

# Structural insights into the recognition of mono- and di-acetylated histones by the ATAD2B bromodomain

## Supporting Information

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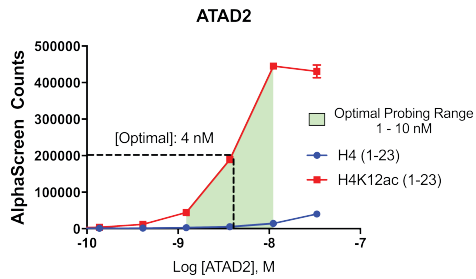
Pg. 8 Supplementary Figure 7. Sequence alignment of the ATAD2 and ATAD2B bromodomains.

Pg. 9-46 HPLC and MS reports for the histone peptides tested by ITC and listed in Table 1.

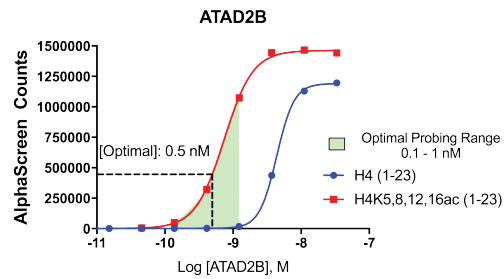
Pg. 47 1D <sup>1</sup>H NMR spectrum of compound 38.

**Supplementary Figure 1. Characterization of the ATAD2/B bromodomain binding curves for the dCypher assay.** (A) The GST-tagged ATAD2 bromodomain (residues 981-1108) and (B) ATAD2B bromodomain (residues 953-1085) were titrated to control biotinylated histone peptides and binding curves determined on AlphaScreen® platform (i.e. dCypher® Phase A: see Methods). X-axes are log(protein concentration (M)) at constant peptide concentration (100 nM); Y-axes are AlphaScreen counts, representing relative strength of binding (n = 2; error bars are S.D.).

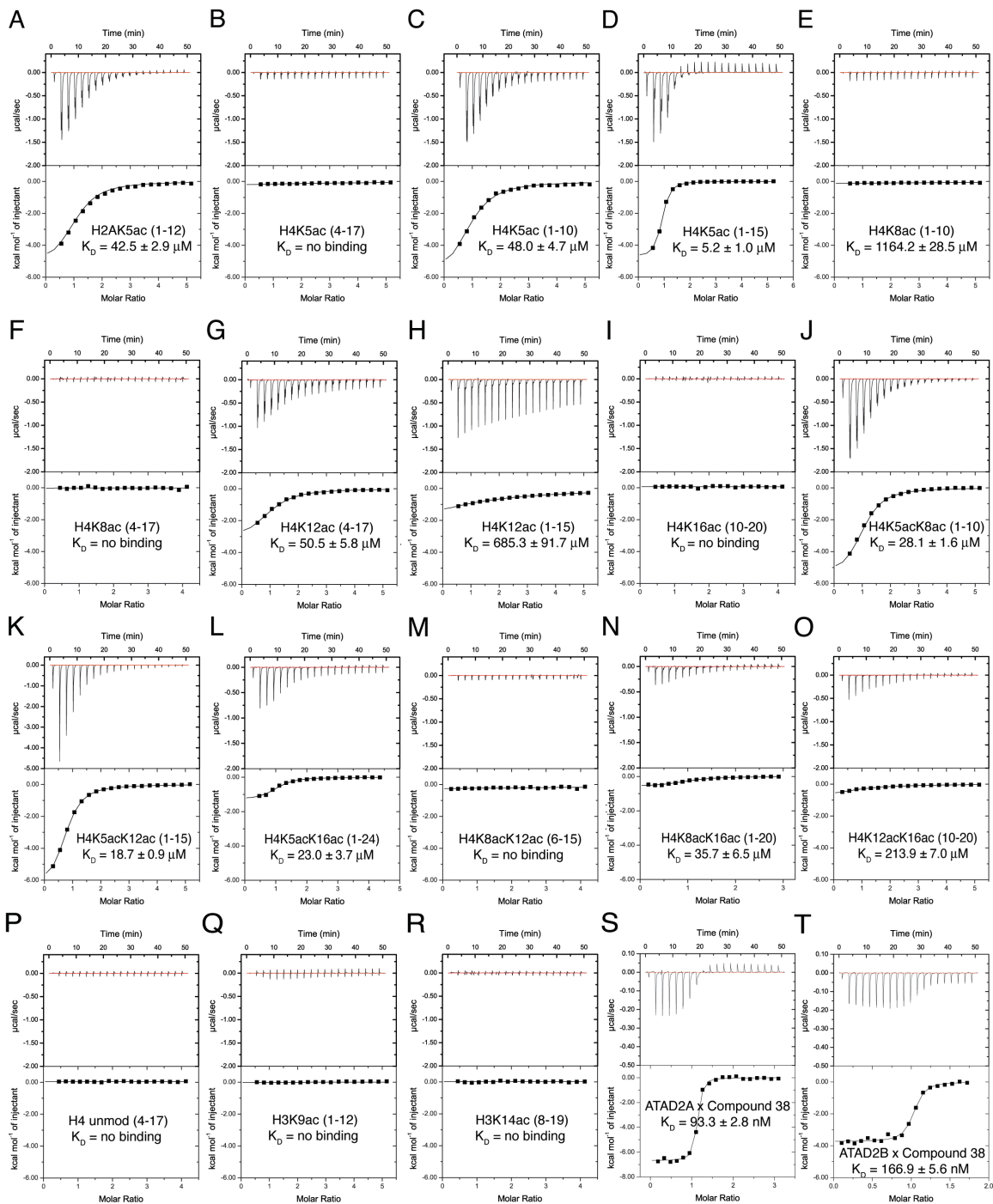
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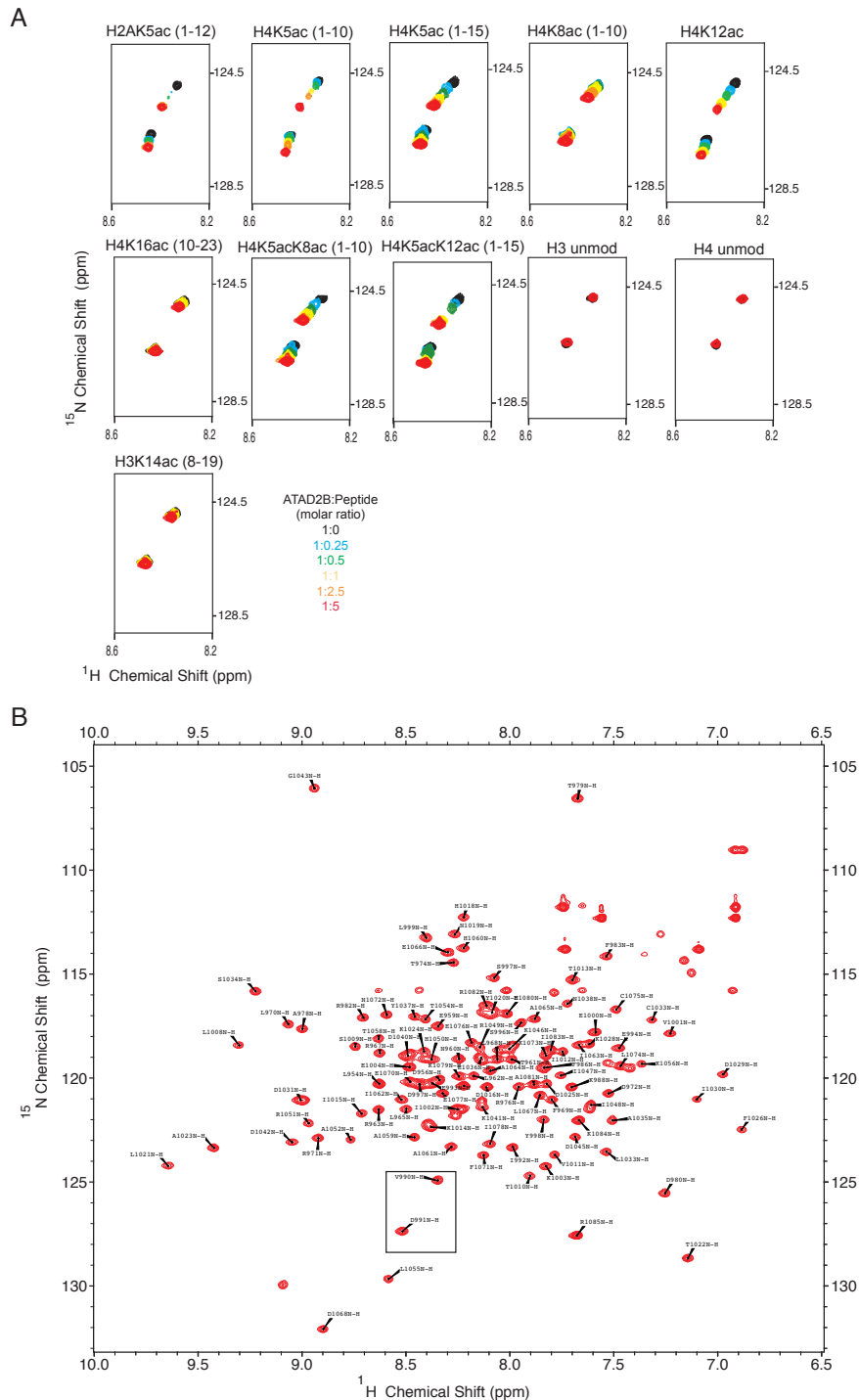
B



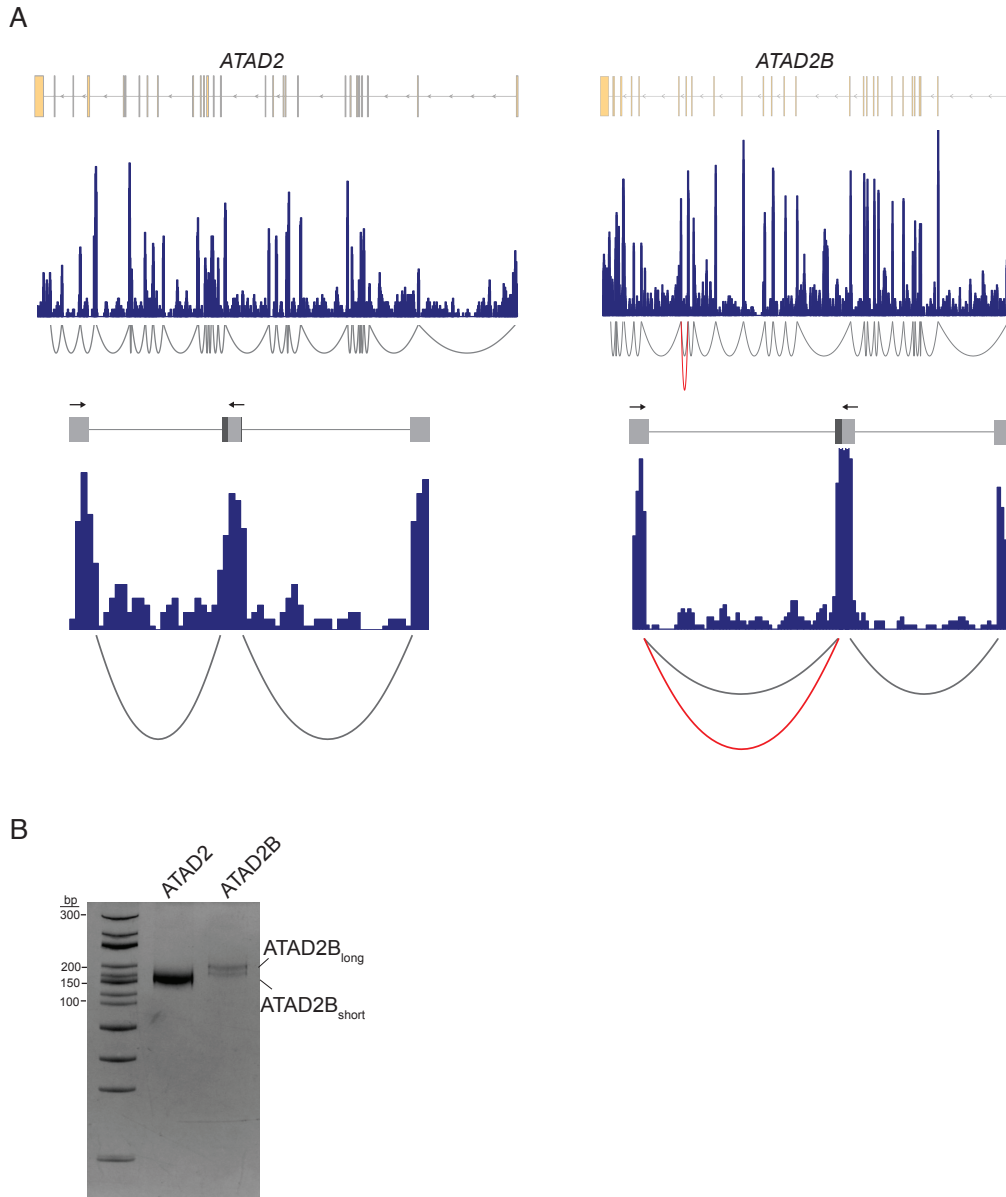
**Supplementary Figure 2. ITC measurements of the interaction between acetylated histone ligands and the ATAD2B bromodomain.** (A-S) Exothermic ITC enthalpy plots for the binding of the ATAD2B bromodomain to unmodified, mono- and di-acetylated histone ligands, as well as the ATAD2 bromodomain inhibitor compound 38. The calculated binding constants are indicated.



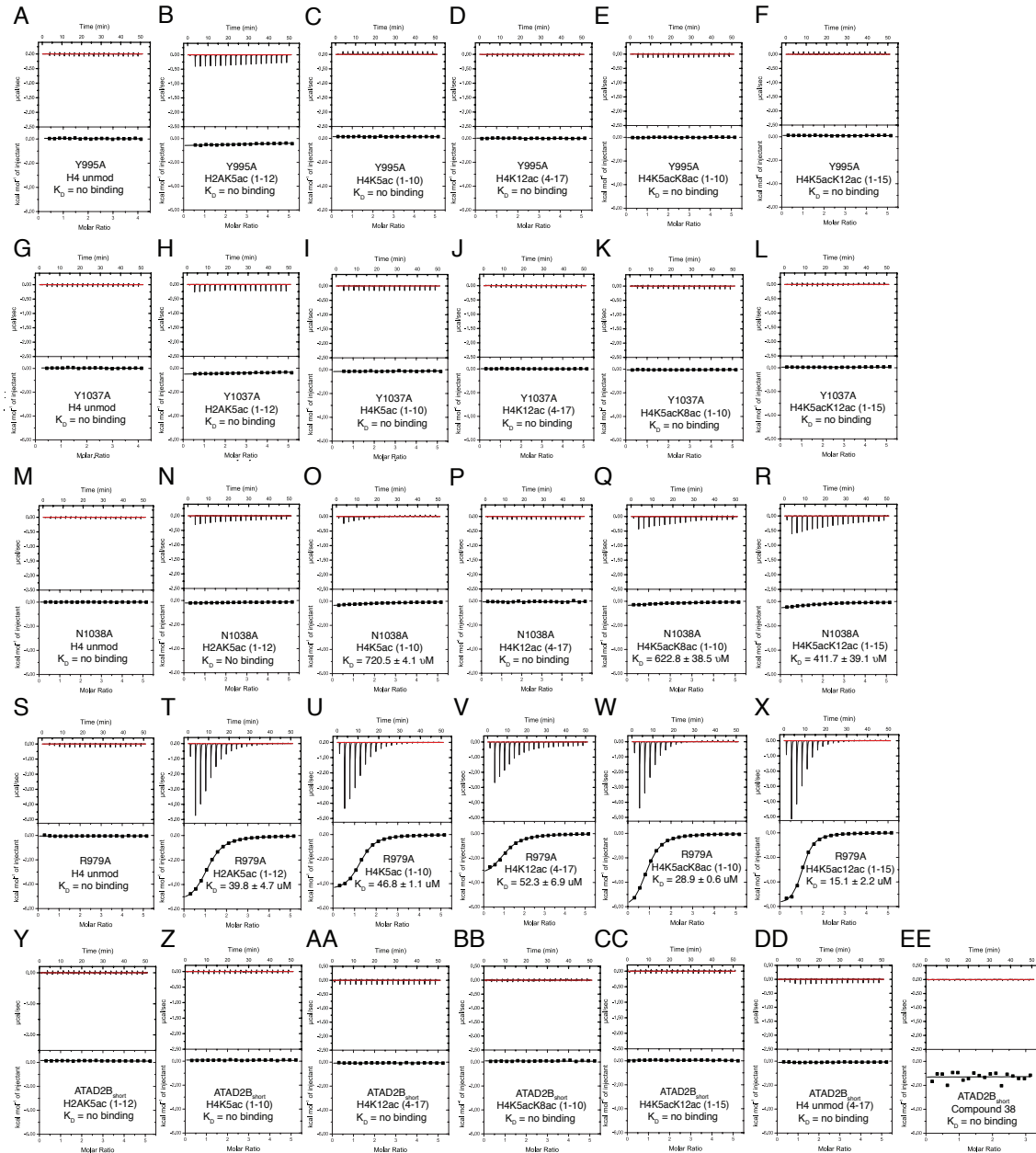
**Supplementary Figure 3. Interaction of the ATAD2B bromodomain with acetylated histone ligands.** (A) Superimposed  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of the ATAD2B bromodomain, collected during titrating in the indicated histone peptides. The spectra are color-coded according to the protein/peptide ratio. (B) 2D  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -labelled ATAD2B bromodomain with the complete HSQC assignments labeled.



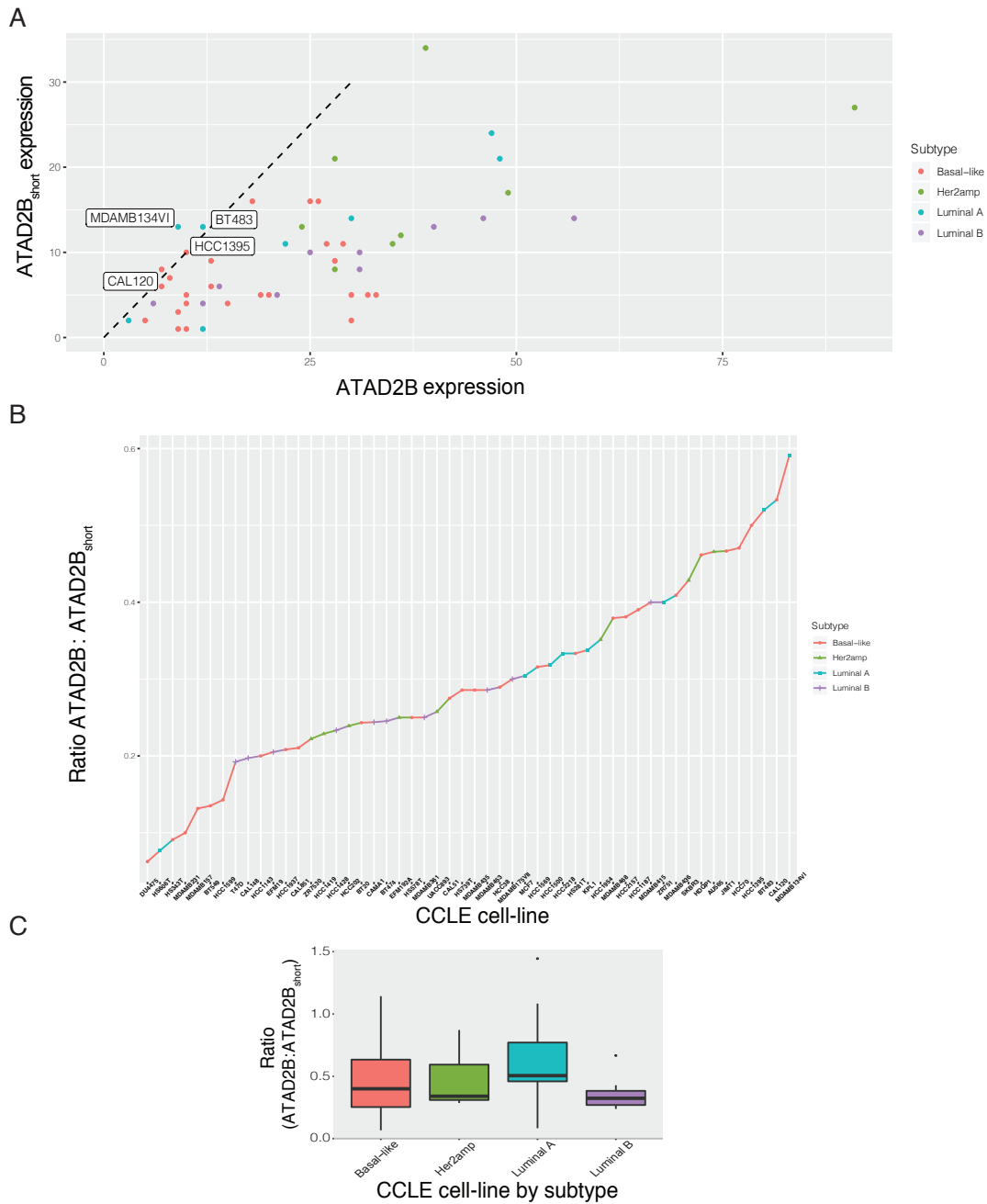
**Supplementary Figure 4.** *ATAD2* and *ATAD2B* gene structures and splicing patterns. (A) Sashimi plots of splice junctions between *ATAD2* and *ATAD2B* are shown. The number of reads spanning each junction is indicated by the size of the sashimi plot curve, and the number of reads spanning novel junctions is indicated by red sashimi plot curves. Coverage plots for total mapped reads are shown (total exon coverages are shown on the y-axis). (B) RT-PCR Analysis of *ATAD2* and *ATAD2B* exon 21-22 splicing patterns.



**Supplementary Figure 5. ITC measurements of the interaction between acetylated histone ligands and mutant ATAD2B bromodomain proteins.** (A-O) Exothermic ITC enthalpy plots for the binding of mutant ATAD2B bromodomain proteins to histone peptide ligands that are unmodified, mono- or di-acetylated. The calculated binding constants are indicated.



**Supplementary Figure 6.** Analysis of ATAD2B splice isoform expression from RNAseq data. (A) Scatterplot comparing the normalized counts of reads that map to ATAD2B (x-axis) and ATAD2B<sub>short</sub> (y-axis) isoforms across human breast cancer datasets from CCLE. Each dataset is colored by the breast cancer molecular subtype, the dashed line represents a 50:50 ratio of each isoform and the names of 4 cell lines with the highest expression of ATAD2B<sub>short</sub> are labeled. (B) A plot showing the ranked expression of ATAD2B: ATAD2B<sub>short</sub> across human breast cancer cell lines. (C) A box plot of the ATAD2B: ATAD2B<sub>short</sub> ratios across human breast cancer molecular subtypes.



**Supplementary Figure 7. Sequence alignment of the ATAD2 and ATAD2B bromodomains.** Sequence alignment of the ATAD2 and ATAD2B bromodomain proteins corresponding to the amino acids in our ATAD2B bromodomain structure (residues 953-1085). Residues colored magenta are involved in hydrogen bonding to compound 38 for the ATAD2B bromodomain, and compound 42 for the ATAD2 bromodomain. Residues colored green are hydrophobic contacts. Residues that were mutated in this study are marked with a red vertical line. Sequence alignment was performed using the T-Coffee software <sup>1</sup>.

```

          962      972      982      992
ATAD2B  953 EDQEENTLRELRLFLRDVTKRLATDKRFNIFSKPVDIEVSDYLEVI 999
ATAD2   979 EEQEEDTFRELRIFLRNVTHRLAIDKRFVFTKPVDPDEVPDYVTVI 1025
          *:***:*:****:***:***:***:***:***:***:***:***:***:***:***:
          1009      1019      1029      1039
ATAD2B 1000 KEPMDLSTVITKIDKHNYLTAKDFLKDIDLICSNALEYNPDKDPCDK 1046
ATAD2  1026 KQPMDLSSVISKIDLHKYLTVKDYLRDIDLICSNALEYNPDRDPCDR 1072
          *:*****:***:***:***:***:***:***:***:***:***:***:***:
          1056      1066      1076
ATAD2B 1047 IIRHRACTLKDTAHAIIAAELDPEFNKLCÉEIKEARIKR 1085
ATAD2  1073 LIIRHACALRDYAYAIKEELDEDFEQLCEEIQESRKKR 1111
          :*****:***:***:***:***:***:***:***:***:***:***:***:

```

1. Notredame, C., Higgins, D.G. & Heringa, J. T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* **302**, 205-17 (2000).



### H2AK5ac (1-12) HPLC report

Sample Name: KG #1

```

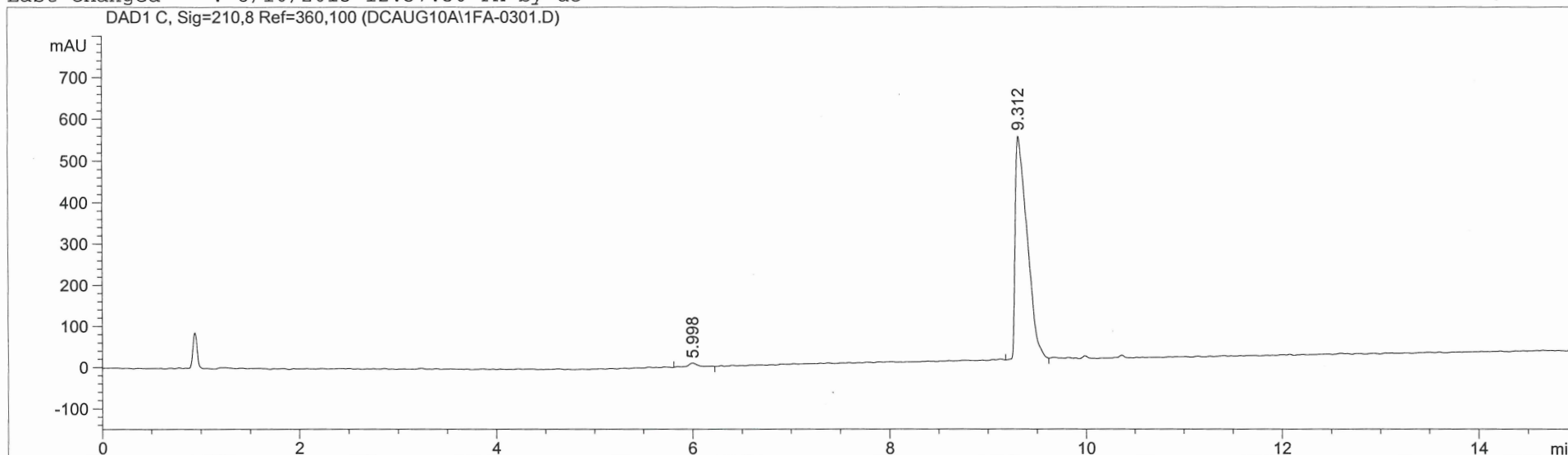
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Acq. Instrument : Instrument 1             Location  : P1-F-01
Injection Date  : 8/10/2015 12:16:58 PM   Inj       :    1
                                           Inj Volume: 5 µl
Different Inj Volume from Sequence !      Actual Inj Volume: 2 µl
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Last changed    : 8/10/2015 12:14:10 PM by dc
Analysis Method : C:\Chem32\1\METHODS\DC1M.m
Last changed    : 8/10/2015 12:37:50 PM by dc
    
```

NH<sub>2</sub>-SGRGGK(Ac)QGGKARA-amide

H2A(1-12)K5ac, purity>98%

8-19-15

MW=1214



#### Area Percent Report

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Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

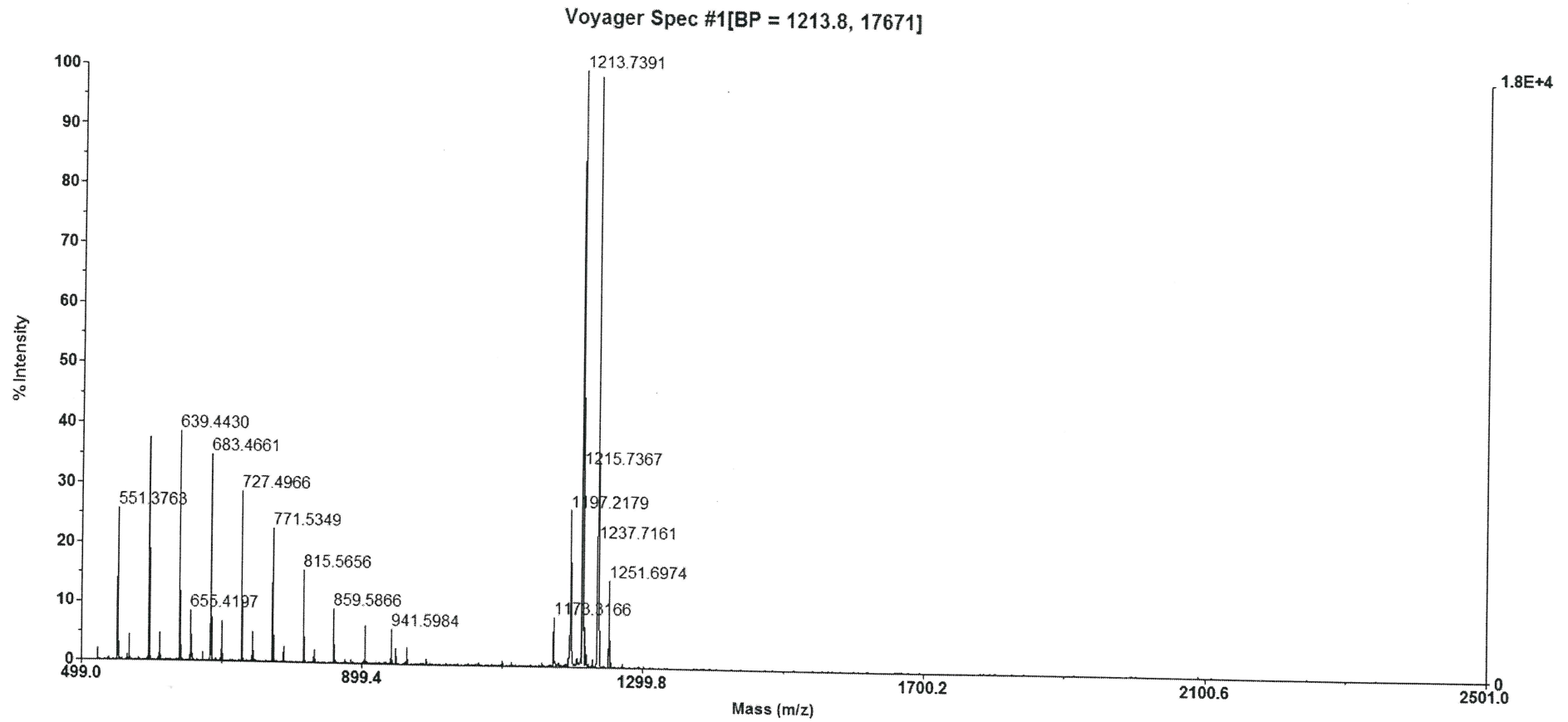
Signal 1: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.998	BB	0.1060	68.84024	9.55910	1.4777
2	9.312	VV	0.1266	4589.64307	543.08765	98.5223

H2AK5ac (1-12) MS report

#1

MW 1214



Sample Name: H 4 PURE

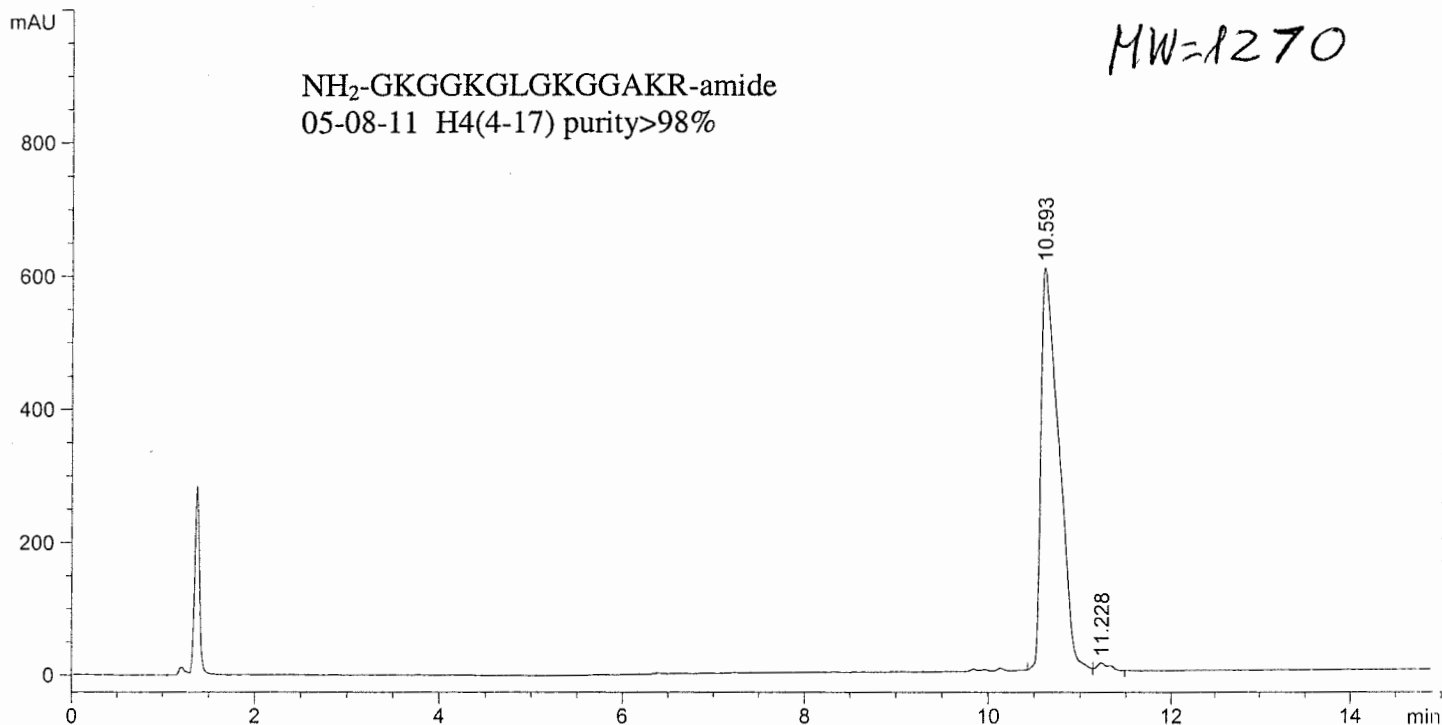
H4 unmodified (4-17) HPLC report

```

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Injection Date  : 5/31/2011 2:33:34 PM    Inj       :    1
                                           Inj Volume: 6 µl

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Last changed    : 5/31/2011 2:31:11 PM by dc
Analysis Method : C:\Chem32\1\METHODS\30MINWASH50C.M
Last changed    : 5/31/2011 3:25:44 PM by wh
    
```

DAD1 C, Sig=210,8 Ref=360,100 (DCMAY31\024-0501.D)



Area Percent Report

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Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.593	BV	0.1955	8200.18457	607.34192	98.6267
2	11.228	VB	0.1292	114.17900	12.19384	1.3733

Totals : 8314.36357 619.53576

\*\*\* End of Report \*\*\*

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**Print Date:** 5/31/2011 3:26:41 PM

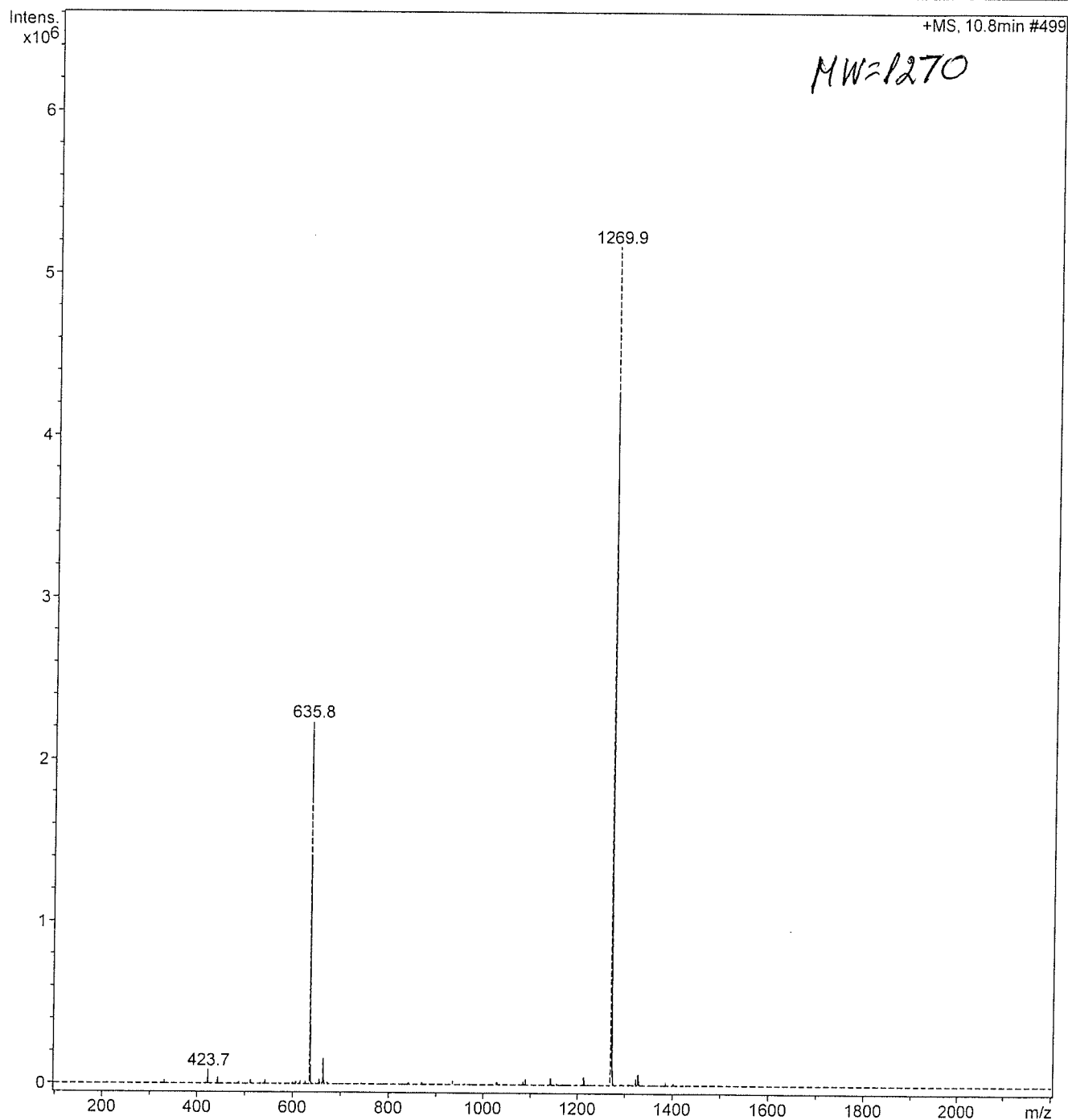
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**Operator:** Hodges

**Acq. Date:** 5/31/2011 2:33:56 PM

**Sample Name:** H 4 PURE

**Analysis Info:**



## H4K5ac (1-15) HPLC report

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 Month-Day-Year Processed :10/30/2019

Pump A : 0.065% trifluoroacetic in 100% water (v/v)  
 Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)  
 Total Flow:1 ml/min  
 Wavelength:220 nm

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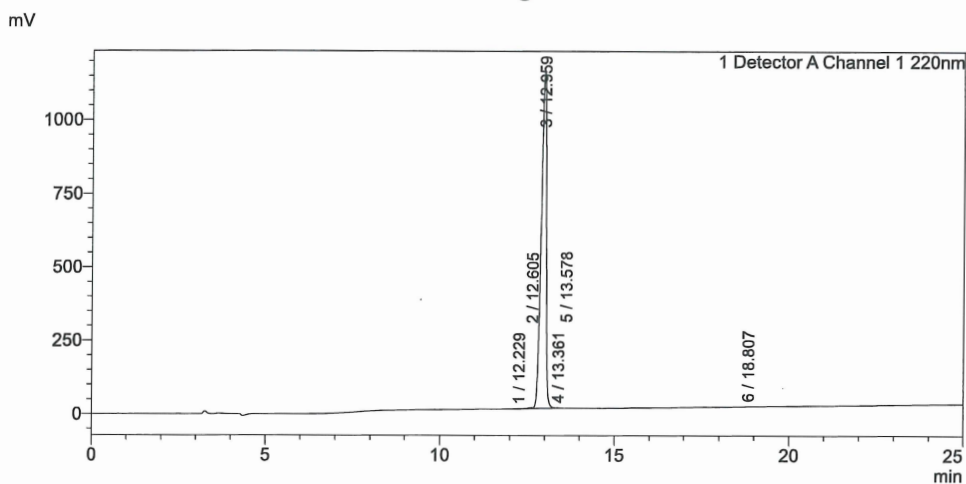
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25.00	Pumps	Pump A B.Conc	65
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31.00	Pumps	Pump A B.Conc	95
31.01	Pumps	Pump A B.Conc	5
40.00	Pumps	Pump A B.Conc	5
40.01	Controller	Stop	

<<Column Performance>>

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Column : AlltimaTM C18 4.6 x 250 mm  
 Equipment:ZJ19010324

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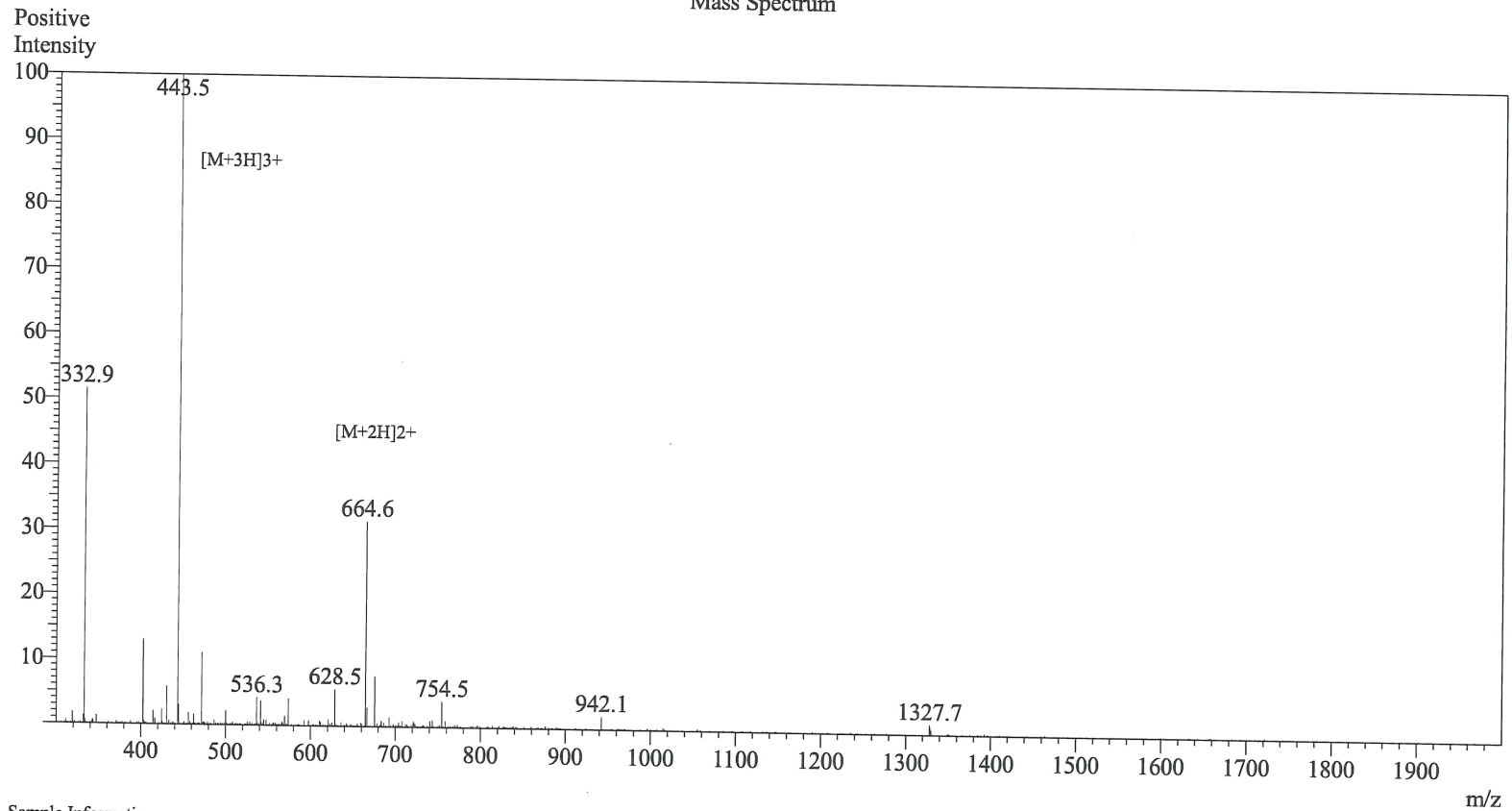
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Detector A Channel 1 220nm

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3	12.959	11807221	1149152	99.563
4	13.361	2802	621	0.024
5	13.578	1550	289	0.013
6	18.807	2072	383	0.017
Total		11859057	1155237	100.000

# H4K5ac (1-15) MS report

## Mass Spectrum



### Sample Information

Acquired by : Gary  
Month-Day Processed : 10/29/19  
Time Processed : 03:13:01 PM  
Injection Volume : 0.3  
Sample Name : H4K5ac (1-15)  
Sample ID : U2819EJ240-1  
Theoretical MW : 1327.49  
Observed MW : 1327.5

Interface : ESI  
Nebulizing Gas Flow : 1.5L/min  
CDL Temp : 250  
Block Temp : 200

Equipment : GK11010007  
Interface Bias : +4.5 kV  
Drying Gas Flow : 5 L/min  
T.Flow : 0.2 ml/min  
B.conc : 50%H2O/50%MeOH

# H4K5ac (1-10) HPLC report

Sample Name: H4K5ac(1-10)  
 Sample ID: U3559EH260-1  
 Time Processed : 8:43:45  
 Month-Day-Year Processed : 09/08/2019

Pump A : 0.065% trifluoroacetic in 100% water (v/v)  
 Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)  
 Total Flow: 1 ml/min  
 Wavelength: 220 nm

<<LC Time Program>>

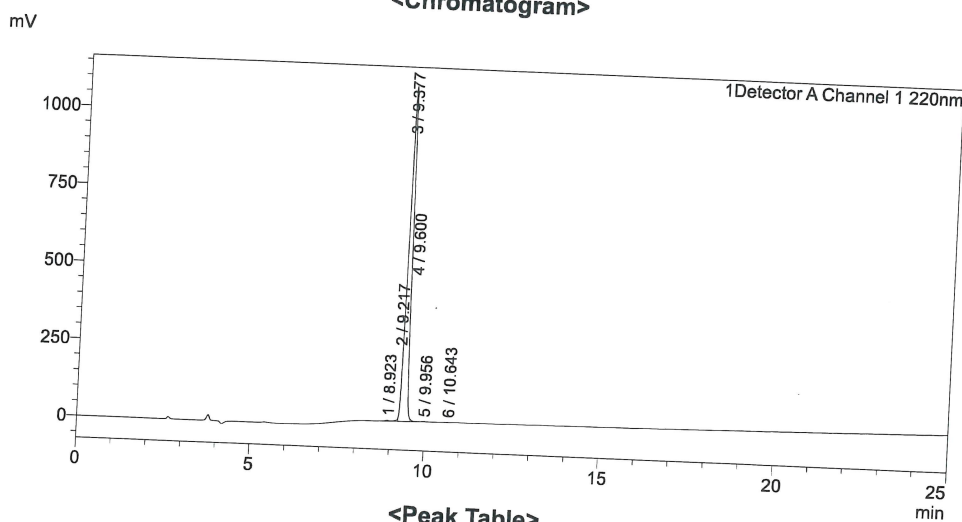
Time	Module	Command	Value
0.01	Pumps	Pump A B.Conc	5
25.00	Pumps	Pump A B.Conc	65
25.01	Pumps	Pump A B.Conc	95
31.00	Pumps	Pump A B.Conc	95
31.01	Pumps	Pump A B.Conc	5
40.00	Pumps	Pump A B.Conc	5
40.01	Controller	Stop	5

<<Column Performance>>

<Detector A>

Column : Inertsil ODS-3 4.6 x 250 mm  
 Equipment: GK12010012

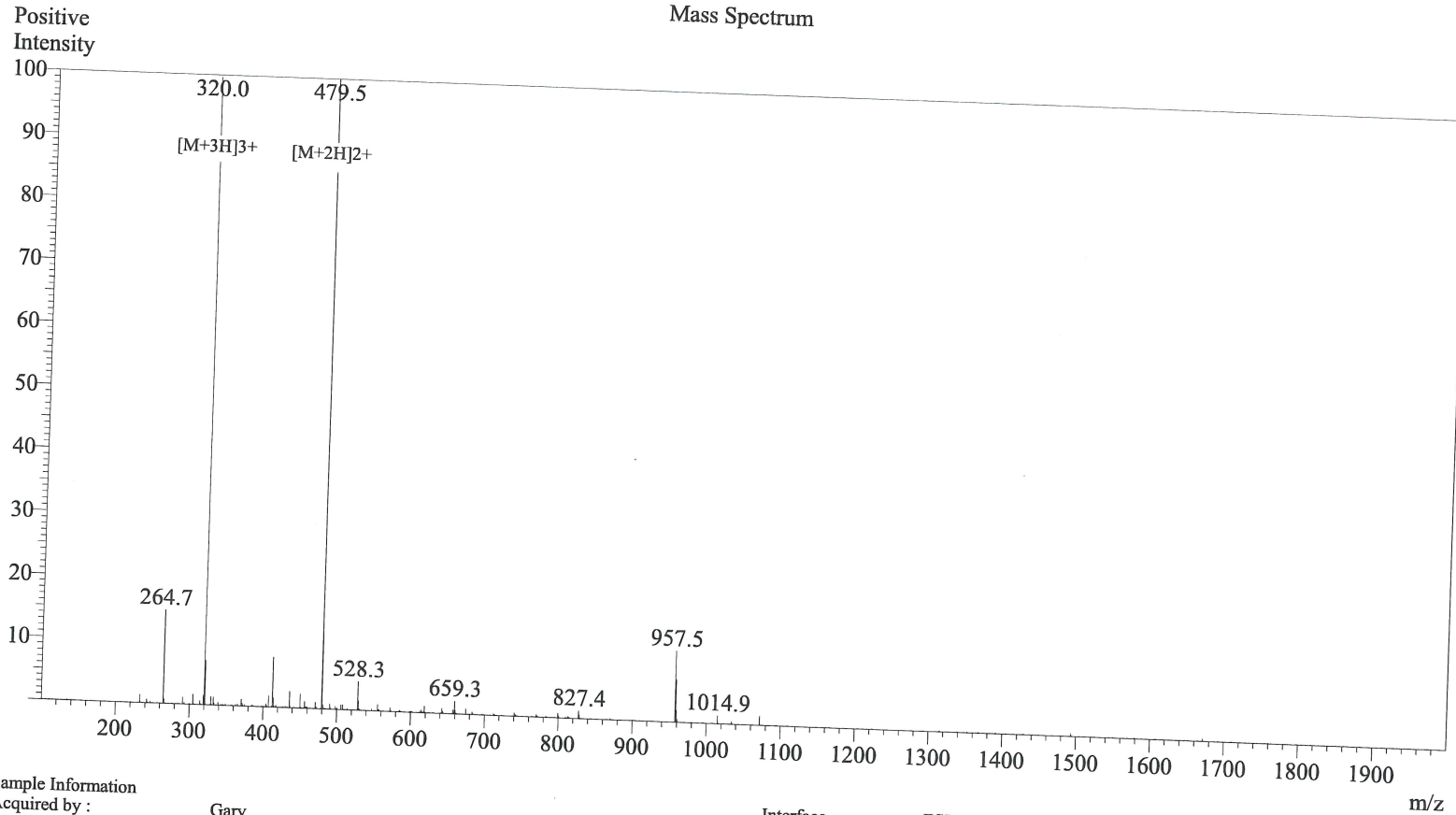
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1	8.923	21854	2574	0.267
2	9.217	33068	5040	0.403
3	9.377	8098036	1075473	98.756
4	9.600	27857	6103	0.340
5	9.956	15390	1260	0.188
6	10.643	3877	572	0.047
Total		8200082	1091022	100.000

# H4K5ac (1-10) MS report



## Sample Information

Acquired by : Gary  
Month-Day Processed : 09/07/19  
Time Processed : 18:01:00  
Injection Volume : 0.3  
Sample Name : H4K5ac(1-10)  
Sample ID : U3559EH260-1  
Theoretical MW : 957.09  
Observed MW : 957.0

Interface : ESI  
Nebulizing Gas Flow : 1.5L/min  
CDL Temp : 250  
Block Temp : 200

Equipment : GK11010007  
Interface Bias : +4.5 kV  
Drying Gas Flow : 5 L/min  
T.Flow : 0.2 ml/min  
B.conc : 50%H<sub>2</sub>O/50%MeOH



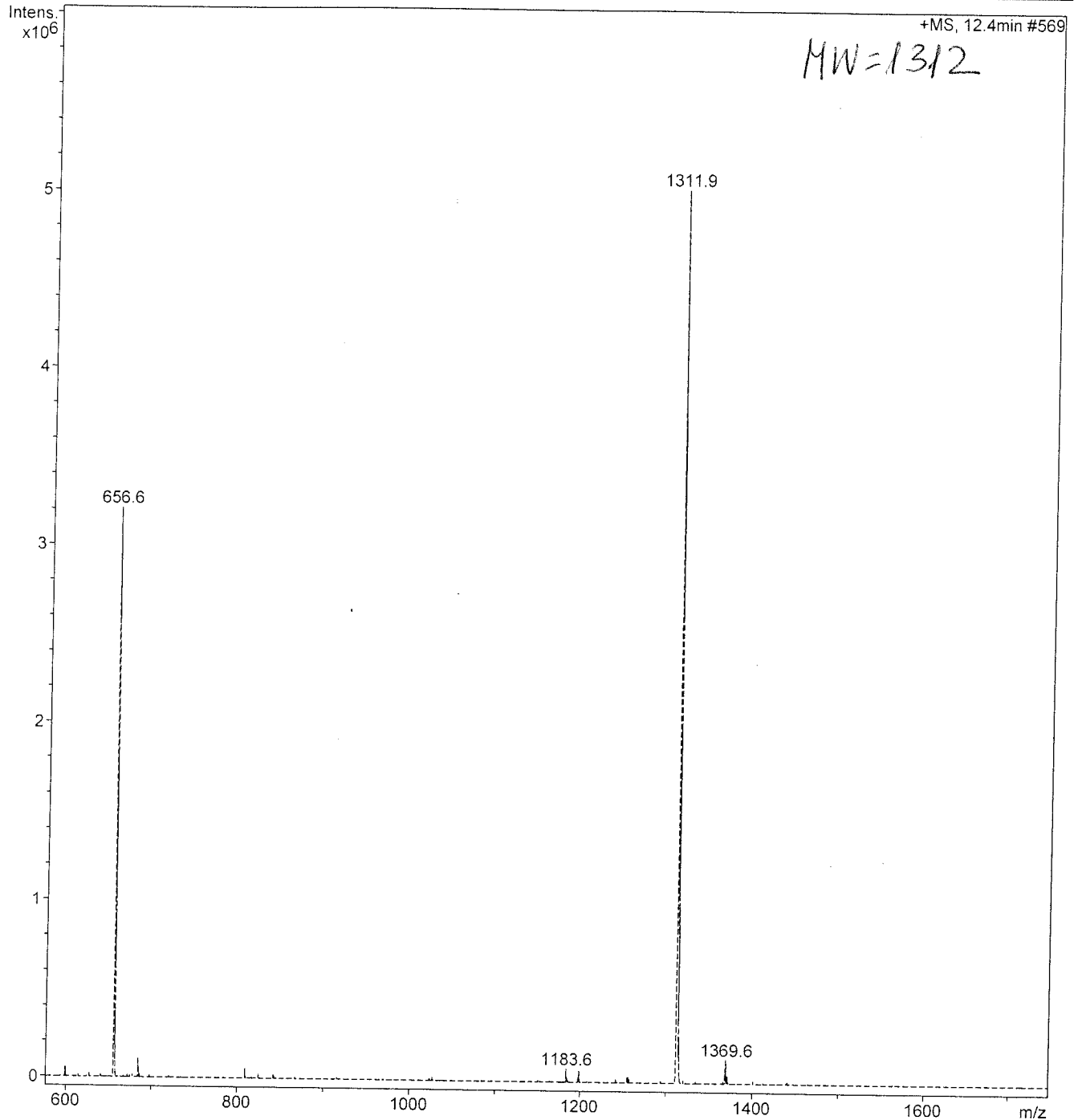


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**Operator:** Hodges

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**Acq. Date:** 5/9/2011 8:21:14 PM



# H4K8ac (1-10) HPLC report

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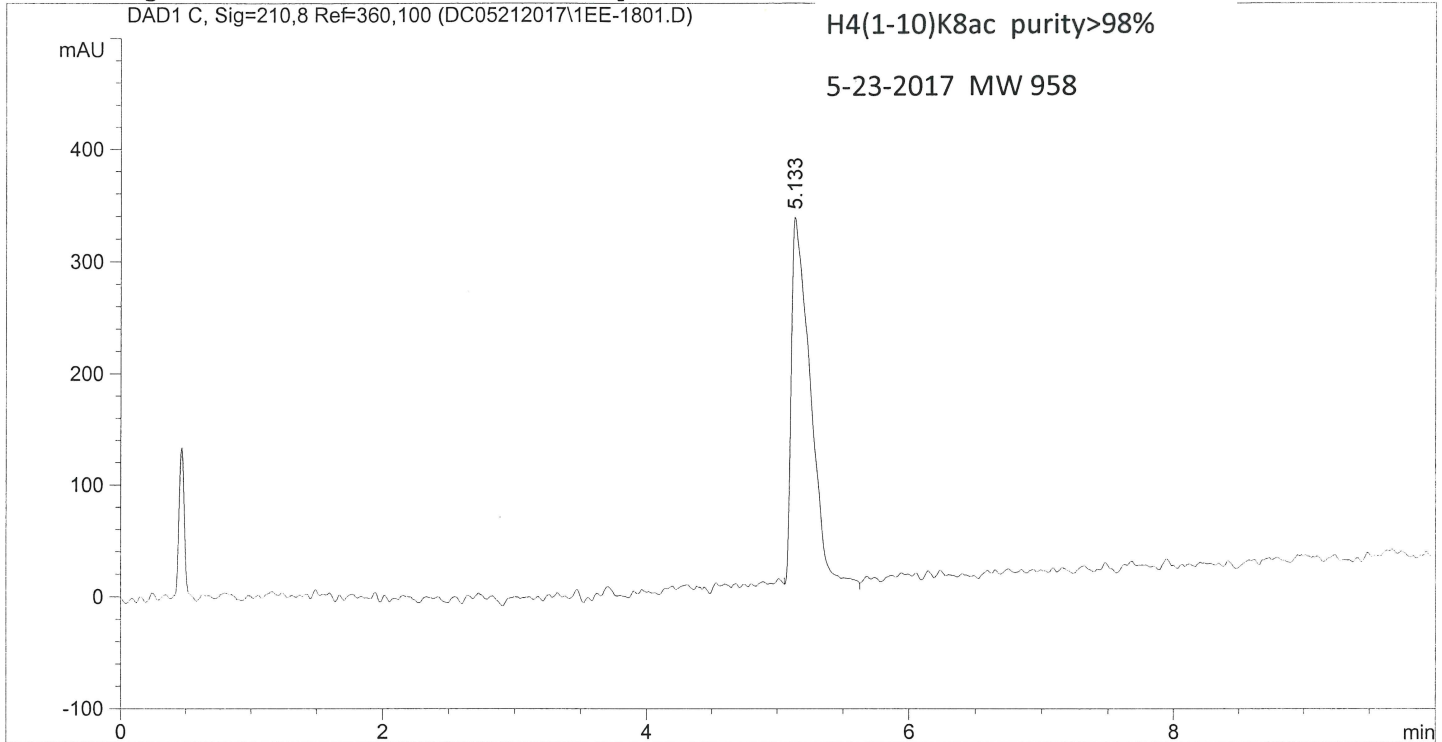
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Last changed    : 5/21/2017 5:42:40 PM by dc
```

NH<sub>2</sub>-SGRGKGGK(Ac)GL-amide

H4(1-10)K8ac purity>98%

5-23-2017 MW 958



## Area Percent Report

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Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
=====
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Signal 1: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	5.133	VV	0.1447	3891.93311	347.34039	100.0000

Totals : 3891.93311 347.34039

\*\*\* End of Report \*\*\*

H4K8ac (1-10) MS report

Display Report - Selected Window Selected Analysis

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Instrument: LC-MSD-Trap-XCT

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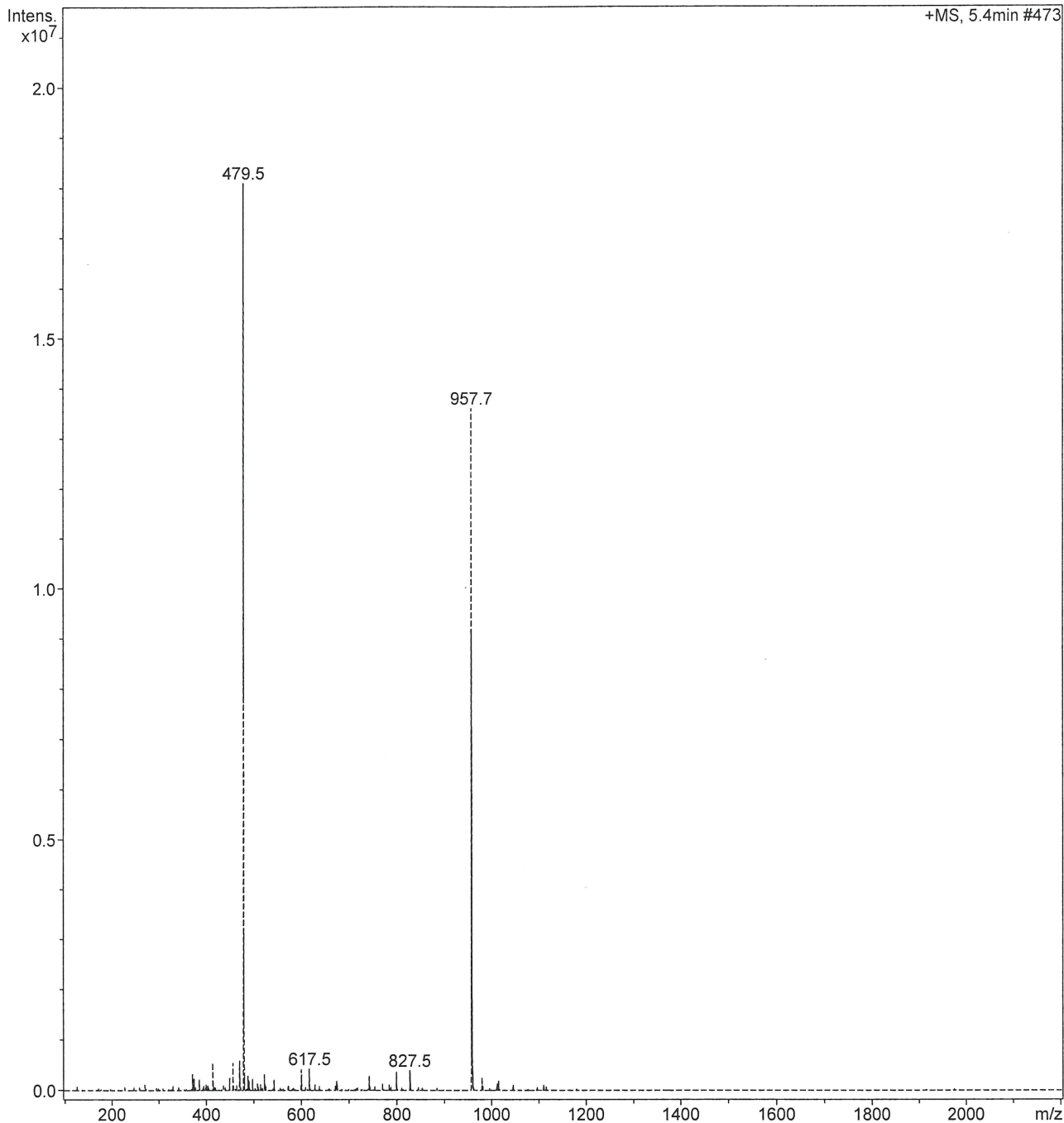
Method: DC2M.M

Operator: Hodges

Acq. Date: 5/21/2017 2:51:17 PM

Sample Name: 67

Analysis Info:





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**Analysis Name:** 016-1101.D

**Instrument:** LC-MSD-Trap-SL

**Print Date:** 5/10/2011 11:11:27 AM

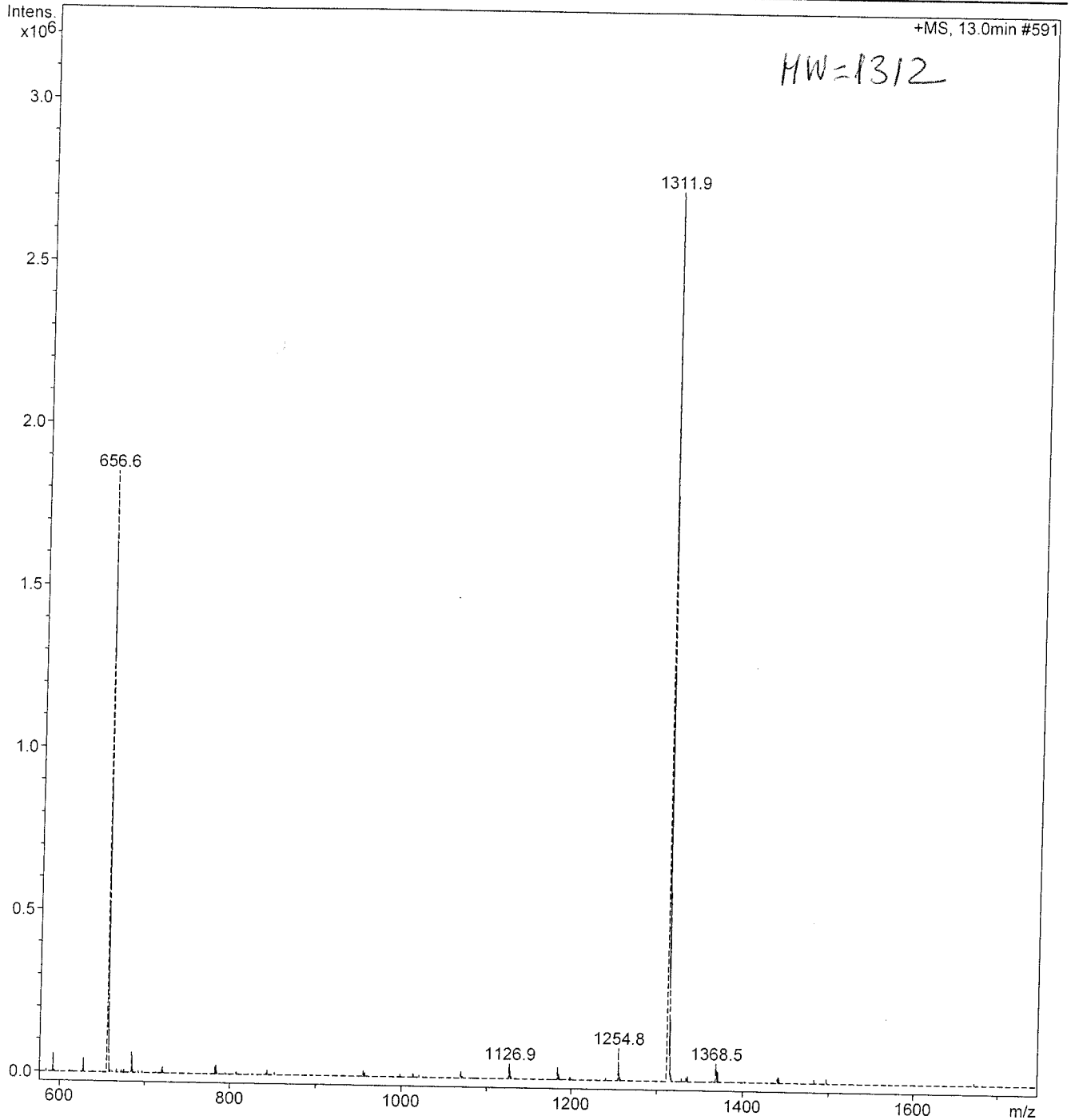
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**Operator:** Hodges

**Acq. Date:** 5/9/2011 9:28:16 PM

**Sample Name:** K8Ac PURE

**Analysis Info:**



# H4K12ac (4-17) HPLC report



**Biomatik**  
Tel: (519) 489-7195, (800) 836-8089  
Fax: (519) 231-0140, (877) 221-3515  
Email: info@biomatik.com  
http://www.biomatik.com

## HPLC REPORT

Sample Description:

Structure: H4(4-17)K12ac GR-14

Lot No: P180117-CL633697

Column: 4.6mm\*250mm, Inertsil ODS-SP

Solvent A: 0.1% Trifluoroacetic in 100% Acetonitrile

Solvent B: 0.1% Trifluoroacetic in 100% Water

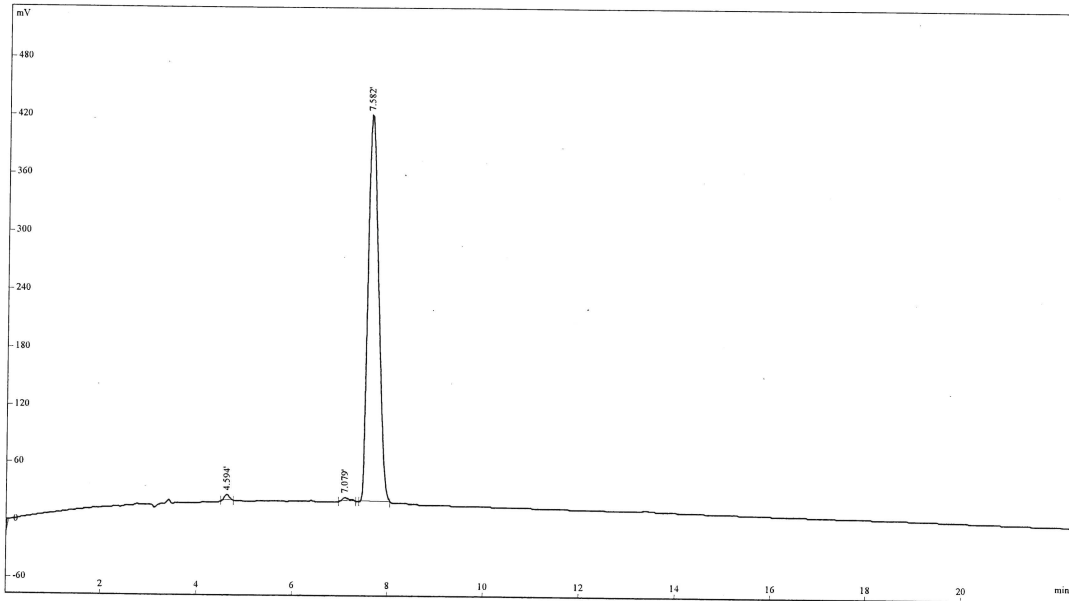
Gradient:

	A	B
0.01min	5%	95%
25.00min	30%	70%
25.01min	100%	0%
30.00min	Stop	

Flow rate: 1.0ml/min

Wavelength: 220nm

Volume: 10 µl



Rank	Time	Conc.	Area	Height
1	4.594	0.7907	49364	6442
2	7.079	0.7209	45008	3761
3	7.582	98.49	6148640	402218
Total		100	6243012	412421

# H4K12ac (4-17) MS report



**Biomatik**

Tel: (519) 489-7195, (800) 836-8089

Fax: (519) 231-0140, (877) 221-3515

Email: info@biomatik.com

http://www.biomatik.com

## MS REPORT

25-Jan-2018

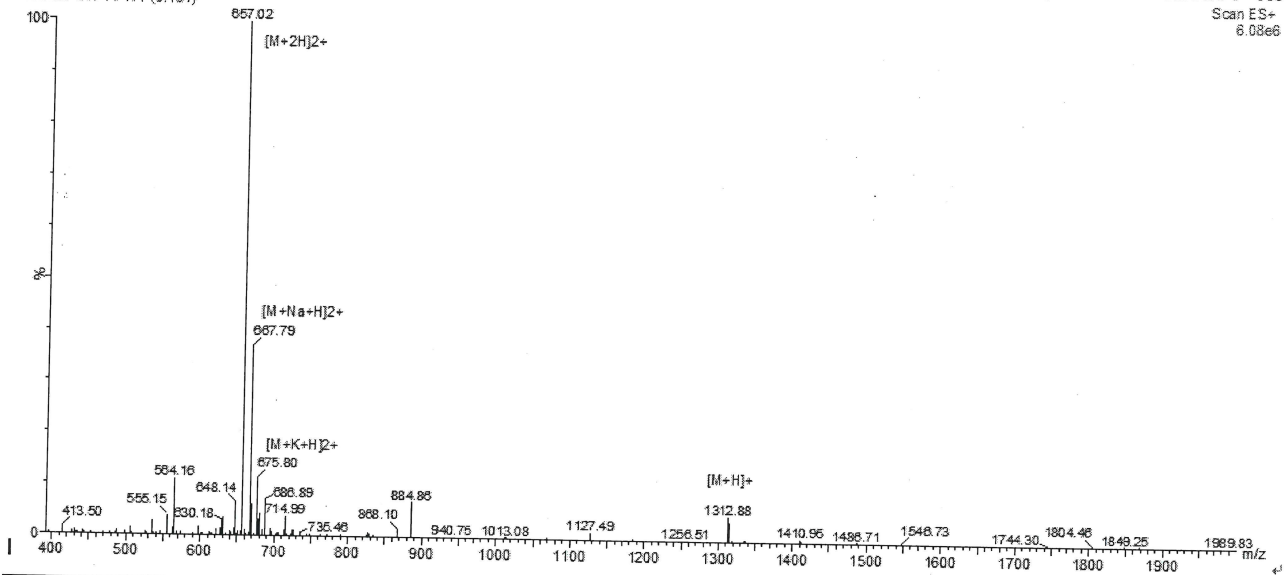
16:55:59

H4(4-17)K12ac P180117-CL633697 MW:1311.56

180125-GR-14 171 (3.161)

Probe: ESI  
Cone: 50v  
Desolvation Temp :350

Capillary:3.00KV  
Extractor: 5v  
Gas Flow : 350  
Scan ES+  
6.08e6







## HPLC Report

Sample Description:

Structure:H4 SA-15

Lot NO. :P170620-MJ589927

Column:250\*4.6mm, Boston Green ODS-AQ

Solvent A:0.1%TFA in 100%water

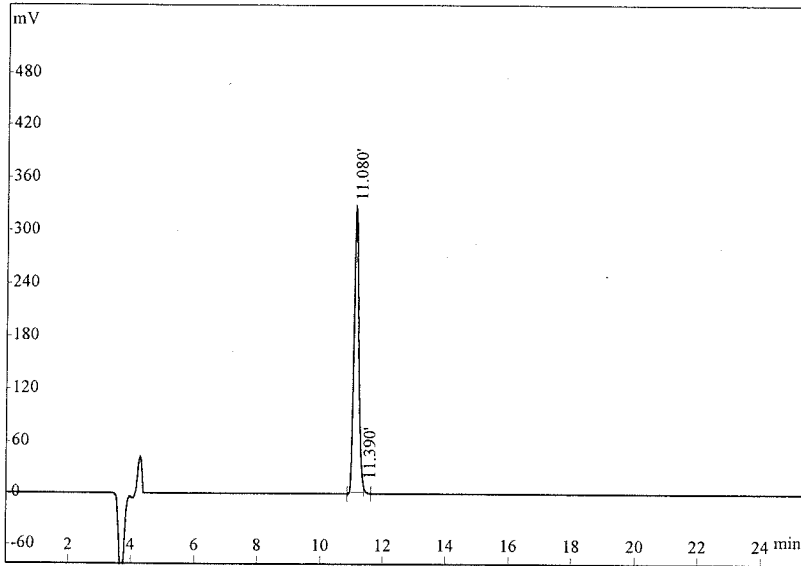
Solvent B:0.1%TFA in 100%acetonitrile

Gradient :	A	B
0.1min	92	8
25min	67	33
25.01min	0	100
30min	0	100

Flow rate:1.0ml/min

Wavelength(nm) :220

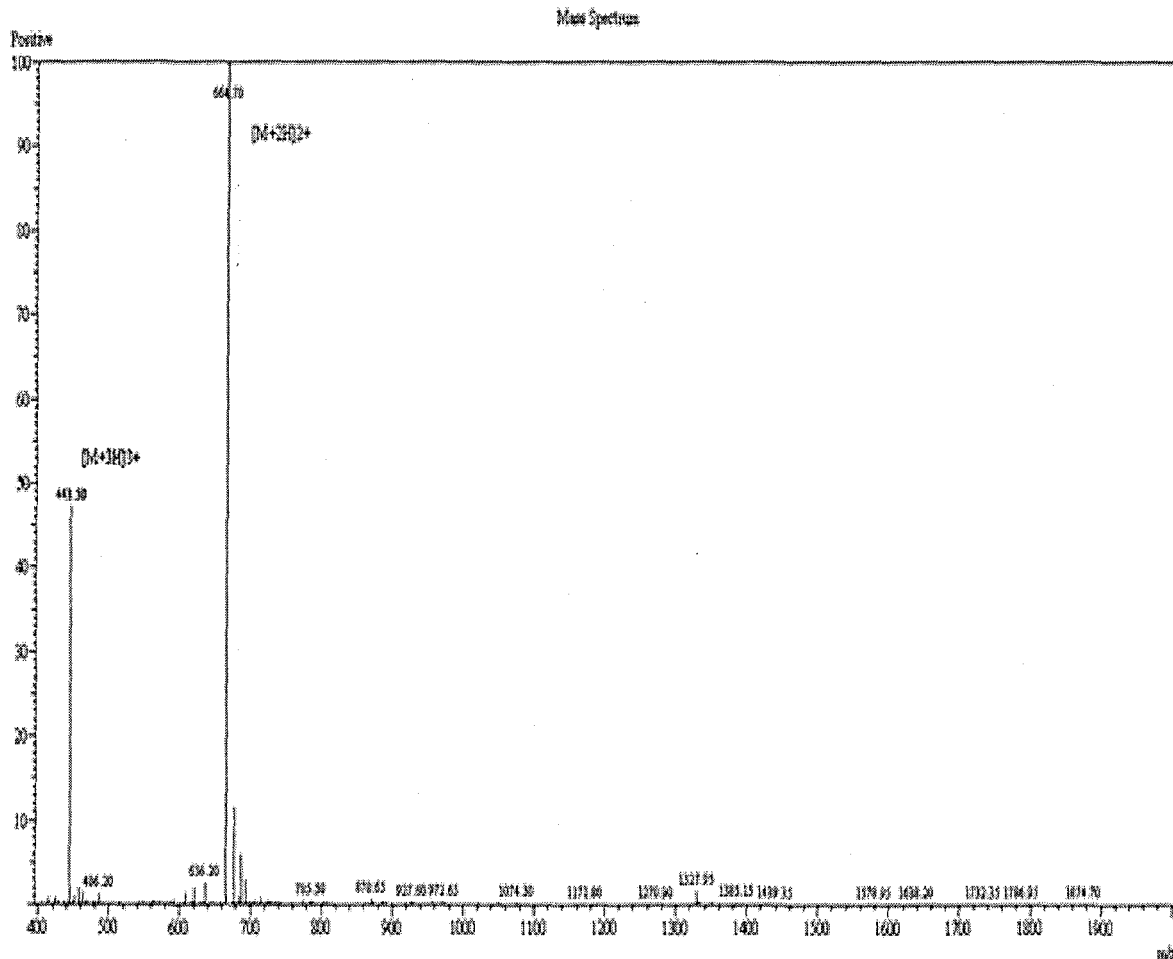
Volume:10ul



Rank	Time	Conc.	Area	Height
1	11.080	99.46	3541495	334837
2	11.390	0.5344	19030	5768
Total		100	3560525	340605



### Mass Spectrometry Report



Sample Information  
 Date and Time: 2017-4-26 10:09:47  
 User: CHAD  
 Sample: H4 SA-15  
 Inj. Volume: 1  
 MW: 1327.52  
 Lot No.: P170620-MJ589927

Probe: ESI      Probe bias: -3 Skv  
 Nebulizer Gas Flow: 1 SL/min      Detector: 1.0kV  
 DL: -20.0v      T. Flow: 0.2nd/min  
 DL Temp: 250°C      B. conc: 50%H2O/50%ACN  
 Block Temp: 200°C

Sample Name: #5

H4K16ac (10-20) HPLC report

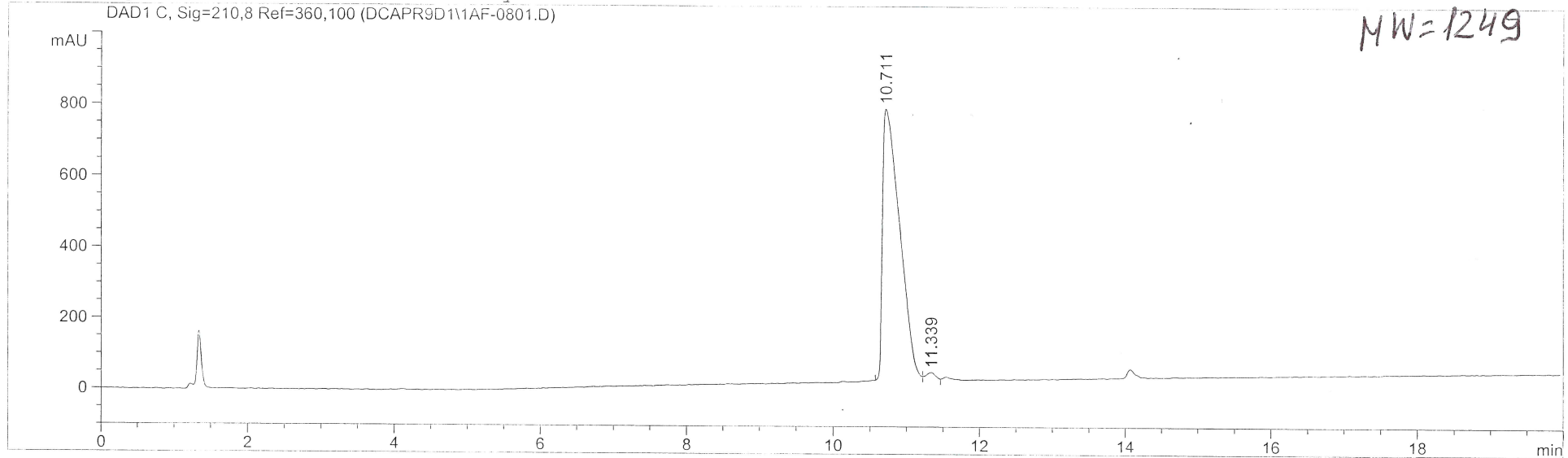
H4K16ac 10-20

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Acq. Instrument : Instrument 1             Location  : P1-A-06
Injection Date  : 4/9/2015 3:13:23 PM    Inj       :    1
                                           Inj Volume: 4 µl
Acq. Method     : C:\Chem32\1\METHODS\DC1M.m
Last changed    : 4/9/2015 3:10:04 PM by dc
Analysis Method : C:\Chem32\1\METHODS\DC1M.m
Last changed    : 4/9/2015 3:36:18 PM by dc
    
```

NH<sub>2</sub>-LGKGGAK(Ac)RHRK-amide

4-09-15 purity >98%



Area Percent Report

```

=====
Sorted By      : Signal
Multiplier     : 1.0000
Dilution       : 1.0000
Use Multiplier & Dilution Factor with ISTDs
    
```

Signal 1: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.711	BV	0.2268	1.25051e4	764.80334	98.2841
2	11.339	VV	0.1414	218.31677	22.85994	1.7159

Totals : 1.27234e4 787.66329

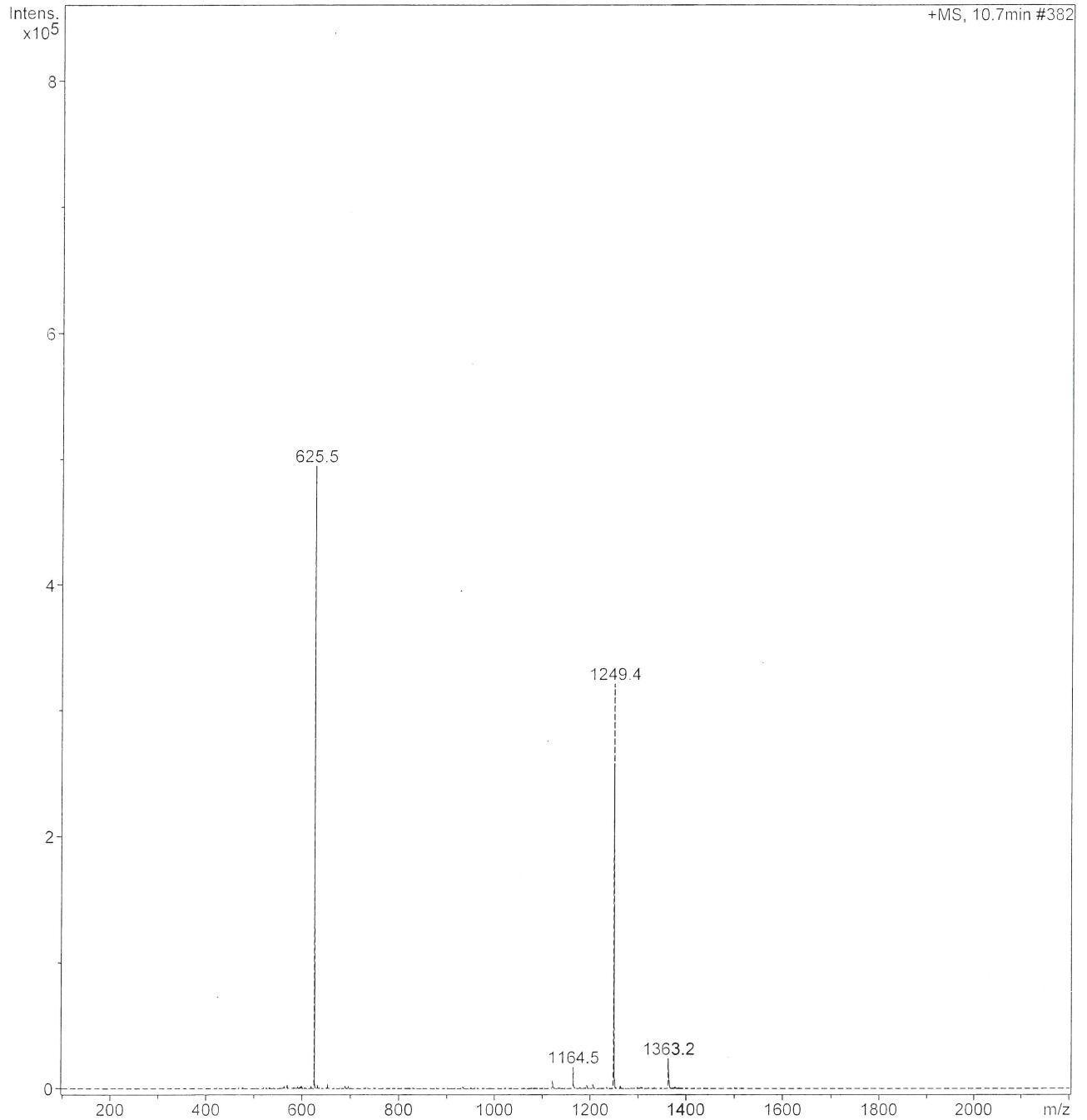
# H4K16ac (10-20) MS report

## Display Report - Selected Window Selected Analysis

**Analysis Name:** 1AF-0801.d  
**Method:** DC1M.m  
**Sample Name:** #5  
**Analysis Info:**

**Instrument:** LC-MSD-Trap-SL  
**Operator:** Hodges

**Print Date:** 4/9/2015 3:58:20 PM  
**Acq. Date:** 4/9/2015 3:12:56 PM



# H4K5acK8ac (1-10) HPLC report

Sample Name :H4K5acK8ac(1-10)  
 Sample ID :U3559EH260-3  
 Time Processed :8:35:14 AM  
 Month-Day-Year Processed :09/04/2019

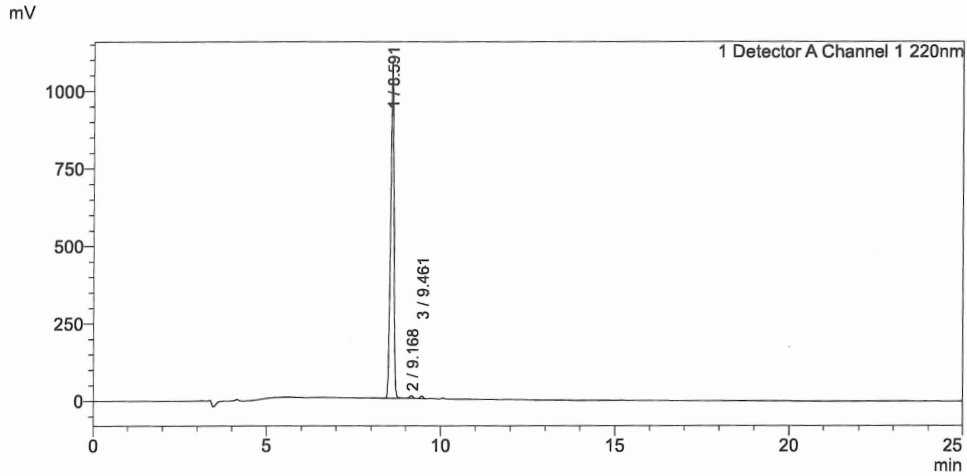
Pump A : 0.065% trifluoroacetic in 100% water (v/v)  
 Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)  
 Total Flow:1 ml/min  
 Wavelength:220 nm

<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	Solvent B Conc.	5
25.00	Pumps	Solvent B Conc.	65
25.01	Pumps	Solvent B Conc.	95
27.00	Pumps	Solvent B Conc.	95
27.01	Pumps	Solvent B Conc.	5
35.00	Pumps	Solvent B Conc.	5
35.01	Controller	Stop	

<<Column Performance>>  
 <Detector A>  
 Column : Inertsil ODS-3 4.6 x 250 mm  
 Equipment:ZJ17010508

## <Chromatogram>

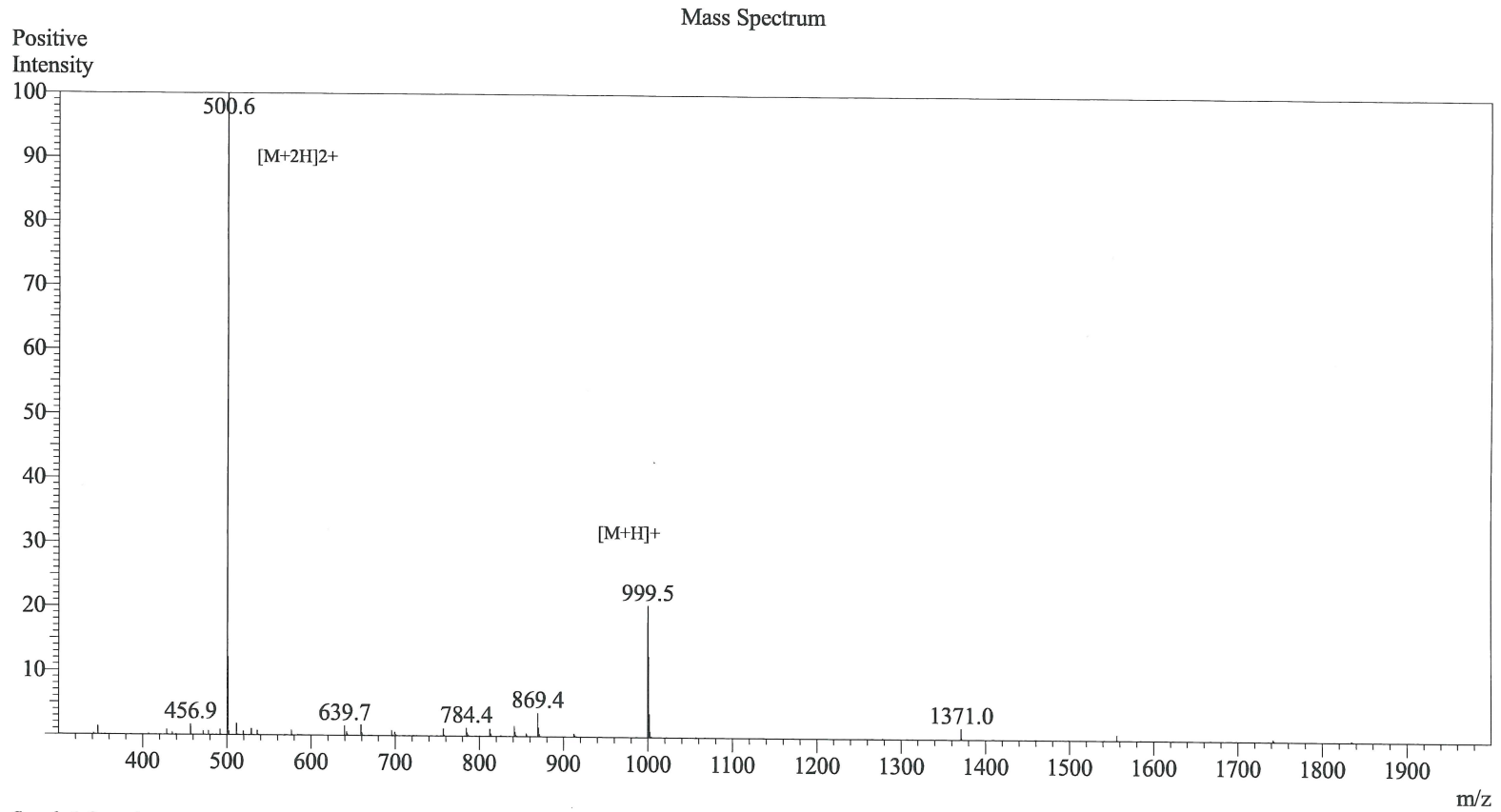


## <Peak Table>

Detector A Channel 1 220nm

Peak#	Ret. Time	Area	Height	Area%
1	8.591	7220115	1087806	98.648
2	9.168	51513	8097	0.704
3	9.461	47441	8344	0.648
Total		7319069	1104247	100.000

# H4K5acK8ac (1-10) MS report



## Sample Information

Acquired by : Gary  
Month-Day Processed : 09/03/19  
Time Processed : 06:45:02 PM  
Injection Volume : 0.4  
Sample Name : H4K5acK8ac(1-10)  
Sample ID : U3559EH260-3  
Theoretical MW : 999.13  
Observed MW : 999.2

Interface : ESI  
Nebulizing Gas Flow : 1.5L/min  
CDL Temp : 250  
Block Temp : 200

Equipment : GK11010007  
Interface Bias : +4.5 kV  
Drying Gas Flow : 5 L/min  
T.Flow : 0.2 ml/min  
B.conc : 50%H2O/50%MeOH

# H4K5ac12ac (1-15) HPLC report

Sample Name :H4(1-15)K5acK12ac-A  
 Sample ID :U291REE170-3  
 Time Processed :2:50:51  
 Month-Day-Year Processed :06/13/2019

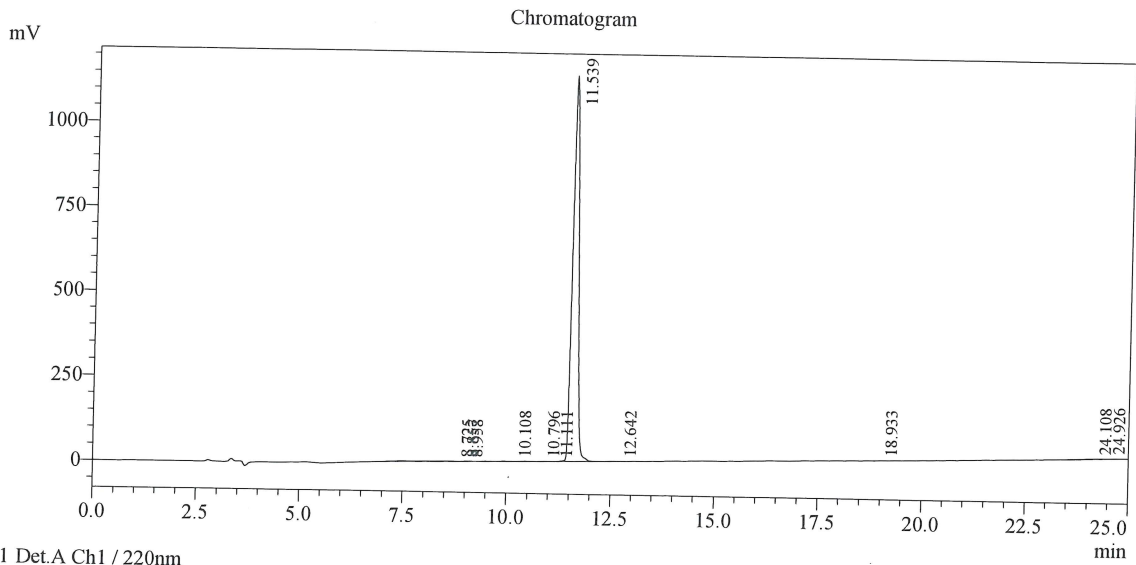
Pump A : 0.065% trifluoroacetic in 100% water (v/v)  
 Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)  
 Total Flow:1 ml/min  
 Wavelength:220 nm

Time	Unit	Command	Value	Comment
0.01	Pumps	Pump B Conc.	5	
25.00	Pumps	Pump B Conc.	65	
25.01	Pumps	Pump B Conc.	95	
31.00	Pumps	Pump B Conc.	95	
31.01	Pumps	Pump B Conc.	5	
40.00	Pumps	Pump B Conc.	5	
40.01	Controller	Stop		

<<Column Performance>>

<Detector A>

Column : Inertsil ODS-3 4.6 x 250 mm  
 Equipment :SS-CM-0310



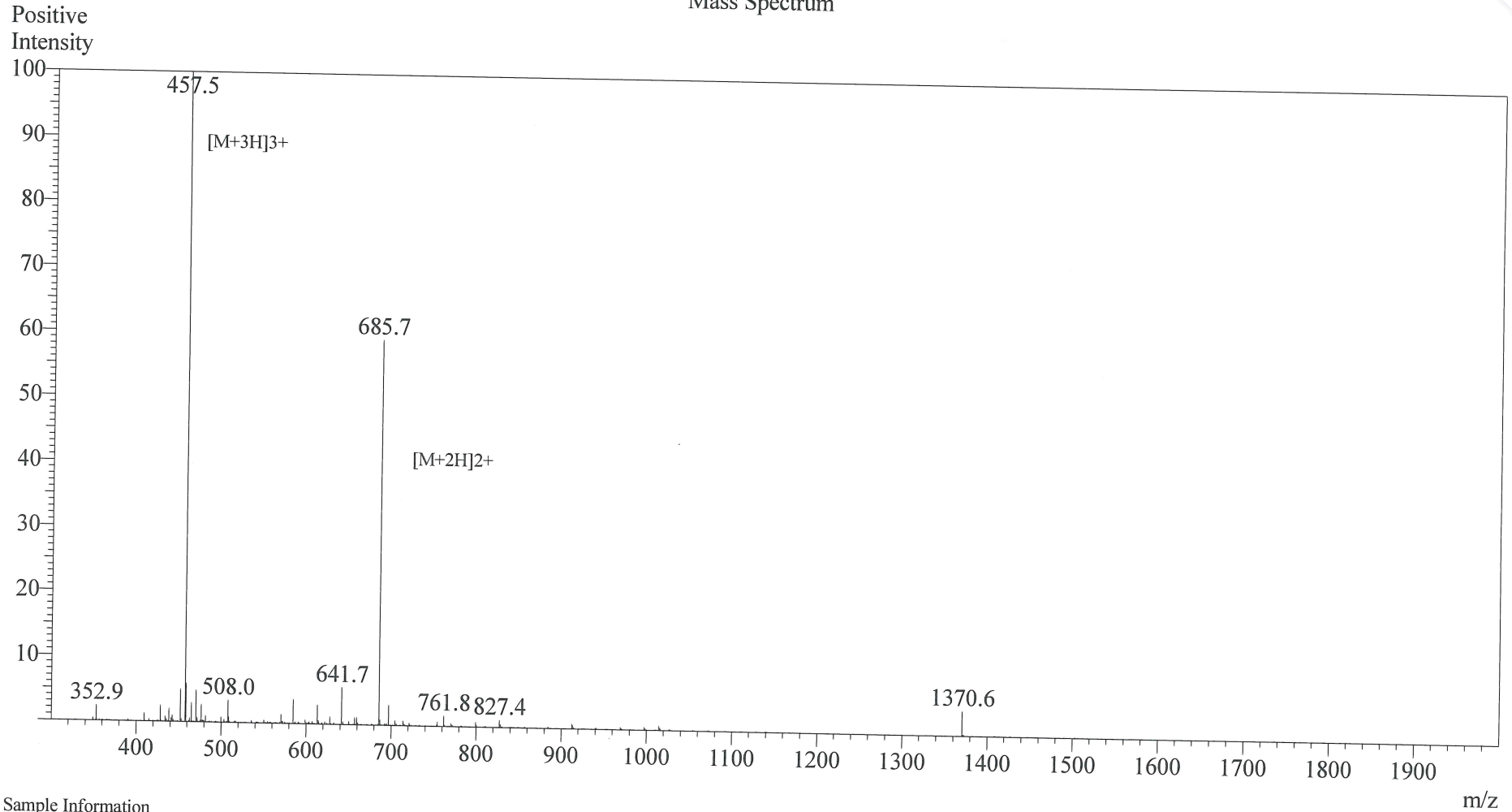
Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	8.725	101018	826	0.874
2	8.857	4764	625	0.041
3	8.958	2870	485	0.025
4	10.108	28279	229	0.245
5	10.796	18063	457	0.156
6	11.111	3432	265	0.030
7	11.539	11331469	1136910	98.067
8	12.642	3893	190	0.034
9	18.933	2296	140	0.020
10	24.108	38861	1287	0.336
11	24.926	19904	473	0.172
Total		11554848	1141887	100.000

# H4K5ac12ac (1-15) MS report

## Mass Spectrum



### Sample Information

Acquired by : Gary  
Month-Day Processed : 06/12/19  
Time Processed : 10:32:49 AM  
Injection Volume : 0.3  
Sample Name : H4(1-15)K5acK12ac-A  
Sample ID : U291REE170-3  
Theoretical MW : 1370.52  
Observed MW : 1369.5

Interface : ESI  
Nebulizing Gas Flow : 1.5L/min  
CDL Temp : 250  
Block Temp : 200

Equipment : GK11010007  
Interface Bias : +4.5 kV  
Drying Gas Flow : 5 L/min  
T.Flow : 0.2 ml/min  
B.conc : 50%H2O/50%MeOH



# H4K5ac16ac (1-24) HPLC report

Sample Name : H4K5acK16ac res 1-24  
 Sample ID : U7933DL110-1  
 Time Processed : 15:31:34  
 Month-Day-Year Processed : 12/22/2018

Sample Information

Pump A : 0.065% trifluoroacetic in 100% waters (v/v)  
 Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)

<<Pump>>  
 Flow : 1.0000 mL/min

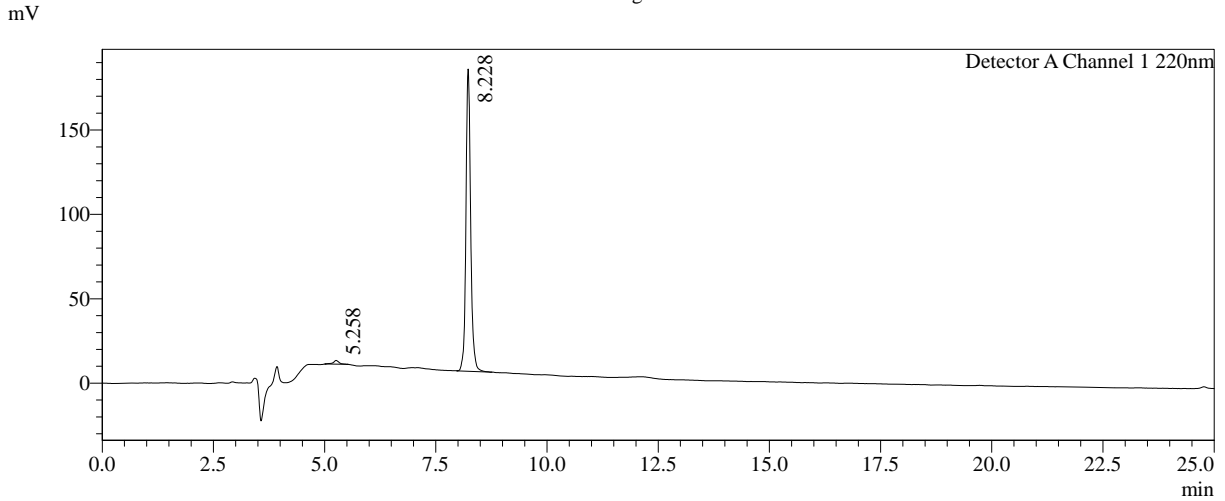
<<Detector A>>  
 Wavelength Ch1 : 220 nm

<<LC Time Program>>

Time	Module	Command	Value	Comment
0.01	Pumps	Solvent B Conc.	5	
25.00	Pumps	Solvent B Conc.	65	
25.01	Pumps	Solvent B Conc.	95	
27.00	Pumps	Solvent B Conc.	95	
27.01	Pumps	Solvent B Conc.	5	
35.00	Pumps	Solvent B Conc.	5	
35.01	Controller	Stop		

<<Column Performance>>  
 <Detector A>  
 Column : Inertsil ODS-3 4.6 x 250 mm  
 Equipment: ZJ17010210

Chromatogram

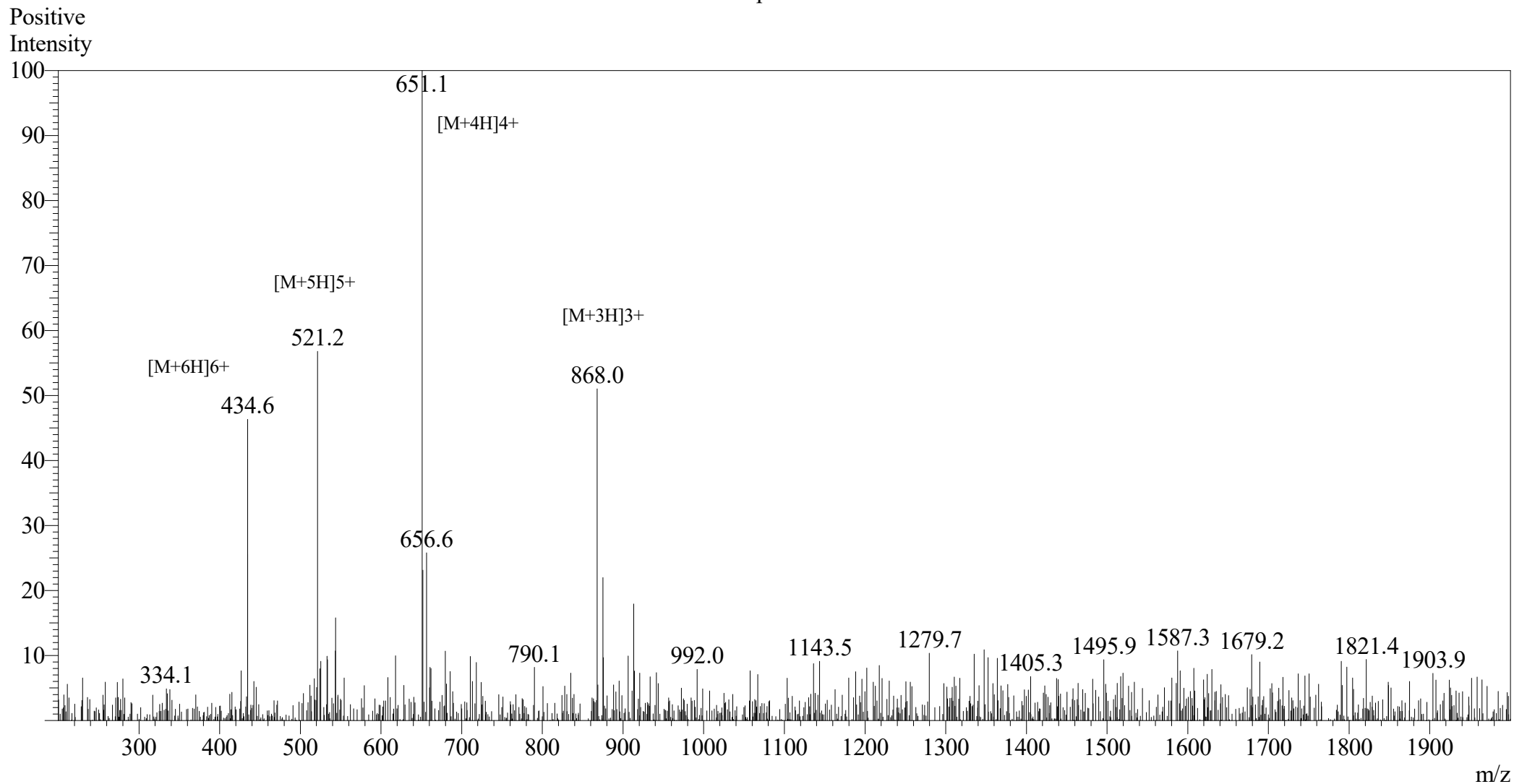


Peak Table

Detector A Channel 1 220nm

Peak#	Ret. Time	Height	Area	Area%
1	5.258	2192	20353	1.513
2	8.228	179137	1324964	98.487
Total		181328	1345317	100.000

H4K5ac16ac (1-24) MS report  
Mass Spectrum



Sample Information

Acquired by : Gary  
Month-Day Processed : 12/21/18  
Time Processed : 08:04:06 PM  
Injection Volume : 0.1  
Sample Name : H4K5acK16ac res 1-24  
Sample ID : U7933DL110-1  
Theoretical MW : 2600.91  
Observed MW : 2600.4

Interface : ESI  
Nebulizing Gas Flow : 1.5L/min  
CDL Temp : 250  
Block Temp : 200

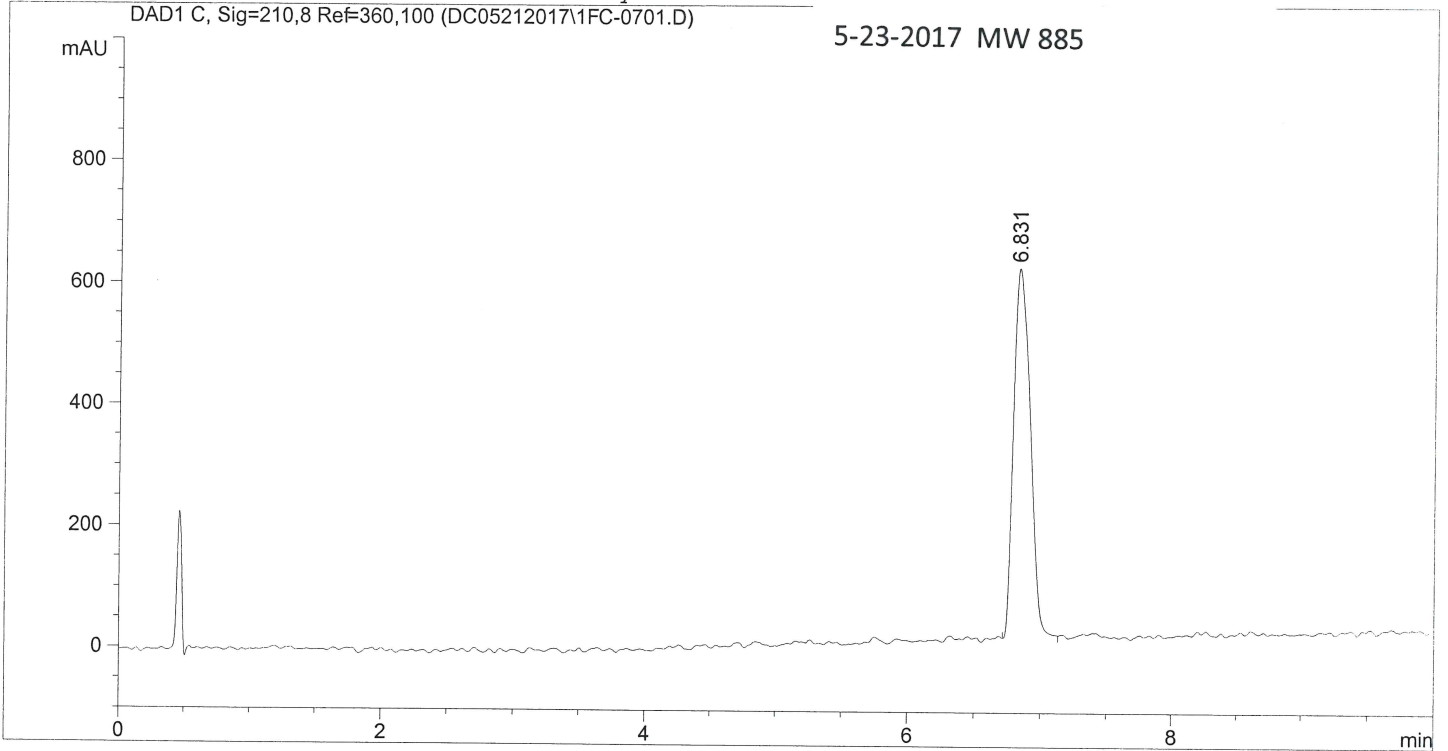
Equipment : GK11010007  
Interface Bias : +4.5 kV  
Drying Gas Flow : 5 L/min  
T.Flow : 0.2 ml/min  
B.conc : 50%H2O/50%MeOH

# H4K8ac12ac (6-15) HPLC report

Data File C:\CHEM32\1\DATA\DC05212017\1FC-0701.D

Sample Name: #4 pure

```
=====
Acq. Operator   : dc                               Seq. Line :    7
Acq. Instrument : Instrument 1                     Location  : P1-F-03
Injection Date  : 5/21/2017 11:45:16 AM           Inj       :    1
                                                    Inj Volume: 3 µl
Acq. Method     : C:\Chem32\1\METHODS\DC2M.m     NH2-GGK(Ac)GLGK(Ac)GGA-amide
Last changed    : 5/21/2017 11:43:28 AM by dc
Analysis Method : C:\Chem32\1\METHODS\DC2M.m     H4(6-15)K8acK12ac, purity>98%
Last changed    : 5/21/2017 5:57:01 PM by dc
=====
```



=====  
Area Percent Report  
=====

Sorted By : Signal  
Multiplier : 1.0000  
Dilution : 1.0000  
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	6.831	VB	0.1313	5988.06836	638.97620	100.0000

Totals : 5988.06836 638.97620

=====  
\*\*\* End of Report \*\*\*

## Display Report - Selected Window Selected Analysis

**Analysis Name:** 1FC-0701.D

**Instrument:** LC-MSD-Trap-XCT

**Print Date:** 5/21/2017 5:58:15 PM

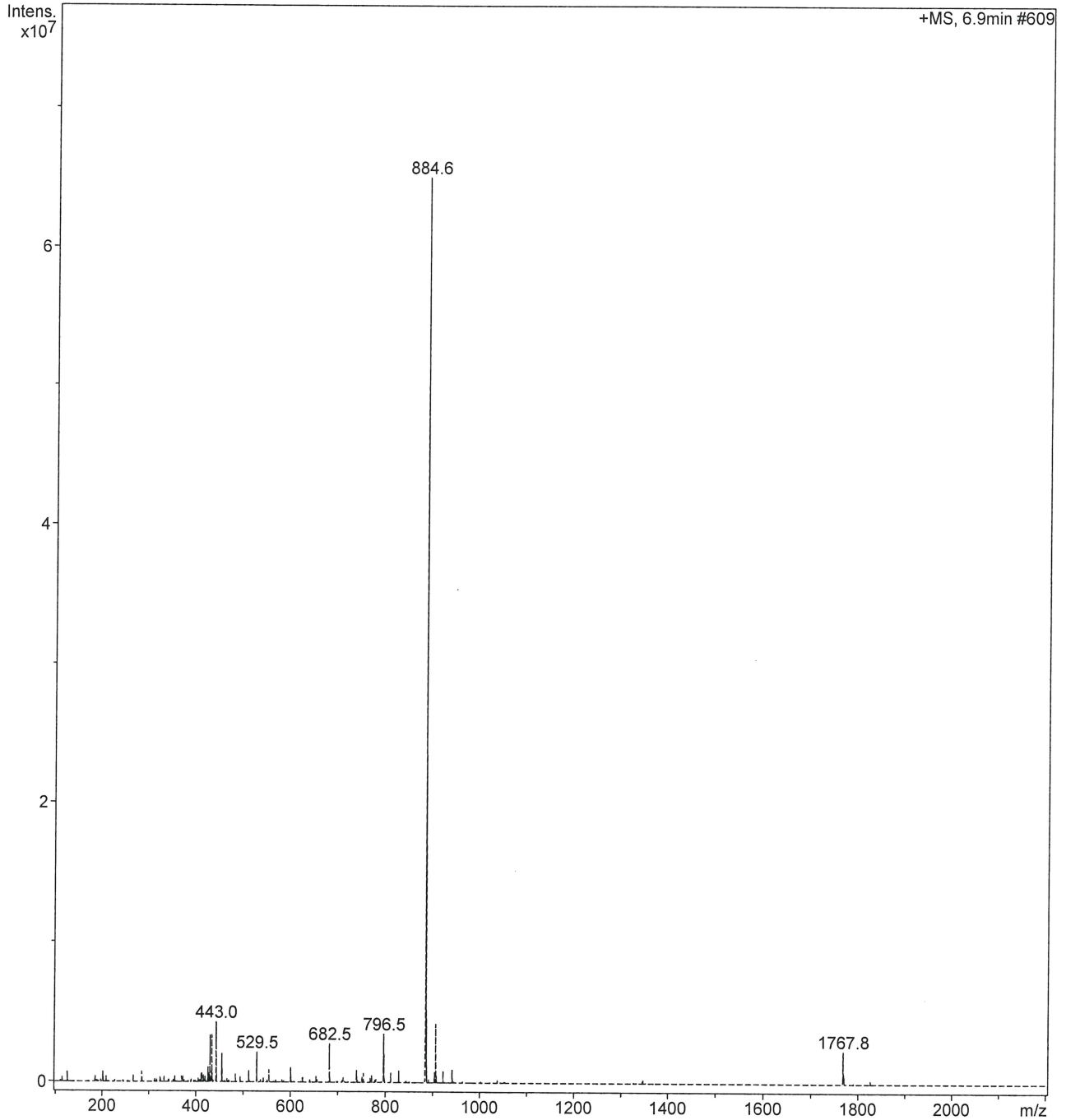
**Method:** DC2M.M

**Operator:** Hodges

**Acq. Date:** 5/21/2017 11:45:14 AM

**Sample Name:** #4 pure

**Analysis Info:**



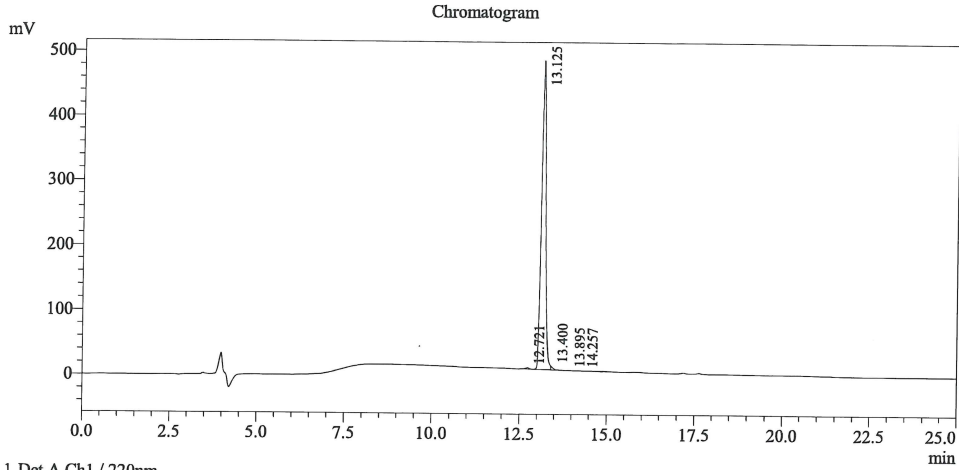
# H4K8ac16ac (1-20) HPLC report

Sample Name: H4K8acK16ac (res 1-20)  
 Sample ID: U931EEK130-1  
 Time Processed :13:17:34  
 Month-Day-Year Processed :11/23/2019

Pump A : 0.065% trifluoroacetic in 100% water (v/v)  
 Pump B : 0.05% trifluoroacetic in 100% acetonitrile (v/v)  
 Total Flow:1 ml/min  
 Wavelength:220 nm

Time	Unit	Command	Value	Comment
0.01	Pumps	Pump A B.Conc	5	
25.00	Pumps	Pump A B.Conc	65	
25.01	Pumps	Pump A B.Conc	95	
32.00	Pumps	Pump A B.Conc	95	
32.01	Pumps	Pump A B.Conc	5	
40.00	Pumps	Pump A B.Conc	5	
40.00	Controller	Stop		

<<Column Performance>>  
 <Detector A>  
 Column : Inertsil ODS-3 4.6 x 250 mm  
 Equipment: GK1101009

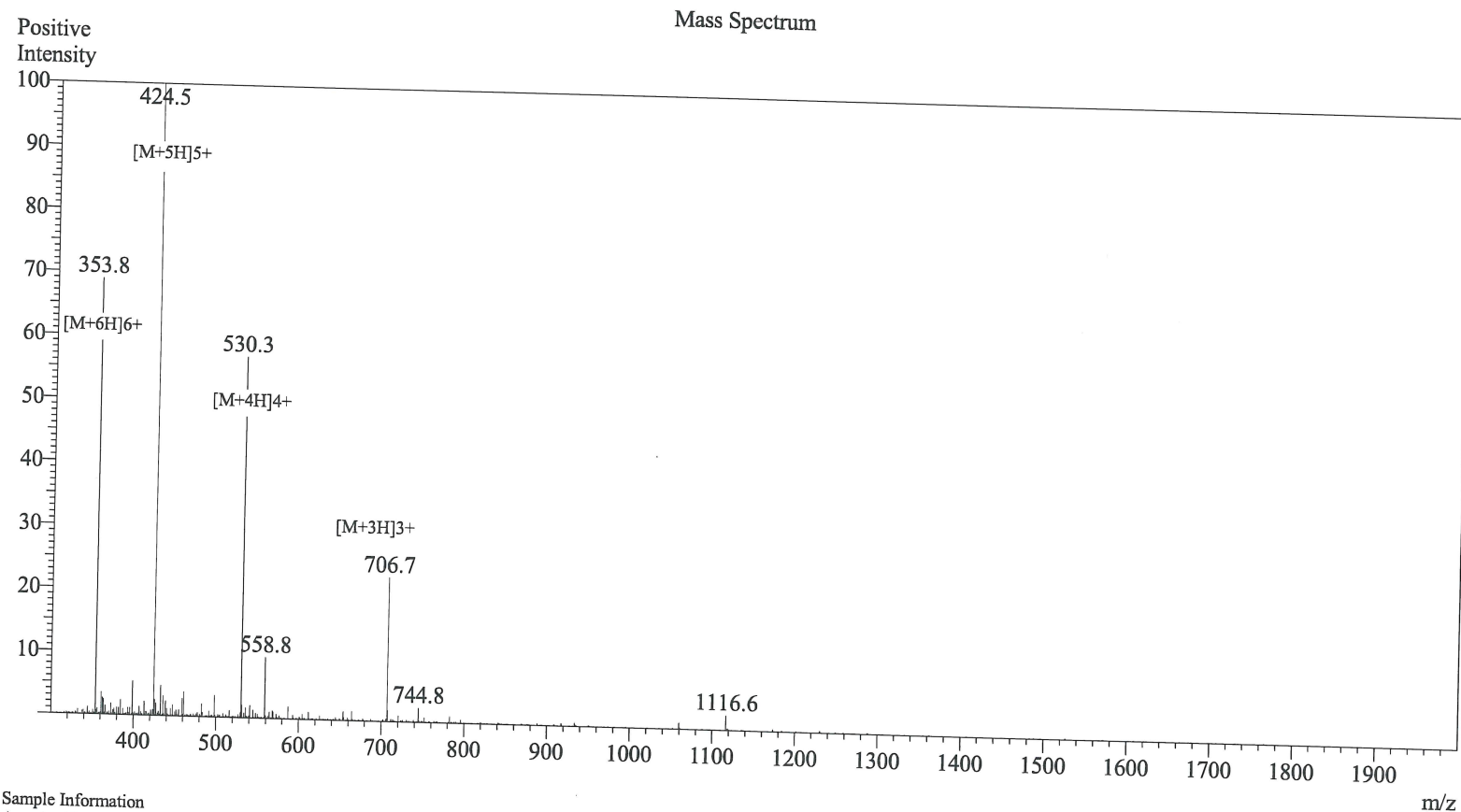


1 Det.A Ch1 / 220nm

Peak Table

Peak#	Ret. Time	Area	Height	Area %
1	12.721	18139	2080	0.434
2	13.125	4133139	476832	98.840
3	13.400	23275	5808	0.557
4	13.895	1355	216	0.032
5	14.257	5738	82	0.137
Total		4181647	485016	100.000

# H4K8ac16ac (1-20) MS report



## Sample Information

Acquired by : Gary  
Month-Day Processed : 11/22/19  
Time Processed : 07:49:24 PM  
Injection Volume : 0.4  
Sample Name : H4K8acK16ac (res 1-20)  
Sample ID : U931EEK130-1  
Theoretical MW : 2117.42  
Observed MW : 2117.5

Interface : ESI  
Nebulizing Gas Flow : 1.5L/min  
CDL Temp : 250  
Block Temp : 200

Equipment : GK11010007  
Interface Bias : +4.5 kV  
Drying Gas Flow : 5 L/min  
T.Flow : 0.2 ml/min  
B.conc : 50%H2O/50%MeOH

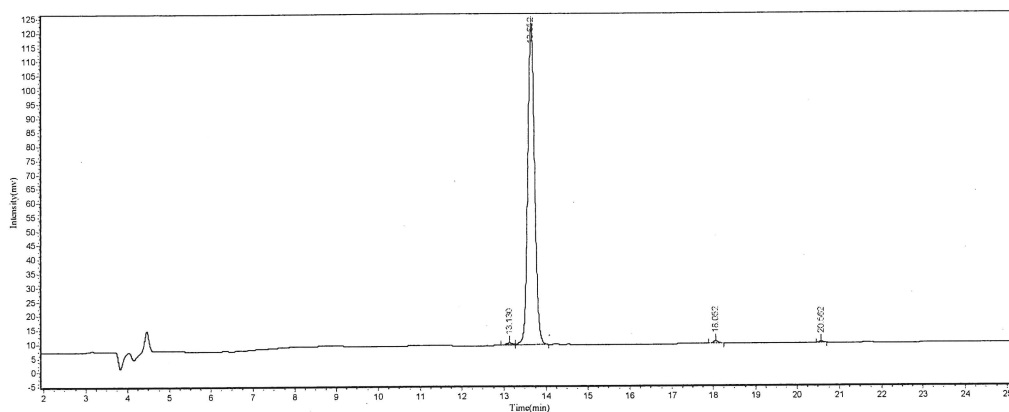


**Biomatik**  
 Tel: (519) 489-7195, (800) 836-8089  
 Fax: (519) 231-0140, (877) 221-3515  
 Email: info@biomatik.com  
 http://www.biomatik.com

## HPLC Report

Structure : H4 LK-11  
 Lot No : P170620-MJ589926  
 Column : 4.6mm\*250mm, Kromasil 100-5 C18  
 Solvent A : 0.1% trifluoroacetic in 100% acetonitrile  
 Solvent B : 0.1% trifluoroacetic in 100% water  
 Gradient :           A           B  
           0.01min  5%           95%  
           25min   30%          70%  
           25.1min 100%         0%  
           30.0min           STOP

Flow rate : 1.0ml/min  
 Wavelength : 220nm  
 Volume : 5ul

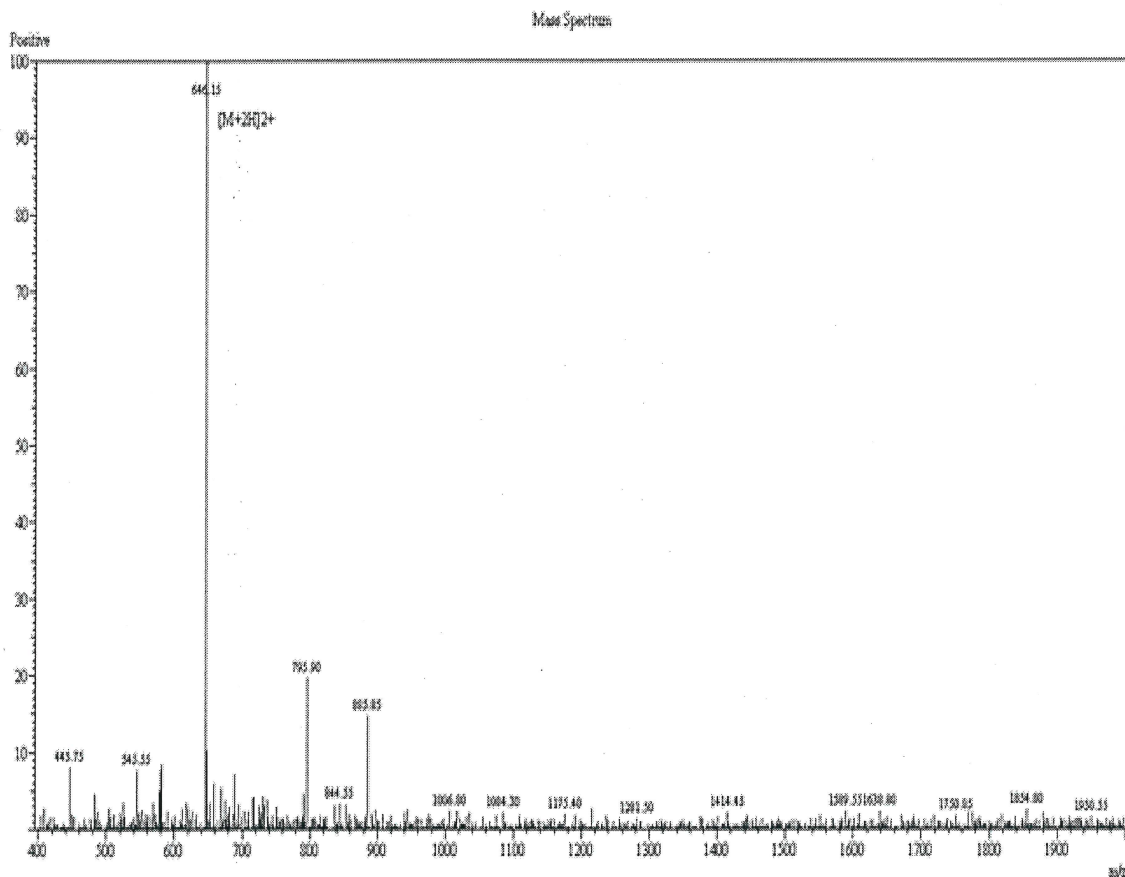


Peak No.	Ret Time	Height	Area	Conc.
1	13.130	804.222	8519.718	0.6521
2	13.652	113478.391	1287781.125	98.5605
3	18.052	857.111	7259.892	0.5556
4	20.562	404.389	3028.215	0.2318
Total				100.0000



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Mass Spectrometry Report



Sample Information  
 Date and Time : 2017-6-27 13:30:21  
 User : CHAO  
 Sample : H4 LK-11  
 Inj. Volume : 1  
 MW : 1290.55  
 Lot No. : P170620-MJ589926

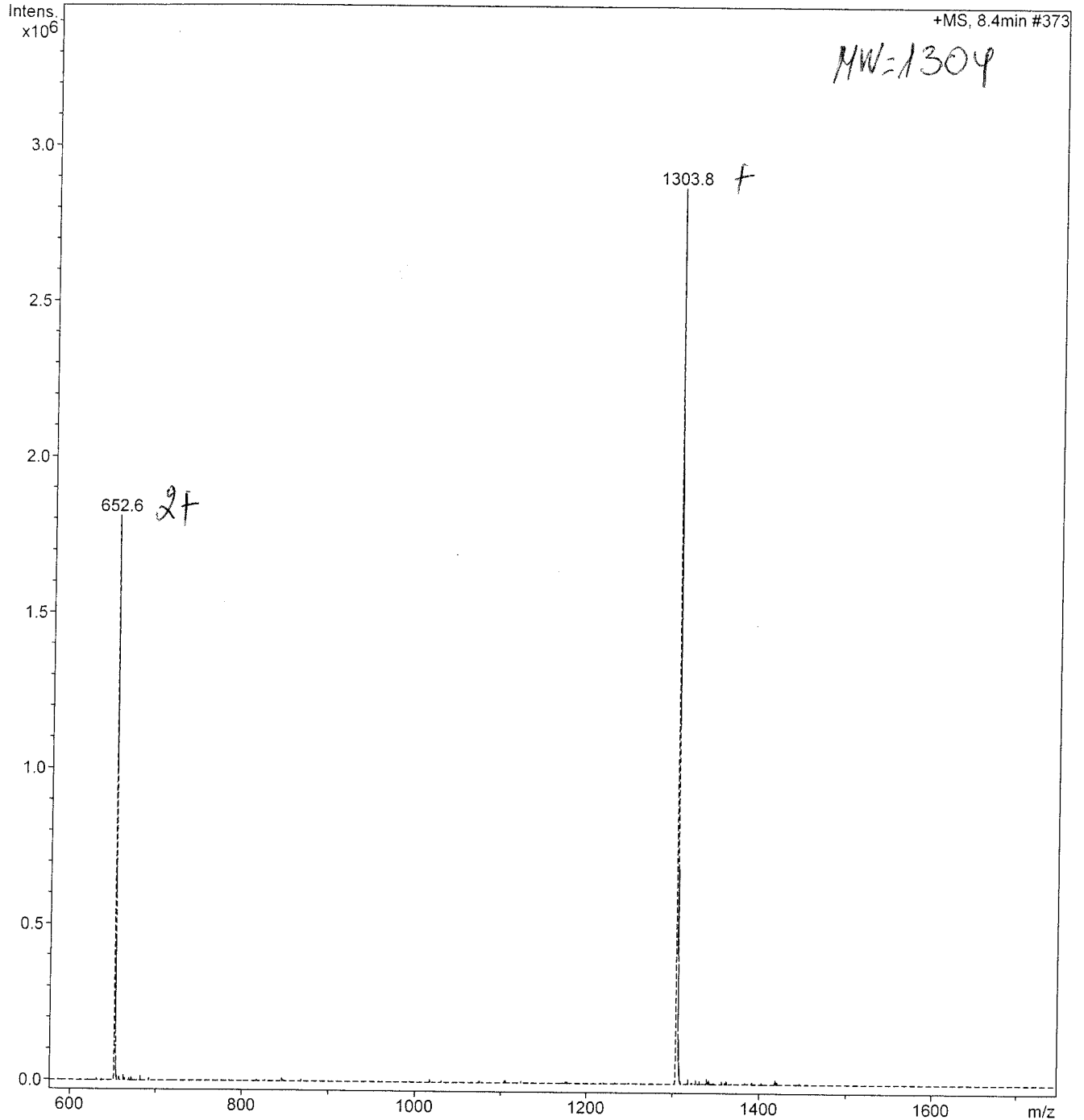
Probe : ESI Probe bias : -3.5kV  
 Nebulizer Gas Flow : 1.5L/min Detector : 1.0eV  
 DL : -20.0v T. Flow : 0.2ml/min  
 DL Temp : 250°C B. conc : 50%H2O/50%ACN  
 Block Temp : 200°C





# Display Report - Selected Window Selected Analysis

**Analysis Name:** 003-0501.D    **Instrument:** LC-MSD-Trap-SL    **Print Date:** 5/9/2011 3:43:22 PM  
**Method:** DC1M.M    **Operator:** Hodges    **Acq. Date:** 5/6/2011 5:01:31 PM  
**Sample Name:** H3 nat. pure  
**Analysis Info:** Zorbax C18 300SB SN USFS003194  
15-30%B over 15min  
WJH3002 Control Peptide for D16



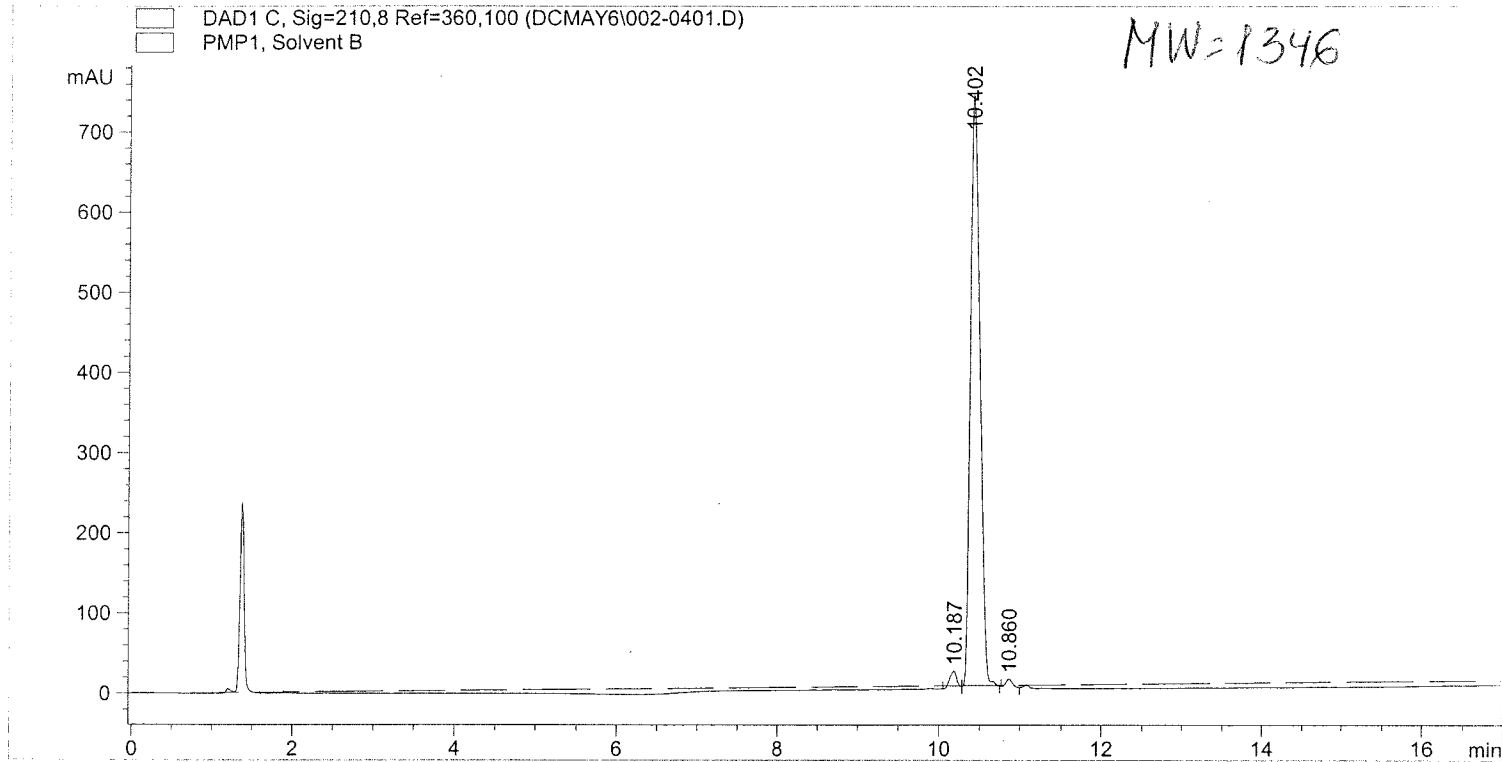
Sample Name: K9Ac pure

H3K9ac (1-12) HPLC report

Acq. Operator : WH Seq. Line : 4  
 Acq. Instrument : Instrument 1 Location : Vial 2  
 Injection Date : 5/6/2011 4:33:07 PM Inj : 1  
 Inj Volume : 6 µl

Acq. Method : C:\Chem32\1\METHODS\DC1M.M  
 Last changed : 5/6/2011 4:29:34 PM by WH  
 Analysis Method : C:\Chem32\1\METHODS\15MINAT50C.M  
 Last changed : 5/9/2011 9:42:28 AM by WH  
 Sample Info : Zorbax C18 300SB SN USFS0031

NH<sub>2</sub>-ARTKQTARKSTG-amide  
 5/06/11 H3(1-12)K9Ac  
 Purity>96%



Area Percent Report

Sorted By : Signal  
 Multiplier : 1.0000  
 Dilution : 1.0000  
 Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=210,8 Ref=360,100

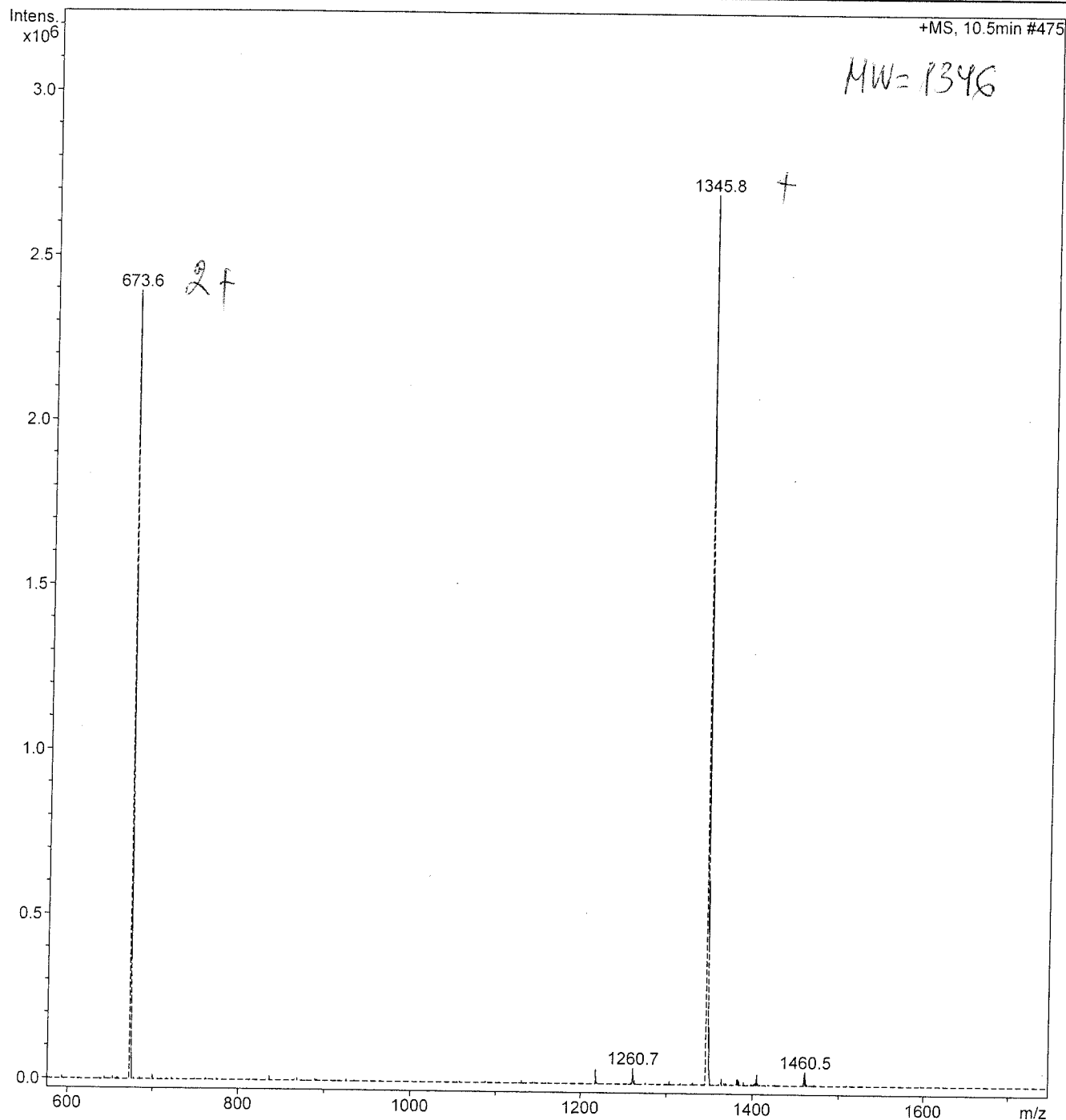
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.187	BV	0.1007	133.63661	22.03868	2.1583
2	10.402	VB	0.1261	5978.96338	741.55469	96.5632
3	10.860	BV	0.0972	79.16403	11.96026	1.2785

Totals : 6191.76402 775.55363

\*\*\* End of Report \*\*\*

# Display Report - Selected Window Selected Analysis

**Analysis Name:** 002-0401.D    **Instrument:** LC-MSD-Trap-SL    **Print Date:** 5/9/2011 3:35:54 PM  
**Method:** DC1M.M    **Operator:** Hodges    **Acq. Date:** 5/6/2011 4:33:01 PM  
**Sample Name:** K9Ac pure  
**Analysis Info:** Zorbax C18 300SB SN USFS003194  
20-80%B over 30min

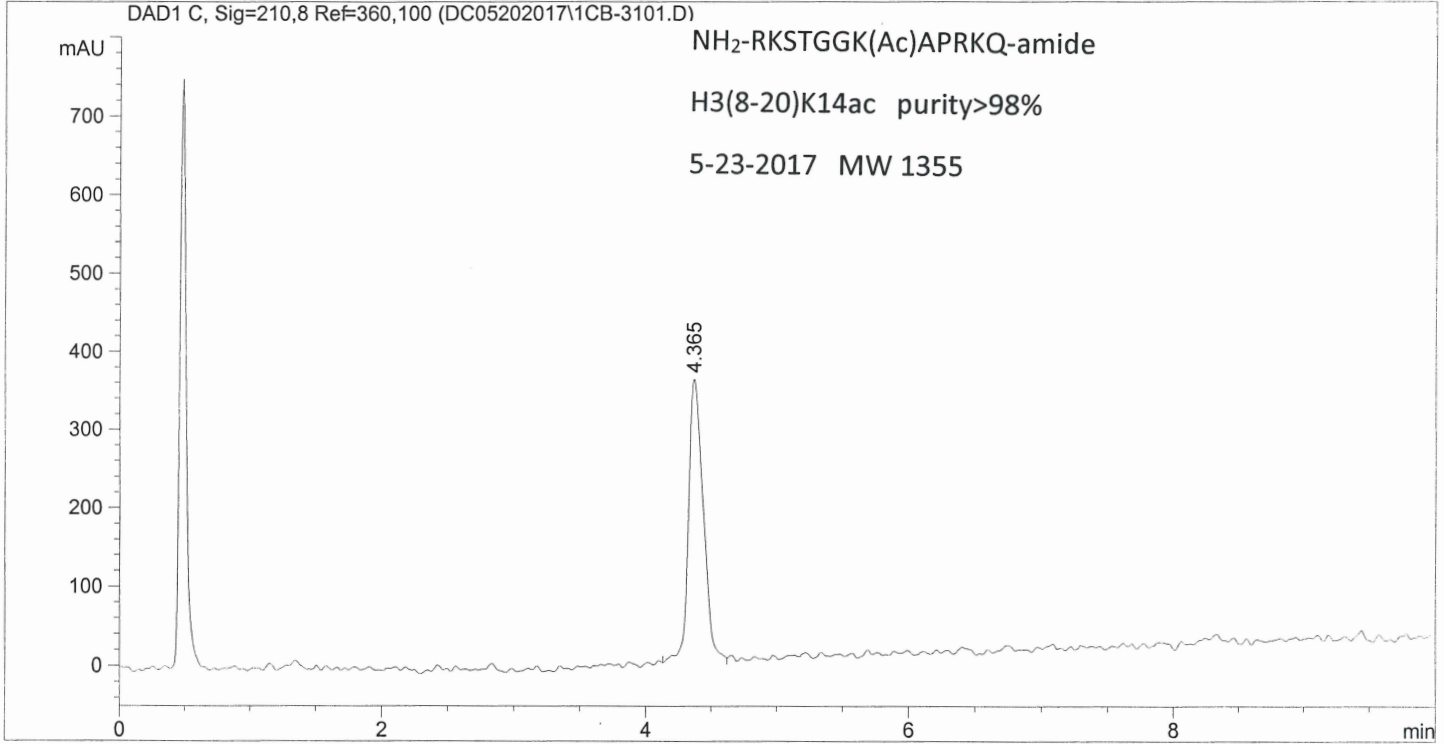


# H3K14ac (8-19) HPLC report

Data File C:\CHEM32\1\DATA\DC05202017\1CB-3101.D

Sample Name: #1 pure

```
=====
Acq. Operator   : dc                               Seq. Line :   31
Acq. Instrument : Instrument 1                     Location  : P1-C-02
Injection Date  : 5/20/2017 7:53:18 PM           Inj       :    1
                                                    Inj Volume: 3 µl
Different Inj Volume from Sequence !      Actual Inj Volume : 5 µl
Acq. Method     : C:\Chem32\1\METHODS\DC2M.M
Last changed    : 5/20/2017 7:50:55 PM by dc
Analysis Method  : C:\Chem32\1\METHODS\30MINWASH50C.M
Last changed    : 5/21/2017 8:24:21 AM by dc
=====
```



=====  
Area Percent Report  
=====

```
Sorted By      :      Signal
Multiplier     :      1.0000
Dilution       :      1.0000
Use Multiplier & Dilution Factor with ISTDs
```

Signal 1: DAD1 C, Sig=210,8 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	4.365	VV	0.1261	3208.54541	373.84125	100.0000

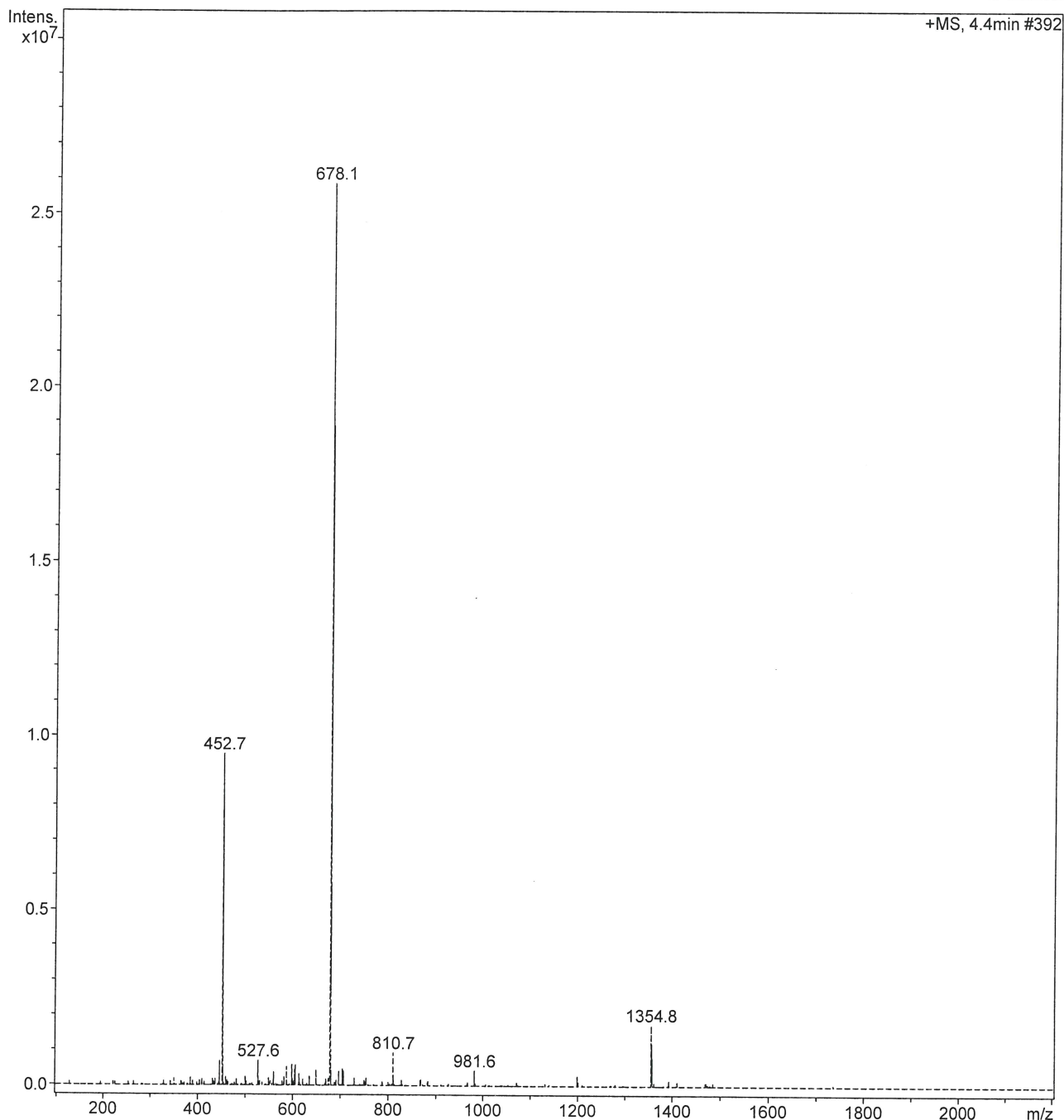
Totals :                    3208.54541   373.84125

=====  
\*\*\* End of Report \*\*\*

# H3K14ac (8-19) MS report

## Display Report - Selected Window Selected Analysis

**Analysis Name:** 1CB-3101.D    **Instrument:** LC-MSD-Trap-XCT    **Print Date:** 5/21/2017 8:25:59 AM  
**Method:** DC2M.M    **Operator:** Hodges    **Acq. Date:** 5/20/2017 7:53:19 PM  
**Sample Name:** #1 pure  
**Analysis Info:**



**$^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ )**

