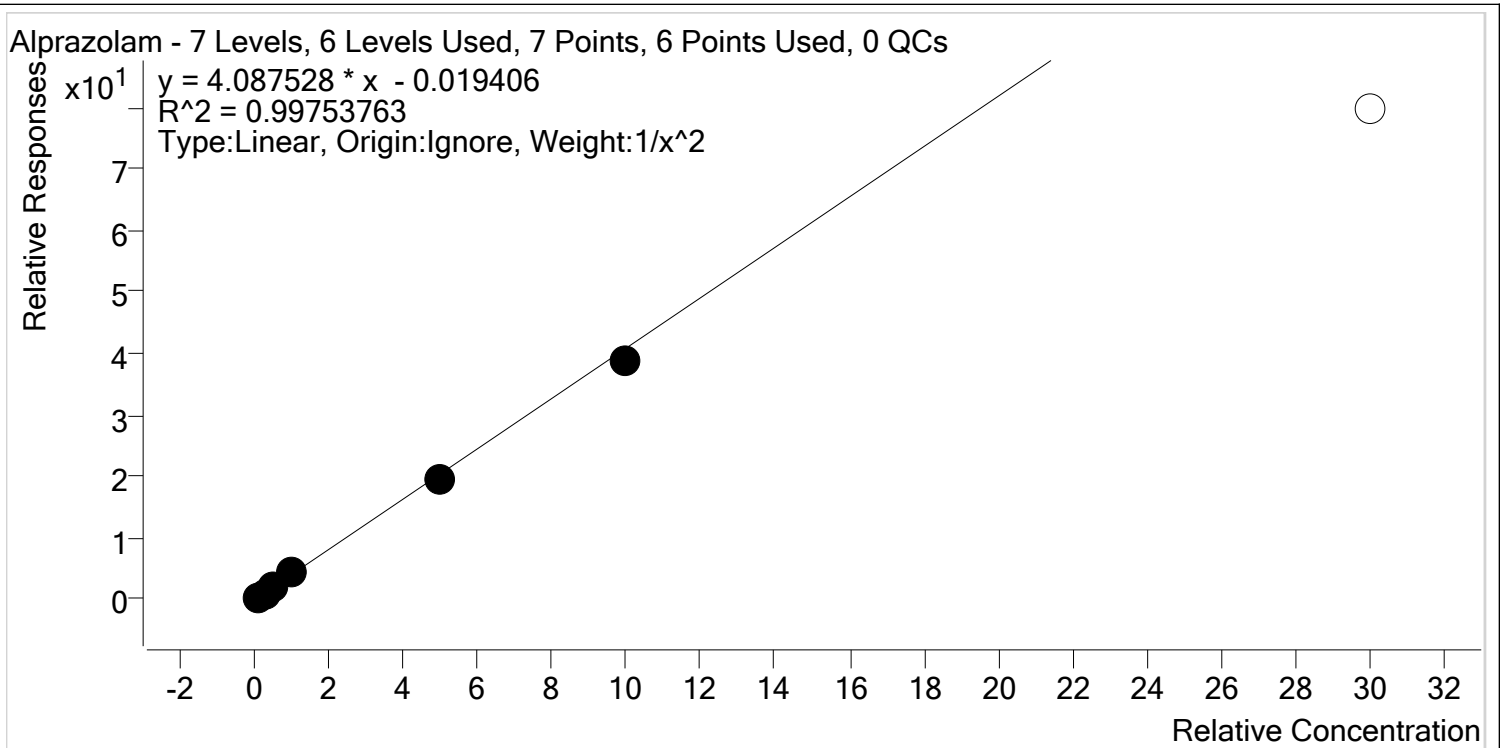


Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	10.0	99.8
Cal 2- 25ng	2	✓	25.0	25.5	102.2
Cal 3 -50ng	3	✓	50.0	47.2	94.5
Cal 4-100ng-reinject	4	✓	100.0	105.3	105.3
Cal 5-500ng-reinject	5	✓	500.0	472.9	94.6
Cal 6-1000ng-reinject	6	✓	1000.0	979.0	97.9
Cal 7-3000ng	7	✓	3000.0	3173.1	105.8



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Alprazolam **Internal Standard** alphahydroxyalprazolam -D5 ISTD



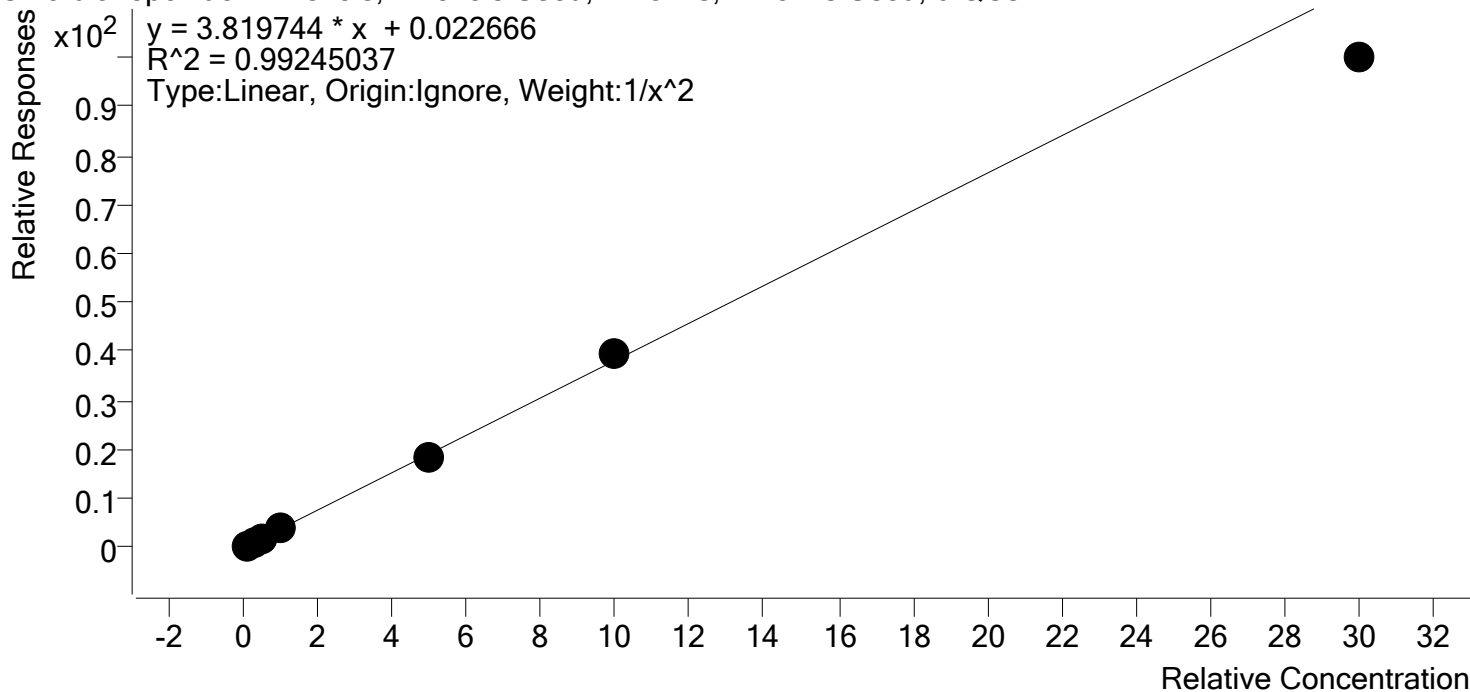
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.8	98.0
Cal 2- 25ng	2	✓	25.0	25.6	102.5
Cal 3 -50ng	3	✓	50.0	51.5	103.0
Cal 4-100ng-reinject	4	✓	100.0	105.3	105.3
Cal 5-500ng-reinject	5	✓	500.0	483.3	96.7
Cal 6-1000ng-reinject	6	✓	1000.0	946.0	94.6
Cal 7-3000ng	7	✗	3000.0	1948.3	64.9



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Chlordiazepoxide **Internal Standard** Nordiazepam-D5 ISTD

Chlordiazepoxide - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



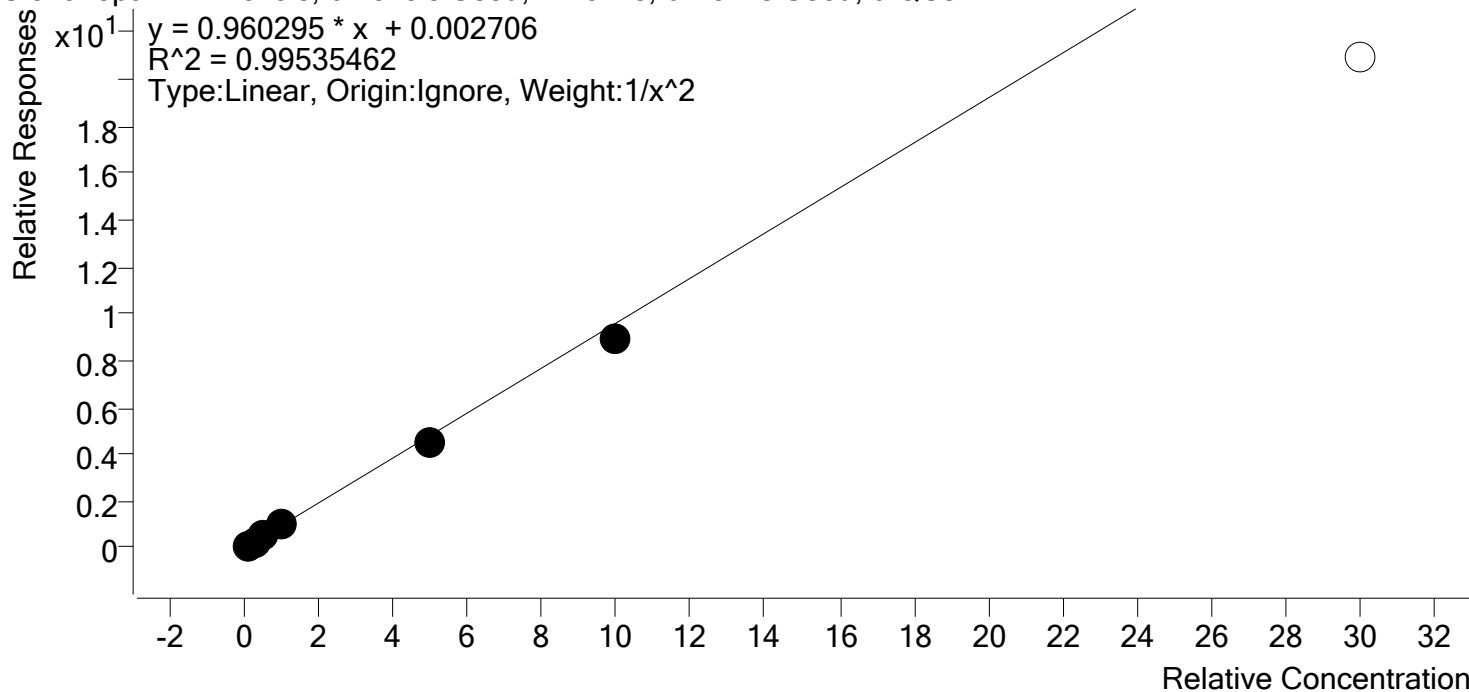
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.6	95.8
Cal 2- 25ng	2	✓	25.0	26.8	107.3
Cal 3 -50ng	3	✓	50.0	51.8	103.7
Cal 4-100ng-reinject	4	✓	100.0	106.8	106.8
Cal 5-500ng-reinject	5	✓	500.0	473.1	94.6
Cal 6-1000ng-reinject	6	✓	1000.0	1045.3	104.5
Cal 7-3000ng	7	✓	3000.0	2617.8	87.3



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Clonazepam **Internal Standard** Clonazepam-D4 ISTD

Clonazepam - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs



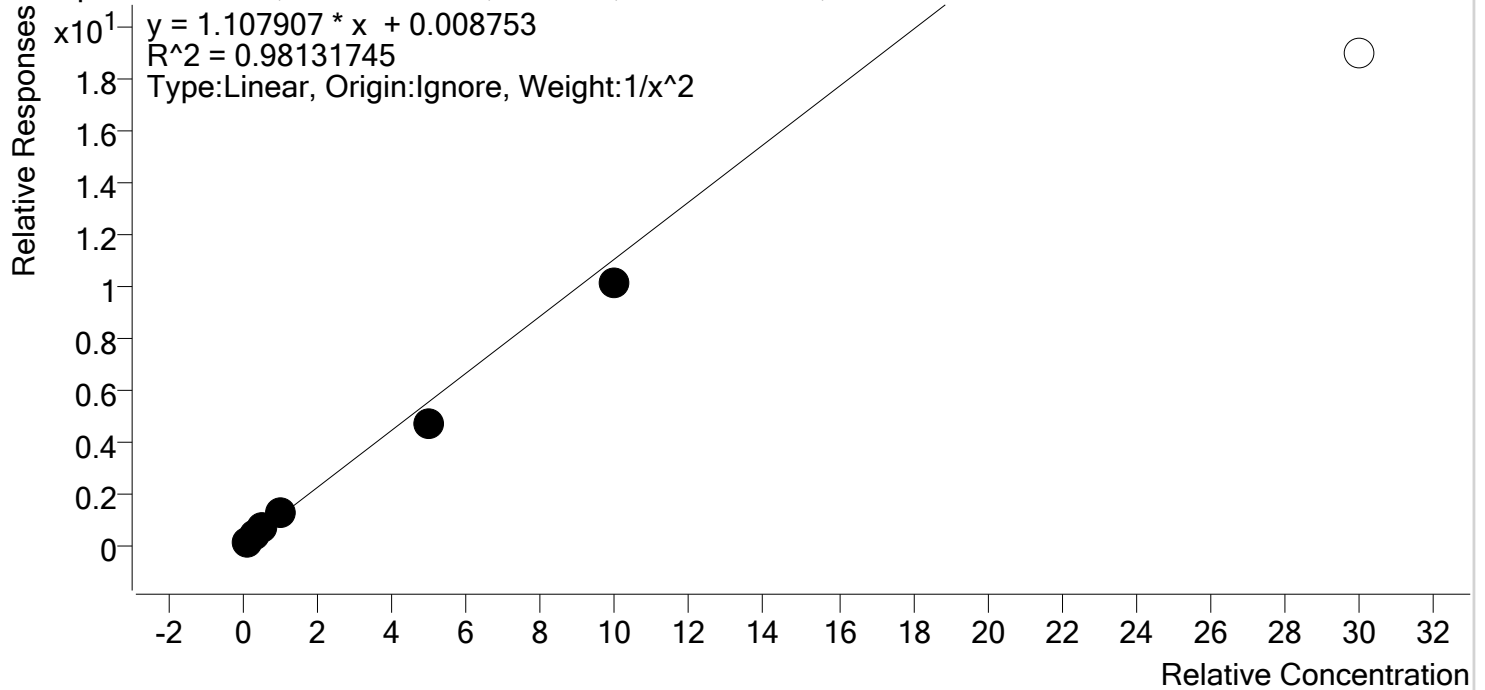
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.6	96.3
Cal 2- 25ng	2	✓	25.0	26.8	107.3
Cal 3 -50ng	3	✓	50.0	50.8	101.7
Cal 4-100ng-reinject	4	✓	100.0	105.7	105.7
Cal 5-500ng-reinject	5	✓	500.0	476.6	95.3
Cal 6-1000ng-reinject	6	✓	1000.0	936.5	93.7
Cal 7-3000ng	7	x	3000.0	2179.8	72.7



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Diazepam **Internal Standard** Diazepam-D5 ISTD

Diazepam - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs



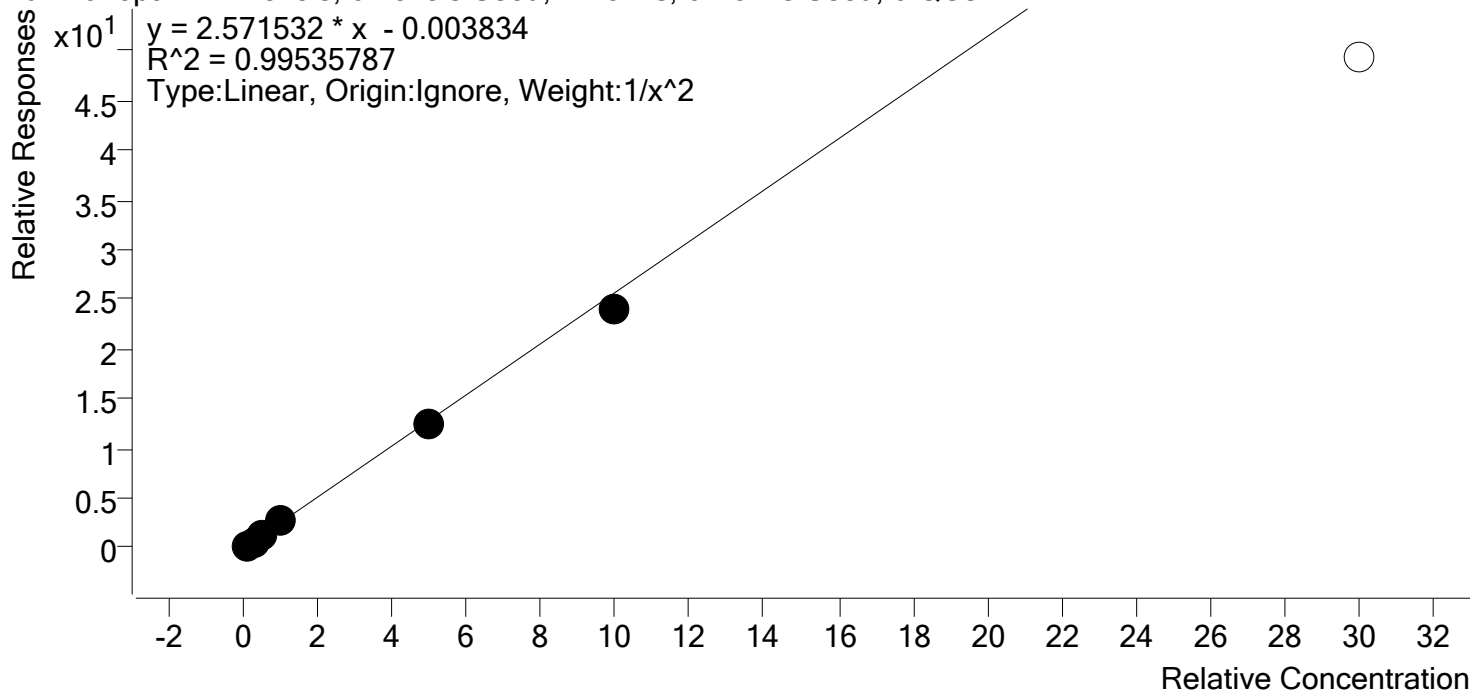
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.2	92.4
Cal 2- 25ng	2	✓	25.0	28.5	114.0
Cal 3 -50ng	3	✓	50.0	53.7	107.5
Cal 4-100ng-reinject	4	✓	100.0	109.0	109.0
Cal 5-500ng-reinject	5	✓	500.0	427.8	85.6
Cal 6-1000ng-reinject	6	✓	1000.0	915.6	91.6
Cal 7-3000ng	7	x	3000.0	1716.7	57.2



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Flunitrazepam **Internal Standard** Clonazepam-D4 ISTD

Flunitrazepam - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs



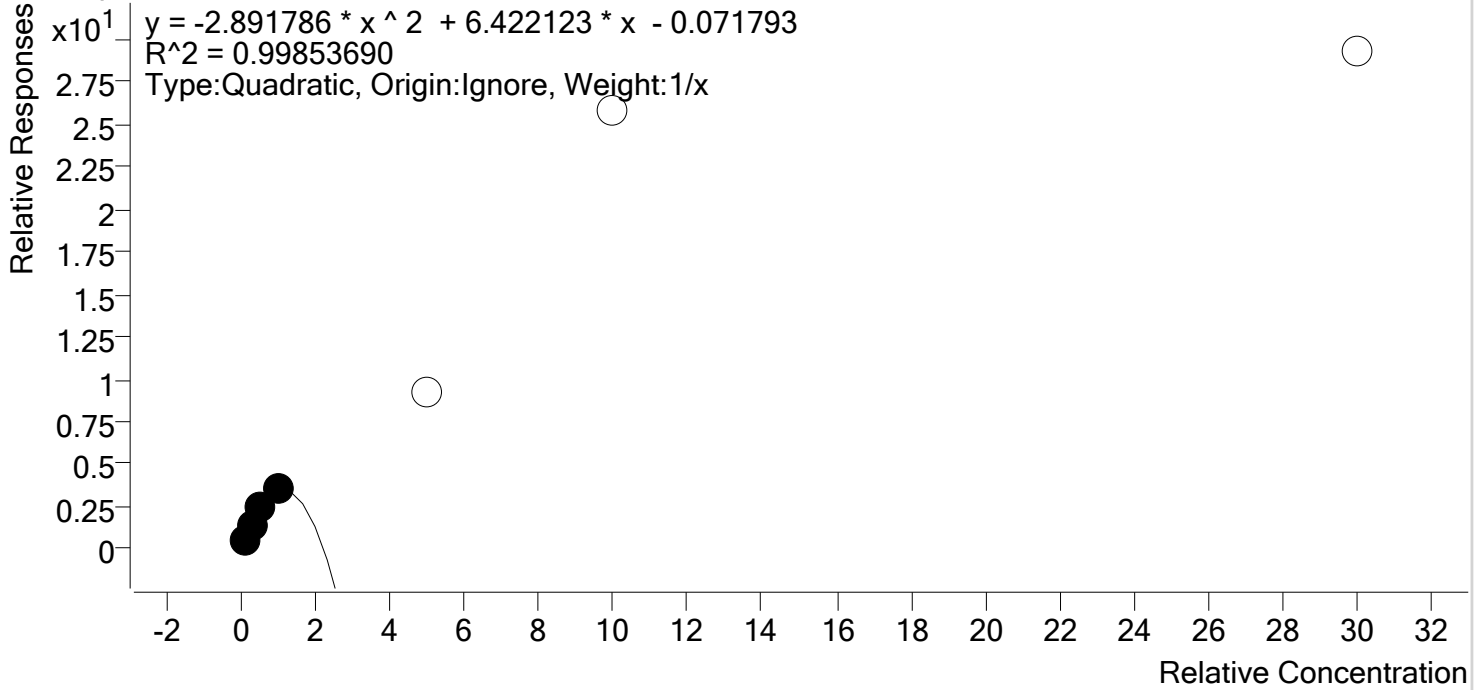
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.8	97.9
Cal 2- 25ng	2	✓	25.0	26.1	104.3
Cal 3 -50ng	3	✓	50.0	49.0	98.1
Cal 4-100ng-reinject	4	✓	100.0	109.4	109.4
Cal 5-500ng-reinject	5	✓	500.0	482.7	96.5
Cal 6-1000ng-reinject	6	✓	1000.0	938.3	93.8
Cal 7-3000ng	7	x	3000.0	1917.8	63.9



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Flurazepam **Internal Standard** Diazepam-D5 ISTD

Flurazepam - 7 Levels, 4 Levels Used, 7 Points, 4 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.8	98.2
Cal 2- 25ng	2	✓	25.0	26.0	104.1
Cal 3 -50ng	3	✓	50.0	48.4	96.8
Cal 4-100ng-reinject	4	✓	100.0	103.1	103.1
Cal 5-500ng-reinject	5	✗	500.0	ND	
Cal 6-1000ng-reinject	6	✗	1000.0	ND	
Cal 7-3000ng	7	✗	3000.0	ND	

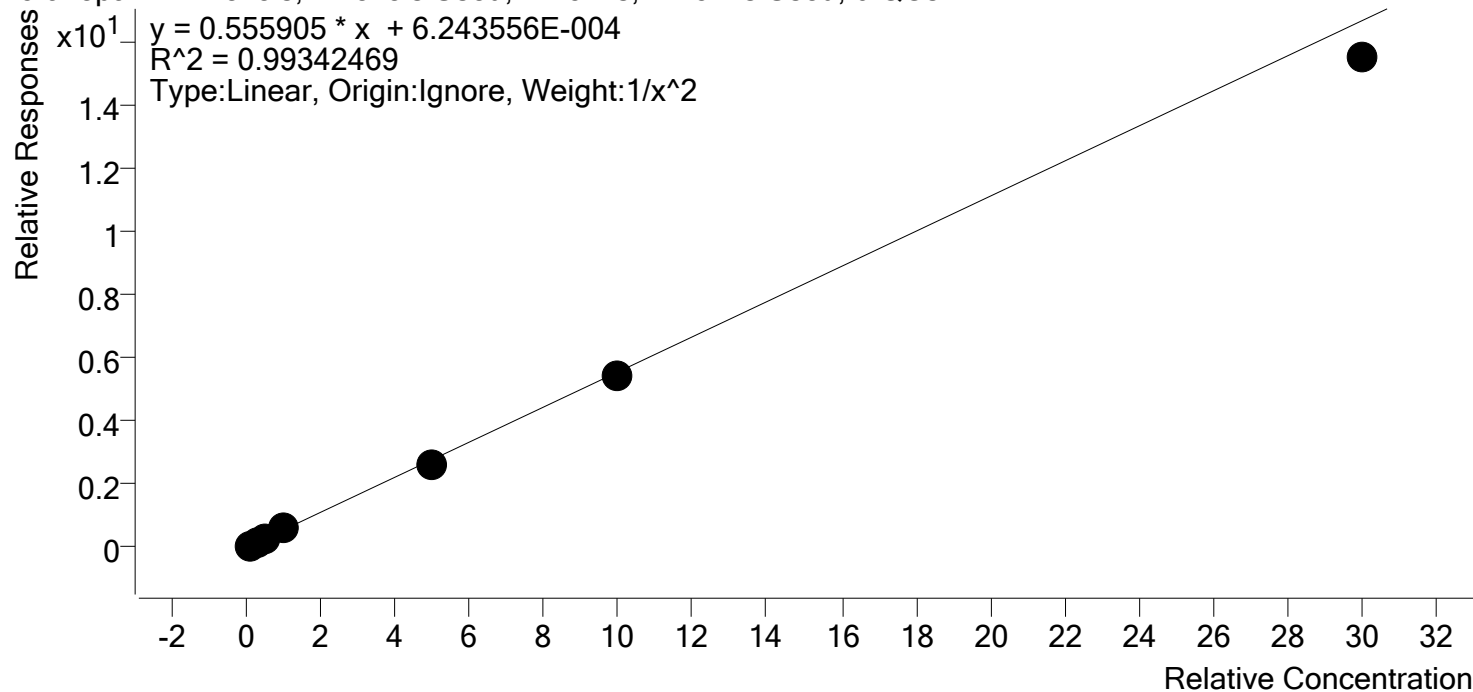




# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Lorazepam **Internal Standard** Nordiazepam-D5 ISTD

Lorazepam - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



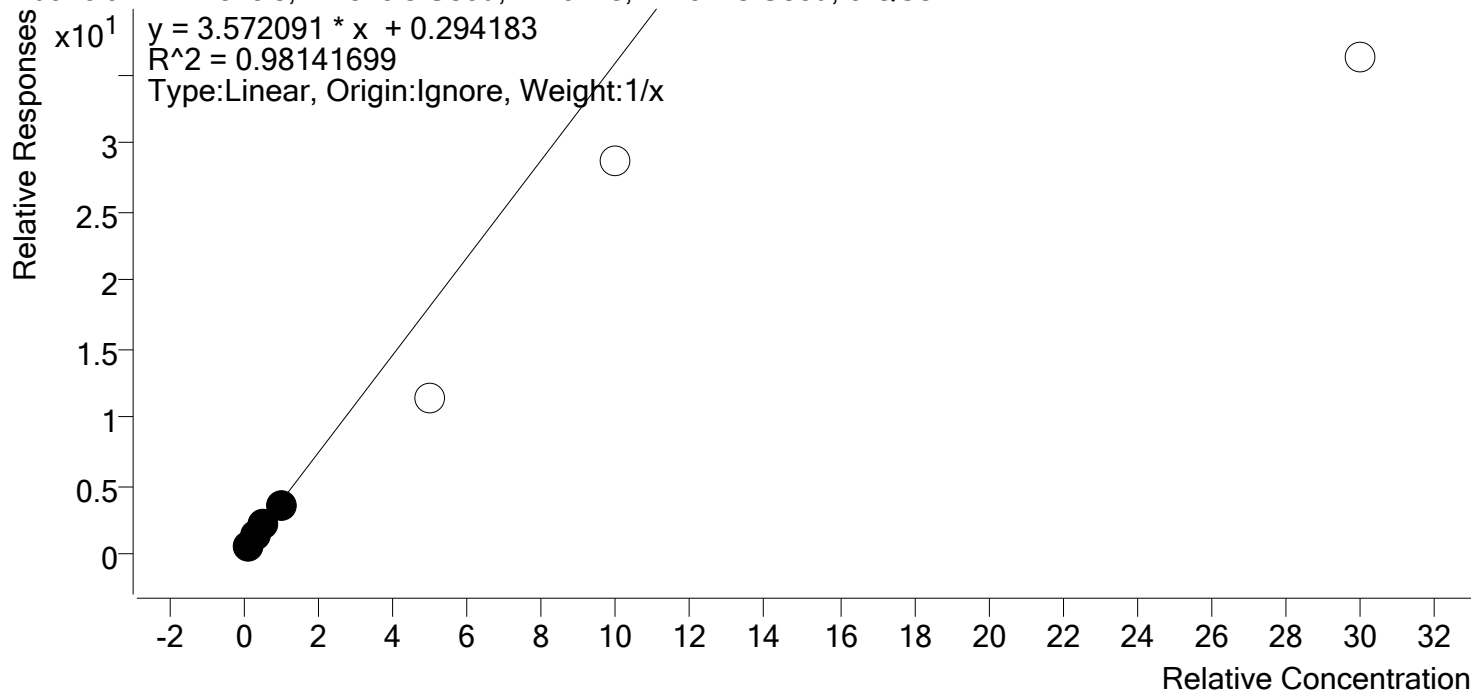
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.5	95.4
Cal 2- 25ng	2	✓	25.0	27.1	108.6
Cal 3 -50ng	3	✓	50.0	50.6	101.1
Cal 4-100ng-reinject	4	✓	100.0	110.6	110.6
Cal 5-500ng-reinject	5	✓	500.0	471.9	94.4
Cal 6-1000ng-reinject	6	✓	1000.0	968.9	96.9
Cal 7-3000ng	7	✓	3000.0	2789.4	93.0



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Midazolam **Internal Standard** Clonazepam-D4 ISTD

Midazolam - 7 Levels, 4 Levels Used, 7 Points, 4 Points Used, 0 QCs



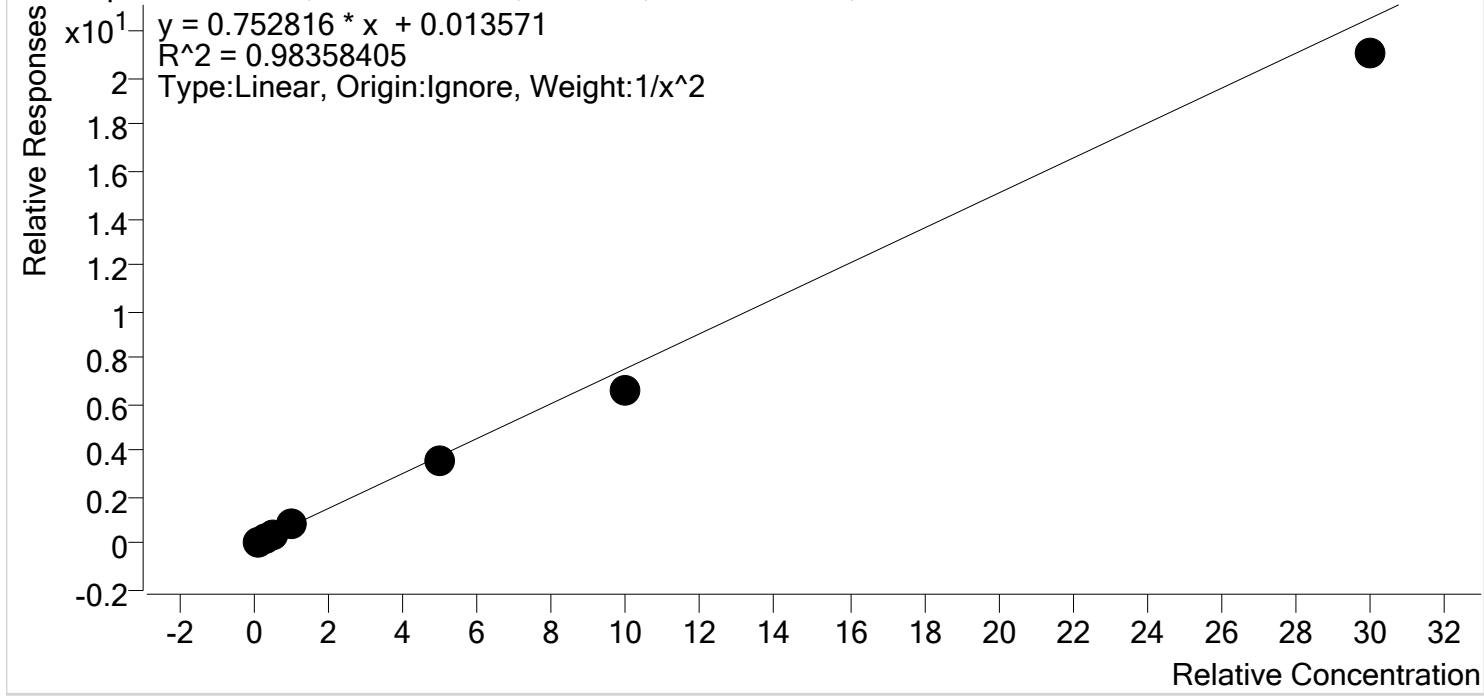
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	8.3	82.7
Cal 2- 25ng	2	✓	25.0	28.6	114.4
Cal 3 -50ng	3	✓	50.0	54.8	109.6
Cal 4-100ng-reinject	4	✓	100.0	93.3	93.3
Cal 5-500ng-reinject	5	✗	500.0	309.5	61.9
Cal 6-1000ng-reinject	6	✗	1000.0	796.3	79.6
Cal 7-3000ng	7	✗	3000.0	1008.4	33.6



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Nitrazepam **Internal Standard** Clonazepam-D4 ISTD

Nitrazepam - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 0 QCs



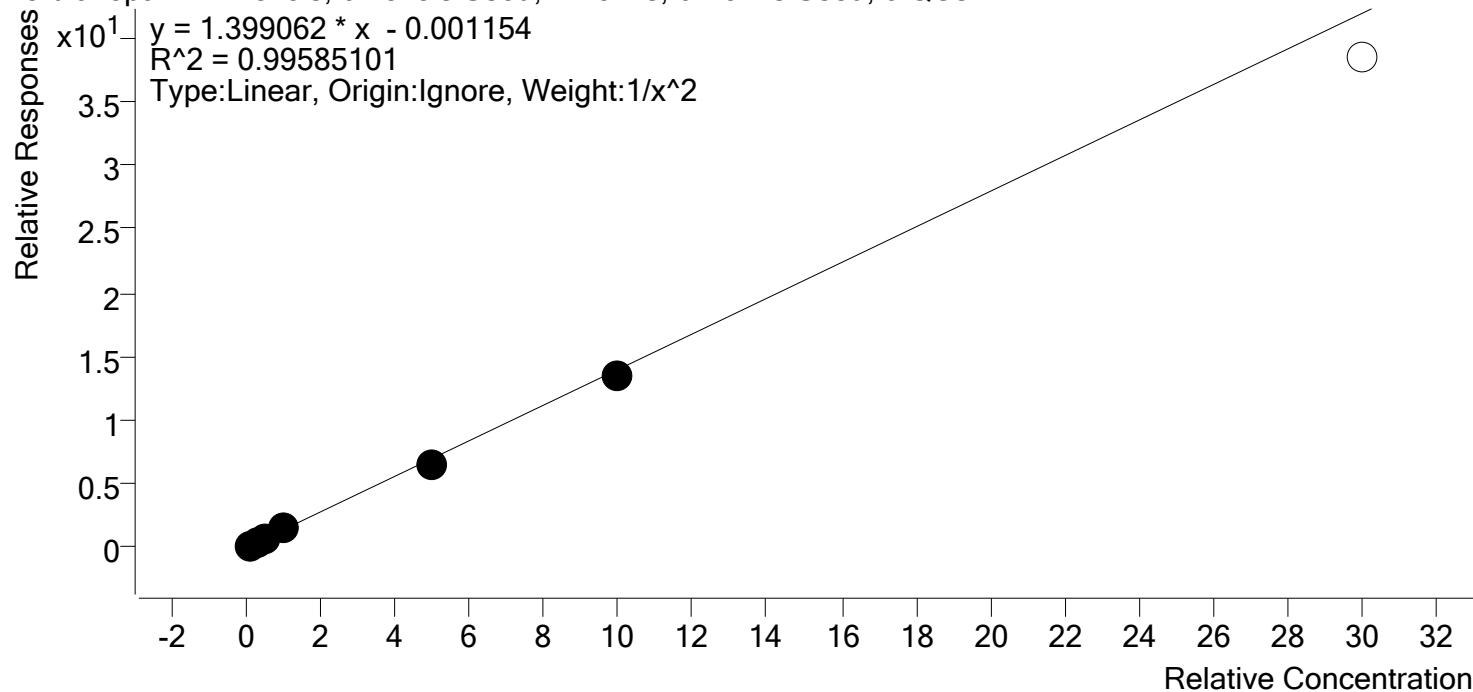
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.1	91.5
Cal 2- 25ng	2	✓	25.0	28.2	112.7
Cal 3 -50ng	3	✓	50.0	58.1	116.3
Cal 4-100ng-reinject	4	✓	100.0	104.9	104.9
Cal 5-500ng-reinject	5	✓	500.0	465.5	93.1
Cal 6-1000ng-reinject	6	✓	1000.0	882.6	88.3
Cal 7-3000ng	7	✓	3000.0	2798.1	93.3



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Nordiazepam **Internal Standard** Nordiazepam-D5 ISTD

Nordiazepam - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs



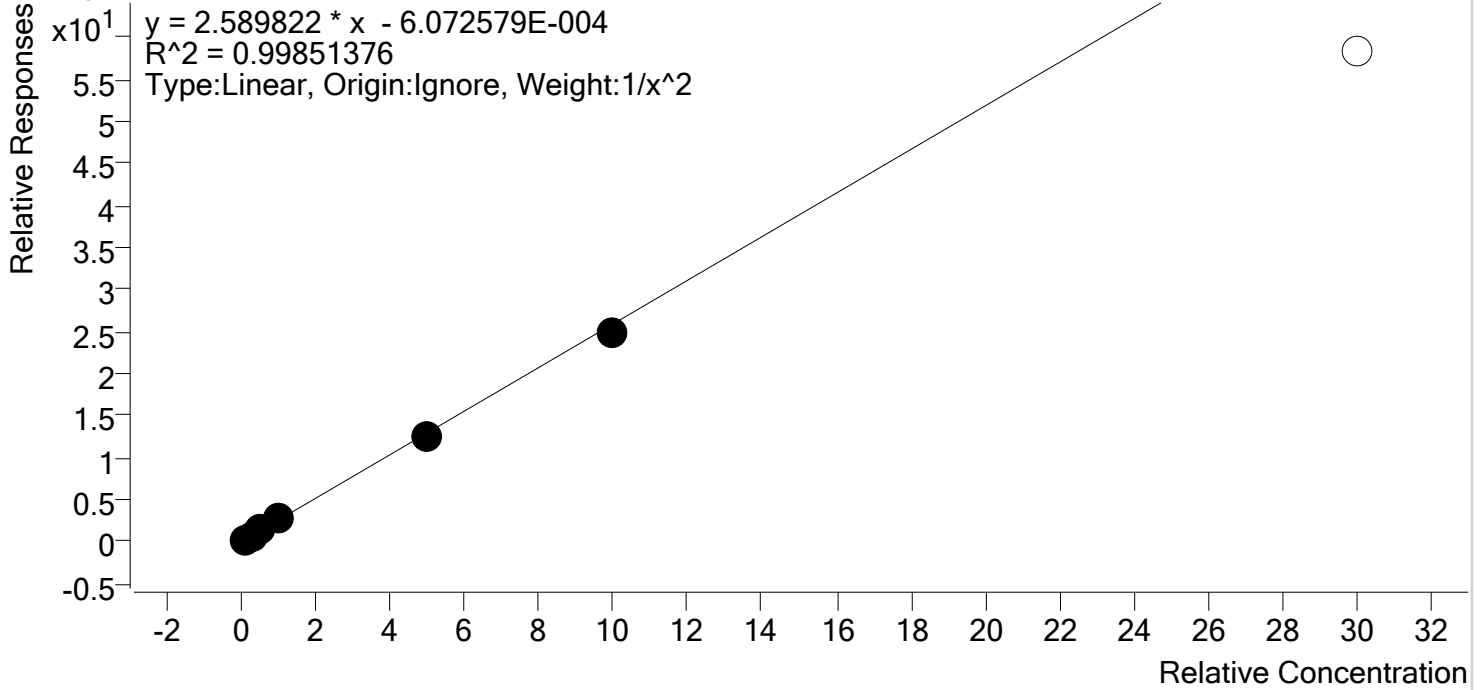
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.7	96.6
Cal 2- 25ng	2	✓	25.0	26.6	106.3
Cal 3 -50ng	3	✓	50.0	51.4	102.9
Cal 4-100ng-reinject	4	✓	100.0	104.9	104.9
Cal 5-500ng-reinject	5	✓	500.0	464.6	92.9
Cal 6-1000ng-reinject	6	✓	1000.0	963.8	96.4
Cal 7-3000ng	7	x	3000.0	2751.3	91.7



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Oxazepam **Internal Standard** Oxazepam-D5 ISTD

Oxazepam - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs



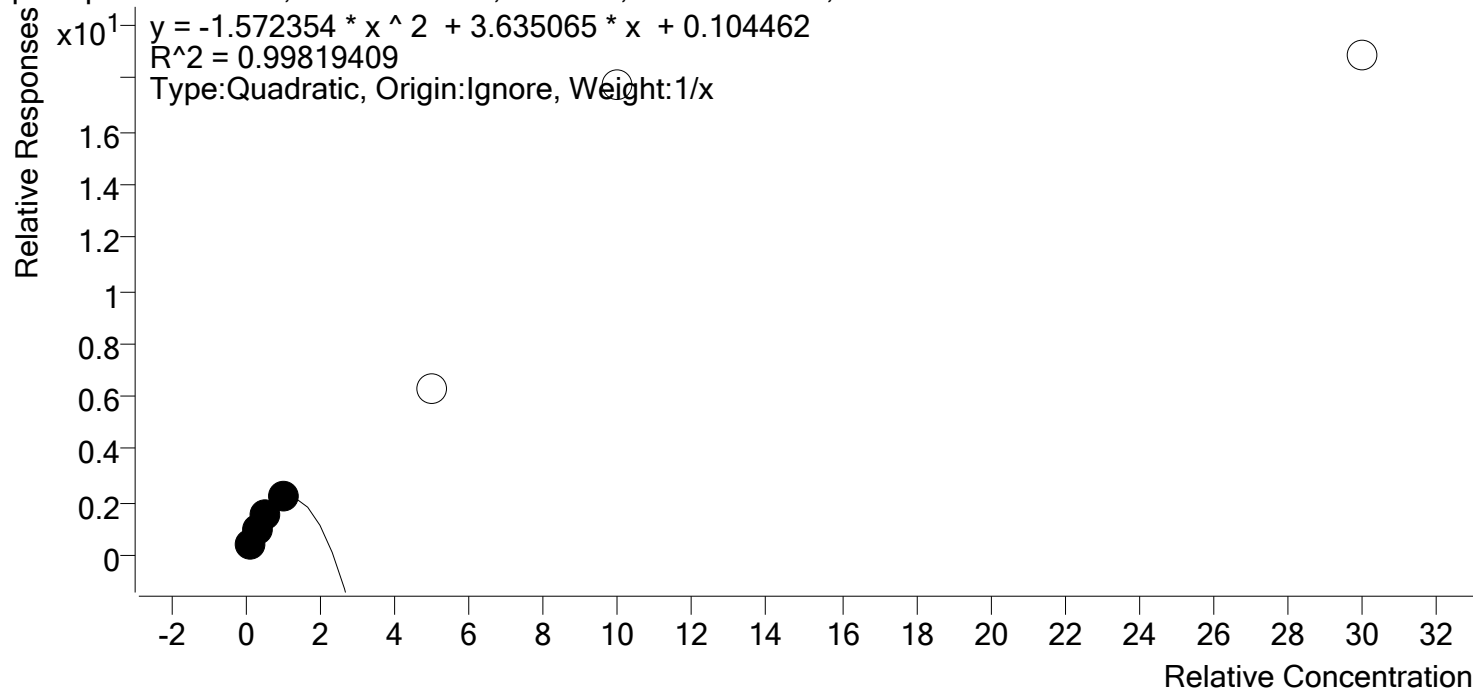
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.8	98.2
Cal 2- 25ng	2	✓	25.0	25.9	103.7
Cal 3 -50ng	3	✓	50.0	49.9	99.8
Cal 4-100ng-reinject	4	✓	100.0	104.2	104.2
Cal 5-500ng-reinject	5	✓	500.0	487.2	97.4
Cal 6-1000ng-reinject	6	✓	1000.0	966.5	96.6
Cal 7-3000ng	7	x	3000.0	2248.9	75.0



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** quetiapine **Internal Standard** Temazepam-D5 ISTD

quetiapine - 7 Levels, 4 Levels Used, 7 Points, 4 Points Used, 0 QCs



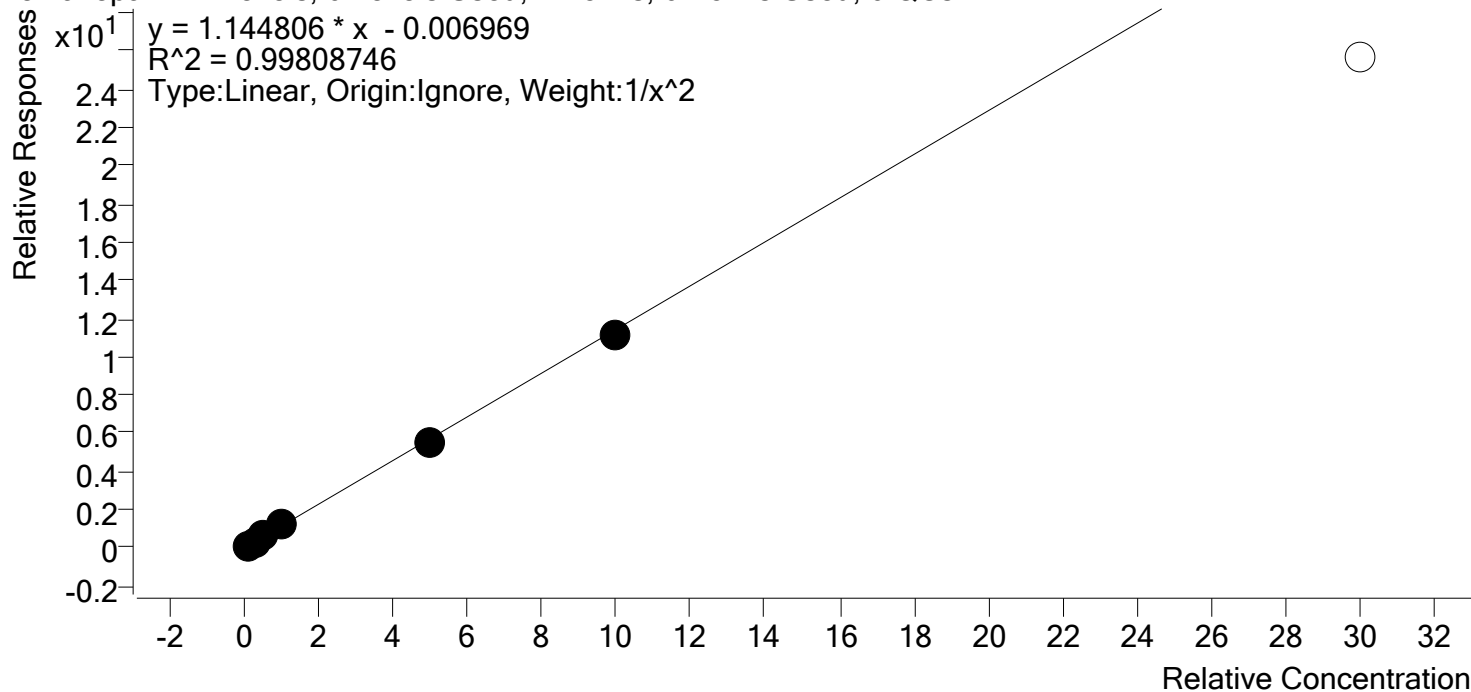
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.8	97.9
Cal 2- 25ng	2	✓	25.0	26.2	104.6
Cal 3 -50ng	3	✓	50.0	48.2	96.5
Cal 4-100ng-reinject	4	✓	100.0	102.4	102.4
Cal 5-500ng-reinject	5	✗	500.0	ND	
Cal 6-1000ng-reinject	6	✗	1000.0	ND	
Cal 7-3000ng	7	✗	3000.0	ND	



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** Temazepam **Internal Standard** Temazepam-D5 ISTD

Temazepam - 7 Levels, 6 Levels Used, 7 Points, 6 Points Used, 0 QCs



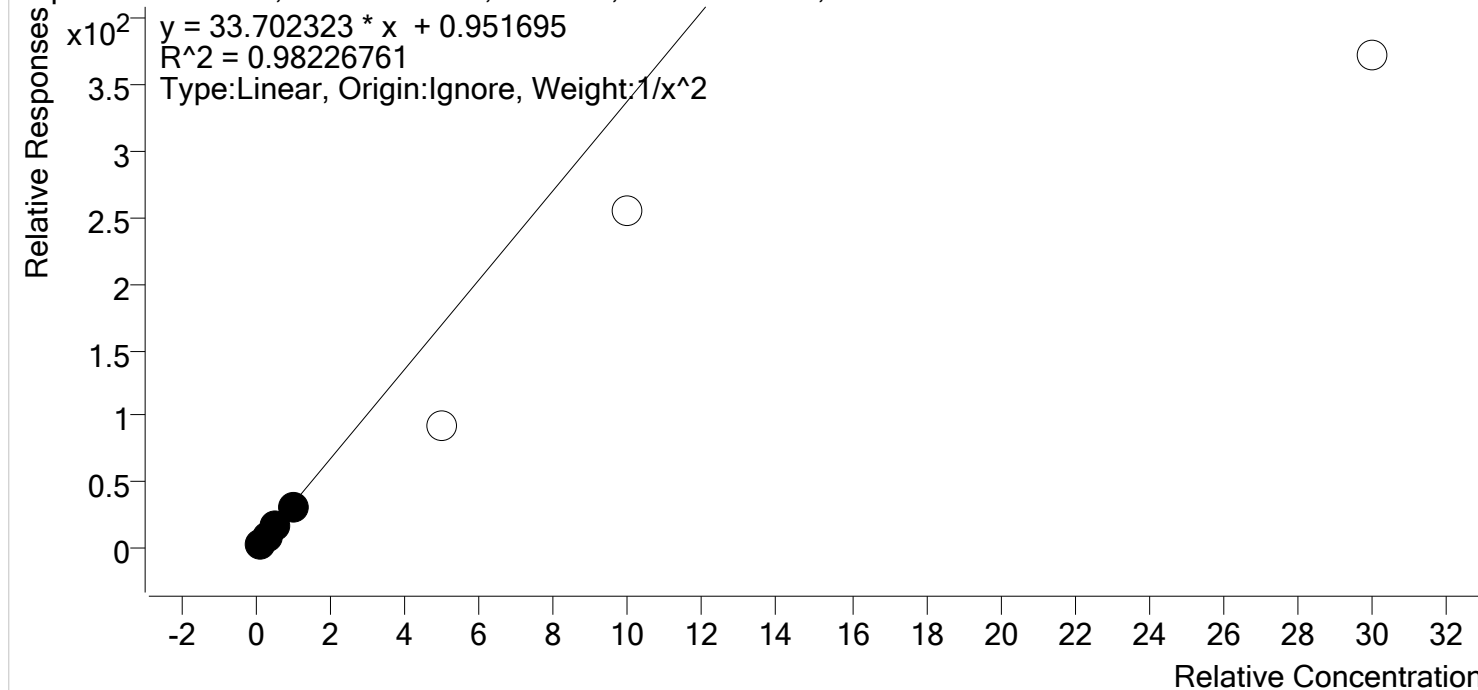
Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.8	97.5
Cal 2- 25ng	2	✓	25.0	26.4	105.7
Cal 3 -50ng	3	✓	50.0	50.0	99.9
Cal 4-100ng-reinject	4	✓	100.0	102.9	102.9
Cal 5-500ng-reinject	5	✓	500.0	480.7	96.1
Cal 6-1000ng-reinject	6	✓	1000.0	977.3	97.7
Cal 7-3000ng	7	x	3000.0	2243.4	74.8



# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** zolpidem **Internal Standard** Nordiazepam-D5 ISTD

zolpidem - 7 Levels, 4 Levels Used, 7 Points, 4 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	✓	10.0	9.6	95.5
Cal 2- 25ng	2	✓	25.0	28.2	112.9
Cal 3 -50ng	3	✓	50.0	50.9	101.7
Cal 4-100ng-reinject	4	✓	100.0	89.9	89.9
Cal 5-500ng-reinject	5	✗	500.0	271.1	54.2
Cal 6-1000ng-reinject	6	✗	1000.0	756.6	75.7
Cal 7-3000ng	7	✗	3000.0	1099.3	36.6

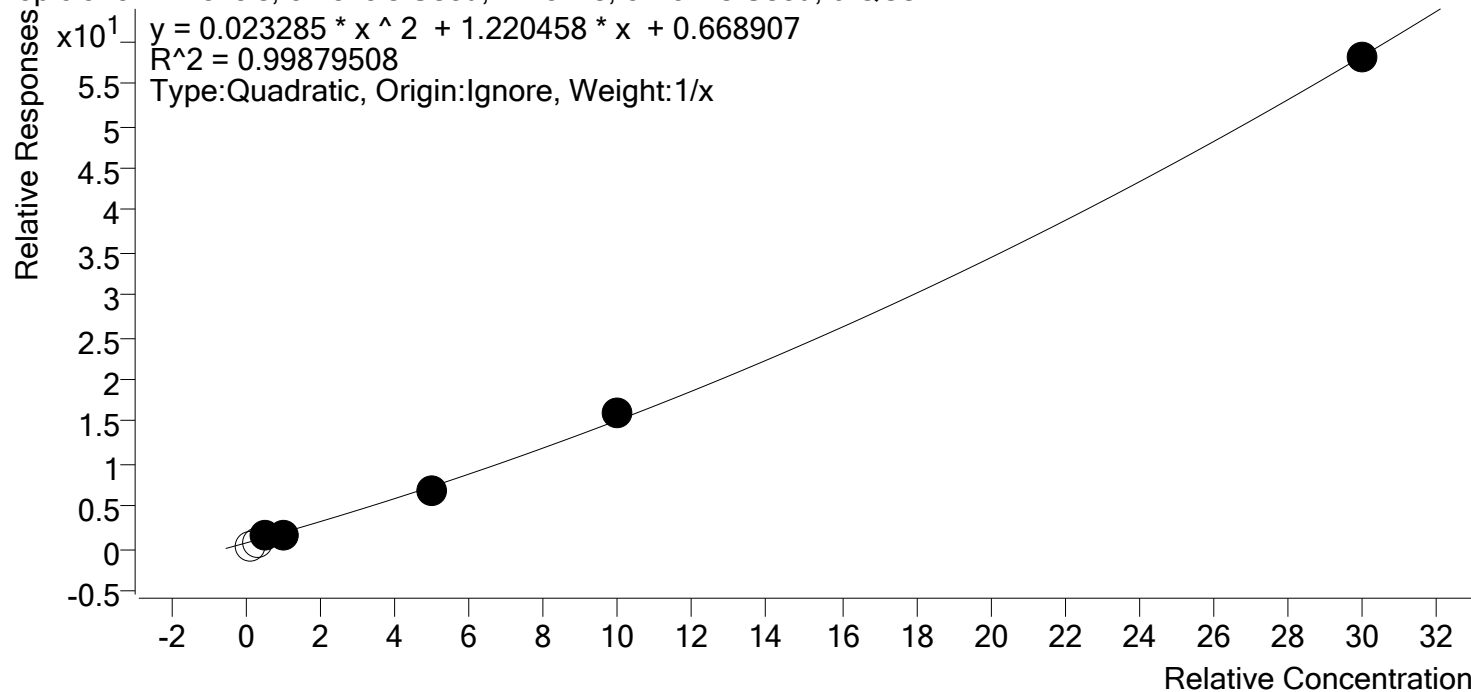




# Compound Calibration Report

**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Last Cal. Update** 7/16/2019 1:39 PM  
**Analyst Name** ISP\datastor  
**Analyte** zopiclone **Internal Standard** Nordiazepam-D5 ISTD

zopiclone - 7 Levels, 5 Levels Used, 7 Points, 5 Points Used, 0 QCs



Sample	Level	Enabled	Expected Concentration	Final Concentration	Accuracy
Cal 1-10ng	1	x	10.0	0.0	0.0
Cal 2- 25ng	2	x	25.0	10.4	41.4
Cal 3 -50ng	3	✓	50.0	59.3	118.6
Cal 4-100ng-reinject	4	✓	100.0	83.6	83.6
Cal 5-500ng-reinject	5	✓	500.0	470.0	94.0
Cal 6-1000ng-reinject	6	✓	1000.0	1039.6	104.0
Cal 7-3000ng	7	✓	3000.0	2994.4	99.8

# AM #13 Benzodiazepines and Z-Drugs



## Batch results

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin

## Calibration Last Update

7/16/2019 1:39:20 PM

## Instrument

Falco

## Data File

Negative Control-reinject2.d

## Type

Sample

## Sample

Negative Control-reinject

## Acq. Method

Benzos\_Z\_Drugs 053019 FINAL.m

## Sample Position

P5-B1

## Comment

Worklist 3536

## Injection Volume

2

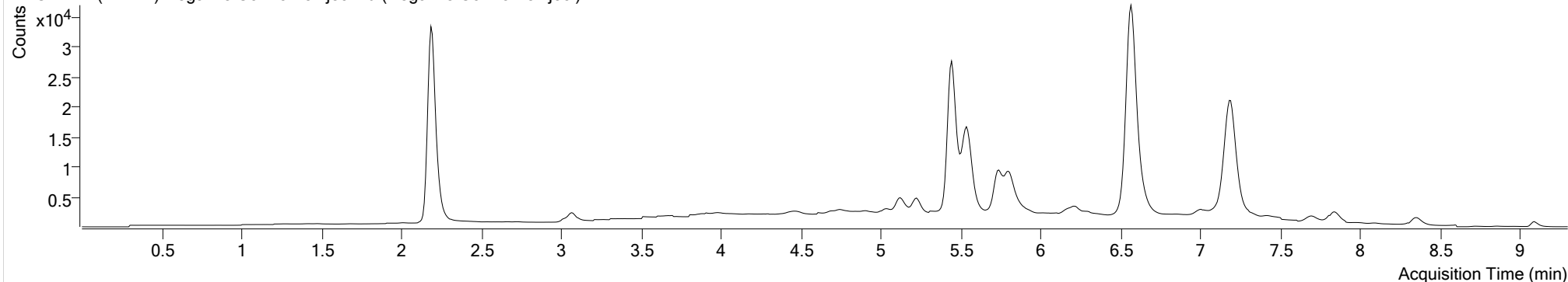
## Acq. Date-Time

7/16/2019 11:51:54 AM

## Sample Info.

## Sample Chromatogram

+ TIC MRM (\*\* -> \*\*) Negative Control-reinject2.d (Negative Control-reinject)



# AM #13 Benzodiazepines and Z-Drugs



**Batch results**

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin

**Calibration Last Update**

7/16/2019 1:39:20 PM

**Instrument**

Falco

**Data File**

Positive Control.d

**Type**

Sample

**Sample**

Positive Control

**Acq. Method**

Benzos\_Z\_Drugs 053019 FINAL.m

**Sample Position**

P5-B2

**Comment**

Worklist 3536

**Injection Volume**

2

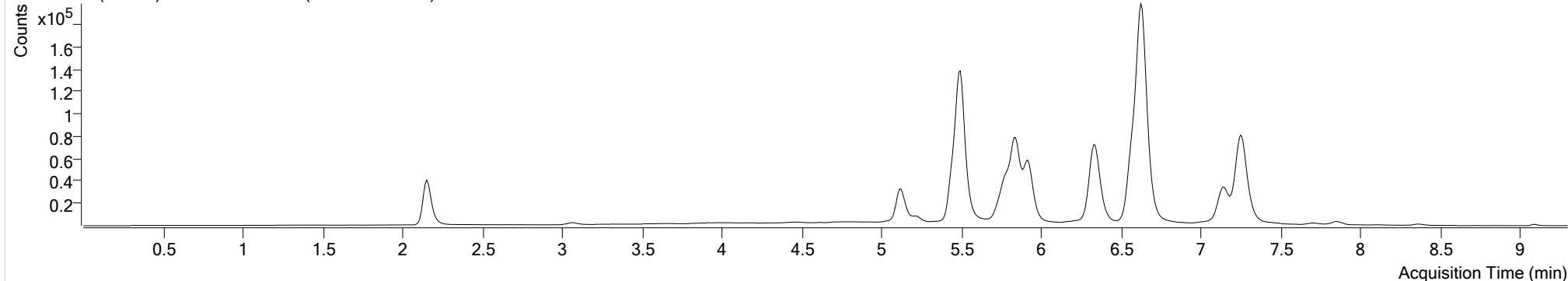
**Acq. Date-Time**

7/15/2019 5:58:04 PM

**Sample Info.**

**Sample Chromatogram**

+ TIC MRM (\*\* -> \*\*) Positive Control.d (Positive Control)



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Alprazolam	5.917	169037	∞	32.3	1026.58	11505	359.9274 ng/ml
Clonazepam	5.781	85700	1394.61	42.5	1012.44	33981	262.3446 ng/ml
Diazepam	7.251	302018	∞	40.1	1159.37	89960	302.2364 ng/ml
Flunitrazepam	6.329	221197	3033.46	43.0	1531.00	33981	253.2838 ng/ml
Lorazepam	5.839	60258	788.00	99.2	2554.22	39816	272.1322 ng/ml
Nitrazepam	5.118	75879	2325.63	52.2	809.73	33981	294.8164 ng/ml
Oxazepam	5.497	319936	10243.12	44.7	774.84	45681	270.4532 ng/ml
Temazepam	6.626	639830	17080.66	33.4	3674.44	209603	267.2548 ng/ml

# AM #13 Benzodiazepines and Z-Drugs

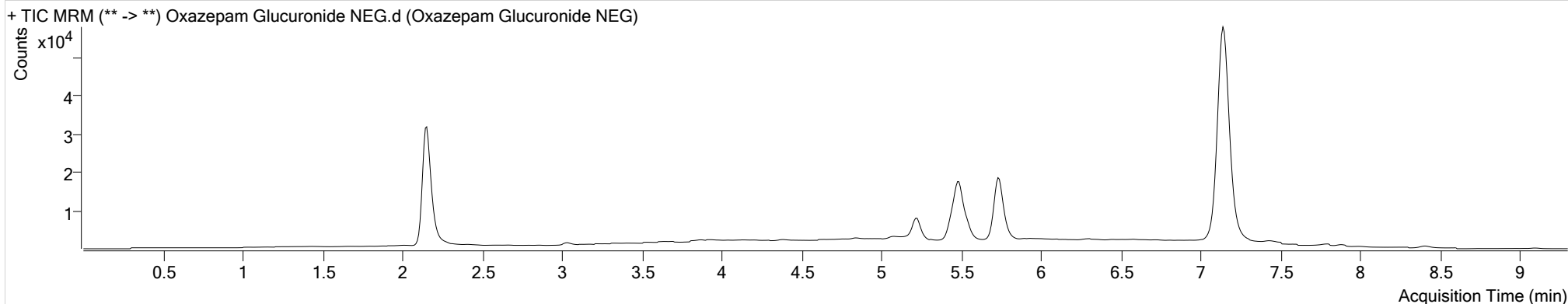


**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Calibration Last Update** 7/16/2019 1:39:20 PM

**Instrument** Falco  
**Type** Sample  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A8  
**Injection Volume** 2  
**Acq. Date-Time** 7/15/2019 5:08:13 PM  
**Sample Info.**

**Data File** Oxazepam Glucuronide NEG.d  
**Sample** Oxazepam Glucuronide NEG  
**Comment** Worklist 3536

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Oxazepam	5.488	10465	351.56	44.5	149.33	6623	61.0375 ng/ml

# AM #13 Benzodiazepines and Z-Drugs

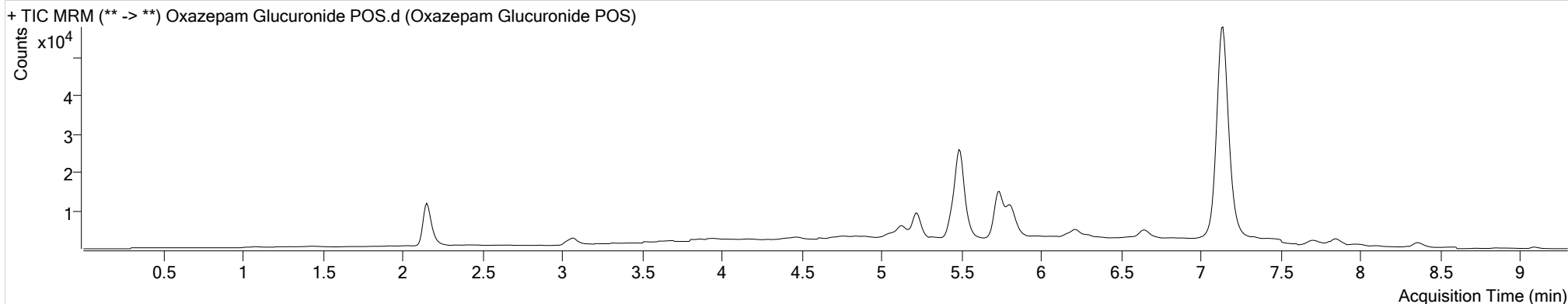


**Batch results** D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
**Calibration Last Update** 7/16/2019 1:39:20 PM

**Instrument** Falco  
**Type** Sample  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A9  
**Injection Volume** 2  
**Acq. Date-Time** 7/15/2019 5:20:41 PM  
**Sample Info.**

**Data File** Oxazepam Glucuronide POS.d  
**Sample** Oxazepam Glucuronide POS  
**Comment** Worklist 3536

## Sample Chromatogram



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
Oxazepam	5.488	36944	1159.93	45.3	∞	5868	243.1429 ng/ml

# AM #13 Benzodiazepines and Z-Drugs



**Batch results**

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin

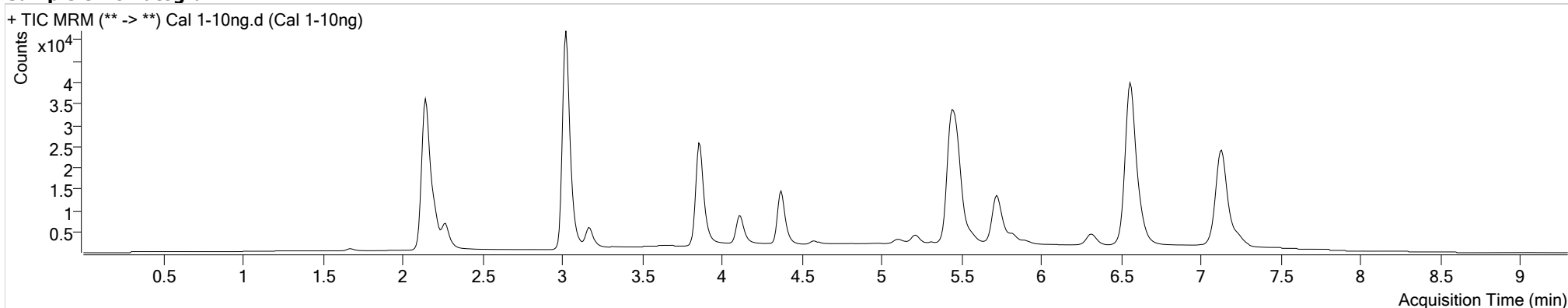
**Calibration Last Update**

7/16/2019 1:39:20 PM

**Instrument Type** Falco  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A1  
**Injection Volume** 2  
**Acq. Date-Time** 7/15/2019 3:28:27 PM  
**Sample Info.**

**Data File** Cal 1-10ng.d  
**Sample** Cal 1-10ng  
**Comment** Worklist 3536

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
alpha-Hydroxyalprazolam	5.248	336	14.81	101.0	6.59	5818	9.3495 ng/ml
alpha-hydroxytriazolam	5.305	1063	67.12	52.7	28.95	31191	9.9820 ng/ml
Alprazolam	5.908	2218	26.14	37.0	12.19	5818	9.8003 ng/ml
Chlordiazepoxide	3.167	12116	439.58	29.9	383.40	31191	9.5758 ng/ml
Clonazepam	5.764	2932	103.00	41.1	63.09	30801	9.6326 ng/ml
Diazepam	7.234	7281	185.75	41.0	118.38	65542	9.2367 ng/ml
Flunitrazepam	6.312	7632	269.48	42.7	116.62	30801	9.7853 ng/ml
Flurazepam	4.368	34800	1145.88	27.5	293.35	65542	9.8196 ng/ml
Lorazepam	5.822	1675	49.70	104.3	56.77	31191	9.5449 ng/ml
Midazolam	4.108	18157	340.64	29.9	312.07	30801	8.2671 ng/ml
Nitrazepam	5.109	2539	114.32	50.9	61.26	30801	9.1455 ng/ml
Nordiazepam	5.566	4179	83.66	55.5	47.43	31191	9.6596 ng/ml
Oxazepam	5.480	8083	385.29	43.0	195.41	31849	9.8226 ng/ml
quetiapine	3.854	56364	5118.11	48.5	3227.36	126539	9.7948 ng/ml
Temazepam	6.617	13246	411.44	35.3	157.59	126539	9.7528 ng/ml
zolpidem	3.024	130096	3518.65	36.7	2063.84	31191	9.5519 ng/ml

# AM #13 Benzodiazepines and Z-Drugs



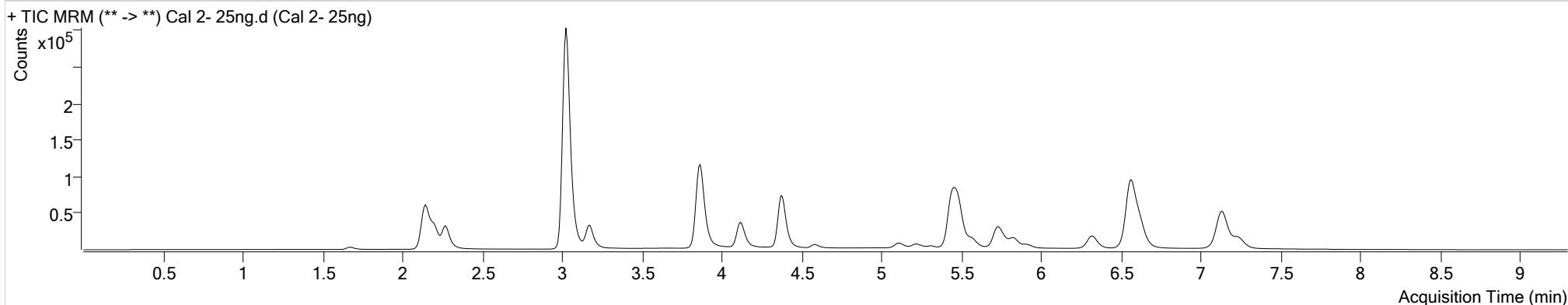
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
7/16/2019 1:39:20 PM

**Instrument** Falco  
**Type** Cal  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A2  
**Injection Volume** 2  
**Acq. Date-Time** 7/15/2019 3:40:55 PM  
**Sample Info.**

**Data File** Cal 2- 25ng.d  
**Sample** Cal 2- 25ng  
**Comment** Worklist 3536

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-Aminoclonazepam	1.678	8131	830.93	35.7	198.36	200041	4.2293 ng/ml
alpha-Hydroxyalprazolam	5.256	2648	58.36	85.2	20.90	14662	27.6477 ng/ml
alpha-hydroxytriazolam	5.305	7071	287.12	55.8	159.19	75520	25.5406 ng/ml
Alprazolam	5.917	15072	135.29	32.1	44.11	14662	25.6245 ng/ml
Chlordiazepoxide	3.176	79108	6201.54	30.0	630.58	75520	26.8303 ng/ml
Clonazepam	5.764	19857	919.43	43.0	293.29	76272	26.8285 ng/ml
Diazepam	7.234	49148	1151.22	40.6	727.29	151429	28.5047 ng/ml
Flunitrazepam	6.320	50852	1855.42	42.5	698.59	76272	26.0760 ng/ml
Flurazepam	4.368	212554	5752.95	26.5	573.17	151429	26.0241 ng/ml
Lorazepam	5.822	11444	513.61	99.1	336.73	75520	27.1473 ng/ml
Midazolam	4.117	100369	∞	29.3	404.91	76272	28.6034 ng/ml
Nitrazepam	5.109	17210	333.18	52.1	609.64	76272	28.1697 ng/ml
Nordiazepam	5.574	27984	543.66	57.0	346.26	75520	26.5685 ng/ml
Oxazepam	5.488	52770	3402.30	42.8	911.05	78654	25.9294 ng/ml
quetiapine	3.862	290352	47489.35	49.4	9881.87	306378	26.1565 ng/ml
Temazepam	6.617	90581	6911.24	34.3	540.65	306378	26.4342 ng/ml
zolpidem	3.024	790029	92307.38	37.3	47786.75	75520	28.2161 ng/ml
zopiclone	2.267	60083	2467.46	61.7	1936.71	75520	10.3598 ng/ml

# AM #13 Benzodiazepines and Z-Drugs



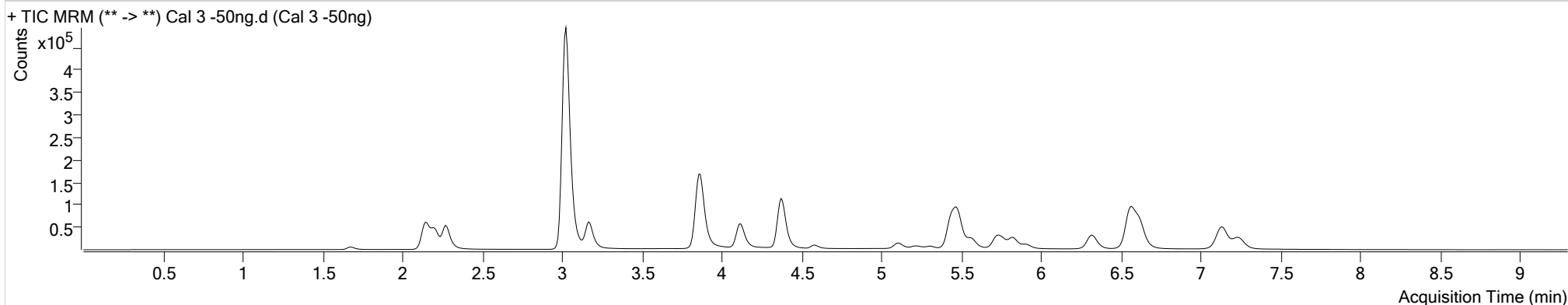
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
7/16/2019 1:39:20 PM

**Instrument** Falco  
**Type** Cal  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A3  
**Injection Volume** 2  
**Acq. Date-Time** 7/15/2019 3:53:24 PM  
**Sample Info.**

**Data File** Cal 3 -50ng.d  
**Sample** Cal 3 -50ng  
**Comment** Worklist 3536

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-Aminoclonazepam	1.678	14814	544.73	36.3	299.73	196754	43.0709 ng/ml
7-Aminoflunitrazepam	2.207	106926	2785.72	34.4	1721.50	196754	8.0591 ng/ml
alpha-Hydroxyalprazolam	5.248	5066	54.86	86.4	75.35	13763	55.5596 ng/ml
alpha-hydroxytriazolam	5.305	13073	512.36	57.1	405.87	73988	47.2413 ng/ml
Alprazolam	5.908	28693	∞	33.8	79.89	13763	51.4768 ng/ml
Chlordiazepoxide	3.167	148178	9168.24	29.7	1264.87	73988	51.8376 ng/ml
Clonazepam	5.764	35588	820.98	44.4	791.54	72502	50.8335 ng/ml
Diazepam	7.242	88011	1338.61	41.3	1263.19	145663	53.7460 ng/ml
Flunitrazepam	6.320	91168	1020.92	42.9	436.00	72502	49.0482 ng/ml
Flurazepam	4.368	343667	5625.01	26.4	27783.00	145663	48.4065 ng/ml
Lorazepam	5.822	20838	872.65	98.4	236.93	73988	50.5514 ng/ml
Midazolam	4.117	163214	∞	29.5	∞	72502	54.7859 ng/ml
Nitrazepam	5.109	32719	713.80	51.5	477.87	72502	58.1439 ng/ml
Nordiazepam	5.566	53167	3125.15	55.9	2186.04	73988	51.4449 ng/ml
Oxazepam	5.480	100019	4206.58	42.8	549.44	77467	49.8769 ng/ml
quetiapine	3.862	443415	29185.20	49.1	16359.29	297180	48.2382 ng/ml
Temazepam	6.617	167898	12741.30	34.4	701.52	297180	49.9595 ng/ml
zolpidem	3.024	1338815	157077.83	37.3	6451.42	73988	50.8667 ng/ml



# AM #13 Benzodiazepines and Z-Drugs



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
zopiclone	2.267	103662	2798.48	62.1	5546.18	73988	59.3182 ng/ml

cg

# AM #13 Benzodiazepines and Z-Drugs



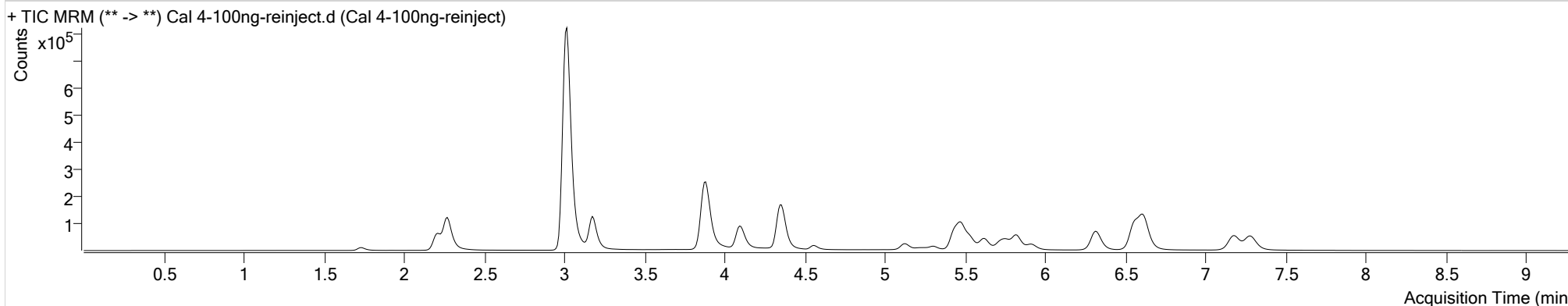
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
7/16/2019 1:39:20 PM

**Instrument Type** Falco  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A4  
**Injection Volume** 2  
**Acq. Date-Time** 7/16/2019 9:17:27 AM  
**Sample Info.**

**Data File** Cal 4-100ng-reinject.d  
**Sample** Cal 4-100ng-reinject  
**Comment** Worklist 3536

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-Aminoclonazepam	1.737	27985	998.47	35.4	594.52	200135	115.4227 ng/ml
7-Aminoflunitrazepam	2.266	177993	4907.68	35.1	2079.21	200135	97.2625 ng/ml
alpha-Hydroxyalprazolam	5.256	10480	876.67	94.2	275.75	15499	101.4216 ng/ml
alpha-hydroxytriazolam	5.297	30233	2188.80	55.4	665.07	75800	105.2882 ng/ml
Alprazolam	5.917	66398	2059.25	31.6	160.38	15499	105.2782 ng/ml
Chlordiazepoxide	3.176	311049	10253.00	30.4	2035.82	75800	106.8373 ng/ml
Clonazepam	5.755	75611	4064.69	43.0	781.15	74280	105.7181 ng/ml
Diazepam	7.284	188789	3657.03	40.3	3227.10	155209	108.9982 ng/ml
Flunitrazepam	6.312	208625	1135.12	42.9	2073.98	74280	109.3691 ng/ml
Flurazepam	4.342	539419	∞	28.3	3818.87	155209	103.0717 ng/ml
Lorazepam	5.822	46658	1690.17	98.3	972.33	75800	110.6165 ng/ml
Midazolam	4.091	269526	∞	29.8	∞	74280	93.3436 ng/ml
Nitrazepam	5.126	59689	716.11	50.9	954.94	74280	104.9389 ng/ml
Nordiazepam	5.625	111201	5822.30	56.0	∞	75800	104.9417 ng/ml
Oxazepam	5.480	222496	7510.98	42.0	2063.93	82460	104.2090 ng/ml
quetiapine	3.879	700647	38065.15	48.0	14201.72	321680	102.4119 ng/ml
Temazepam	6.617	376823	∞	34.9	3026.86	321680	102.9336 ng/ml
zolpidem	3.016	2368307	145782.64	37.0	82543.43	75800	89.8829 ng/ml

# AM #13 Benzodiazepines and Z-Drugs



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
zopiclone	2.267	129228	1871.23	61.6	2266.22	75800	83.5512 ng/ml

# AM #13 Benzodiazepines and Z-Drugs



**Batch results**

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin

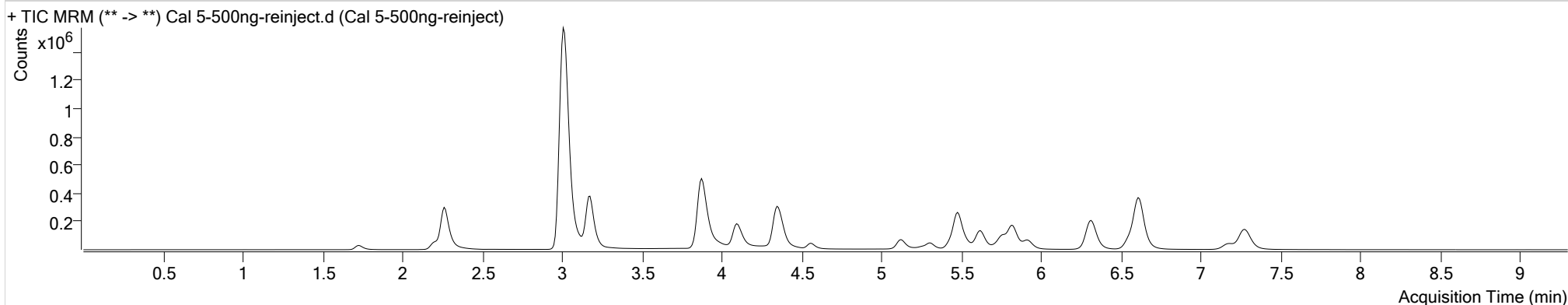
**Calibration Last Update**

7/16/2019 1:39:20 PM

**Instrument Type** Falco  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A5  
**Injection Volume** 2  
**Acq. Date-Time** 7/16/2019 8:47:54 AM  
**Sample Info.**

**Data File** Cal 5-500ng-reinject.d  
**Sample** Cal 5-500ng-reinject  
**Comment** Worklist 3536

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-Aminoclonazepam	1.728	80531	10702.38	36.0	927.68	151414	554.8941 ng/ml
7-Aminoflunitrazepam	2.266	407686	∞	35.1	3370.70	151414	562.2531 ng/ml
alpha-Hydroxyalprazolam	5.256	35226	∞	87.7	260.16	10626	494.3276 ng/ml
alpha-hydroxytriazolam	5.305	99586	1426.94	56.7	1421.78	55151	472.8691 ng/ml
Alprazolam	5.917	209723	485.00	32.4	379.30	10626	483.3340 ng/ml
Chlordiazepoxide	3.176	997856	44726.13	29.7	27459.73	55151	473.0837 ng/ml
Clonazepam	5.755	232130	4149.47	43.0	7510.22	50688	476.6102 ng/ml
Diazepam	7.276	549693	5826.31	40.6	3190.37	115766	427.7938 ng/ml
Flunitrazepam	6.312	629027	∞	43.0	3550.12	50688	482.7314 ng/ml
Lorazepam	5.822	144703	12203.63	101.4	2702.46	55151	471.8704 ng/ml
Midazolam	4.091	575366	∞	30.1	∞	50688	309.5365 ng/ml
Nitrazepam	5.126	178335	3687.24	51.6	5035.82	50688	465.5458 ng/ml
Nordiazepam	5.625	358416	10784.09	55.7	∞	55151	464.5969 ng/ml
Oxazepam	5.480	705077	37709.63	42.4	11964.86	55879	487.2381 ng/ml
Temazepam	6.609	1247369	49316.15	34.6	27234.43	226931	480.7494 ng/ml
zolpidem	3.008	5091806	305873.35	37.1	121876.38	55151	271.1196 ng/ml
zopiclone	2.258	381624	62682.16	61.0	30353.37	55151	470.0161 ng/ml

# AM #13 Benzodiazepines and Z-Drugs



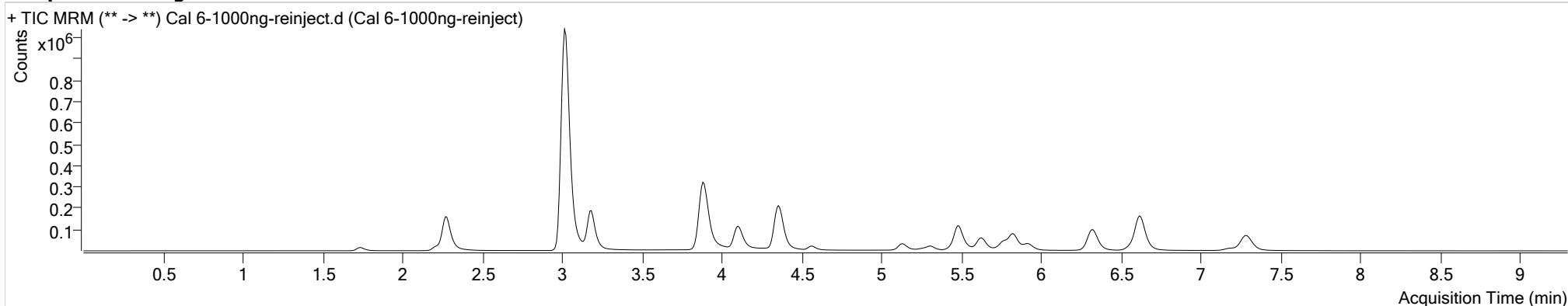
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
7/16/2019 1:39:20 PM

**Instrument** Falco  
**Type** Cal  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A6  
**Injection Volume** 2  
**Acq. Date-Time** 7/16/2019 9:41:42 AM  
**Sample Info.**

**Data File** Cal 6-1000ng-reinject.d  
**Sample** Cal 6-1000ng-reinject  
**Comment** Worklist 3536

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-Aminoclonazepam	1.737	40541	1536.67	35.5	615.69	51360	843.5456 ng/ml
7-Aminoflunitrazepam	2.274	242536	5241.65	35.3	4634.11	51360	1085.6687 ng/ml
alpha-Hydroxyalprazolam	5.256	16500	257.09	89.6	564.28	2548	964.9463 ng/ml
alpha-hydroxytriazolam	5.305	44732	999.14	54.9	918.51	11951	979.0202 ng/ml
Alprazolam	5.917	98470	124.84	31.9	472.20	2548	946.0050 ng/ml
Chlordiazepoxide	3.184	477468	28382.19	30.4	20781.97	11951	1045.3247 ng/ml
Clonazepam	5.764	109044	3837.58	43.2	455.71	12121	936.5283 ng/ml
Diazepam	7.284	269903	33243.70	39.9	11467.41	26583	915.6477 ng/ml
Flunitrazepam	6.320	292427	2049.48	43.5	1482.99	12121	938.3132 ng/ml
Lorazepam	5.822	64378	1902.00	100.8	5650.08	11951	968.8980 ng/ml
Midazolam	4.100	348343	∞	30.2	3694.72	12121	796.2866 ng/ml
Nitrazepam	5.134	80702	1269.20	52.1	3514.13	12121	882.5919 ng/ml
Nordiazepam	5.625	161137	22655.18	55.2	1372.45	11951	963.7899 ng/ml
Oxazepam	5.488	320816	10431.41	43.3	1995.61	12818	966.4619 ng/ml
Temazepam	6.617	558386	28096.00	34.2	7407.98	49938	977.3310 ng/ml
zolpidem	3.016	3058916	241373.45	37.1	50451.50	11951	756.6200 ng/ml
zopiclone	2.275	189715	7204.20	62.1	2364.44	11951	1039.6440 ng/ml

# AM #13 Benzodiazepines and Z-Drugs



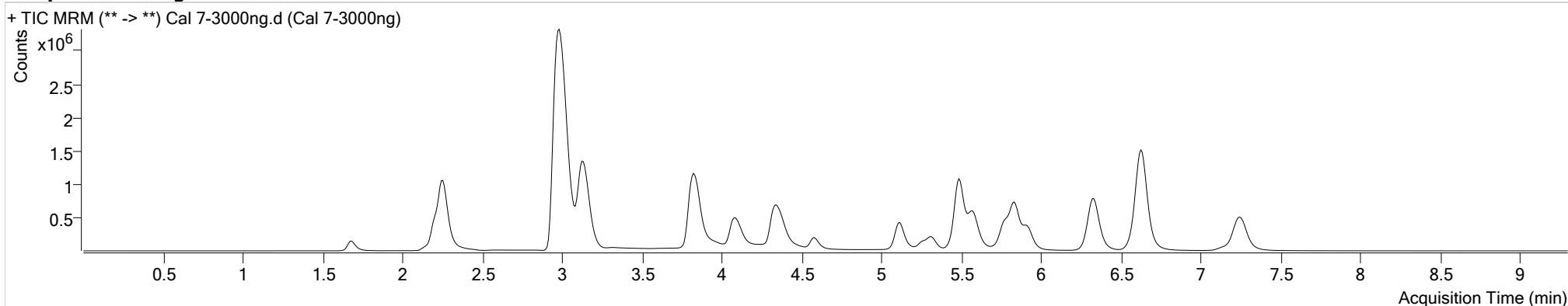
**Batch results**  
**Calibration Last Update**

D:\MassHunter\Data\2019\AM 13\AM 13 worklist 3536\QuantResults\am 13 worklist 3536.batch.bin  
7/16/2019 1:39:20 PM

**Instrument** Falco  
**Type** Cal  
**Acq. Method** Benzos\_Z\_Drugs 053019 FINAL.m  
**Sample Position** P5-A7  
**Injection Volume** 2  
**Acq. Date-Time** 7/15/2019 4:43:18 PM  
**Sample Info.**

**Data File** Cal 7-3000ng.d  
**Sample** Cal 7-3000ng  
**Comment** Worklist 3536

**Sample Chromatogram**



Name	RT	Resp.	S/N	Ratio	S/N	ISTD Resp.	Final Conc.
7-Aminoclonazepam	1.678	404169	7258.61	36.4	4012.58	144553	3093.0668 ng/ml
7-Aminoflunitrazepam	2.207	1448287	∞	34.9	∞	144553	2451.5998 ng/ml
alpha-Hydroxyalprazolam	5.256	246748	∞	87.1	∞	13918	2640.3834 ng/ml
alpha-hydroxytriazolam	5.305	504714	∞	55.2	∞	41573	3173.1339 ng/ml
Alprazolam	5.917	1108069	∞	33.0	∞	13918	1948.2700 ng/ml
Chlordiazepoxide	3.134	4157919	103658.45	29.9	136313.69	41573	2617.7903 ng/ml
Clonazepam	5.772	1104394	50135.77	42.4	15901.37	52753	2179.7920 ng/ml
Diazepam	7.242	2194615	∞	40.4	∞	115338	1716.6516 ng/ml
Flunitrazepam	6.329	2601401	∞	42.8	∞	52753	1917.7899 ng/ml
Lorazepam	5.831	644658	26358.79	99.8	43204.32	41573	2789.3507 ng/ml
Midazolam	4.083	1915757	∞	30.0	12962.13	52753	1008.4102 ng/ml
Nitrazepam	5.118	1111956	39309.99	52.5	16178.62	52753	2798.1468 ng/ml
Nordiazepam	5.574	1600208	83641.19	56.7	32271.62	41573	2751.3395 ng/ml
Oxazepam	5.488	3193494	177505.61	43.3	69977.55	54831	2248.9129 ng/ml
Temazepam	6.626	5931153	238617.75	34.1	65055.42	231002	2243.4118 ng/ml
zolpidem	2.982	15441145	429678.20	36.9	264612.41	41573	1099.2514 ng/ml
zopiclone	2.250	2415055	317325.07	61.4	96032.56	41573	2994.3900 ng/ml