
Efficient genetic code expansion without host genome modifications

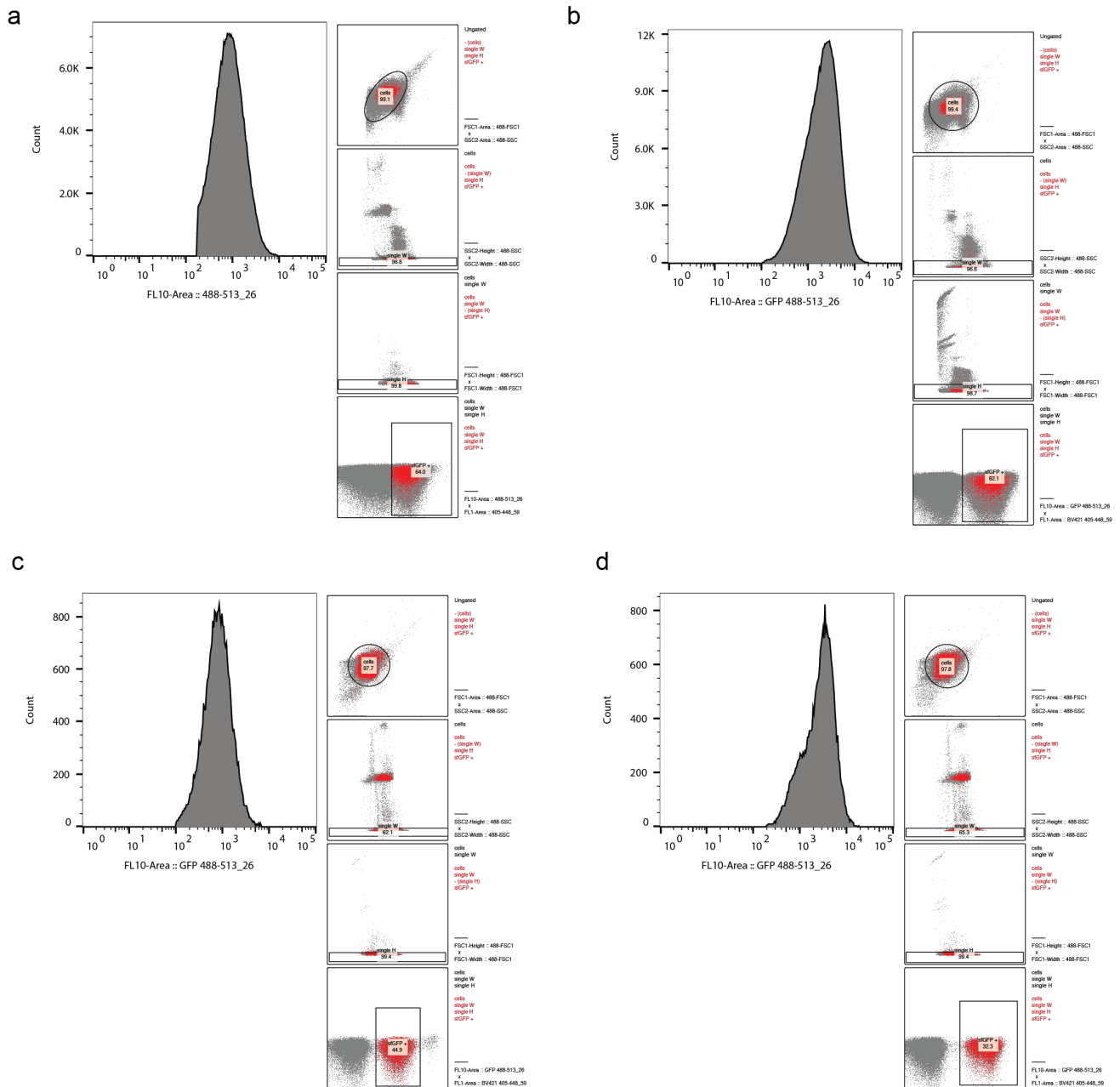
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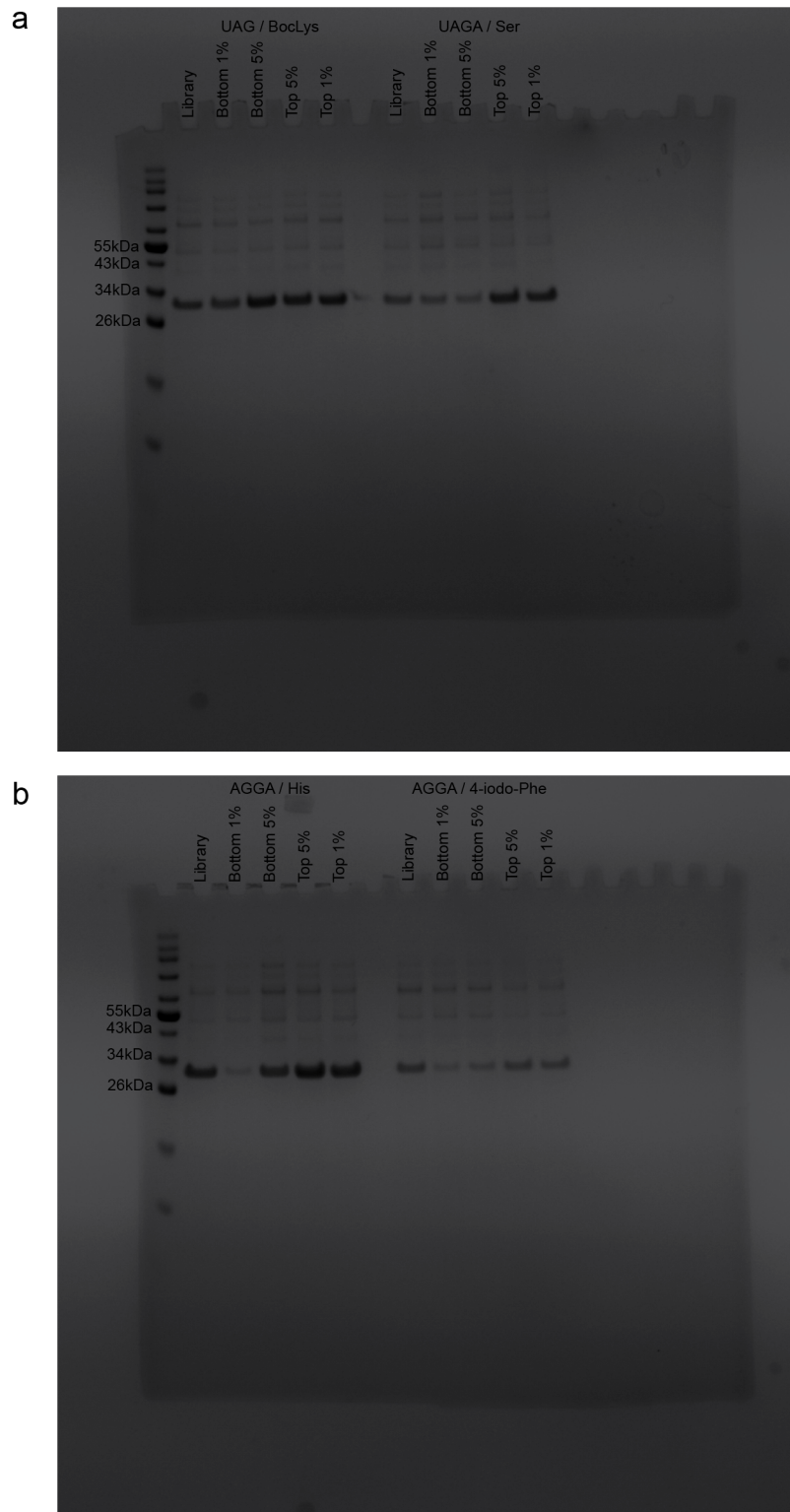
Supplementary References



Supplementary Figure 1 | Gating Strategy for sfGFP Positive Cell Sorting.

E. coli S3489 cells carrying genetic code expansion plasmids and sfGFP libraries were first gated based on cell size (FSC-A by SSC-A), then single cells (SSC-H x SSC-W, and FSC-H x FSC-W). Next, cells were gated based on sfGFP fluorescence (excitation at 488 nm, emission at 513 nm). Histograms and scatter plots are shown for IPTG-induced libraries with the cognate ncAA as appropriate, and each plot includes a back-gating summary with events used in final analysis highlighted in red:

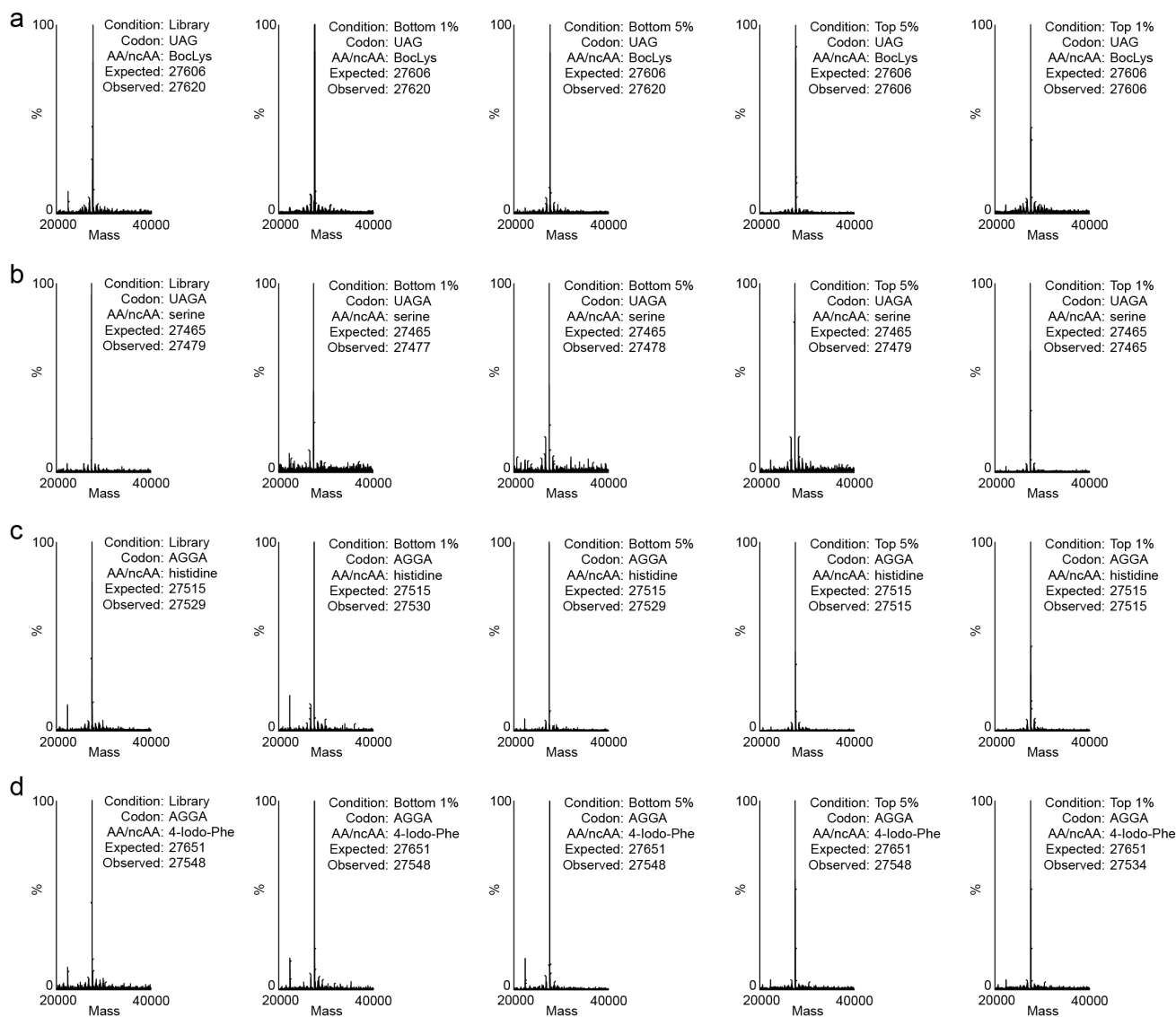
- Ec* tRNA^{Ser}_{UAGA};
- Ec* tRNA^{His}_{AGGA};
- Af*tRNA^{Tyr}_{AGGA} + *Af*TyrRS(G5) with 1 mM *para*-iodophenylalanine (pIF);
- Mb* tRNA^{Pyl}_{UAG} + *Mb*PylRS with 1 mM N6-(tert-butoxycarbonyl)-lysine (BocK).



Supplementary Figure 2 | Ni-NTA-Purified sfGFP-His6 Products from Library Populations.

- a)** Nickel-NTA-purified sfGFP(Y151UAG)-His6 from UAG synonymous codon library populations grown in the presence of 1 mM BocLys and sfGFP(Y151UAGA)-His6 from UAGA synonymous codon library populations. Molecular weight marker present in left most column.
- b)** Nickel-NTA-purified sfGFP(Y151AGGA)-His6 from AGGA synonymous codon library populations and sfGFP(Y151AGGA)-His6 from AGGA synonymous codon library populations grow in the presence of 1 mM 4-iodo-Phe. In all cases the sorted population is indicated and naming is consistent with other figures. Molecular weight marker present in left most column.

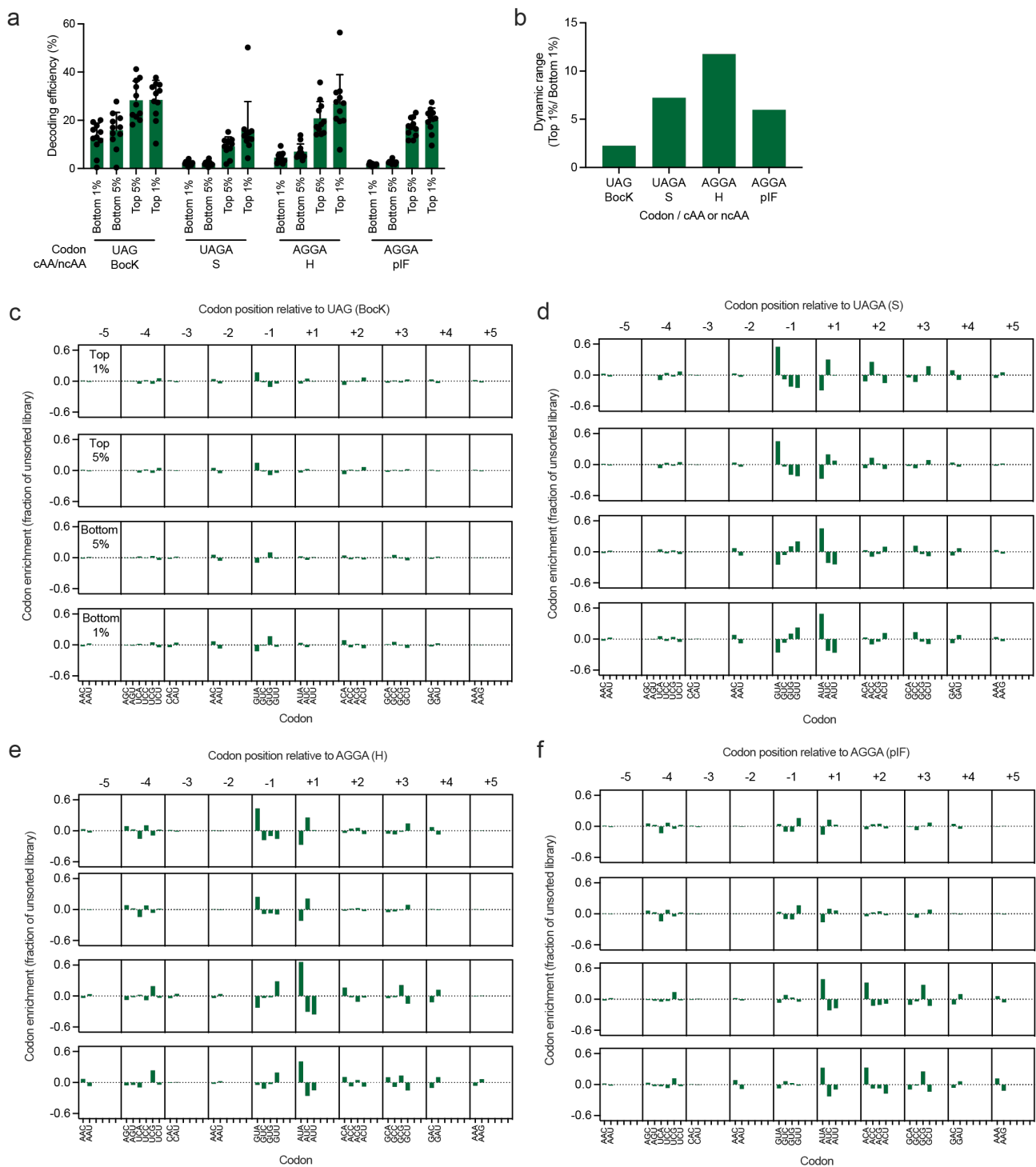
For both gels, samples are derived from a heterogenous cultures of sorted cells from the flow-cytometry analysis and sorting in Figure 1a. The samples correspond to those used to prep HTS in Figure(s) 1b-e.



Supplementary Figure 3 | Mass Spectrometry Analysis of sfGFP-His6 Library Products.

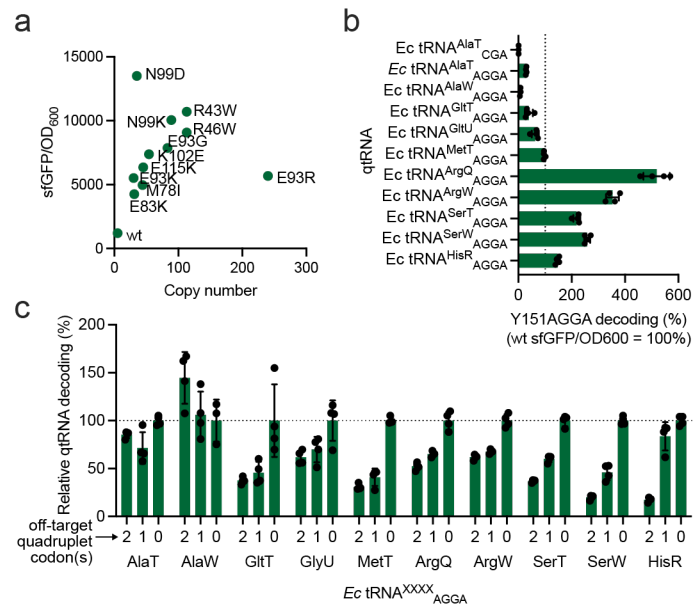
- Time-of-flight mass spectrometry (TOF-MS) of Ni-NTA-purified sfGFP(Y151UAG)-His6 in the presence of 1 mM BocLys for sorted populations from the UAG synonymous codon library.
- TOF-MS analysis of Ni-NTA-purified sfGFP(Y151UAGA)-His6 for sorted populations from the UAGA synonymous codon library using a serine-encoding qtRNA.
- TOF-MS analysis of Ni-NTA-purified sfGFP(Y151AGGA)-His6 for sorted populations from the AGGA synonymous codon library using a histidine-encoding qtRNA.
- TOF-MS analysis of Ni-NTA-purified sfGFP(Y151AGGA)-His6 in the presence of 1 mM 4-iodo-Phe for sorted populations from the AGGA synonymous codon library.

In all cases, the sorted population is indicated and naming is consistent with other figures. The codon substitution at sfGFP Y151, target amino acid or ncAA, expected, and observed masses are indicated. Whereas the correct mass is observed in the higher % bins for most samples (indicating correct translation), the 4-iodo-Phe-incorporating samples did not show the correct mass in the top bin. We attribute this to the poor ncAA incorporation efficiency (see **Supplemental Data 1** below) and artifacts of library generation (see **Methods**).



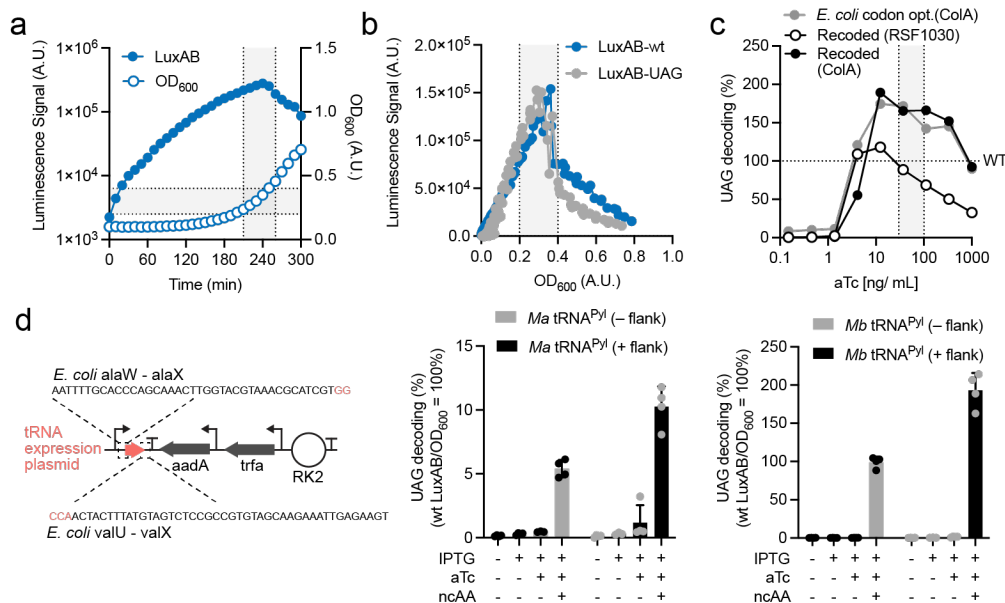
Supplementary Figure 4 | Codon Usage Near Quadruplet Codons Impacts Decoding Efficiency.

- Single clones from each sorted population were assayed for sfGFP activity after FACS: bottom 1%, bottom 5%, top 5%, and top 1% ($n = 16$ biological replicates from each population). Error represents the standard deviation of clones in each pool.
 - Mean dynamic range between top 1% and bottom 1% for each library strain, calculated from (a).
- For c-f, sorted libraries were subjected to NGS and codon enrichment was calculated relative to the unsorted library populations at positions -5 to $+5$ of the UAG/quadruplet decoding site for:
- Mb* tRNA^{Pyl}_{UAG} with *Mb*PylRS incorporating N6-(tert-butoxycarbonyl)-lysine (BocK);
 - Af*tRNA^{Tyr(A01)}_{AGGA} with *Af*TyrRS(G5) incorporating para-iodophenylalanine (pIF);
 - Ec* tRNA^{Ser(evo2)}_{UAGA} incorporating serine;
 - Ec* tRNA^{His}_{AGGA} incorporating histidine.



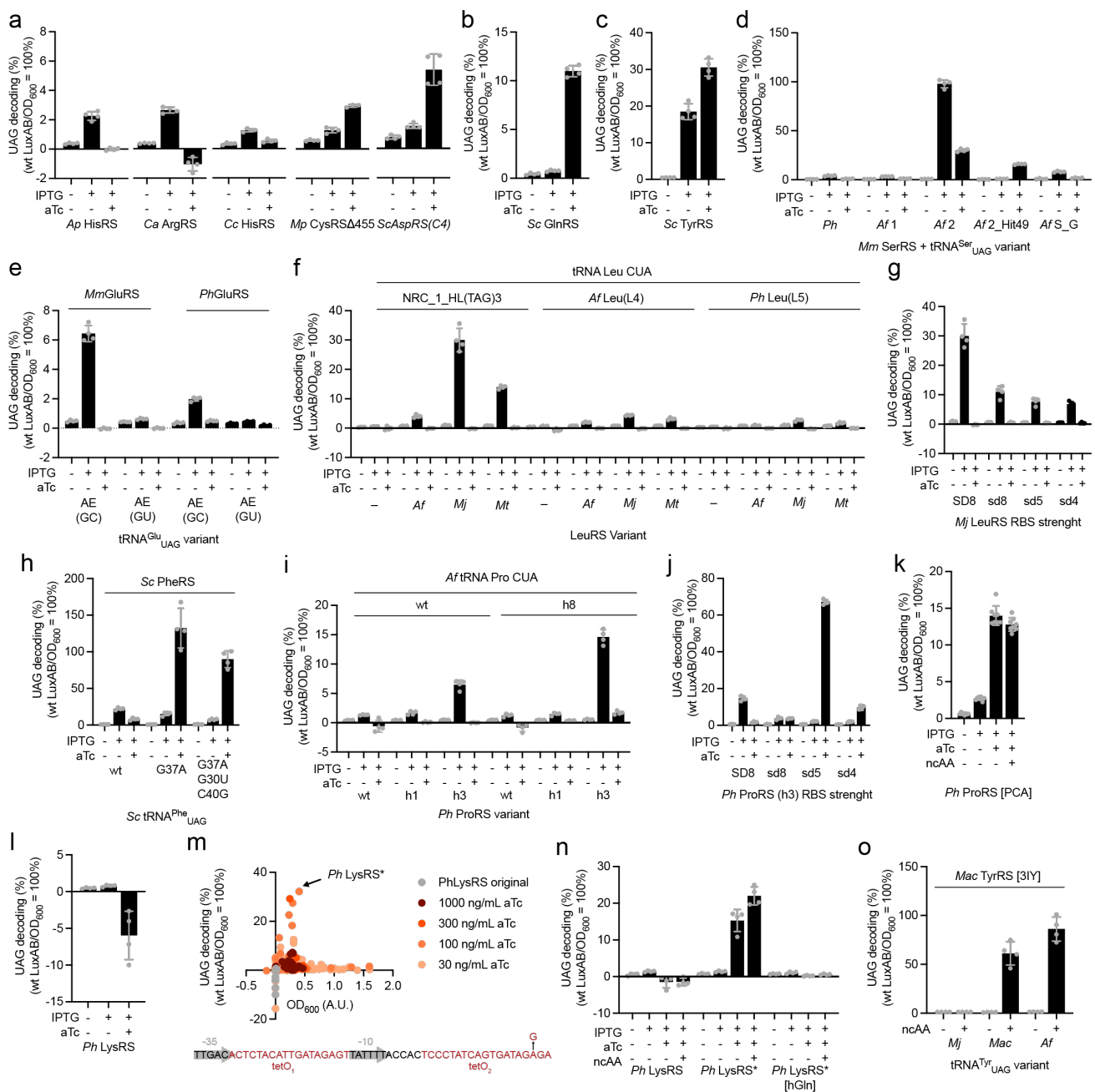
Supplementary Figure 5 | High mRNA Expression and Removal of Off-Target Quadruplet Codons Improves Apparent Quadruplet Decoding Efficiency.

- Increasing SC101 copy number (x-axis) through RepA mutations¹ improves sfGFP signal upon quadruplet decoding (y-axis). In all cases, *Ec* qtRNA^{His}_{AGGA} is expressed from a low copy RK2 origin of replication (4-7 copies/cell) using the P_{proK}-lacO promoter (n = 6). SC101 RepA^{N99D} is used for quadruplet decoding experiments from this point forward.
- New AGGA-decoding qtRNAs were engineered by swapping their anticodon sequences for UCCU, resulting in variable decoding efficiencies using a Y151AGGA sfGFP reporter. *Ec* tRNA^{AlaT}_{GCA} was used as a negative control. For each engineered qtRNA, data shows mean and standard deviation (n = 4 biological replicates).
- Using plasmids that have been fully recoded to use only 20 high usage codons, introduction of AGG codons at positions R96 and R122 reduce apparent decoding at Y151AGGA. For each engineered qtRNA, data shows mean and standard deviation (n = 4 biological replicates). Each set is normalized to AGGA decoding efficiency of each qtRNA without additional AGG codons in sfGFP.



Supplementary Figure 6 | Optimization of LuxAB UAG Decoding Circuit.

- Kinetic analysis of fully recoded wild-type bacterial luciferase (LuxAB) reporter in *E. coli* S3489 cells. Luminescence values (left y-axis) and cell density (OD_{600} ; right y-axis) were quantified in 10 min intervals. Peak luminescence signal occurs in early exponential growth ($OD_{600} = 0.2-0.4$). The grey shaded areas represent time and cell density conditions used for subsequent analyses and UAG/quadruplet decoding is reported as a percentage of this reporter's luminescence activity ($n = 4$).
- Kinetic analysis of *Mb* tRNA^{Pyl}_{UAG} decoding (with *MbPylRS* and BockK) as compared to wild-type LuxAB translation in *E. coli* S3489 cells ($n = 2$).
- Testing the impact of synthetase plasmid copy number and *MbPylRS* codon usage on UAG decoding. Fully recoded and *E. coli* codon optimized *MbPylRS* genes behave similarly using the medium copy number origin ColA (20-40 copies/cell), but the higher origin RSF1030 (>100 copies/cell) can reduce cell fitness and apparent UAG decoding. *MbPylRS* is expressed from an aTc-controlled promoter (P_{tetAP}) and the grey shaded area indicates the aTc concentration used in subsequent assays (0.15-1000 ng/mL). Data represents the mean ($n = 2$ biological replicates). Values are normalized to a wild-type LuxAB reporter under the same growth conditions (WT).
- Ma* tRNA^{Pyl}_{UAG} (left) and *Mb* tRNA^{Pyl}_{UAG} (right) decoding activities were evaluated with and without the previously described *E. coli* MG1655 derived intergenic sequences *alaW-alaX* and *valU-valX*². *MbPylRS* and BockK used in both cases. Red text indicates the first and last bases of the tRNA. Data represents the mean and standard deviation ($n = 4$ biological replicates). Values are normalized to a wild-type LuxAB reporter under the same growth conditions.

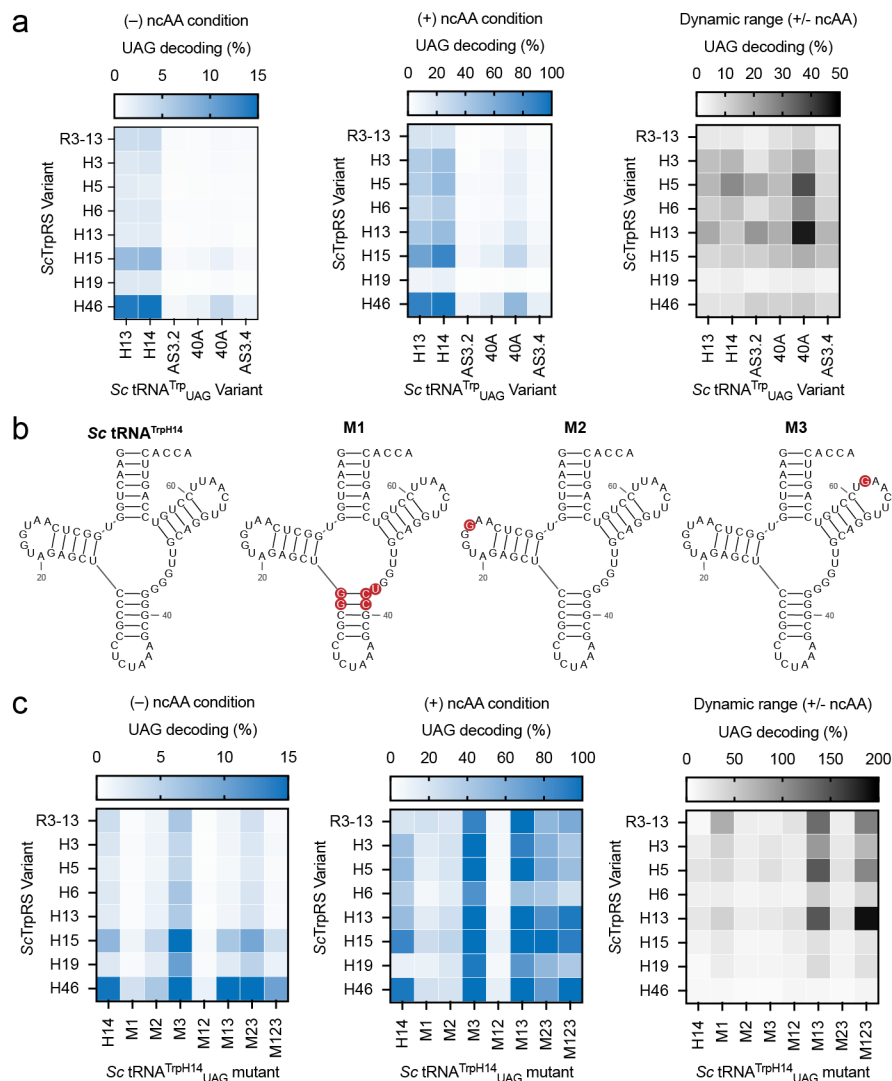


Supplementary Figure 7 | Investigating the Impact of tRNA–Synthetase Identity, Expression, Host Tolerance, and Substrate Scope on Decoding Efficiency.

Diverse tRNA–synthetases pairs were mined from literature sources and evaluated for UAG decoding efficiency. All genes have been fully recoded and tested data as follows: no additive, tRNA induction (IPTG only), tRNA–synthetase co-induction (IPTG and aTc), tRNA–synthetase co-induction with ncAA. Cognate tRNAs were used unless otherwise indicated. All values reported relative to wild-type LuxAB:

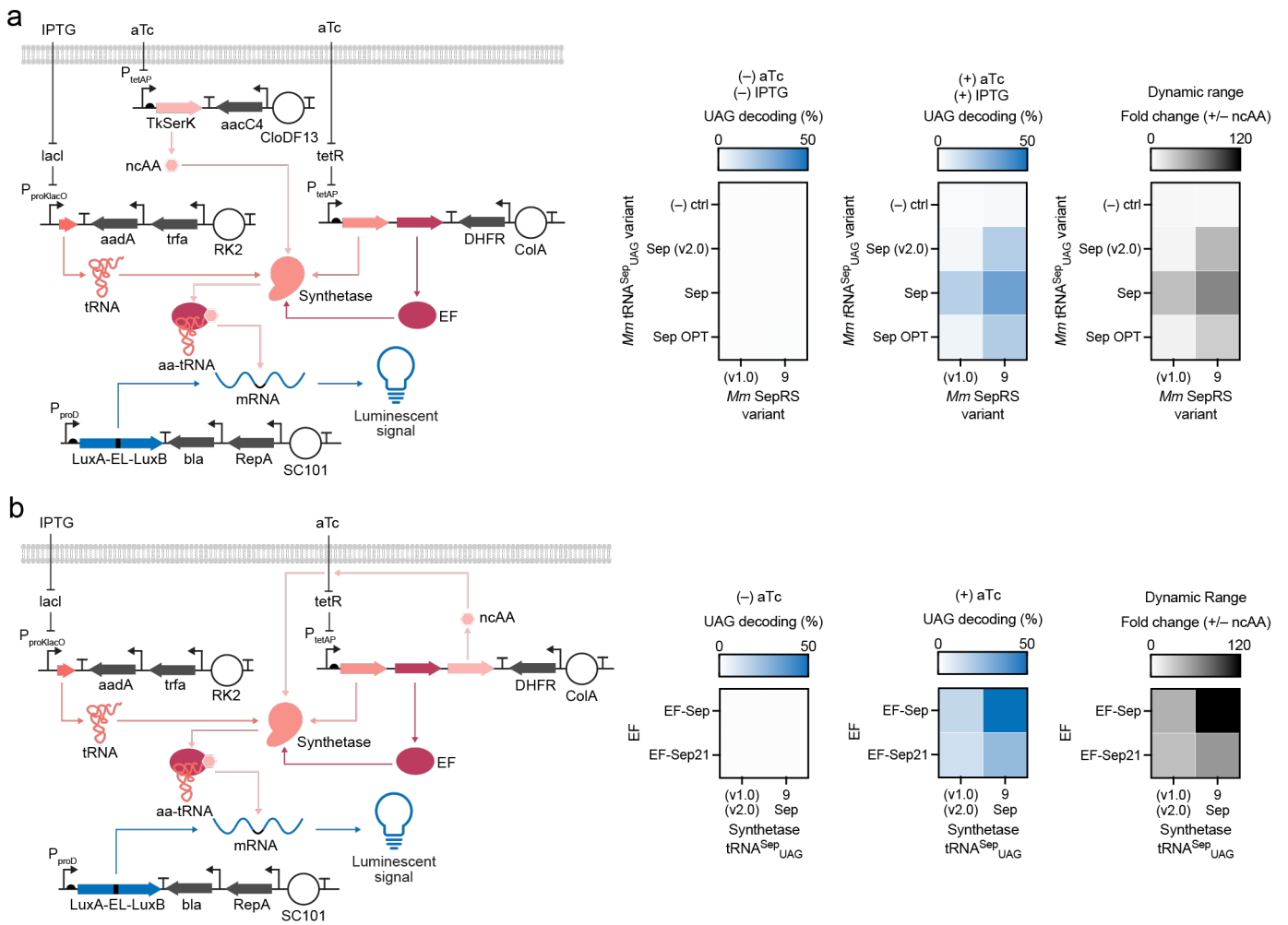
- Ap*HisRS, *Ca*ArgRS, *Cc*HisRS, *Mp*CysRS, and *Sc*AspRS. Data represents the mean and standard deviation (n = 4 biological replicates);
- Sc*GlnRS. Data represents the mean and standard deviation (n = 4 biological replicates);
- Sc*TyrRS. Data represents the mean and standard deviation (n = 4 biological replicates);
- Mm*SerRS alongside tRNA variants. Data represents the mean and standard deviation (n = 4 biological replicates);
- Mm*GluRS and *Ph*GluRS alongside tRNA variants. Data represents the mean and standard deviation (n = 4 biological replicates);
- Af*LeuRS, *Mj*LeuRS, and *Mt*LeuRS alongside tRNA variants. Data represents the mean and standard deviation (n = 4 biological replicates);

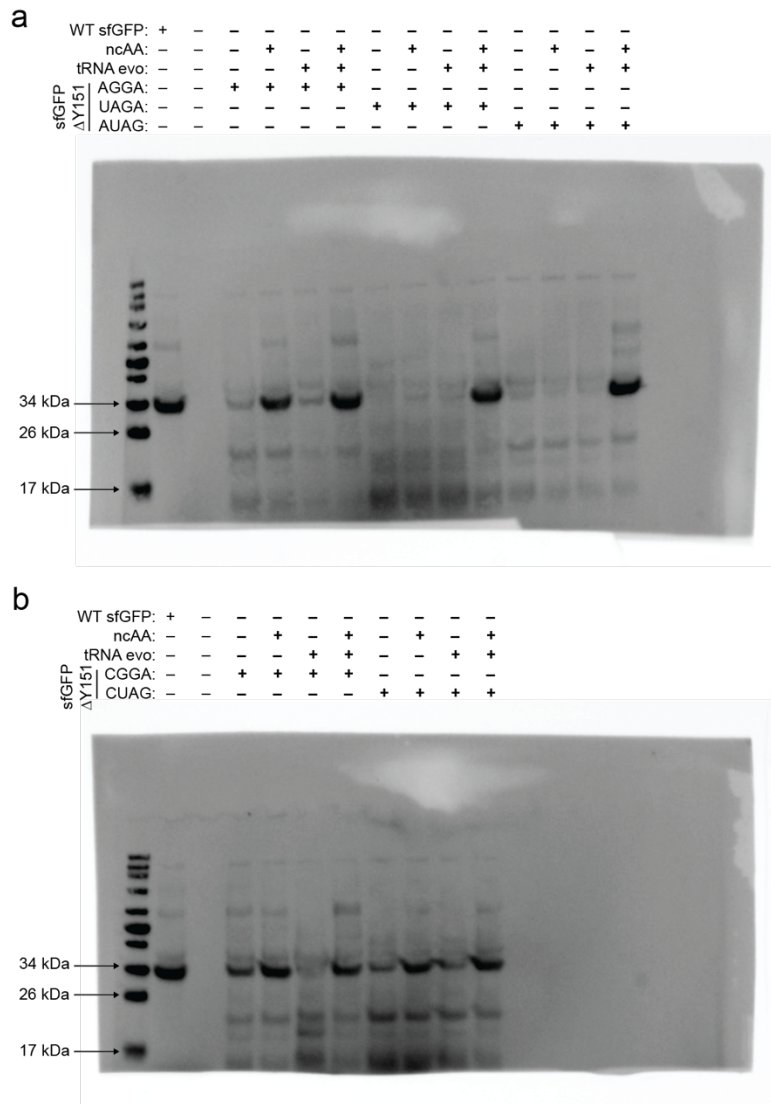
- g)** Tuning RBS strength driving *MjLeuRS*. Data represents the mean and standard deviation (n = 4 biological replicates);
- h)** *ScPheRS* alongside tRNA variants. Data represents the mean and standard deviation (n = 4 biological replicates);
- i)** *PhProRS* variants alongside tRNA variants. Data represents the mean and standard deviation (n = 4 biological replicates);
- j)** Tuning RBS strength driving *PhProRS(h3)*. Data represents the mean and standard deviation (n = 4 biological replicates);
- k)** Active site mutant of *PhProRS(h3)* reported to incorporate PCA (pipercolinic acid). Data represents the mean and standard deviation (n = 4 biological replicates);
- l)** *PhLysRS* shows toxicity upon synthetase induction. Data represents the mean and standard deviation (n = 4 biological replicates);
- m)** *PhLysRS* expression plasmid was plated on agar containing increasing aTc concentrations. A consensus mutation in the tetO₂ (below) was found and renamed *PhLysRS**;
- n)** *PhLysRS** improves synthetase-dependent aminoacylation but a reported active site variant is incapable of incorporating the cognate ncAA homoglutamine (hGln). Data represents the mean and standard deviation (n = 4 biological replicates);
- o)** *MacTyrRS* alongside tRNA variants. Data represents the mean and standard deviation (n = 4 biological replicates).



Supplementary Figure 8 | Rational Engineering of *Sc* tRNA^{Trp}_{UAG} Improves Decoding Efficiency and Dynamic Range.

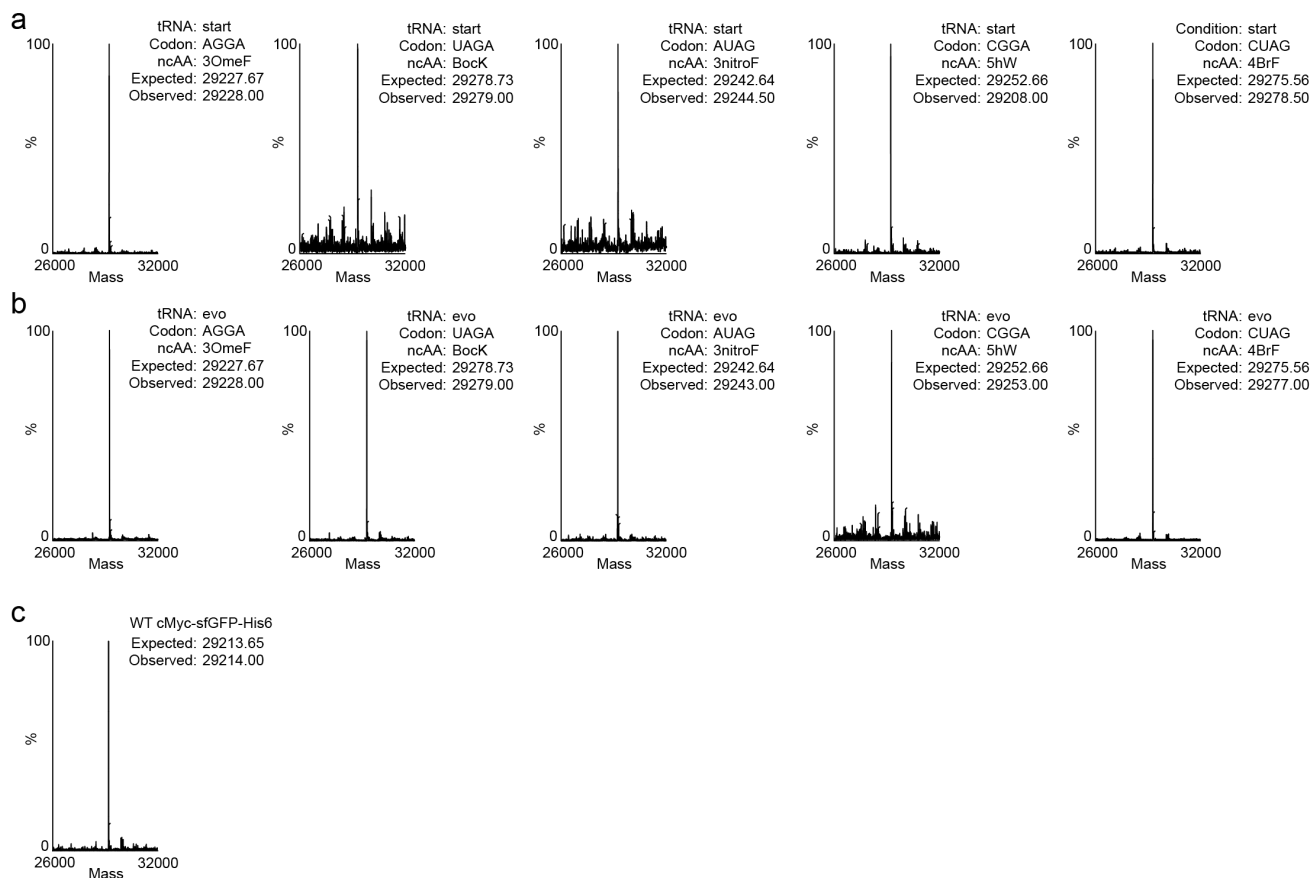
- Recoded genetic circuits were created using previously reported *Sc*TrpRS–*Sc* tRNA^{Trp} variants were evaluated in the absence (–) or presence (+) of the pooled cognate substrates: 1-methyltryptophan (1meW), 6-methyltryptophan (6meW), 5-hydroxytryptophan (5hW), 1-naphthylalanine (1NapA). Normalized dynamic range is shown. In all cases UAG decoding activity is normalized to a wildtype LuxAB reporter ($n = 4$ biological replicates).
- Secondary structures of the prioritized *Sc* tRNA^{TrpH14}_{UAG}, as well as previously reported mutations introduced into this scaffold (in red). We refer to the mutant sets as M1³, M2⁴, and M3⁴, respectively. tRNA 2D representations were predicted with RNAfold⁵ and tRNA schematics were drawn with RNACanvas⁶.
- All possible mutational combinations were introduced into *Sc* tRNA^{TrpH14}_{UAG} and evaluated against all reported *Sc*TrpRS variants as in part (a). In all cases UAG decoding activity is normalized to a wildtype LuxAB reporter ($n = 4$ biological replicates). *Sc* tRNA^{Trp(M13)}_{UAG} was used for all assays from this point forward, which contains the combined mutations M1 and M3 in the *Sc* tRNA^{TrpH14}_{UAG} scaffold.





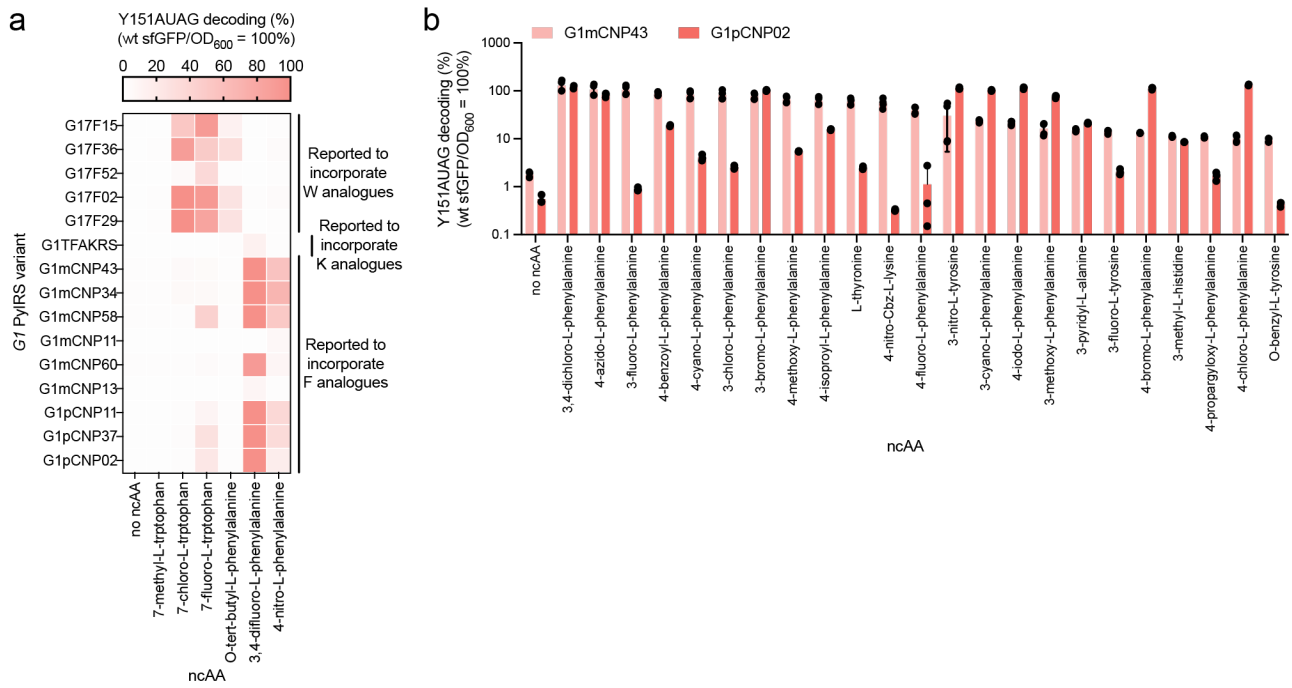
Supplementary Figure 10 | Western Blot Analysis of Quadruplet Codon Decoding Using Starting and Evolved qtRNAs.

In each case, sfGFP has been tagged N-terminally using a cMyc-tag and includes the indicated quadruplet codon at position Y151. Failure to decode the quadruplet codon results in a truncated product with a lower molecular weight. For the UAGA reporter, truncation will occur at Y151UAGA due to the in-frame stop codon (18 kDa) and will shift the frame to +1 for the other quadruplet codons (AGGA, AUAG, CGGA, CUAG) and terminate 5 amino acids later (19 kDa). This analysis was carried out for all quadruplet codons and is visualized across two blots: **(a)** AGGA, UAGA, and AUAG, and **(b)** CGGA and CUAG. The tRNAs \pm evolution (tRNA evo) are the same as described in main text (**Figure 3**). In all cases, four colonies were picked to grow independent cultures, pooled, and lysed to carry out the Western Blot analysis.



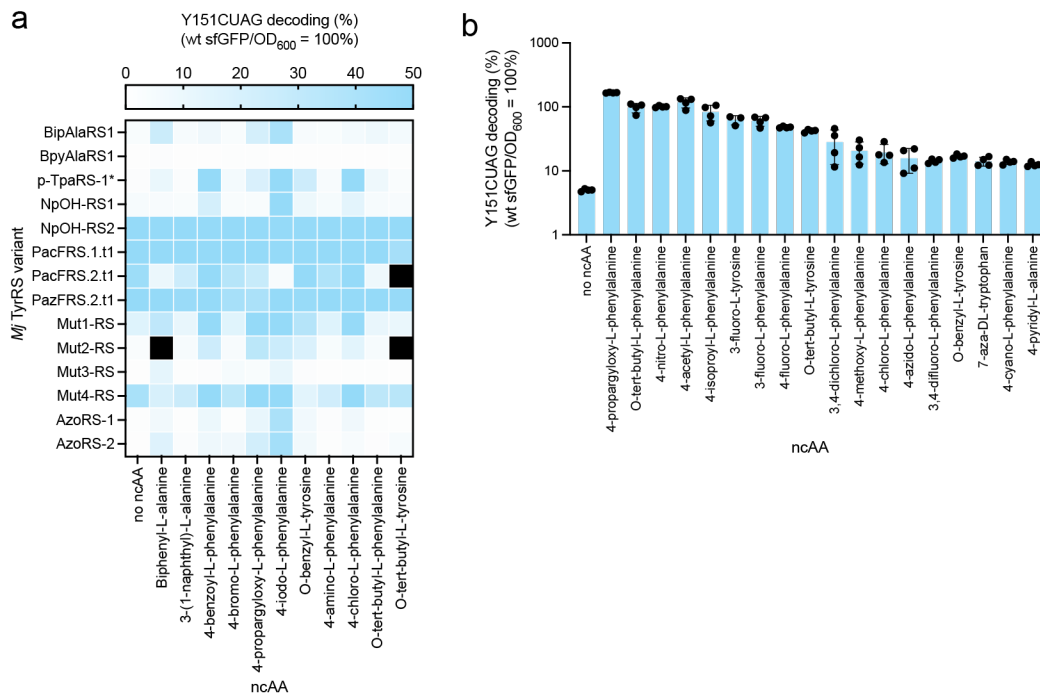
Supplementary Figure 11 | TOF-MS Analysis of Quadruplet Codon Decoding Using Starting and Evolved qtRNAs.

Ni-NTA-purified cMyc-sfGFP(Y151quad)-His6 was subjected to electron spray ionization time-of-flight mass spectrometry (ESI-TOF MS). The data includes the starting **(a)** and evolved **(b)** qtRNAs. **(c)** Analogous ESI-TOF MS data using wild-type cMyc-sfGFP-His6. LC traces and m/z spectra can be found in **(Supplemental Data 2)**.



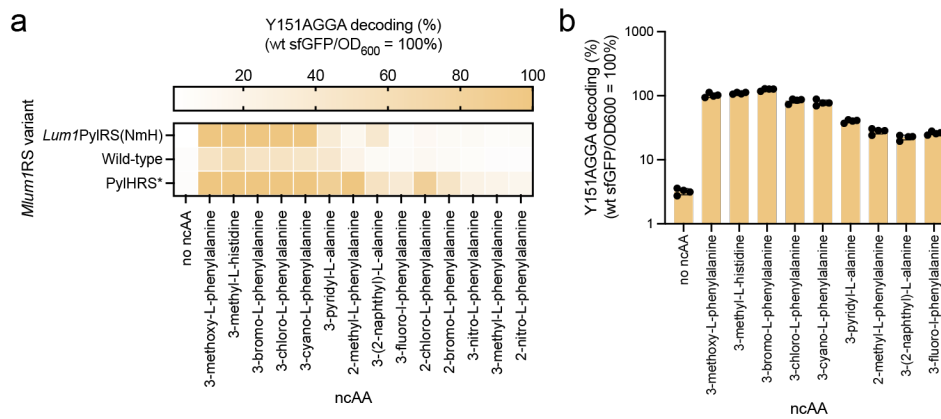
Supplementary Figure 12 | Screening *G1PylRS* Active Site Variants with Broad ncAA Substrate Scope.

- Previously reported *G1PylRS* active site variants were screened against a representative set of six ncAAs that include tryptophan, phenylalanine, and lysine analogs ($n = 4$). This analysis nominated G1pCNP02 as the lead candidate for further exploration due to its low background activity and robust ncAA incorporation. All active site mutations are described in **Supplementary Table 6**.
- Broader exploration of the ncAA scope of G1mCNP43 and G1pCNP02, which can use a large repertoire of related *meta*- and *para*-substituted phenylalanine and tyrosine derivatives. Data represents the mean and standard deviation for G1mCNP43 ($n = 4$ biological replicates), and G1pCNP02 ($n = 3$ biological replicates). G1mCNP43 has the broadest ncAA scope but G1pCNP02 has lower background activity and less overlap with other synthetase-tRNA pairs in this study.



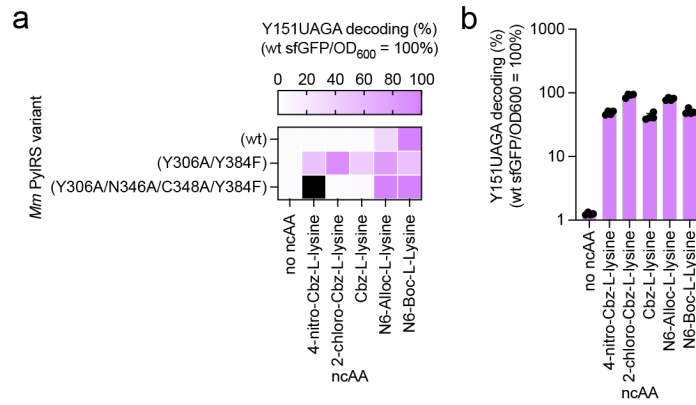
Supplementary Figure 13 | Screening *MjTyrRS* Active Site Variants with Broad ncAA Substrate Scope.

- a)** Previously reported *MjTyrRS* active site variants were screened against a representative set of 11 ncAAs that include bulky phenylalanine analogs ($n = 4$ biological replicates). This analysis nominated *p*-TpaRS-1* as the lead candidate for further exploration due to its low background activity and robust ncAA incorporation. Black squares were not tested. All active site mutations are described in **Supplementary Table 7**.
- b)** Broader exploration of the ncAA scope of *p*-TpaRS-1*, which uses diverse *para*-substituted and *para*-/*meta*-disubstituted phenylalanine derivatives including bulky adducts. Data represents the mean and standard deviation ($n = 4$ biological replicates).



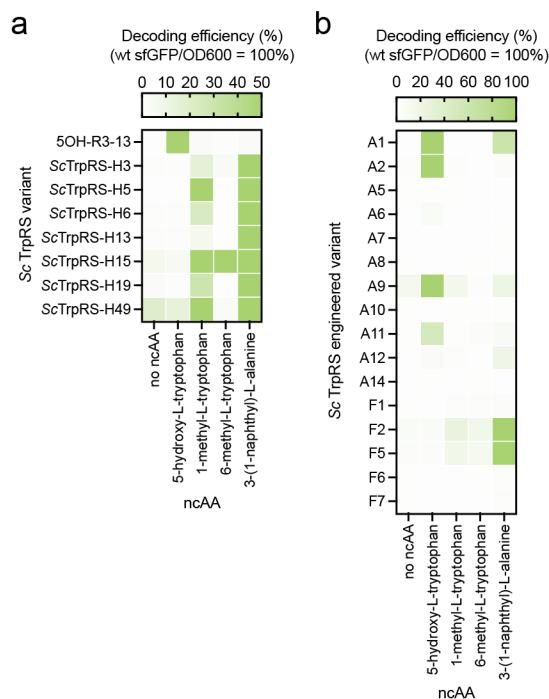
Supplementary Figure 14 | Screening *MlumIRS* Active Site Variants with Broad ncAA Substrate Scope.

- a) Previously reported *MlumIRS* active site variants were screened against a set of 28 ncAAs enriched in phenylalanine analogs ($n = 4$ biological replicates). This analysis nominated PylHRS* as the lead candidate for further exploration due to its broader ncAA scope. All active site mutations are described in **Supplementary Table 8**.
- b) Detailed ncAA scope of PylHRS*, which charges derivatives of histidine, thiophene, naphthalene, as well as *meta*- or *ortho*-substituted phenylalanine. Data represents the mean and standard deviation ($n = 4$ biological replicates).



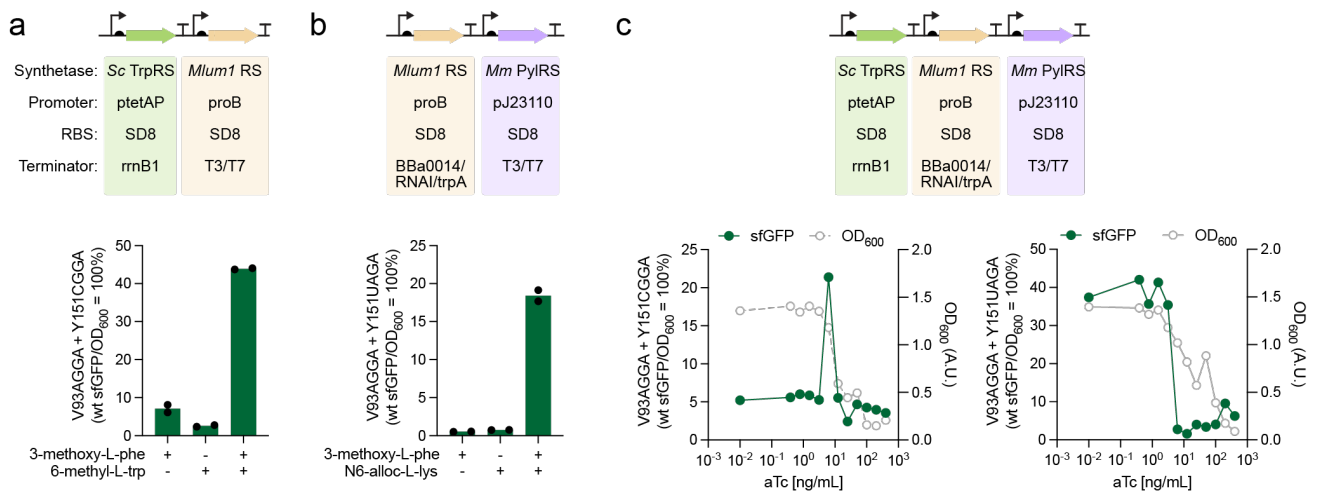
Supplementary Figure 15 | Screening *Mm*PylRS Active Site Variants with Broad ncAA Substrate Scope.

- a) Previously reported *Mm*PylRS active site variants were screened against a representative set of 11 ncAAs enriched in lysine analogs ($n = 4$ biological replicates). The black square was not tested. All active site mutations are described in **Supplementary Table 9**.
- b) This analysis nominated PylRS(Y306A/Y384F) as the lead candidate for further exploration due to its focused ncAA scope and more robust signal. Data represents the mean and standard deviation ($n = 4$ biological replicates).



Supplementary Figure 16 | Screening *ScTrpRS* Active Site Variants with Broad ncAA Substrate Scope.

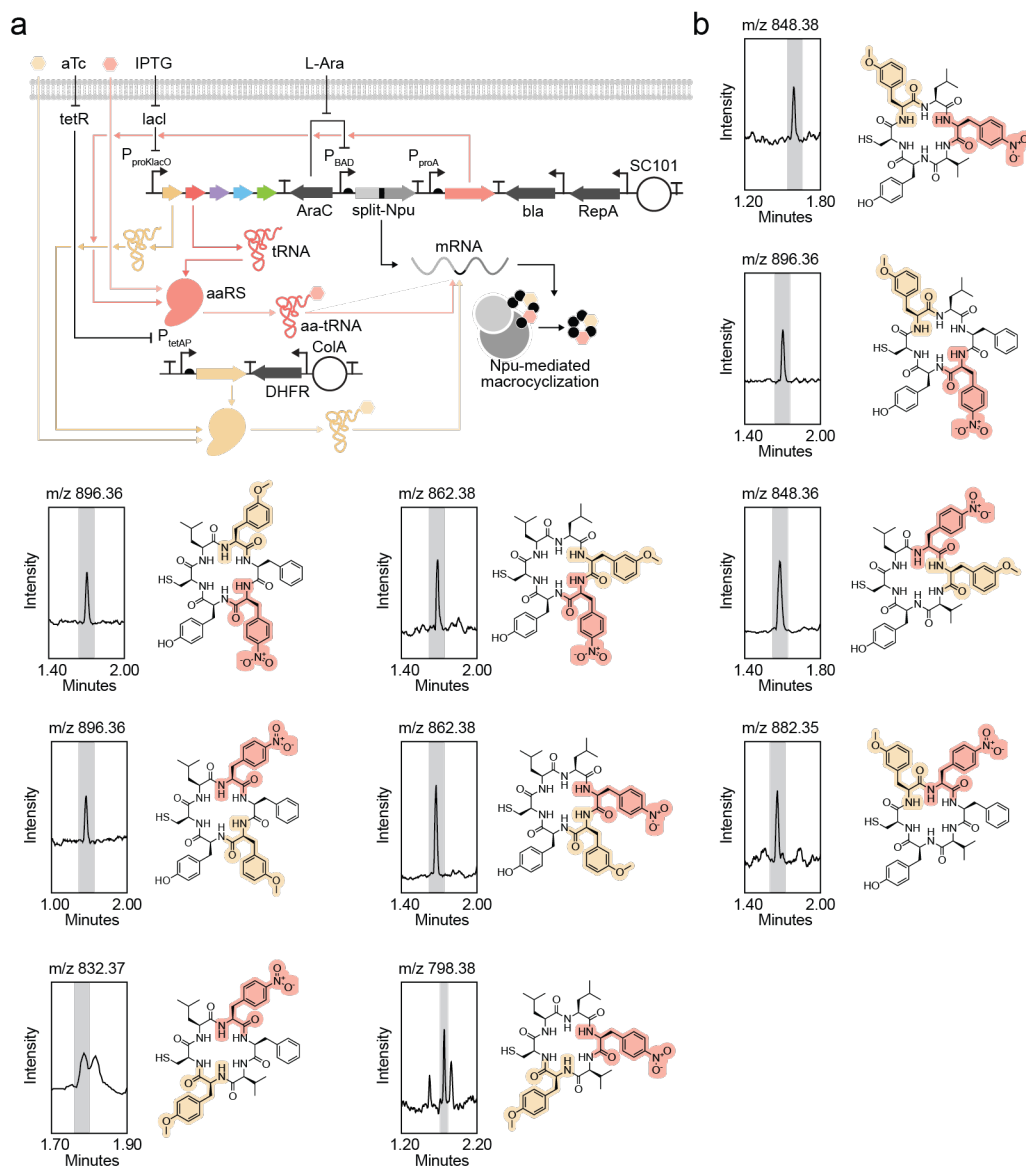
- a) Previously reported *ScTrpRS* active site variants were screened against a representative set of four tryptophan analog ncAAs ($n = 4$ biological replicates). This analysis nominated *ScTrpRS*-H15 as the lead candidate for further exploration since it had the broadest ncAA scope. All active site mutations are described in **Supplementary Table 10**.
- b) Guided by 5OH-R3-13 and *ScTrpRS*-H15, we rationally engineered combinations of their active site mutations that may provide access to all 4 ncAAs. While this yielded synthetases with altered ncAA scope (e.g., A1*), no engineered variant could incorporate all four ncAAs ($n = 4$ biological replicates).



Supplementary Figure 17 | Multi-synthetase Expression Plasmid for Multiple ncAA Incorporation.

Synthetase gene, promoter, RBS, and terminators are indicated in all panels for multi-synthetase expression.

- Synthetase expression cassette architecture for AGGA + CGGA decoding in sfGFP, data represents the mean with standard deviation. Assay conditions: 1 mM ncAA, 1 mM IPTG (tRNA expression), 100 ng mL⁻¹ aTc (synthetase expression where ptetAP is used). Data represents the mean (n = 2 biological replicates).
- Synthetase expression cassette architecture for AGGA + UAGA decoding in sfGFP, data represents the mean with standard deviation. Assay conditions: 1 mM ncAA, 1 mM IPTG (tRNA expression), 100 ng mL⁻¹ aTc (synthetase expression where ptetAP is used). Data represents the mean (n = 2 biological replicates).
- Synthetase expression cassette architecture for AGGA + CGGA or AGGA + UAGA decoding in sfGFP, data represents the mean with standard deviation. Assay conditions: 1 mM ncAA, 1 mM IPTG (tRNA expression), titration of aTc 400-0.4 ng mL⁻¹ (synthetase expression where ptetAP is used). Synthetase variants used for triple ncAA incorporation into macrocycles (Fig. 6): *Sc*TrpRS (*Sc*TrpRS-H15), *Mlum1*PylRS (PylHRS*), *Mm*PylRS (wt). Data represents the mean (n = 2 biological replicates).



Supplementary Figure 18 | Biosynthesis of Peptide Macrocycles Encoding Multiple ncAAs.

- Schematic representation of the macrocycle biosynthesis pipeline for multiplexed ncAA incorporation. The inducible promoters and extraction strategy are identical to main text **Figure 5**.
- Incorporation of two unique ncAAs in cyclo-CLLFVY using combinations of AGGA+AUAG codons. Extracted ion chromatogram (XIC) at the anticipated mass +H are shown. In total, 46 macrocycles encoding two unique ncAAs were generated through quadruplet decoding, 10 by AGGA+AUAG decoding (**Supplemental Data 5**).

Scaffold	Library	Identifier	Sequence (5'-3')
<i>Ma</i> qtRNA ^{Pyl} _{AGUA}	Anticodon stem	MD03	GGGGGACGGTCCGGGCGACCAGGCAGGCTTACTAACCAGGGGCGCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MC03	GGGGGACGGTCCGGGCGACCAGGCAGGCTTACTAACCAGGGGCGCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MA02	GGGGGACGGTCCGGGCGACCAGCGTGCCTTACTAAGCATCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MA03	GGGGGACGGTCCGGGCGACCAGCGTGCCTTACTAAGCATCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MG02	GGGGGACGGTCCGGGCGACCAGCGTGCCTTACTAAGCATCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		ME02	GGGGGACGGTCCGGGCGACCAGCGTGGCTTACTAACCAGGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MF02	GGGGGACGGTCCGGGCGACCAGCTGAGCTTACTAAGCTTAGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
<i>Ma</i> qtRNA ^{Pyl} _{CGAA}	Anticodon stem	MA12	GGGGGACGGTCCGGGCGACCAGCCAGTCTTTCGAAACTAGGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
	Anticodon loop	MB07	GGGGGACGGTCCGGGCGACCAGCGGGTATTTTCGAAACCTAGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
<i>Ma</i> qtRNA ^{Pyl} _{AUAG}	Anticodon stem	MB11	GGGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MC10	GGGGGACGGTCCGGGCGACCAGCGTGCATCTATACGCACTGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		ME10	GGGGGACGGTCCGGGCGACCAGGCGGGATCTATACCCTGTGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		ME11	GGGGGACGGTCCGGGCGACCAGCGCACATCTATACGTGCAGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
	Anticodon loop	MD01	GGGGGACGGTCCGGGCGACCAGCGGGTATCTATACACCTAGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
	Acceptor stem	MA03	GGGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MA08	GCCAGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTGGCGCCA
		MB02	GGGGAACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCCCGCCA
		MB03	GGGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MB04	GTGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCCCGCGCCA
		MB05	GGGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MB12	GGGCGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MC02	GGGGAACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCCCGCCA
		MC04	GGGAGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCCCCGCCA
		MC05	GTGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCCTCGCCA
		MD02	GTCTGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCAGACGCCA
		MD12	GAAGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		ME02	GTGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCCCGCGCCA
		MF01	GTCGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCGACGCCA
		MF02	GGGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA
		MF03	GGGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCCCCGCCA
	MF05	GGGGAACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCTCTCGCCA	
	MG08	GTGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCCTCGCCA	
MG12	GCCGAACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTACGGCGCCA		
MH01	GGGGGACGGTCCGGGCGACCAGCAGGCCTCTATAAGCCTCGCCTTGCAGGGGTTTCGACACCCCGGTCCCCGCCA		

Supplementary Table 1 | Sequences of Evolved *Ma* qtRNA^{Pyl}_{AGUA}, *Ma* qtRNA^{Pyl}_{CGAA}, and *Ma* qtRNA^{Pyl}_{AUAG} Variants.

The evolved mutant B11 (MB11; highlighted) was designated as the best variant used for all assays.

Scaffold	Library	Identifier	Sequence (5'–3')
<i>Af</i> qtRNA ^{Tyr} _{CUAG}	Anticodon stem	M1	CCCGCCCTAGCTCAGAGGTAGAGCGTGCCTCTCTAGAAATGCATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCA
		M2	CCCGCCCTAGCTCAGAGGTAGAGCGTGCCTCTCTAGAAGGGCATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCA
		M3	CCCGCCCTAGCTCAGAGGTAGAGCGTGCCTCTCTAGAATAGCATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCA
		M4	CCCGCCCTAGCTCAGAGGTAGAGCGTGCACCTCTAGAAATGCATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCA
		M5	CCCGCCCTAGCTCAGAGGTAGAGCGTGCCTCTCTAGAATTGCATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCA
		M6	CCCGCCCTAGCTCAGAGGTAGAGCGTGCCTCTCTAGAATTGCATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCA
		M8	CCCGCCCTAGCTCAGAGGTAGAGCGTGTCTCTAGAAAAACATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCA
		M9	CCCGCCCTAGCTCAGAGGTAGAGCGTGCCTCTCTAGAAGGGCATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCA

Supplementary Table 2 | Sequences of Evolved *Af* qtRNA^{Tyr}_{CUAG} Variants.

The evolved mutant 9 (M9; highlighted) was designated as the best variant used for all assays.

Scaffold	Library	Identifier	Sequence (5'-3')
<i>Int</i> qtRNA ^{Pyl} _{AGGA}	Anticodon stem	M1	GGTGTTCTGGTCCGGGACCACCGTGCCTTCCTAAGCCTCGGTTAGCCGGGTTCAACTCCCGGGAACATCGCCA
		M3	GGTGTTCTGGTCCGGGACCACCGTGCCTTCCTAAGCCTCGGTTAGCCGGGTTCAACTCCCGGGAACATCGCCA
		M13	GGTGTTCTGGTCCGGGACCACCGGGCCTTCCTAAGCCTCGGTTAGCCGGGTTCAACTCCCGGGAACATCGCCA
		M5	GGTGTTCTGGTCCGGGACCACCGAGCCTTCCTAAGCTCCGGTTAGCCGGGTTCAACTCCCGGGAACATCGCCA
	Anticodon loop	M5.1	GGTGTTCTGGTCCGGGACCACCGAGCCTTCCTATGCTCCGGTTAGCCGGGTTCAACTCCCGGGAACATCGCCA
		M5.2	GGTGTTCTGGTCCGGGACCACCGAGCATTCTACGCTCCGGTTAGCCGGGTTCAACTCCCGGGAACATCGCCA
		M5.3	GGTGTTCTGGTCCGGGACCACCGAGCATTCTAAGCTCCGGTTAGCCGGGTTCAACTCCCGGGAACATCGCCA
		M5.4	GGTGTTCTGGTCCGGGACCACCGAGCCCTCCTATGCTCCGGTTAGCCGGGTTCAACTCCCGGGAACATCGCCA

Supplementary Table 3 | Sequences of Evolved *Int* qtRNA^{Pyl}_{AGGA} Variants.

The evolved mutant 5.2 (M5.2; highlighted) was designated as the best variant used for all assays.

Scaffold	Library	Identifier	Sequence (5'-3')	
<i>Spe</i> qtRNA ^{Py₁} _{UAGA}	Anticodon stem	MD01	GGAAATCTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGGTTCCGCCA	
		MG02	GGAAATCTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGGTTCCGCCA	
		MA01	GGAAATCTGATCATGTAGATCGAAGCGGCTTCTAATCCAACCTCAGCCGGGTTAGATTCCCAGGGTTCCGCCA	
		MG05	GGAAATCTGATCATGTAGATCGAAGCGGCTTCTAATCCAACCTCAGCCGGGTTAGATTCCCAGGGTTCCGCCA	
		MB06	GGAAATCTGATCATGTAGATCGAAGCGGCTTCTAATCCAACCTCAGCCGGGTTAGATTCCCAGGGTTCCGCCA	
		ME04	GGAAATCTGATCATGTAGATCGATGCGGCTTCTAATCCAACCTCAGCCGGGTTAGATTCCCAGGGTTCCGCCA	
	Acceptor stem 1	MA02	GGATTCGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGATCCGCCA	
		MA03	GGACGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		MA07	GCAGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		MA08	GCAGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		MB07	GGACGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		MC07	GGACCTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGGGTCCGCCA	
		MD07	GAAGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		MD07*	GGACGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		ME07	GCAGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		MF07	GGACGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
	Acceptor stem 2	MA01	GGATTGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCAATCCGCCA	
		MA05	GGACGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGACGTCGCCA	
		MA09	GGAGGCGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		MB05	GGAGTGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCACTCCGCCA	
		MB06	GGATCGTTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGACGATCCGCCA	
		MD01	GGAGTGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCACTCCGCCA	
		MD06	GGAGGCGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
		MD10	GGAGTGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCCGCTCCGCCA	
		ME01	GGAGGCATGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGTGCTCCGCCA	
		ME06	GGAGCGTTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGACGCTCCGCCA	
		MG05	GGATCCTTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGAGGATCCGCCA	
		D-loop	MA01	GGACGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA
			MA09	GGACGTTTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGAGCGTCCGCCA
	MB09		GGACGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
	MC09		GGACGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGGCGCTCCGCCA	
	Ψ-stem	MA02	GGAGTGGTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGGTTAGATTCCCAGTCACTCCGCCA	

Supplementary Table 4 | Sequences of Evolved *Spe* qtRNA^{Py₁}_{UAGA} Variants.

The evolved mutant C07 (MC07; highlighted) was designated as the best variant used for all assays.

Scaffold	Library	Identifier	Sequence (5'-3')
<i>Sc</i> qtRNA ^{Trp} _{CGGA}	Anticodon stem	MA04	GAACTGGTGGCTCAATGGTAGAGCTGCGCGCTTCCGAACGCGTTGGTTGCAGGTTCAAGTCCTGTCCAGTTCACCA
		MA11	GAACTGGTGGCTCAATGGTAGAGCTGCACGCTTCCGAACGTGTTGGTTGCAGGTTCAAGTCCTGTCCAGTTCACCA
		MB06	GAACTGGTGGCTCAATGGTAGAGCTAGGCACTTCCGAATGCCGTGGTTGCAGGTTCAAGTCCTGTCCAGTTCACCA
		MB09	GAACTGGTGGCTCAATGGTAGAGCTGGGGGCTTCCGAAACCCCTGGTTGCAGGTTCAAGTCCTGTCCAGTTCACCA
		MB11	GAACTGGTGGCTCAATGGTAGAGCTCTGCCCTTCCGAATGCAGTGGTTGCAGGTTCAAGTCCTGTCCAGTTCACCA
		MD01	GAACTGGTGGCTCAATGGTAGAGCTAGGCACTTCCGAATGCCGTGGTTGCAGGTTCAAGTCCTGTCCAGTTCACCA
<i>Sc</i> qtRNA ^{Trp} _{CGGC}		MG01	GAACTGGTGGCTCAATGGTAGAGCTGCGGGCTGCCGAACCCGTTGGTTGCAGGTTCAAGTCCTGTCCAGTTCACCA
		MG07	GAACTGGTGGCTCAATGGTAGAGCTCTCGGCTGCCGAATCGAGTGGTTGCAGGTTCAAGTCCTGTCCAGTTCACCA

Supplementary Table 5 | Sequences of Evolved *Sc* qtRNA^{Trp}_{CGGA} and *Sc* qtRNA^{Trp}_{CGGC} Variants.

The evolved mutant A11 (MA11; highlighted) was designated as the best variant used for all assays.

Synthetase	Published Name	L124	Y125	N165	V167	Y204	A221	W237	DOI
<i>G/PyIRS</i>	G17F15	S	F	A	F	W	G	Y	10.1021/acssensors.1c02467
	G17F36	Q	F	A	F	W	G	Y	10.1021/acssensors.1c02467
	G17F52	L	Y	A	F	W	G	Y	10.1021/acssensors.1c02467
	G17F02	G	F	G	F	W	G	Y	10.1021/acssensors.1c02467
	G17F29	H	F	A	A	W	G	Y	10.1021/acssensors.1c02467
	G1TFAKRS	I	L	N	A	W	C	W	10.1021/jacs.1c10104
	G1mCNP43	A	F	N	V	F	S	Y	10.1002/anie.202114154
	G1mCNP34	A	L	N	A	W	S	W	10.1002/anie.202114154
	G1mCNP58	A	L	N	C	W	S	W	10.1002/anie.202114154
	G1mCNP11	R	C	S	C	W	A	W	10.1002/anie.202114154
	G1mCNP60	A	M	N	S	W	C	W	10.1002/anie.202114154
	G1mCNP13	G	R	N	V	W	C	W	10.1002/anie.202114154
	G1pCNP11	A	F	N	V	W	A	Y	10.1002/anie.202114154
	G1pCNP37	A	F	N	V	W	S	Y	10.1002/anie.202114154
	G1pCNP02	S	F	N	V	W	S	Y	10.1002/anie.202114154

Supplementary Table 6 | *G/PyIRS* Active Site Variants with Altered ncAA Substrate Scopes.

Published synthetase names, active site mutations, and DOIs are given in each case.

Synthetase	Published Name	Y32	L65	H70	K90	E107	F108	Q109	Q155	D158	I159	H160	Y161	L162	V164	A167	DOI
<i>MjTyrRS</i>	BipAlaRS1	H	H	H	K	E	W	M	Q	G	I	H	Y	K	V	A	10.1002/anie.200703397
	BpyAlaRS1	G	Y	A	K	E	F	Q	E	G	W	H	Y	S	V	A	10.1002/anie.200703397
	p-TpaRS-1*	I	I	H	K	E	F	M	Q	G	I	H	Y	V	G	A	10.1021/ja104350y
	NpOH-RS1	E	T	H	K	E	F	Q	Q	S	A	P	T	Q	V	W	10.1021/bc400168u
	NpOH-RS2	E	V	H	E	E	F	Q	Q	D	A	W	G	Q	V	I	10.1021/bc400168u
	PacFRS.1.t1	L	L	H	K	E	F	Q	Q	G	C	H	Y	R	V	D	10.3389/fbioe.2022.913057
	PacFRS.2.t1	L	V	H	K	E	F	Q	Q	G	C	H	Y	R	V	D	10.3389/fbioe.2022.913057
	PazFRS.2.t1	L	L	H	K	E	F	Q	Q	S	M	H	Y	K	V	H	10.3389/fbioe.2022.913057
	Mut1-RS	L	V	H	K	E	F	Q	Q	G	A	H	Y	E	V	H	10.3389/fbioe.2022.913057
	Mut2-RS	L	V	H	K	E	F	Q	Q	G	M	H	Y	S	V	H	10.3389/fbioe.2022.913057
	Mut3-RS	G	V	H	K	E	F	Q	Q	G	Y	H	Y	S	V	F	10.3389/fbioe.2022.913057
	Mut4-RS	L	V	H	K	S	V	S	Q	G	Y	H	Y	S	V	F	10.3389/fbioe.2022.913057
	AzoRS-1	L	V	H	K	N	V	L	Q	G	Y	H	Y	S	V	F	10.3389/fbioe.2022.913057
	AzoRS-2	G	V	H	K	E	F	Q	Q	G	Y	H	Y	R	V	A	10.3389/fbioe.2022.913057
	AzoRS-3	L	V	H	K	N	V	L	Q	G	Y	H	Y	S	V	F	10.3389/fbioe.2022.913057
	AzoRS-4	G	V	H	K	E	F	Q	Q	G	Y	H	Y	R	V	A	10.3389/fbioe.2022.913057

Supplementary Table 7 | *MjTyrRS* Active Site Variants with Altered ncAA Substrate Scopes.

Published synthetase names, active site mutations, and DOIs are given in each case. The published sequence for *p-TpaRS-1* includes Y32L, whereas our variant contains Y32I. All *MjTyrRS* variants also include mutations to allow bindings and aminoacylation of qtRNA⁷: Y230K, C231K, P232K, H283Q, D286S.

Synthetase	Published Name	L121	L125	Y126	M129	V168	Y205	DOI
<i>MlumIRS</i>	<i>Lum1PylRS</i> (NmH)	M	I	F	A	F	Y	10.1038/s41557-020-0472-x
	Wild-type	L	L	Y	M	V	Y	10.1038/s41557-020-0472-x
	<i>PylHRS*</i>	L	I	F	G	F	F	10.1021/cb500032c

Supplementary Table 8 | *MlumIRS* Active Site Variants with Altered ncAA Substrate Scopes.

Published synthetase names, active site mutations, and DOIs are given in each case. We note that *PylHRS* was developed using *MmPylRS*, and that the discovered mutations were transplanted onto the *MlumIRS* sequence. *PylHRS** has therefore not been previously described.

Synthetase	Published Name	Y306	N346	C348	Y384	DOI
<i>MmPylRS</i>	Wild-type	Y	N	C	Y	10.1016/j.chembiol.2008.10.004
	PylRS(Y306A/Y384F)	A	N	C	F	10.1016/j.chembiol.2008.10.004
	PylRS(Y306A/N346A/C348A/Y384F)	A	A	A	F	10.1021/bc500361d

Supplementary Table 9 | *MmPylRS* Active Site Variants with Altered ncAA Substrate Scopes.

Published synthetase names, active site mutations, and DOIs are given in each case.

Synthetase	Published Name	Y106	T107	E141	T233	I253	P254	C255	F263	DOI
<i>ScTrpRS</i>	5OH-R3-13	Y	C	E	T	I	T	A	F	10.1038/nbt.2714
	<i>ScTrpRS</i> -H3	L	T	P	T	C	P	C	W	10.1002/anie.201301094
	<i>ScTrpRS</i> -H5	L	T	A	C	V	P	C	C	10.1002/anie.201301094
	<i>ScTrpRS</i> -H6	V	T	P	C	V	P	C	G	10.1002/anie.201301094
	<i>ScTrpRS</i> -H13	L	T	P	V	V	P	C	Y	10.1002/anie.201301094
	<i>ScTrpRS</i> -H15	V	T	P	C	C	P	C	F	10.1002/anie.201301094
	<i>ScTrpRS</i> -H19	I	T	P	T	I	P	C	A	10.1002/anie.201301094
	<i>ScTrpRS</i> -H49	V	T	P	S	V	P	C	Y	10.1002/anie.201301094
	A1*	V	C	E	T	I	T	A	F	–
	A2*	V	C	E	C	I	T	A	F	–
	A3*	V	C	E	T	C	T	A	F	–
	A4*	V	C	E	C	C	T	A	F	–
	A5*	Y	C	P	T	I	T	A	F	–
	A6*	Y	C	P	C	I	T	A	F	–
	A7*	Y	C	P	T	C	T	A	F	–
	A8*	Y	C	P	C	C	T	A	F	–
	A9*	Y	C	E	C	I	T	A	F	–
	A10*	Y	C	E	T	C	T	A	F	–
	A11*	Y	C	E	C	C	T	A	F	–
	A12*	V	C	P	T	I	T	A	F	–
	A13*	V	C	P	C	I	T	A	F	–
	A14*	V	C	P	T	C	T	A	F	–
	F1*	V	C	P	C	C	P	C	F	–
	F2*	V	T	P	C	C	T	C	F	–
	F3*	V	T	P	C	C	P	A	F	–
	F4*	V	C	P	C	C	T	C	F	–
	F5*	V	T	P	C	C	T	A	F	–
	F6*	V	C	P	C	C	P	A	F	–
	F7*	V	C	P	C	C	T	A	F	–

Supplementary Table 10 | *ScTrpRS* Active Site Variants with Altered ncAA Substrate Scopes.

Published synthetase names, active site mutations, and DOIs are given in each case. We note that all *ScTrpRS* variants with created through rational mutagenesis based on published mutants, and have therefore not been previously described.

#	Name	CAS #
1	7-fluoro-L-tryptophan	53314-95-7
2	4-benzoyl-L-phenylalanine	104504-39-4
3	4-pyridyl-L-alanine	178933-04-5
4	7-chloro-L-tryptophan	73945-46-7
5	L-thyronine	1596-67-4
6	3-(2-Oxo-1,2-dihydro-4-quinoliny)-DL-alanine	4876-14-6
7	Biphenyl-L-alanine	155760-02-4
8	O-benzyl-L-tyrosine	16652-64-5
9	7-aza-DL-tryptophan	7303-50-6
10	4-cyano-L-phenylalanine	167479-78-9
11	4-methoxy-L-phenylalanine	01-11-6230
12	O-tert-butyl-L-tyrosine	18822-59-8
13	4-fluoro-L-phenylalanine	1132-68-9
14	3-fluoro-L-phenylalanine	19883-77-3
15	3-fluoro-L-tyrosine	403-90-7
16	4-acetyl-L-phenylalanine	20299-31-4
17	4-isopropyl-L-phenylalanine	98708-79-3
18	4-propargyloxy-L-phenylalanine	1080496-42-9
19	O-tert-butyl-L-phenylalanine	82372-74-5
20	4-nitro-L-phenylalanine	949-99-5
21	4-chloro-L-phenylalanine	14173-39-8
22	4-azido-L-phenylalanine	33173-53-4
23	3-amino-L-tyrosine	23279-22-3
24	3,4-dichloro-L-phenylalanine	52794-99-7
25	3,4-difluoro-L-phenylalanine	32133-36-1
26	4-bromo-L-phenylalanine	24250-84-8
27	3-nitro-L-phenylalanine	19883-74-0
28	4-iodo-L-phenylalanine	24250-85-9
29	3-(2-naphthyl)-L-alanine	58438-03-02
30	3-cyano-L-phenylalanine	57213-48-6
31	3-bromo-L-phenylalanine	82311-69-1
32	3-methoxy-L-phenylalanine	33879-32-2
33	3-methyl-L-histidine	368-16-1
34	3-chloro-L-phenylalanine	80126-51-8
35	3-pyridyl-L-alanine	64090-98-8
36	2-methyl-L-phenylalanine	80126-53-0
37	2-chloro-Cbz-L-lysine	42390-97-6
38	Cbz-L-lysine	1155-64-2
39	N6-alloc-L-lysine	147529-99-5
40	N6-Boc-L-lysine	2418-95-3
41	4-nitro-Cbz-L-lysine	3557-90-2
42	3-(1-naphthyl)-L-alanine	55516-54-6
43	3-benzothienyl-L-alanine	72120-71-9
44	1-methyl-L-tryptophan	21339-55-9
45	6-methyl-L-tryptophan	33468-34-7
46	5-hydroxy-L-tryptophan	08-09-4350

Supplementary Table 11 | Identifiers and CAS Numbers for All Tested ncAAs.

Identifier	Sequence (5'-3')
<i>valU-valX</i>	ACTACTTTATGTAGTCTCCGCCGTGTAGCAAGAAATTGAGAAGT
<i>glyX-glyY</i>	AAATTTGAAAGTGCTGTAAGGCACAGACCACCCAA
<i>argY-argZ</i>	TCTCTTACTTGATATGGCTTTAGTAGCGGTATCAATATCAGCAGTAAAATAAATTTCCCGAT
<i>alaW-alaX</i>	AATTTTGCACCCAGCAAACCTTGGTACGTAAACGCATCGT
<i>glyW-cysT</i>	GTTTAAAAGACATCGGCGTCAAGCGGATGTCTGGCTGAAAGGCCTGAAGAATTT
<i>ileV-alaV</i>	AATTTGCACGGCAAATTTGAAGAGGTTTAACTACATGTTAT
<i>valX-valY</i>	CTTTCGCGCCAGCTAAATTTCTTGTA AAAATGTGAAGTACCGAAGT
<i>metZ-metW</i>	ATTAAAATTTGATGAAGTAAAGCAGTACGGTGA
<i>metW-metV</i>	ATCAAATTTGATGAAGTAAAGCAGTACGGTGA
<i>argX-hisR</i>	TTTAGTCCCGGCGCTTGAGCTGCGGTGGTAGTAATACCGCGTAACAAGATTTGTAGT
<i>hisR-leuT</i>	TTATTAGAAGTTGTGACAAT
<i>leuT-proM</i>	CGACTTTAAAGAATTGAACTAAAAATTCAAAAAGCAGTATTT
<i>ileT-alaT</i>	AATTTGCACGGCAAATTTGAAGAGGTTTAACTACATGTTAT
<i>glyV-glyX</i>	AAATTTGAAAAGTGCTGCAAAGCACAGACCACCCAA
<i>leuQ-leuP</i>	AAAACCACGTTGATATTGCTCGCACTGG
<i>leuP-leuV</i>	AACGAGGCGATATCAAAAAAAGTAAGATGACTGT
<i>ileU-alaU</i>	AATTTGCACGGCAAATTTGAAGAGGTTTAACTACATGTTAT
<i>leuW-glnU</i>	TTCACCAGAAAGCGTTGTACGGA
<i>glnU-glnW</i>	TCTTCTCGAGTAAGCGGTTCCACGCCCGGTTAT
<i>metU-glnV</i>	AATTCTGAATGTATCGAATATGTTCCGGCAAATTCAAAACCAATTTGT
<i>glnV-glnX</i>	ATTTATTCAAGACGCTTACCTTGTAAGTGCACCCAGT

Supplementary Table 12 | Mined *E. coli* Inter-tRNA Sequences Used to Improve qtRNA Production.

Panel	Description	ncAA(s)	Chemical Formula	Calculated [M+H] ⁺	Found [M+H] ⁺
Figure 6b	C[AGGA]LF[AGGA]Y	3OmeF	C ₄₇ H ₅₆ N ₆ O ₉ S	881.3908	881.389
Figure 6b	CL[AGGA][AGGA]VY	3OmeF	C ₄₃ H ₅₆ N ₆ O ₉ S	833.3908	833.3907
Figure 6b	C(AGGA)(AGGA)F(AGGA)Y	3OmeF	C ₅₁ H ₅₆ N ₆ O ₁₀ S	945.3857	945.3856
Figure 6b	C(AGGA)(AGGA)F(AGGA)Y	3OmeF	C ₄₇ H ₅₆ N ₆ O ₉ S	881.3908	881.3884
Figure 6c	C(AGGA)LF(CGGA)Y	3OmeF 6meW	C ₄₉ H ₅₇ N ₇ O ₈ S	904.4068	904.4029
Figure 6c	CLL(AGGA)(CGGA)Y	3OmeF 6meW	C ₄₆ H ₅₉ N ₇ O ₈ S	870.4224	870.4216
Figure 6c	C(AGGA)LF(CGGA)Y	3meH 6meW	C ₄₆ H ₅₅ N ₉ O ₇ S	878.4023	878.4023
Figure 6c	CLL(AGGA)(CGGA)Y	3meH 6meW	C ₄₃ H ₅₇ N ₉ O ₇ S	844.418	844.4155
Figure 6c	C(AGGA)(UAGA)FVY	3CNF AllocK	C ₄₆ H ₅₆ N ₈ O ₉ S	897.3969	897.3951
Figure 6c	C(AGGA)LF(UAGA)Y	3CNF AllocK	C ₄₇ H ₅₈ N ₈ O ₉ S	911.4126	911.4085
Figure 6c	CL(AGGA)FV(UAGA)	3CNF AllocK	C ₄₃ H ₅₈ N ₈ O ₈ S	847.4177	847.4153
Figure 6c	CLLF(AGGA)(UAGA)	3CNF AllocK	C ₄₄ H ₆₀ N ₈ O ₈ S	861.4333	861.4319
Figure 6d	CL(AGGA)(CGGA)(UAGA)Y	3OmeF 6meW AllocK	C ₅₀ H ₆₄ N ₈ O ₁₀ S	969.4544	969.4542
Figure 6d	CL(AGGA)F(CGGA)(UAGA)	3OmeF 6meW AllocK	C ₅₀ H ₆₄ N ₈ O ₉ S	953.4595	953.4554
Figure 6d	C(AGGA)(UAGA)F(CGGA)Y	3OmeF 6meW AllocK	C ₅₃ H ₆₂ N ₈ O ₁₀ S	1003.4388	1003.4387
Figure 6d	C(AGGA)(UAGA)FV(CGGA)	3OmeF 6meW AllocK	C ₄₉ H ₆₂ N ₈ O ₉ S	939.4439	939.4423
Figure 6d	C(AGGA)L(UAGA)(CGGA)Y	3OmeF 6meW AllocK	C ₅₀ H ₆₄ N ₈ O ₁₀ S	969.4544	969.4533
Figure 6d	CL(AGGA)F(UAGA)(CGGA)	3OmeF 6meW AllocK	C ₅₀ H ₆₄ N ₈ O ₉ S	953.4595	953.4565
Figure 6d	CLL(AGGA)(UAGA)(CGGA)	3OmeF 6meW AllocK	C ₄₇ H ₆₆ N ₈ O ₉ S	919.4752	919.4734
Figure 6d	CLL(CGGA)(AGGA)(UAGA)	3OmeF 6meW AllocK	C ₄₇ H ₆₆ N ₈ O ₉ S	919.4752	919.4729
Figure 6d	CL(AGGA)(CGGA)(UAGA)Y	3BrF 6meW AllocK	C ₄₉ H ₆₁ BrN ₈ O ₉ S	1017.3544	1017.3526
Figure 6d	CLL(CGGA)(AGGA)(UAGA)	3BrF 6meW AllocK	C ₄₆ H ₆₃ BrN ₈ O ₈ S	967.3751	967.3742

Supplementary Table 13 | High Resolution Mass Spectrometry (HRMS) Analysis of Macrocycles.

HRMS was carried out for all macrocycles with multiple ncAA incorporation events. The table indicates the panel in which the XIC trace is shown in the Main Text, a description of the peptide, the ncAA(s) used, the chemical formula, calculated and found masses in each case.

Classification	Plasmid ID	Description	Sequence (5'-3')
Synthetase expression plasmid	pAB228i16	ColA BBa_B0014 tetAp SD8 G1_PylRS(rc) [mCNP43] T3Te-T7Te DHFR(rc)	<pre> aaagctctagaagatgccaggaggatactagcagagagacaataagccggagcgaagccgttttccataggtccgccccctgacgaacatcacgaaatctg acgctcaaatcagtggtggcgaaccgacaggactataaagataaccaggcgtttcccctgatggctccctctgctcctctgttcccctgctggcgctccgtgtf gtggtggagcgtttaccaatcaccacgtcccgctccgtgtagacagttcgtccaagctgggctgtgtgcaagaacccccctgaccccactgctgctgcttacc cgtaactatcatcttgagtcacaaccggaagacacgacaaaacgccactggcagcagccattggaactgagaattagtggtattagatcagagacttgaagtg gtggcctaacagaggctacactgaaaggacagatattggatctgctcctcaaaagccagttaccagggttaagcagttccccactgacttaacctogatacaaccg cctccccaggcgtttttcgtttacagagcagagattacgacgatcgtaaaaggatcctcaagaagatcAttacggattcccgaaccaaaggagacatccggttca cactggctcacttcgggtggcctttctgctgtttatatactagagagagaataaaaaagccagattataatccggctttttatttagacttagggaccctcgggcc gccattaatcctaattttgtgacactctatcattgatagagttatttaccactccctatcagtagatagagaaaagtaaatgaatagacctgaggtgagtaagagg aaaaaaaATGGTTGTTAAATTCACCGATTCTCAGATTTCAGCATTGATGGAATACGGTGATAACGATTG GTCTGAAGCGGAATTCGAAGATGCGGCGCGCGTGATAAAGAATTCTCTTCTCAGTTCTCTAAATTG AAATCTGCGAACGATAAAAGGTTTGAAGATGTTATTGCGAACCCCGGTAACGATTTGACCGAATTTGG AAAACAAAATTCGTGAAAAATGGCGGCGCGTGGTTTCATTGAAGTTCATACCCCGATTTCGTTTCT AAATCTGCGTTGGCGAAAATGACCATTACCGAAGATCATCCGTTGTTCAAACAGGTTTTCTGGATTG ATGATAAACGTGCGTTGCGTCCGATGCATGCGATGAACGCGTTCAAAGTTATGCGTGAATTGCGTGA TCATACCAAAGGTCCGGTTAAAATTTTCGAAATTTGTTTTCGTTAAAGAATCTAAATCTTCTA CCCATTTGGAAGAATTCACCATGTTGAACTTGGTTGAAATGGGTCCGGATGGTGATCCGATGGAACA TTTGAAAATGTACATTGGTGATATTATGGATGCGGTTGGTGTGAAATACACCACCTCTCGTGAAGAA TCTGATGTTTTCGTTGAAACCTTGGATGTTGAAATTAACGGTACCGAAGTTGCGTCTGGTTCTGTTGG TCGCATAAAATTTGATCCGCGCATGATGTTTCATGAACCGTACGCGGTTATTGGTTTCGGTTTGGAA CGTTTGTGATGTTGAAAAACGGTAAATCTAACGCGCGTAAAACCGGTAATCTATTACCTACTTGA ACGGTTACAAAATTTGGATTAActtaataaacggcactcctcagctgtaaccaactcactggctcacttcacgggtgAgccttcttcgcaagaaa ggccccccgaaggtgagccagtgattacattatgccatggcgfTTAACCTTTCTGCCAAATCTGGTAAAGAGTAGTTAATGT TAGACGCGAAATCTTGGTGAAAACCGGACGGAAGTTAGACGGAATTTCCGGGAAGTAAACATCAC CTTCCGGTTCAATATCAATGGTAGAAATATGCAAGGTATCAACCTGATCAATCAAAGATTTGTAAT TTCACCACCACGAAAACAATAACATGATCGGTAATTTTTTCAAGTTGGTCAACGCATCTTTAATAG ACGGGAAAATCAAACGTTTTTCGTTATCAGAGGTGAAAAGAAGAACGGGTAACAACCCGCGTATTTAC GGTTCGGCAACGCACCCATAGAACCAGGTTTTACGACCAACCAACAACCACTGGTTGTAGGTAAT CGCTTGAACAACAACCTGTTACCTTTTCGCAGACCACGGAATATCCGGACCGTTACCAATAACACCG TTTTTAGAAATCGCAACCATCAAAGACAATTTCAtaattctcctcagaggttaacattttagtaagcatgctcgtttgtcaccgcgt gatgttaccgttaataataatcactgttataatagactttctctaaaaatacaagacactctgtattacaatctgctgctgctctctctgttgaagccaaat aggcgt </pre>
	pAB228i24	ColA BBa_B0014 tetAp SD8 G1_PylRS(rc) [pCNP02] T3Te-T7Te DHFR(rc)	<pre> aaagctctagaagatgccaggaggatactagcagagagacaataagccggagcgaagccgttttccataggtccgccccctgacgaacatcacgaaatctg acgctcaaatcagtggtggcgaaccgacaggactataaagataaccaggcgtttcccctgatggctccctctgctcctctgttcccctgctggcgctccgtgtf gtggtggagcgtttaccaatcaccacgtcccgctccgtgtagacagttcgtccaagctgggctgtgtgcaagaacccccctgaccccactgctgctgcttacc cgtaactatcatcttgagtcacaaccggaagacacgacaaaacgccactggcagcagccattggaactgagaattagtggtattagatcagagacttgaagtg gtggcctaacagaggctacactgaaaggacagatattggatctgctcctcaaaagccagttaccagggttaagcagttccccactgacttaacctogatacaaccg cctccccaggcgtttttcgtttacagagcagagattacgacgatcgtaaaaggatcctcaagaagatcAttacggattcccgaaccaaaggagacatccggttca cactggctcacttcgggtggcctttctgctgtttatatactagagagagaataaaaaagccagattataatccggctttttatttagacttagggaccctcgggcc gccattaatcctaattttgtgacactctatcattgatagagttatttaccactccctatcagtagatagagaaaagtaaatgaatagacctgaggtgagtaagagg aaaaaaaATGGTTGTTAAATTCACCGATTCTCAGATTTCAGCATTGATGGAATACGGTGATAACGATTG GTCTGAAGCGGAATTCGAAGATGCGGCGCGCGTGATAAAGAATTCTCTTCTCAGTTCTCTAAATTG AAATCTGCGAACGATAAAAGGTTTGAAGATGTTATTGCGAACCCCGGTAACGATTTGACCGAATTTGG AAAACAAAATTCGTGAAAAATGGCGGCGCGTGGTTTCATTGAAGTTCATACCCCGATTTCGTTTCT AAATCTGCGTTGGCGAAAATGACCATTACCGAAGATCATCCGTTGTTCAAACAGGTTTTCTGGATTG ATGATAAACGTGCGTTGCGTCCGATGCATGCGATGAACCTTTTCAAAGTTATGCGTGAATTGCGTGA TCATACCAAAGGTCCGGTTAAAATTTTCGAAATTTGTTTTCGTTAAAGAATCTAAATCTTCTA CCCATTTGGAAGAATTCACCATGTTGAACTTGGTTGAAATGGGTCCGGATGGTGATCCGATGGAACA TTTGAAAATGTACATTGGTGATATTATGGATGCGGTTGGTGTGAAATACACCACCTCTCGTGAAGAA TCTGATGTTTTGGTTGAAACCTTGGATGTTGAAATTAACGGTACCGAAGTTGCGTCTGGTTCTGTTGG </pre>

			<p>AACCATTTGGAAGAATTCACCATGTTGAACTTGTTCGAATTGGCGCCGACGGCGGATGCGACCGAAC GTTTGAAAAGAACATATTGCGACCGTTATGAACGCGGTTGGTTTGGCGTACGAATTGGTTGTTGAAGG TTCTGAAGTTTTCCGGTACCACCATTGATGTTGAAGTTGATGGTGTGTAATTGGCGTCTGGTGCGGTTG GTCCGTTGCCGATGGATAAACCCGATGGTATTACCGAACCGTGGGCGGGTGTGGTTCCGGTTTGGGA ACGTATTGCGTTGATGCGTACCAAGAACAGAACATTAAGAAAGTTGGTCGTTCTTTGGTTTACGTT AACGGTGGCGGATTGATATTTAAacttaattaacggcactcctcagctgtaaccaactcactggctcaggggtgAgcctttctcgcg cagaagggccaccggaaggtgagccagtgattacattatgccatggcgTAAACCTTTCTGCCAAATCTGGTAAAGTAGTTA ATGTTAGACGCGAAATCTGGGTGAAAACCGGACGGAAGTTAGACGGAATTTCCGGGAAGTAAACA TCACCTTCCGGTTCAATATCAATGGTAGAAATATGCAAGGTATCAACCTGATCAATCAAAGATTTGT AAATTCACCACCAGAAAACAATAACATGATCGGTAATTTTTTCAAGTTGGTCAACGCATCTTTA ATAGACGGGAAAAATCAAAACGTTTTTCGTTATCAGAGGTGAAAGAAGAACGGGTAACAACCGCTTAT TTACGGTTCGGCAACGCACCCATAGAACCGAAGGTTTTACGACCAACCAACAACCCTGGTTGTAGG TAATCGCTTTGAACAACAACCTGTTACCTTTTCGACAGACCACGGAATATCCGGACCGTTACCAATAAC ACCGTTTTTAGAAATCGCAACCATCAAGACAATTTCAaattctctcagaggttaacattttatagtaagcatgctctgtttg accgctgatgcttaccgtaataataataactgttataatagacttctctaaaaatacaagacactctgttattacaatcgtcgtgcccgtctactctgctgaag gccaataaggccgt</p>
pAB228p	ColA BBa_B0014 tetAp SD8 MmPylRS(rc) [wt] T3Te-T7Te DHFR(rc)		<p>aaacgtcctagaagatgccaggaggatactagcagagagacaataaggccggagcgaagccgttttccataggtccgccccctgacgaacatcacgaaatctg acgctcaaatcagtggtgccaaccgacaggactataaagatacagcgtttcccctgatgctccctctgctcctctctctgctccctgctgcccgtccgctccggtt gtggtggagcctttacccaatcaccacgtcccgttccgtgtagacagttcgtccaagctgggctgtgtgcaagaaccccccttgcagcccactgctgccccttacc cgtaactatcatcttgagtcacaaccggaaagacacgacaaaacggcactggcagcaccattgtaactgagaattagtgattatgatcagagatctgaagtg gtggcctaacagaggctactgaaaggacagattttggtatctgctcctcaaaagccagttaccaggttaagcagttccccactgactaaccttcatcaaacg cctccccagcggcttttctgttccagagcagagattacgacatcgtaaaaggatcctcaagaagatcAttacggattcccgaacaaagagacatccggttca cactggctcacttccgggtggccttctgctttatatactagagagagaataaaaaagccagattataatccggctttttatttagacttagggacccttccggcc gccattaatcctaattttgtgacactctatcattgataggttatttaccactccctacagtgatagagaaaagtgaatgaataAactgcaggtgagtaaggagg aaaaaaaaATGGATAAAAAACCGTTGAACACCTTGATTTCTGCGACCGGTTTGGGATGTCTCGTACCGG TACCATTCATAAAATTAACATCATGAAGTTTCTCGTTCTAAAAATTTACATTGAAATGGCGTGTGGTG ATCATTTGGTTGTTAACAACCTCTCGTTCTTCTCGTACCGCGCGTGCCTTGCCTCATCAATAAACCGT AAAACCTGTAACCGTTGTCGTGTTCTGATGAAGATTTGAACAAATCTTGACAAAGCAACGAAG ATCAGACCTCTGTAAAGTTAAAGTTGTTTCTGCGCCGACCGTACCAAAAAAGCGATGCCGAAATC TGTTGCGCGTGCGCCGAAACCGTTGAAAAACCGAAGCGGCGCAGGCGCAGCCGTTCTGGTTCTAA ATTCTCTCCGGGATTCCGGTTTCTACCCAGGAATCTGTTTCTGTTCCGGCGTCTGTTTCTACCTCTAT TTCTTCTATTTCTACCGGTGCGACCGCTCTGCGTTGGTTAAAGGTAACACCAACCCGATTAACCTCTA TGTCGCGCCGGTTCAGGCGTCTGCGCCGGGTTGACCAAAATCTCAGACCGATCTTTGGAAGTTTG TTGAACCCGAAAGATGAAATTTCTTTGAACTCTGGTAAACCGTTCCGTGAATTGGAATCTGAATTGTT GTCTCGTCGTAATAAAGATTTGCAGCAGATTTACGCGGAAGAACGTGAAAACACTTGGGTAATTTG GAACGTGAAATACCCGTTTCTTCGTTGATCGTGGTTTCTTGAAATTAATCTCCGATTTTGATTC GTTGGAATACATTGAACGTATGGGTATTGATAACGATACCGAATTGTCTAAACAGATTTCCGTGTT GATAAAAACTTCTGTTTGCCTCCGATGTTGGCGCCGAACTTGTACAACACTTGCCTAAATTTGGATCG TGCGTTGCCGGATCCGATTAATAATTTTCGAAATTTGGTCCGTGTTACCGTAAAGAACTGATGGTAAA GAACATTTGGAAGAATTCACCATGTTGAACTTCTGTCAGATGGGTTCTGGTTGTACCCGTGAAAACCT GGAATCTATTATTACCGATTTCTGAACCATTTGGGTATTGATTTCAAAATTTGGTGTATTCTGTAT GGTTACGGTGATACCTTGGATGTTATGCATGGTGATTGGAAATTTGCTTCTCGGTTTGGTCCGA TTCCGTTGGATCGTGAATGGGGTATTGATAAACCGTGGATTGGTGCGGGTTCCGGTTTGGAACGTTT TTGAAAGTTAAACATGATTTCAAAAAACATTAACCGTGCGGCGGTTCTGAATCTTACTACAACGGTA TTTCTACCAACTTGTAaacttaattaacggcactcctcagctgtaaccaactcactggctcacttccaggggtgAgcctttctcgcgagaaagggccc accggaaggtgagccagtgattacattatgccatggcgTAAACCTTTCTGCCAAATCTGGTAAAGTAGTTAATGTTAG ACGCGAAATCCTGGGTGAAAACCGGACGGAAGTTAGACGGAATTTCCGGGAAGTAAACATCACCTT CCGTTCAATATCAATGGTAGAAATATGCAAGGTATCAACCTGATCAATCAAAGATTTGTAATTTT ACCACCACCAGAAAACAATAACATGATCGGTAATTTTTTCAAGTTGGTCAACGCATCTTTAATAGAC GGGAAAATCAAAACGTTTTTCGTTATCAGAGGTGAAAGAAGAACGGGTAACAACCCGTTATTACCG TTCGGCAACGCACCCATAGAACCGAAGGTTTTACGACCAACCAACAACCCTGGTTGTATGTAATCG CTTTGAACAACAACCTGTTACCTTTTCGACAGACCACGGAATATCCGGACCGTTACCAATAACACCGTT</p>

			<p>CGATAAATACAAATCTGGTGAATTGTTGCTGGTGAATGAAAAAATTGTGTATTGAAACCTTGCAG GAATTCGTTAAAGCGTTCCAGGAACGTCGTGCGCAGGTTGATGAAGAAAACCTTGGATAAATTCATGG TTCCGCATAAATTGGTTTGGGGTGAAAAAGAACGTTTGGTTGCGCCGAAACCGAAAACCAAACAGG AAAAAATAAacttaattaacggcactcctcagctgaaccaactcactgctcactcactcaggggtgAgcctttctcgcgcagaagcccccgaag gtgagccagtggtattacattatgccatggcgtTAAACCTTTCTGCCAAATCTGGTAAGAGTAGTAAATGTTAGACGCG AAATCCTGGGTGAAAACCGGACGGAAGTTAGACGGAATTTCCGGGAAGTAGAAACATCACTTCCGGT TCAATATCAATGGTAGAAAATATGCAAGGTATCAACCTGATCAATCAAAGATTTGTAAATTTACCAC CACCAGAAACAATAACATGATCGGTAATTTTTTCAAGTTGGTCAACGCATCTTAATAGACGGGAA AATCAAAACGTTTTCTGTTATCAGAGGTGAAAGAAGAACGGGTAACAACCGCGTATTTACGGTTCGGC AACGCACCCATAGAACCGAAGGTTTTACGACCAACAACCAGTGGTTGATAGGTAATCGCTTTGA ACAACAACCTGTTACCTTTTCGACGACACCGGAATATCCGGACCGTTACCAATAAACACCGTTTTAGA AATCGCAACCATCAAAGACAATTTCAaattctctcagaggttaacattttatagtaagcatgctgctttgaccccgatgcttaccgtt aattaataatcactgtattaaatagaacttcttaaaaaatacagacactctgtattacaacatgctgctgcttaccctgcttcaagggcaataaaggcct</p>
pAMC070n1a12	ColA Bba_B0014 tetAp SD8 ScTrpRS rrmB1 proB SD8 LumIPylRS(rc) BbaB10014RNAlrpA+ J23100 SD8 MmPylRS(rc) T3Te-T7Te DHFR(rc)		<p>aaacgtctagaagatgccaggaggatacttagcagagagacaataaggccggagcgaagccgtttttccatagggctccgccccctgacgaacatcacgaaatctg acgctcaaatcagtggtggcgaacccgacaggactataaagataaccaggcgtttccccctgatggctccctcttgcgctcctctgttccccctgctgagccgctggt gtggtggagccttaccacaatcaccacgtccccctgctgtagacagttcctccaagctggcgtggtgcaagaacccccctcagccctcagcccttacc cgtaactatcatctgagtcacccggaagacacgacaaaacgccactggcagcagccattgtaactgagaattagtgattatgatcagagactctgaaagtg gtggcctaacagagctcactgaaaggacagattttggtatctgcgctccactaaagccagttaccaggttaagcagttccccactgacttaacctgatcaaacccg cctccccaggcggttttctgttacagagcaggagattacgacgatcgtaaaaggatcgaagaagatcAttacgattcccgaaccaagagacatccgggtca cactggctcaActcgggtggcctttctcgtttatatactagagagagaatataaaaagccagattattaaccggctttttattatagacttagggaccctgtgcttc tcaaatgctgagggcagttgctcaggctctccccgtggaggaataattgacgatgatcagtgacggcctaactaaggcctgctgactttctgccgatcaaaa ggcattttgctaaaggattgacgagggcgtatctgcccagtaagataattgtagcggatacaatagcagacaagatAATTTTGCACCCAGCAA ACTTGGTACGTAAACGCATCGTGGcGaacTGGTCCGGGACCACCgaGCaTtctAAGCtCGGtAGCCGGGT TCAACTCCCGGGtCgTCGCCAAAATTTGAAAGTGCTGTAAGGCACAGACCACCCAGCCGAGCGGTC CGGCGACCAGCAGGCCTctatAAGCCTCGCCTTGCGGGTTCGACACCCCGTCTCTCGCCAGTTAAA AGACATCGGCGTCAAGCGGATGTCTGGCTGAAAGGCCTGAAGAAATTTGGACCTGTGATCATGTAGAT CGAGAGGCTTCTAATCCATATCAGCCGGGTAGATTTCCCGCGGGTCCGCCATCTCTTACTGTATATG GCTTAGTAGCGGTATCAATATCAGCAGTAAAATAAATTTCCCGATCCCGCCCTCAGAGGTTAG AGCGTGCcTCTtagAAggGCATGGTCCCGGTTCAAATCCTGGGGGCGGGACCAACTACTTTATGTAGT CTcGCCGTGTAGCAAGAAATTGAGAAGTGAAGTGGTGGCTCAATGGTAGAGCTGCACGCTTCCGAA CGTGTGGTTGCAGGTTCAAGTCTGTCCAGTTCACCAATTTATTCAAGACGCTTACCTTGTAAGTGC ACCCAGTtaggatccgtcagactcagatccttagcgaagaagtaaggatttttttaagcttctgcatcaccagacttagggaccctgcccggccattaatc ctaatttgtgacactctatcattgatagattttttaccactccctatcagtgatagagaaaagtgaatgaatagacctcaggtcagtaaggagaaaaaaaA TGCTAACGATGAAACCGTTGAAAAAGTTACCCAGCAGGTTTCTGAATTGAAATCTACCGATGTTAA AGAACAGGTTGTTACCCCGTGGGATGTTGAAGGTGGTGTGATGAACAGGGTCGTGCGCAGAACATT GATTACGATAAATGATTAACAGTTCGGTACCAAACCGGTTAACGAAGAAACCTTGAACGTTTCA AACAGGTTACCGGTCGTGAACCGCATCTTTCTTGGTAAAGGTTGTTCTTCTTGAACGTTGATTTT ACCAAAATTTTGGATTTGTACGAACAGGGTAAACCGTTCTTCTTggtaccGGTCTGGTCCGTCTTCTG ATTCTATGCATTTGGGTCATATGATTCGGTTCGTTTTACCAAATGGTTGCAGGAAGTTTTCGATGTT CCGTTGGTTATTcggTTGACCGATGATGAAAAATTTCTGTTCAAAACATAAATGACCATTAACGATGTT AAAACTTCGCGCGTGAACCGGAAAGATATTATTGCGGTTGGTTTCGATCCGAAAAACACCTTCA TTTTCTGATTTGACGATACATGGGTGGTGGTCTACGAAACCGTTGTTCTGCTCTCTCGTCAATTA CCGGTTCTACCGGAAAAGCGGTTTTCGGTTTCAACGATTCTGATTGATTGGTAAATTCATTTCCGG TCTATTAGATTGCGgtGCGTTCCCGTCTTCTTCCCGAACGTTTGGGTTTGCCGGATAAAACCCCGT GTTTgtgctcgtGCGATTGATCAGGATCCGTACTTCCGTGTTTGTGCTGATGTTGCGGATAAATGAAAT ACTCTAAACCGGCGTTGTTGCATTTCTGTTTCTTCCCGCGTTGCAGGTTTCTACCACCAAAATGTCT GCGTCTGATGATACCACCGGATTTTCTATGACCGATACCCCGAAACAGATTACAGAAAAAATTAACA AATACGCTTCTCTGGTGGTCAAGTTCGCGAATTTGCATCGTGAATTGGGTGGTAACCCGGATGTT GATGTTGCGTACCAGTACTTGTCTTTCTTCAAAGATGATGATGTTTTCTTGAAGAATGTTACGATAA ATACAAATCTGGTGAATGTTGTCTGGTGAATGAAAAATTTGTGATTGAAACCTTGCAGGAATTC GTTAAAGCGTTCCAGGAACGTCGTGCGCAGGTTGATGAAGAAACCTTGGATAAATTCATGGTTCCGC ATAAATGGTTTTGGGGTGA AAAAGAACGTTTGGTTGCGCCGAAACCGAAAACCAAACAGGAAAAAA</p>

			<p>AATAAactaattaacggcactcaataaaacgaaggctcagtcgaaagactgggaccttctgttctgagggagacttagggaccctcacagtaacaccacgtcgtccctatctgctccctaggtctatgagtggttctGGATAACTTTACGGGCATGCATAAAGGCTCGTAATATATATTCAGGgagaccacaacggttccctctacaataattttttaactttacctgcaggtgcagtaagaggagaaaaaaATGGATACCCGTTTGACCCCGGCGCAGGCGCAGCGTATTCGTGAAATGGGTGGTACCGTTGATCCGTCTTTGGCGTTCTCTTGAAGCGGAACGTGAATCTGCGTTCCAGCGTATTTCTGCGGATTTGCAGGGTGCAGAACTTGGCGAAAAATTCGTCGTTGTGCGGAAGCGCCGGAACGTTCATCCGATTGGTTCTTTGAAAACACCTTGGCGGTGCGTTGGCGAAAGGTTTCATTGAAGTTAAAACCCCGATGATGATTCCGGCGGATGGTTTGGTTAAAATGGGTATTGATGAATCTCATCCGTTGTGGAACCAGGTTTTCTGGGTTGGTCCGAAAAAAGCGTTGCGTCCGATGTTGGCGCCGAACATTTTCTTCTGGGTCGTCATTTGCGTCGTTCTGTTCCGGCGCCGTTGTTGTTGTCGAAATGGTCCGTTTCCGTAAGAATCTCGTGGTTCTAACCATTTGGAAGAATTCACCATGTTGAACTTGTTCGAATTTGGCGCCGACGGCGGATGCGACCGAACGTTTGAAGAACATATTGCGACCGTTATGAACGCGTTGGTTTGCCTACGAATTGGTTGTTGAAGTTCTGAAGTTTCCGTACCACCATGATGTTGAAGTTGATGGTGTGAATTGGCGTCTGGTGGGTTGGTCCGTTGCCGATGGATAAACCCGATGTATTACCGAACCGTGGGCGGGTGTGGTTCCGTTTGGAAACGTATTGCGTTGATGCGTACCAAAGAACAGAACAATAAAAAAGTTGGTCTTTGGTTTACGTTAACGGTGCAGGATTTAAcggagccaatgctcagegtcacactggctcacttcgggtgggaccttctgctttatatactagagagagaataaaaaagccagattattaacccgctttttattattgga tccgatccggcaacaaccaccggtgtagcggtgtttttttgaccgtaaaaaaagcccgctattagcgggctgggtatgattgctggtgacTTGACGGCTAGCTCAGTCCTAGGTACAGTGCTAGCggtgcagtaaggagaaaaaaATGGATAAAAAACCGTTGAACACCTGATTTCTGCGACCGGTTTGTGGATGTCGTACCGTACCATTCAAAAATAAACACTCGTAAGTTCTCGTTCTAAAATTTACATTGAAATGGCGTGTGGTATCATTGGTTGTTAACAACTCTCGTTCTTCTCGTACCAGCGGTGCGTTCATCATAAATACCGTAAAACCTGTAAACGTTGTCGTGTTCTGATGAAGATTTGAACAAATTTTGACCAAAGCGAACGAAGATCAGACCTCTGTTAAAGTTAAAGTTGTTTCTGCGCCGACCCGTACCAAAAAAGCGATGCCGAAATCTGTTGCGCGTGCGCCCAACCCGTTGGA AACACCGAAGCGGCGCAGGCGCAGCCGTCTGTTTCTAAAATTTCTCTCCGCGCTCCGCGTTTCTACC CAGGAATCTGTTTCTGTTCCGGCGTCTGTTTCTACCTCTATTTCTTCTATTTCTACCGGTGCGACCGGCTGCGTTGGTTAAAGGTAACACCAACCCGATTACCTCTATGTCGCGCCGTTACAGGCGTCTGCGCC GGCGTTGACCAAACTCAGACCGATCGTTTGGAAAGTTTGGTTGAACCCGAAAGATGAAATTTCTTTG AACTCTGGTAAACCGTTCCGTAATTTGGAATCTGAAATTTGTTGTCGTCGTAATAAAAGATTTGAGC AGATTTACGCGGAAGAACGTGAAAACACTTGGGTAATTTGGAACGTGAAATTTACCCGTTTCTTCGT TGATCGTGGTTTCTTGGAAATTAATCTCCGATTTGATTCCGTTGGAATACATTGAACGTATGGGTA TTGATAACGATACCGAATTGTCTAAAACAGATTTCCGTTGTTGATAAAAACTTCTGTTTGCCTCCGATG TTGGCGCCGAACCTGTACAACACTTGCCTAAATTTGGATCGTGCCTTGCCTGATCCGATTAATAATTT CGAAATTTGGTCCGTTTACCCTAAAAGAAATCTGATGGTAAAGAACATTTGGAAGAATTCACCATGTTG AACTTCTGTCAGATGGGTTCTGGTTGTACCCGTGAAAACCTTGGAAATCTATTATTACCGATTTCTTGAA CCATTTGGGATTTGATTTCAAAATTTGTTGGTATTCTGTATGGTTACGGTGATACCTTGGATGTTAT GCATGGTGATTTGGAATTTGCTTCTGCGGTTGTTGGTCCGATTCCGTTGGATCGTGAATGGGGTATTG ATAAAACCGTGGATTGGTGCGGGTTTCCGTTTGGAAACGTTTGGTTGAAAGTTAAACATGATTTCAAAAA CATTAAACGTGCGGCGCGTTCTGAATCTTACTACAACGGTATTTCTACCAACTTGTAAactaattaacggcact cctcagctgaaccaactcactggctcacttcacgggtgAgcccttctcgcgcagaaaggccaccggaaggtgagccagtgattacattatgccatggcgt TTAACCTTTCTGCCAAATCTGGTAAGAGTAGTTAATGTTAGACGCGGAAATCTGGGTGAAAACCGGA CGGAAGTTAGACGGAATTTCCGGGAAGTAAACATCACCTTCCGTTCAATATCAATGGTAGAAATAT GCAAGGTATCAACCTGATCAATCAAAGATTTGTAATTTTACCACCACCAGAAACAATACATGATC GGTAATTTTTTCAAGTTGGTCAACGCATCTTAATAGACGGGAAAAATCAAACGTTTTCTGTTATCAG AAGTAAAAGAAGAACGGGTAACAACCGGATTTACGGTTCGGCAACGCACCCATAGAACCGAAGG TTTTACGACCAACCAACAACCACTGGTTGTAGGTAATCGCTTTGAACAACAACCTGTTCACTTTCCGCA GACCAGGAATATCCGGACCGTTACCAATAACACCGTTTTTGAAGTTCGCAACCATCAAGACAATT TCA Taatttctcctcagaggttaacattttatagtaagcatgctcgtttgtcaccgctgatgctaccgtaataataatacactgtattaaatagactttctctaaaaa tacaagacactctgtattacaatcgtcgtgcccgtctctcgttcaaggccaataggccgt</p>
sfGFP and tRNA operon	pAMC042z21	RepA(rc)(N99D) SC101 T0-BS7 proD SD8 sfGFP(rc) (V93AGGA Y151UAGA) ProK 5 tRNA operon tetA-orfL bla(rc)	<p>TTAAATACGACCGTATTTCCGCCAAAATGTTTTCCAAGGTGGTACGCTGTTTCTGGGTCAACCAAGAG AAAGAACCGTTCAAATCATGTTTAGACTGCATACGACAAGAATTTGCTTCCGAAAGAGGTCAACT GAATTTTCCGCGTCAACGCATCATGCAAGGTTTTTACGCAAACCGTTACGCAAGTAAGAATCAGAGGT AATGGTGGTCCGAATTTTATCACCGTTCATTTAATCTGGTTGTTTTCCAATTCGGTAACCAATCCA</p>

		<p>TCTGACGATCCAATTCAACCTGGAAAATCAAGGTATCGGTCCGACGACCACGTTTATCAACAACCAA TTTCATGTTAGAGTAGGTGTTCAAATCTTTAGAAAATCGGTTTCAAAAACCCACTGGTTCAAACGTTTGA ATTCATGGTAGTTGTTTTCCAACATCAACATGAATTTGAATTCATCCAAAGAAAATTTCAATGTTTCGCT TTATGGGTTTTTTCTGGGTCAATTCCTTCAACAACCATTCGTAATAACGCATAGAGTATTTGTTTTCG AAAGATTTAACATGTTCCAAGTTGTATTTAATGAATTTTTTCAACTGGAACAAGTACGGCAAAAATTTTC TTCAGAGAAAACCAATTCCAATTTTCAGAAGAGAATTTTCGCGTAATTCGGTCCACTGGAAAATTTTCG AAACCTTTAACCAACGGGTTACGAATTTCAACGGTACGGGTCAATTCACGGGTTCGTTTCGCCA AAACACCGTACGCGTTTTCACGAGAAAATGTTTCATCATCTGCGCGTACTGGTTGTAGGTGAAAAGAAAC GGTACGTTCTTACGGGTTCGGGTTTTCAATGGTTCGGGTTCAACAACGCAACACAACAACAAAATCAAT TTGGTTTCATGTTTCGGTCAAATCGTAACGAGAAAATCGCCAATTCGTTTCGCTTGA AAAACAACCAATTC AGACATAcactcCaattgctagggatTTtaacACTATAccaattgagatgggctAGTCAAtgataaactagctcttcttctgagttgggtatctg taaattctgctagaccttctggaanaactgtaaattctgtagacctctgtaaattccgctagacctttgtgtttttttgtttatattcaagttggtataattatagaataaa gaaagaataaaaaagataaaaaagatagatcccagccctgtgtataactcactacttttagtcagttcccgagttattcaaaaaggatgctgcaaacgcgtttgtctcct acaaaaagaccttaaacctaaaggcttaagtagcacctctgcaagctcgggcaaatcgctgaatattcctttgtctccgacctagggacctgagtcgctgctt ttcgtgacattcagttcgtcgtcgtcacggctctgacagtgaaatgggggtaaatggcactacagggccctttatgattcatgcaaggaactaccataacaaga aagcccgtcagggcttctcagggctttatgcccgtctctatgtggtctctatctgacttttctgctgtcagcagttctgcccctctgatttccagctgaccactcgg attatcccgtgacaggtcattcagactggctaatgcaccagtaaggcagcggatcatcaacaggttaccctcttactgtcaagagacatccggtttgtcagaac gctcggtctgcaaccgggctttttcttctgtgagtcctatgactgaataataaaaaacagccgttccgcaaaagagggcagcgctttttattttagacttaggga ccctCACAGCTAACACCACGTCGTCCTATCTGTGCCCTAGGTCTATGAGTGGTCTGATAACTT ACGGGCATGCATAAGGCTCGTATAATATATTCAGGGAGACCACAACGGTTTCCCTCTACAAAATAATT TTGTTAACTTTacctgcaggtgcagtaaggaggaaaaaaaATGTCATAAAGGTGAAGAATTGTTACCGGTGTTGTT CCGATTTTGGTTGAATTGGATGGTGTATGTTAACGGTCATAAATTCTCTGTTCGTGGTGAAGGTGAAG GTGATGCGACCAACGGTAAATTGACCTTGAATTCATTTGTACCACCGGTAATTTGCCGGTTCGGTG GCCGACCTTGGTTACCACTTGACCTACGGTGTTCAGTGTTTCTCTCGTTACCCGGATCGATGAAAAC AGCATGATTTCTTCAAATCTGCGATGCCGGAAGGTTACaggaCAGGAACGTACCATTTCTTTCAAAGAT GATGTTACCTACAAAACCGTGCAGGAAAGTTAAATTCGAAGGTGATACCTTGGTTAACCGTATTGAAT TGAAAGGTATTGATTTCAAAGAAGATGGTAAACATTTTGGGTCAATAATTGGAATACAACCTTCAACTC CTATAACGTTtagaATTACCGCGGATAAACAGAAAAACGGTATTAAAGCGAACTCAAAATTCGTCAT AACGTTGAAGATGGTTCTGTTCAAGTTGGCGGATCATTACCAGCAGAACACCCCGATTGGTGATGGTC CGGTTTTGTTGCCGATAAACCATTACTTGTCTACCCAGTCTGCGTTGTCTAAAAGATCCGAACGAAAAA CGTGATCATATGGTTTTGTTGGAATTCGTTACCGCGGCGGGTATTACCCATGGTATGGATGAATTGTA CAAAGTTCTCATCATCATCATCATATAaaactaattaacggcactcaataaaaacgaaaggctcagtcgaaagactggccttctgtttg ctgaggagacttagggacccttctgcttcaaatgcctgagggcagtttgcacaggtctccccgtggaggtaataatgacgatatgacagtcgacggcctaactaag cgccctgctgactttctcggatcaaaaaggcatttctatataaggattgacgagggctatctgctgagtaagataattgtgagcggataacaattagcagacaaga tAATTTTGCACCCAGCAAACCTTGGTACGTAAACGCATCGTGGcGaacTGGTCCGGGACCACCgaGCATtctc AAGTcCGGttAGCCGGGTTCAACTCCCCGGGttCgTCGCCAAAATTTGAAAAGTGTGTAAAGGCACAGACC ACCCAAAGGGGGACGGTCCGGCGACCAGCAGGCCctatAAGCCTCGCCTTGCGGGGTTCGACACCCCG GTCTCTCGCCAGTTTAAAAGACATCGGGCTCAAGCGGATGTCTGGCTGAAAGGCCTGAAGAAATTTGG ACCTGTGATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGTTAGATTCCCGGGCGGGTCCG CCATCTCTTACTTGATATGGCTTTAGTAGCGGTATCAATATCAGCAGTAAAATAAAATTTCCCGATCCC GCCcTAGCTCAGAGGTAGAGCGTGCcTCTctagAAggGCATGGTCCCCGGTTCAAATCCTGGGGCGGG ACCAACTACTTTATGTAGTCTcGCCGTGTAGCAAGAAATTGAGAAGTGAACCTGGTGGTCAATGGT AGAGCTGCACGCTTCCGAACGTGTTGGTTGCAGGTTCAAGTCTGTCCAGTTCACCAATTTATTCAAG ACGCTTACCTTGTAAAGTGCACCCAGTtaggatccgtcagctcagatccttagcgaagctaaggatttttttaagcttctgccatcatccgac ttaattaaggcactcctcagaaataatgacctcttgataaccaagaggcatttttaagcccagtcgtTTACCAATGTTTAAATCAAAGA CGCACCAATTTCCGCAATCTGACGGTTACGTTTACCATGGTTCGCTGAGAACCCTGGTGGTGAATA ACAACAATACGAGACGGTTTACCATCCGGACCCAACGCCGCAATAATACCACGAGAACCACGTTCA CCCGCACAGATTTATCCGCAATGAACCAACCCGCCGGCAACGCAGAACGCAACAACGGACCCGCA ACTTTATCCGCTTCCATCAATCAACTGCTGACGAGACGCCAAGGTCAACAATTCACCGGTCA ACAATTTACGCAAGGTGGTTCGCATCGCAACCGGCATGGTGGTATCACGTTCAACCTCGAATTCG TTTCGTTCAATTCGGTTCCCAACGATCCAAACGGgTAACATGaTACCCCATGTTATGCAAGAACCGG TCAATCTTTTCGGACCACCAATGGTGGTCAACAACAAGTTCGCCGGGTGTTATCAGACATGGTAAT</p>
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			<p>CGCCGCAGAACACAATTCACGAACGGTCATACCATCGGTCAAATGTTTTTCGGTAACCGGAGAGTAT TCAACCAAATCGTTCTGAGAGTAATGAATACGACGACCCAACCTGTTCCGACCCGCATCAATACGAG ACAAAACCGCACACACAACAAAACCTTTGAAGGTAGACATCATCGGGAAACGTTCTTCCGGACGGA AAGATTCCAAAATTTTACCAGAGTCAAATCCAATTCATGTAACCAACACGCGCACCCAACCTGATC TTCCGCATCTTTAACTTTAACCAAGGTTTCCGGATGCGCGAAAAACCGGCAAACAGAACGCCGCGAAG AACGGAATCAACGCAACACGGAAATGCTGAATAGACA Tactctctctttcaatattattgaagcattatcagggfattgtctcatg agcggatacatatttgaatgtatttagaaaaataaaggccaaataAgccgt</p>
<p>Macrocycle biosynthesis and tRNA operon</p>	<p>pAAP00312</p>	<p>RepA(rc)(N99D) SC101 T0-BS7 araC(rc) pBAD SD8 NpuC-C[AGGA]LFVY-NpuN-SsrA(rc) ProK 5 tRNA operon tetA-orfL bla(rc)</p>	<p>TTAAATACGACCGTATTTTCGCCAAAATGTTTTCCAAGGTGGTACGCTGTTTTCTGGGTCAACCAAGAG AAAGAACCGTTCAAATCATGTTTAGACTGCATATCAGACAAGAATTTTCGTTTCGAAAAGAGGTCAAAT GAATTTTCGCGGTCAACGCATCATGCAAGGTTTTACGCAAACCGTTACGCAAGTAAGAATCAGAGGT AATGGTGGTCGGAATTTATCACCGTTCATTTAATCTGGTTGTTTTCCAATTCGGTAACCAAAATCCA TCTGACGATCCAATTC AACCTGGAAAATCAAGGTATCGGTTCGGACGACCACGTTTATCAACAACCAA TTTCATGTTAGAGTAGGTGTTCAAATCTTTAGAAAATCGGTTTCAAACCCACTGGTTCAAACGTTTGA ATTCATGGTAGTTGTTTTCCAACATCAACATGAAATTTGAATTCATCCAAAGAAAATTTCAATGTTTCGCT TTATGGGTTTTTTCTGGGTCAAATTTTCAACAACCAATTCGTAATAACGATAGAGTATTTGTTTTCTC AAAGATTTAACAATGTTCCAAGTTGATTTAATGAAATTTTTTCAACTGGAACAAGTACGGCAAAAATTTTC TTCAGAGAAAACCAATTC AATTTTTTCAGAAGAGAATTTTCGCGTAATTCGGTCCACTGAAAATTTTCG AAACCTTTAACCAACGGGTTACGAATTTCAACGGTACGGGTCAATTCACGGGTTCGTTTTTCGCCA AAACACCGTACGCGTTTTCACGAGAAAATGTTTCATCATCTGCGCGTACTGGTGTAGTAAAAGAAAAC GGTACGTTCTTTACGGGTCCGGTTTTCAATGGTCCGGTTCAACAACGCAACACAACCAAAAATCAAT TTGGTTTCATGTTTCGGTCAAATCGTAACGAGAAAATCGCCAATTCGTTTCGTTTTGAAAACAACCAATTC AGACATAcactcaattgtctaggtgatttaacACTATAc aattgagatggcctAGTCAAAtgataactatgctctttcttggattggtgattctg taaattctgtagaccttctggtgaaaactgttaattctgtagaccctctgtaattccgctagacctttgtgtttttttttatattcaagtggtataatttagaataaa gaaagaataaaaaagataaaaaagaaatagatcccagccctgtgtataactcactacttagtctagctccgagattatacaaaaagagctgtttgtctctct acaaaaagacacttaaacctaaaggcttaagtagcaccctcgaagctcgggcaaatcgtgaatattcctttgtctccgacctcaggcaccctgagtcgctgcttt ttcgtgacattcagttcgtcgtcagcggctcgtcagtgatgaggggtaaatggcactcagcgcctttatggattatgcaaggaactaccataatacaagaa aagcccgtcagcggcttctcagcggcttttatgctggctgctgctatggtgctgctgctttttgtctgacagcttctgctctgatttccagctgaccactcgg atfatccgtgacaggtcattcagactggtatgcaaccagtaagcagcgggtatcatcaacaggttaccctctactgtcaagaggacatccggtttgtcagaac gctcggcttgcacacggcgtttttctgtgagctcatgacactgaaataaataaaaaacagcgttgcagaaaagagcagcggctgtttttattttagacttaggga cccttaagacaatttaaccgaacatgtaacttttttcacaaccgcaggaattcagacggagacgaccggtacatttttgaaacacgagagaagtacaact gatcatcgaaccaactttacgaccaacggctcgaatcggcctacgggtgtagacaacaacaatttcgctgagaataacgctgatctcagcccaagacaaca gaaataccaactgctgacggaacaatagacaacgagacggagacaacaacaatcgtcgcgaacagacgaatatacgaagtgaatccgcaaatgatca gaaatgtactgacacgcttcacgaacagcgtttatccatcggcggatgcaagattcgttaatcgttccatacagcgaacaacaactgtccaacaagtaatcgccea caattcagagtaacgacctcaccctgaccgcgttaataatcgaaccgaacaatcagagaatcggctgatgcttccatccggaggaagaacccggtgttcgc gaaaatagacggcagttcaaccattcatgccagtagcacgcggaggaagtaaacctgatggtaccattcacgcgttccggatgacgaccgtaatgatgaa ttcaccggcgggaacaacaaatatacccgagacaacgaattcagaccctggttttaacaacaccctgaccaggaatggtcaagttcaaatgtaaccttc ataccaacggacgatcaatgaagaatccaagtaaccgttcctcaatcggggtcaaacccgcaaccaaatgcccgttgaagagtaaccggcaacaacggat cgttctcgcctccgcaactttcatactccaccattcagagaagaacaattgtccatattcatcagacattgcccactcgtcttttactggtctctctcgtaac ccaaccgtaaccccgttataaaagcattctgtaacaaagcgggaccaaaagccatgacaaaacgcgtaacaaaagtgtctataatcagcgcagaaaagtccacat tgattttgacggcgtcacactttgctatgccatagcattttatccataagattagcggatcctactgacgctttttatcgaactctctactgtttccataccggtttac ctgaggtgcagtaaggaggaaaaaaaATGATTAATAATTGCGACCCGTAATAACTTGGGTAAACAGAACGTTTACG ATATTGGTGTGAAACGTGATCATAACTTCGCGTTGAAAAACGGTTTCACTTGGCTCAACTGTAGGATT GTTCTGTTACTGTTTGTCTTACGAAACCGAAAATTTTGACCGTTGAATACGGTGTGTTGCCGATTGGTA AAATGTTGAAAAACGTATTGAATGTACCGTTTACTCTGTTGATAACAACGGTAACATTTACACCCA GCCGTTGCGCAGTGGCATGATCGTGGTGAACAGGAAGTTTTTGAATACTGTTTGGAAAGATGGTTCT TTGATTCGTGCGACCAAAGATCATAAATTCATGACCGTTGATGGTACAGATGTTGCCGATTGATAAA TTTTCGAACGTGAATTTGATTTGATGCGTGTGATAAATTGCGGAACGGTACC CGCGGCAACGATGA AAACTACGCGTTGGCGGCTAAacttaattaacggcactcaataaaacgaaaggctcagtcgaaagactggcctttctgttctgaggagact tagggaccctgtgcttcaaatgctgagccagttgtcagcgtctcccgtgaggttaataatgacgatatgatgacagcgtactaaagcggcctgctga ctttctcggatcaaaagcattttgcttaaggattgacgagggcgtatctcgcagtaagataattgtgagcgatacaattagcagacaagatAATTTT GCACCCAGCAAACCTTGGTACGTAACGCATCGTGGcGaacTGGTCCGGGACCAACgaGCaTcttAAAGtC GGttAGCCGGGTTCAACTCCCGGttCgTCGCCAAAATTTGAAAGTGTGTAAGGCACAGACCACCAA</p>

			<p>GGGGGACGGTCCGGCGACCAGCAGGCCTctatAAGCCTCGCCTTGCGGGGTTTCGACACCCCGGTCTCTC GCCAGTTTAAAAGACATCGGCGTCAAGCGGATGTCTGGCTGAAAAGCCTGAAGAATTTGGACCTGTG ATCATGTAGATCGAGAGGCTTCTAATCCATATCAGCCGGTTAGATTCCCAGCGGGTCCGCCATCTC TACTTGATATGGCTTTAGTAGCGGTATCAATATCAGCAGTAAAATAAATTTCCCGATCCCGCCcTAG CTCAGAGGTAGAGCGTGCcTCTctagAAggGCATGGTCCCCGGTTCAAATCCTGGGGGCGGGACCAACT ACTTTATGTAGTCTcGCCGTGTAGCAAGAAATTGAGAAGTGAACCTGGTGGCTCAATGGTAGAGCTG CACGCTTCCGAACGTGTTGGTTGCAGGTTCAAGTCTGTCCAGTTCACCAATTTATTCAAGACGCTTA CCTTGTAAGTGCACCCAGTaggatccgtcgacctgcagatccttagcgaaagctaaggattttttaagctctgccaatccgacttaattaacgge actcctcagcaataataatgacctcttgataaccaagaggcatttttaatgccatggcgtTTACCAATGTTTAAATCAAAGACGCACCA ATTTCCGCAATCTGACGGTTACGTTTCATCCATGGTCGCCTGAGAACCAGGTGGTGTAAATAACAACAA TACGAGACGGTTTACCATCCGGACCCAACGCCGAATAATACCACGAGAACCACGTTACCCCGCACC AGATTTATCCGCAATGAACCAACCCGCCGGCAACGCAGAACGCAACAACGGACCCGCAACTTTATC CGCTTCCATCCAATCAATCAACTGCTGACGAGACGCCAAGGTCAACAATTCACCGGTCAACAATTTA CGCAAGGTGGTCCCATCGCAACCGGCATGGTGGTATCACGTTTCATCGTTCCGGAATCGCTTCGTTCA ATTCCGGTTCCCAACGATCCAAACGGgTAACATGaTCACCCATGTTATGCAAGAACGCGGTCAATTCT TTCGGACCACCAATGGTGGTCAACAACAAGTTCGCCCGGGTGTATCAGACATGGTAATCGCCGAG AACACAATTCACGAACGGTCAATACCATCGGTCAAATGTTTTTCGGTAACCGGAGAGTATTCAACCAA ATCGTTCTGAGAGTAATGAATACGACGACCCAACCTGTTCTGACCCGCATCAATACGAGACAAAAAC GCACCACACAACAAAACCTTGAAGGTAGACATCATCGGAAACGTTCTTCCGGACGGAAAGATTCC AAAATTTTACCAGAGTTCAAATCCAATTCAATGTAACCAACACGCGCACCCAACCTGATCTTCCGCAT CTTTAACTTTAACCAAGGTTTCCGGATGCGCGAAAACCGGCAAACAGAACCCGCGAAGAACGGAA TCAACGCAACACGGAAATGCTGAATAGACATactcttccctttcaatatttagaagcattatcagggtattgtctcatgacggatacat atthaatgtatttagaaaataaaggccaataAgcgt</p>
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Supplementary Table 14 | Key Plasmids From this Study for Plug-and-Play Genetic Code Expansion.

Plasmid classification, ID, description, and sequence are given for the prioritized plasmids generated in this study.

Supplementary Data 1 | Mass Spectrometry Data for Supplementary Figure 3.

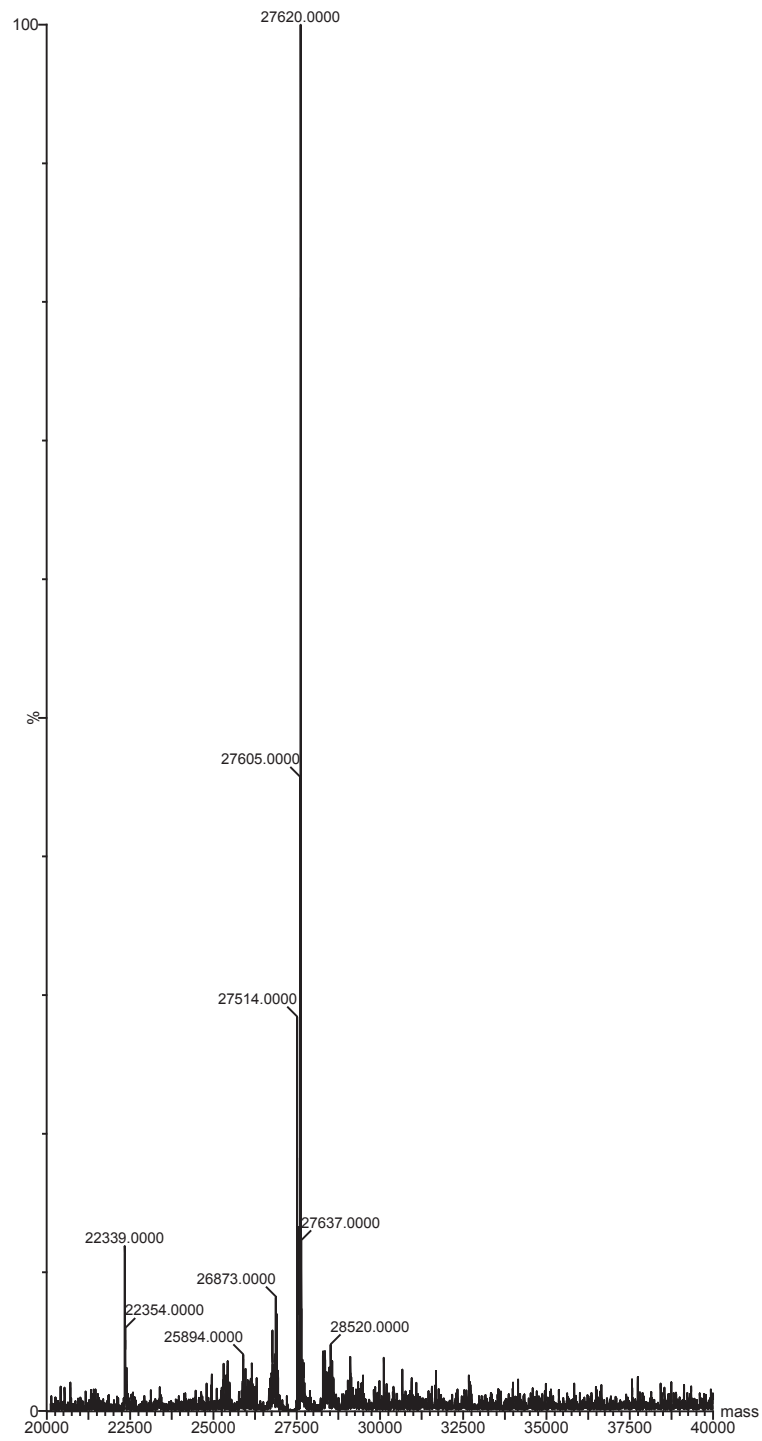
Condition: Library

Codon: UAG

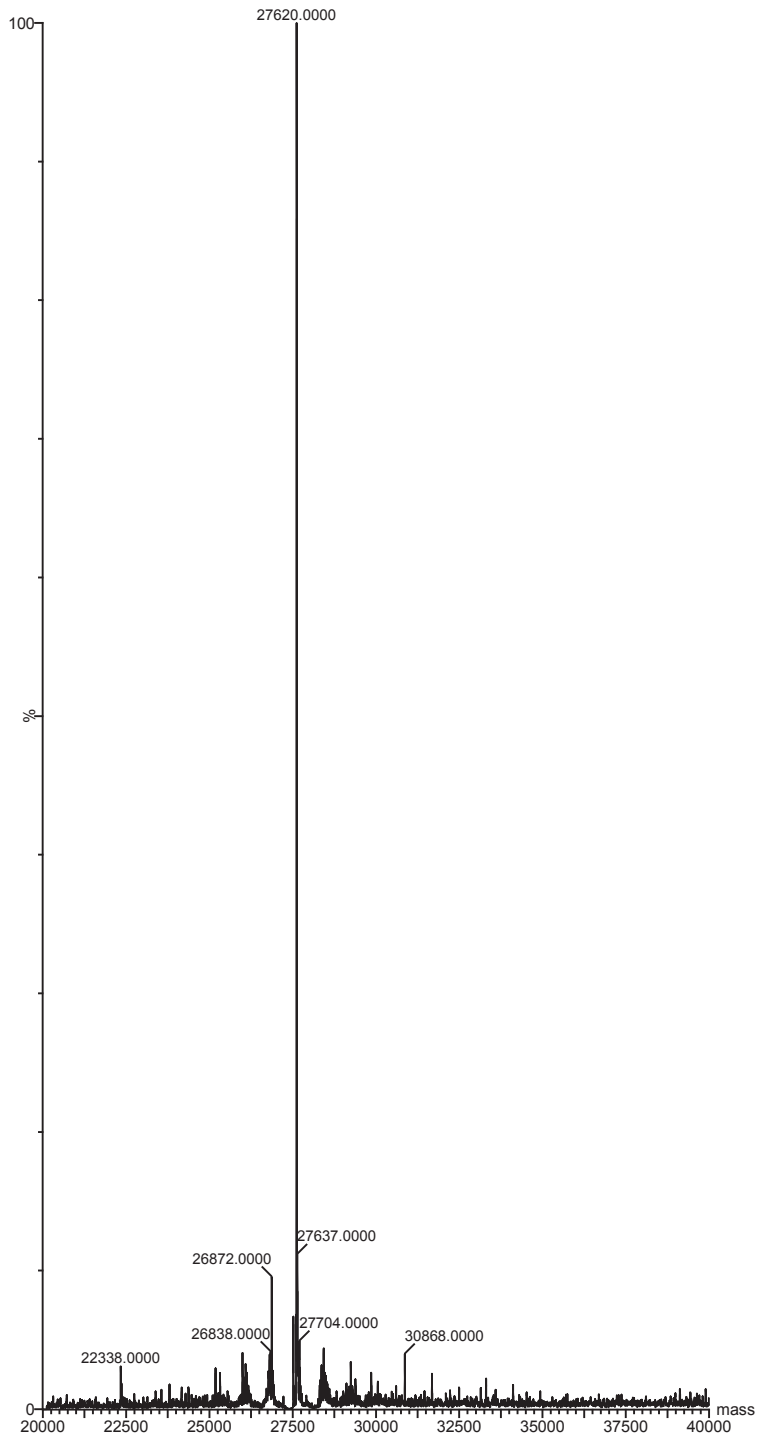
AA/ncAA: N6-Boc-L-lys

Expected mass: 27606

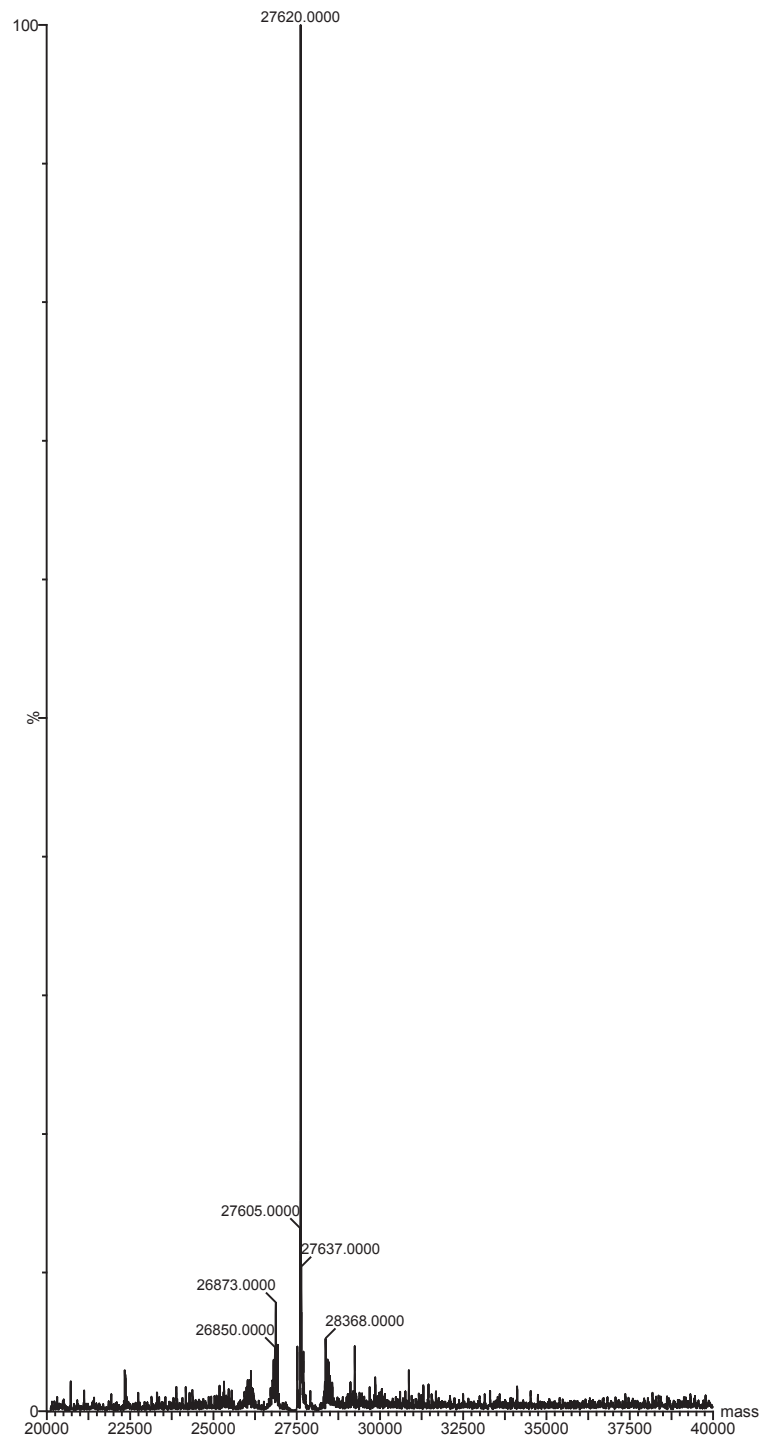
Observed mass: 27620



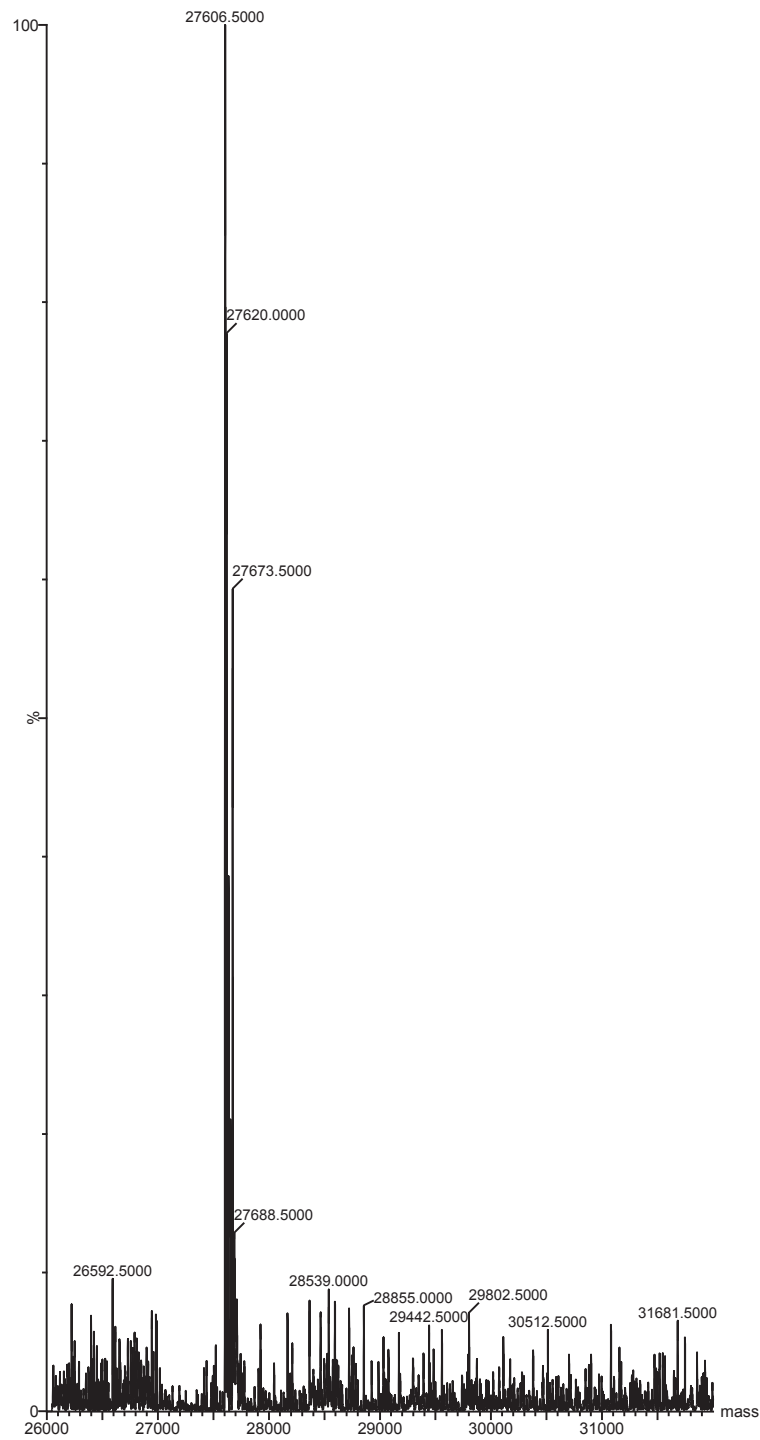
Condition: Bottom 1%
Codon: UAG
AA/ncAA: N6-Boc-L-lys
Expected mass: 27606
Observed mass: 27620



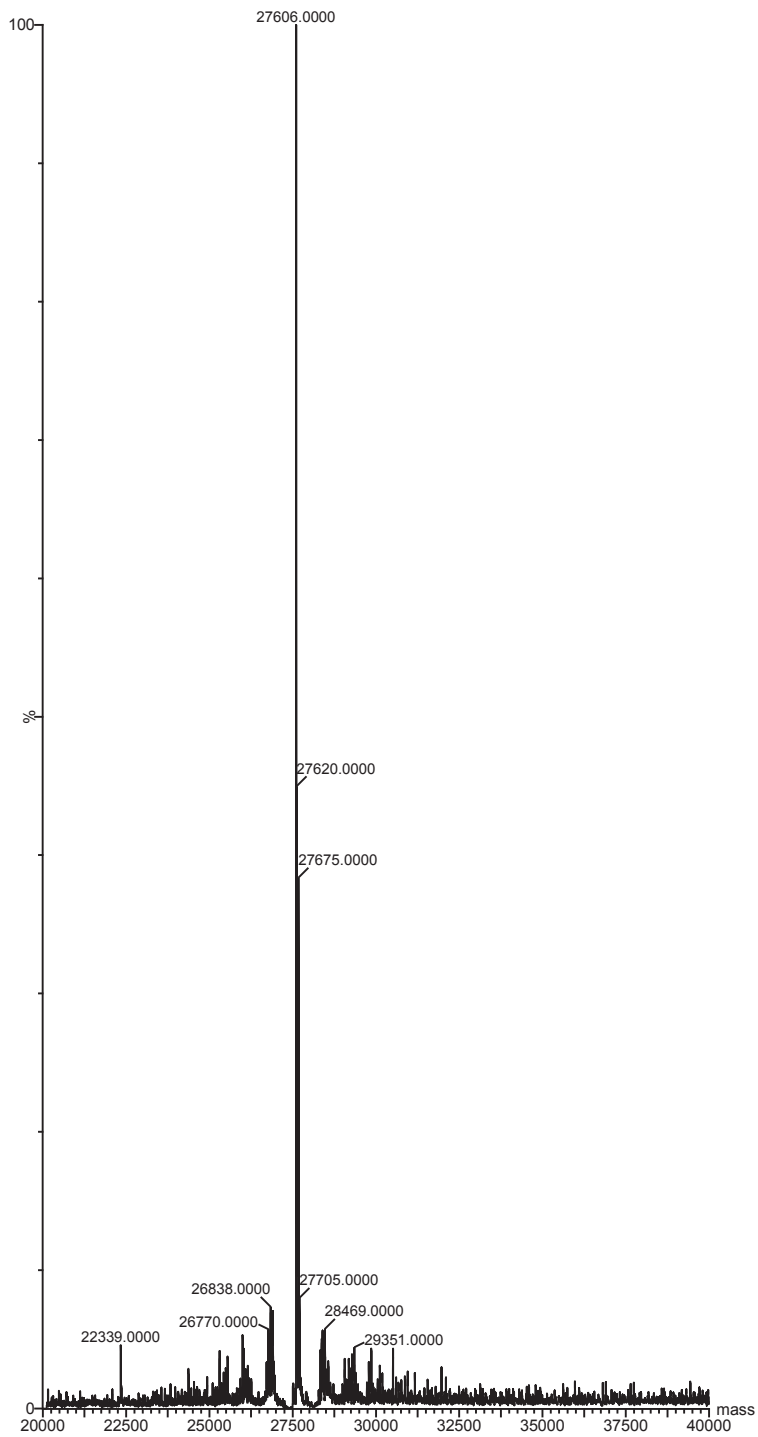
Condition: Bottom 5%
Codon: UAG
AA/ncAA: N6-Boc-L-lys
Expected mass: 27606
Observed mass: 27620



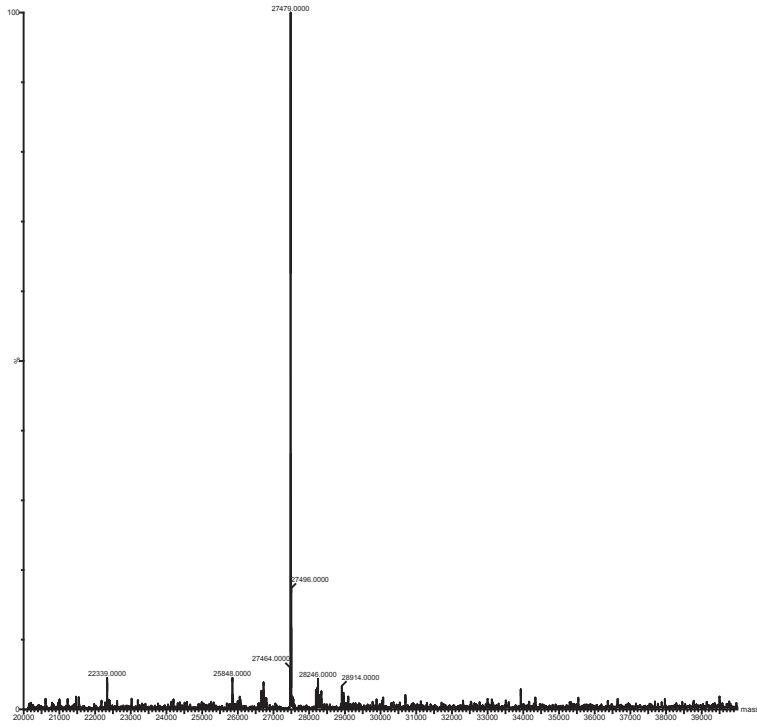
Condition: Top 5%
Codon: UAG
AA/ncAA: N6-Boc-L-lys
Expected mass: 27606
Observed mass: 27606.5



Condition: Top 1%
Codon: UAG
AA/ncAA: N6-Boc-L-lys
Expected mass: 27606
Observed mass: 27606



Condition: Library
Codon: UAGA
AA/ncAA: L-ser
Expected mass: 27465
Observed mass: 27479



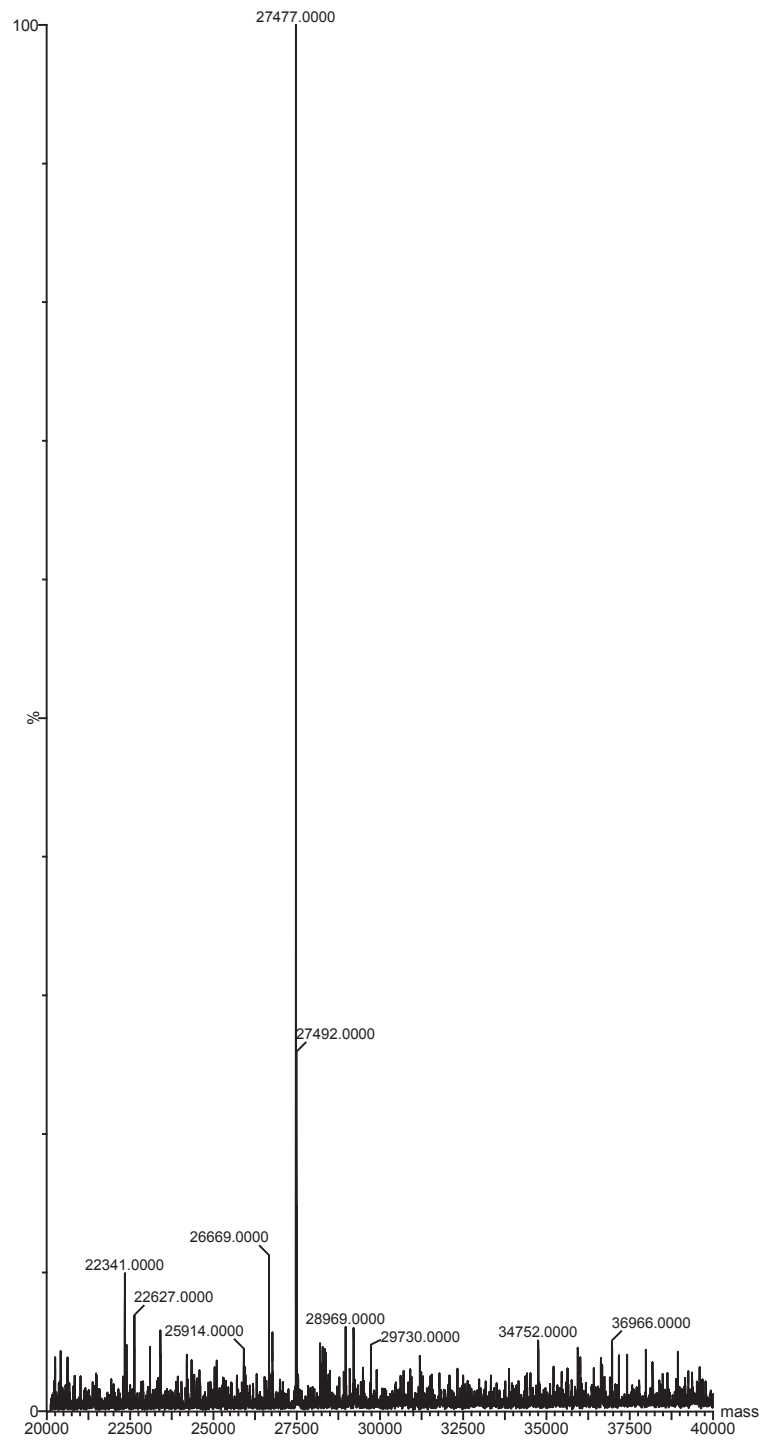
Condition: Bottom 1%

Codon: UAGA

AA/ncAA: L-ser

Expected mass: 27465

Observed mass: 27477



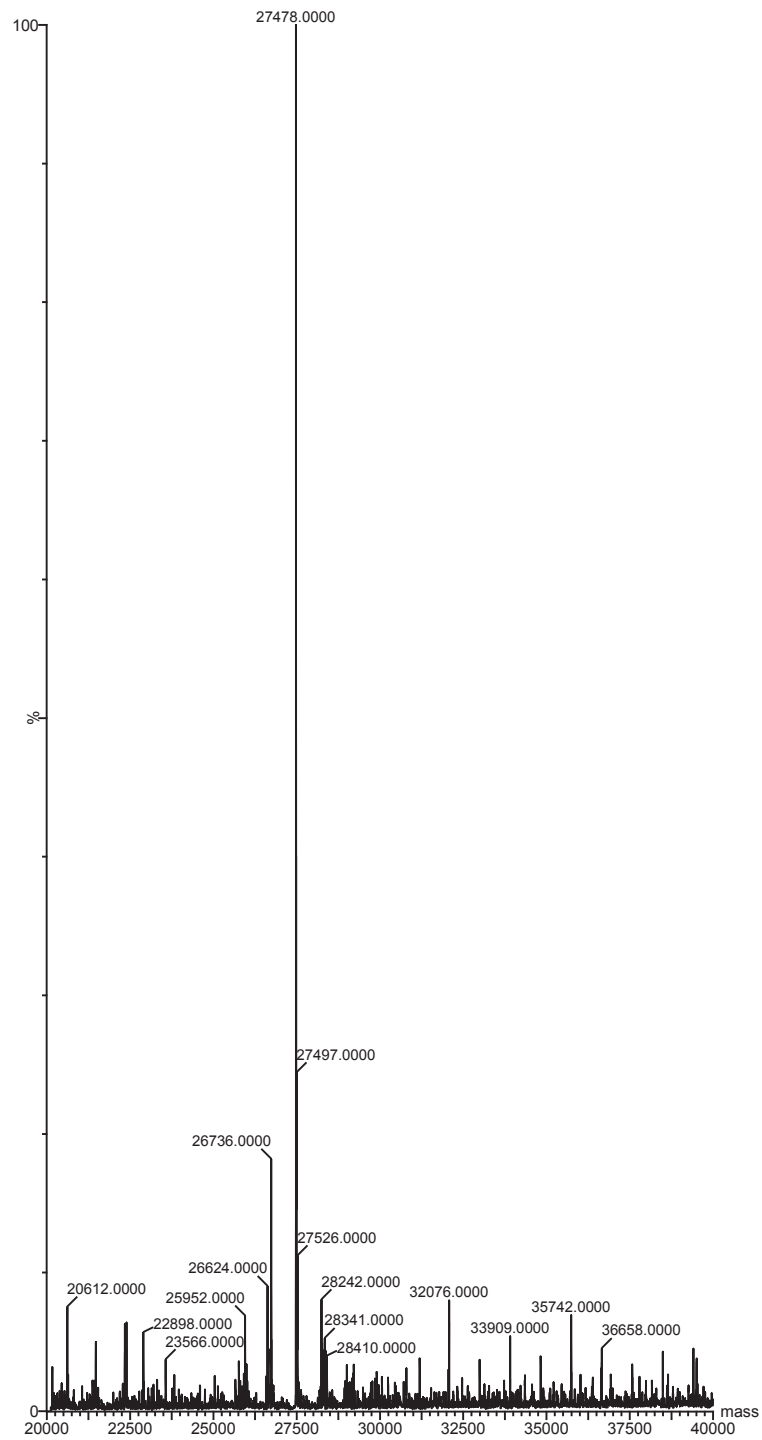
Condition: Bottom 5%

Codon: UAGA

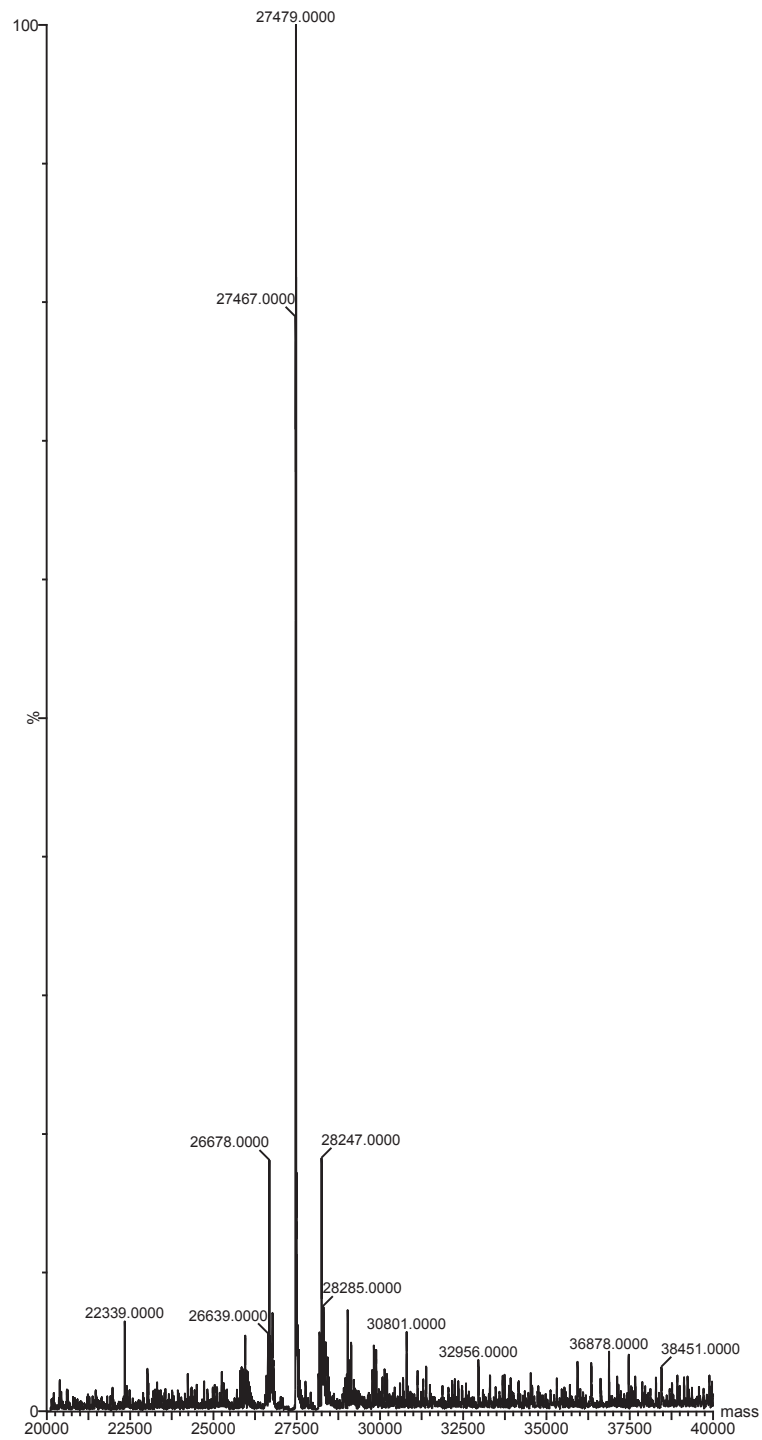
AA/ncAA: L-ser

Expected mass: 27465

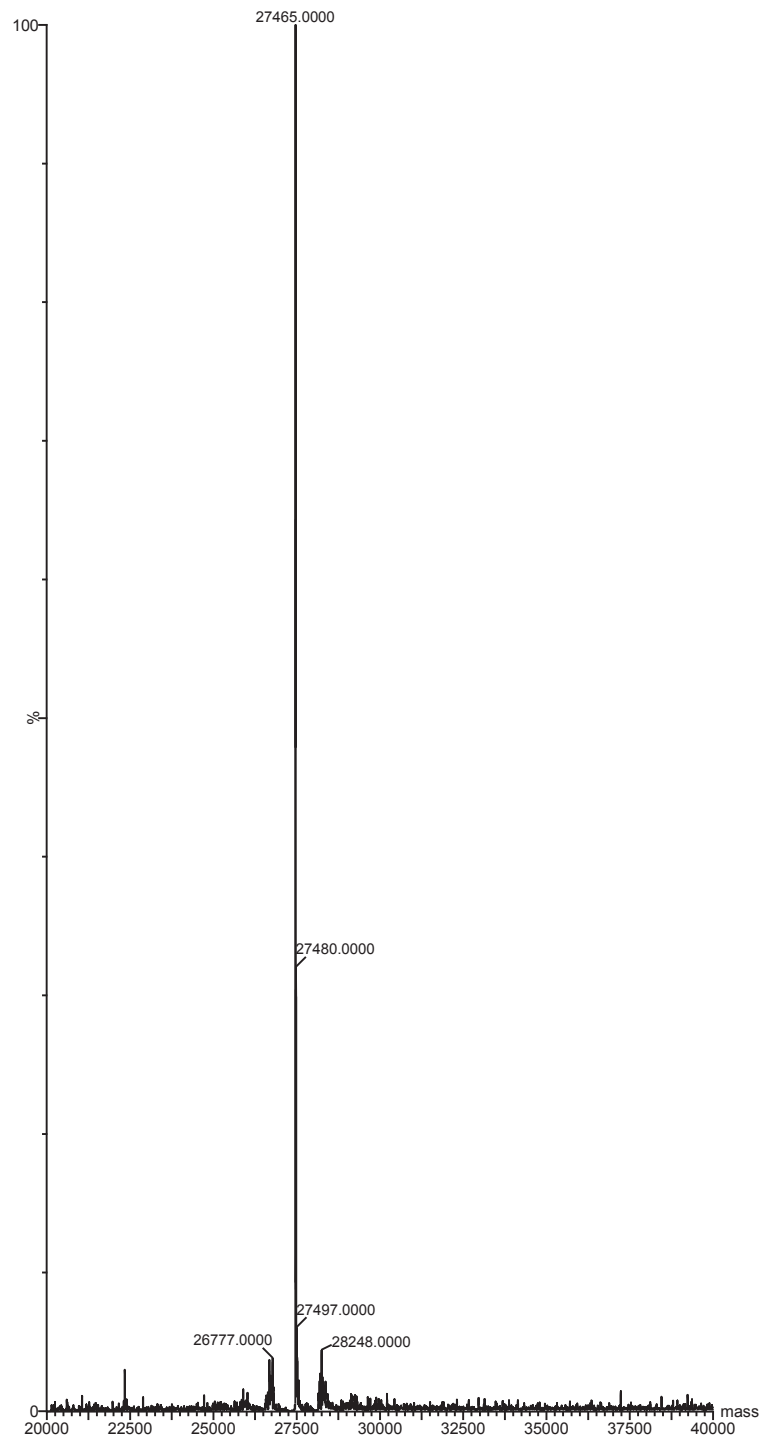
Observed mass: 27478



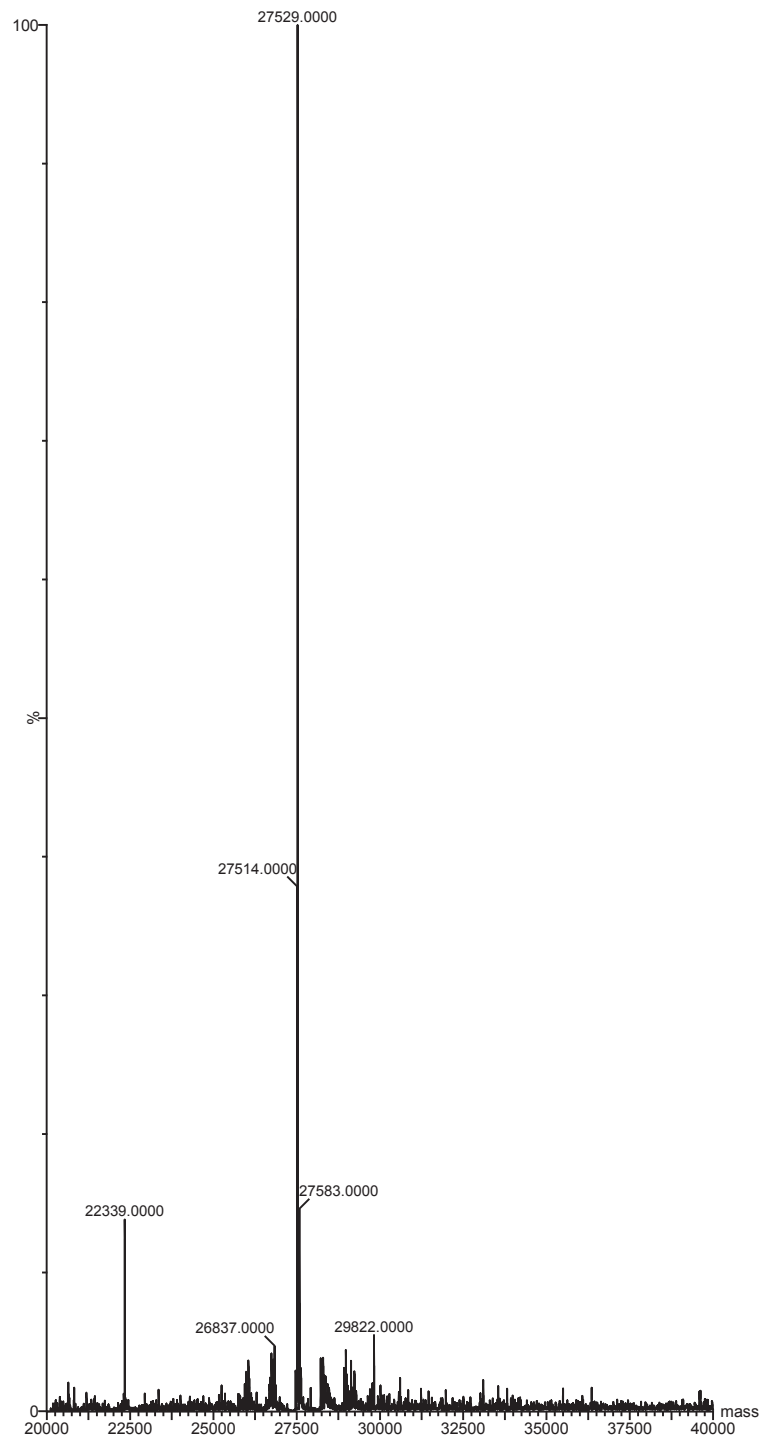
Condition: Top 5%
Codon: UAGA
AA/ncAA: L-ser
Expected mass: 27465
Observed mass: 27479



Condition: Top 1%
Codon: UAGA
AA/ncAA: L-ser
Expected mass: 27465
Observed mass: 27465



Condition: Library
Codon: AGGA
AA/ncAA: L-his
Expected mass: 27515
Observed mass: 27529



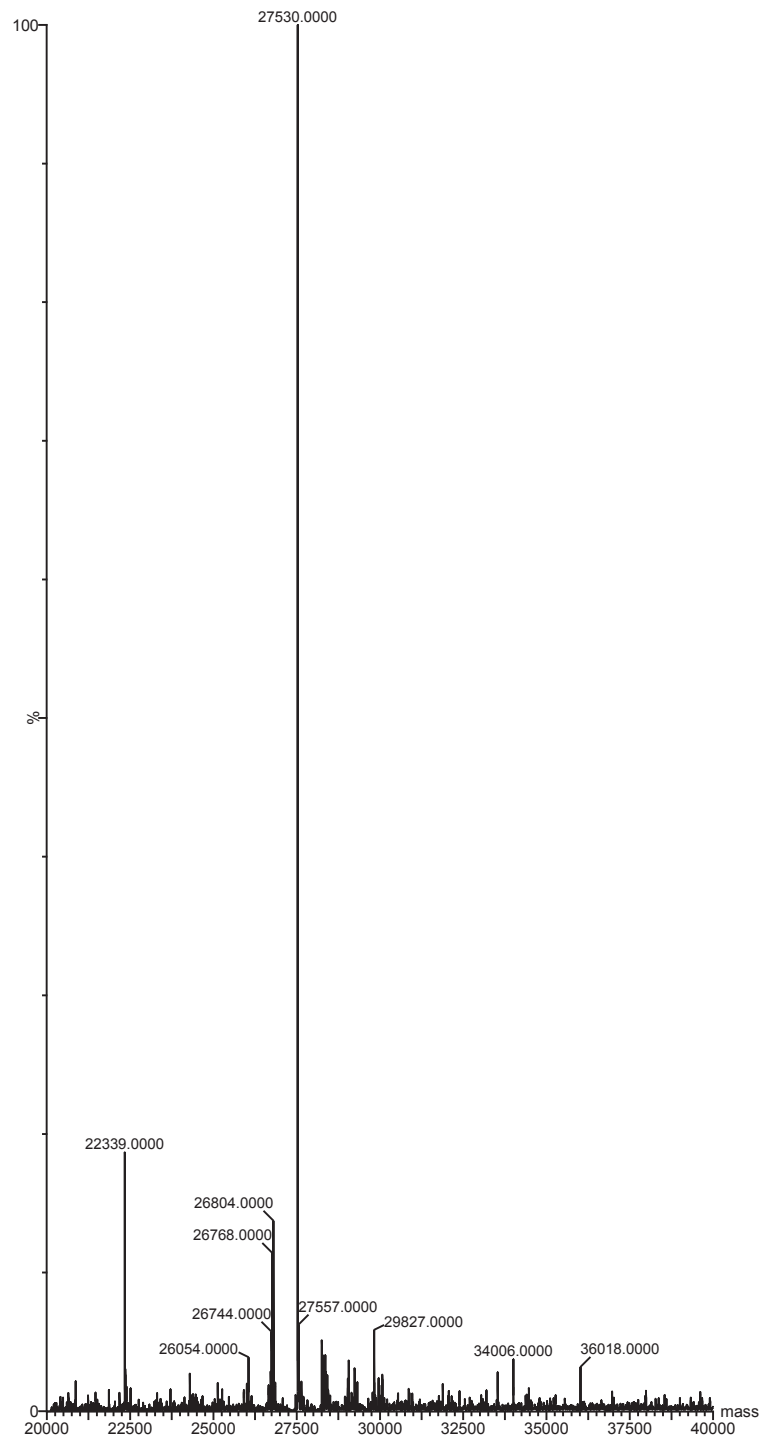
Condition: Bottom 1%

Codon: AGGA

AA/ncAA: L-his

Expected mass: 27515

Observed mass: 27530



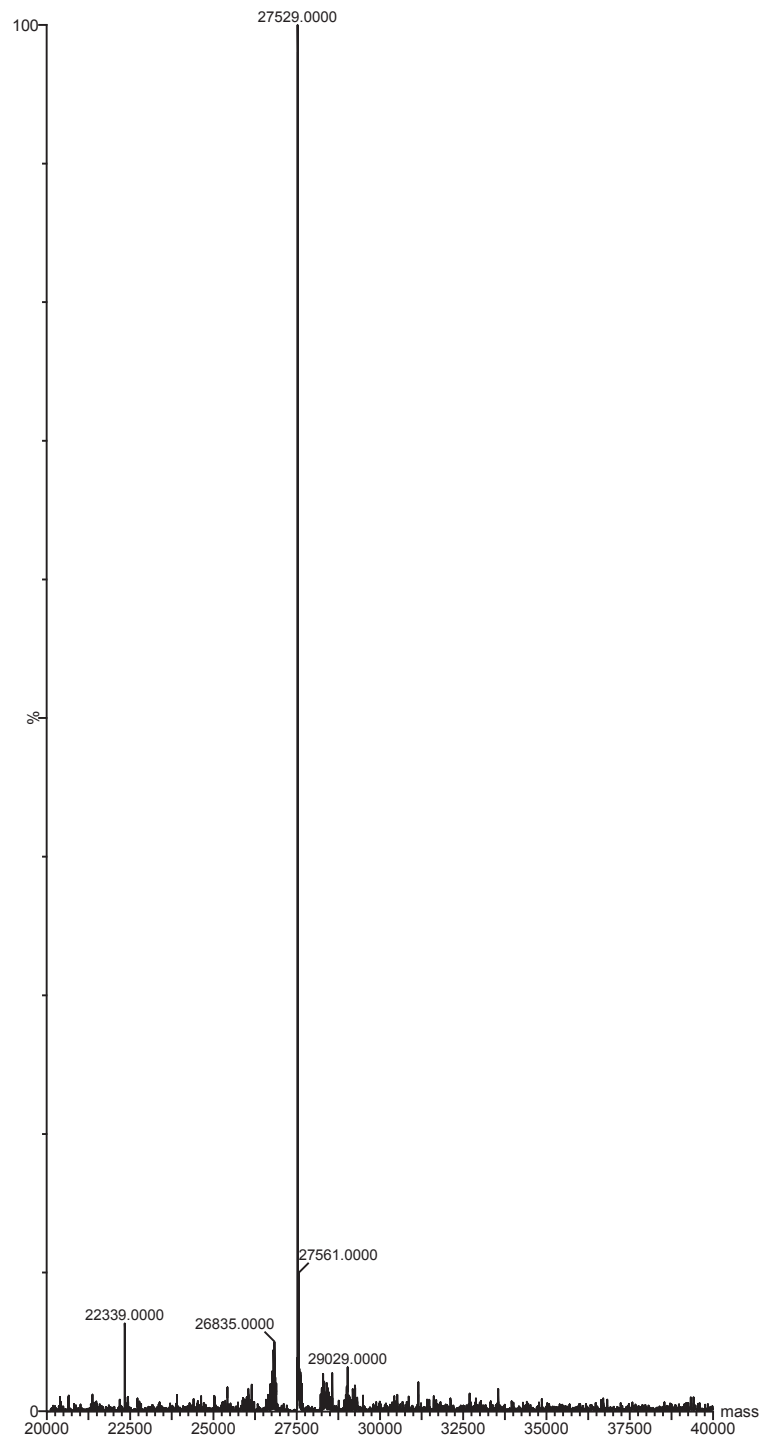
Condition: Bottom 5%

Codon: AGGA

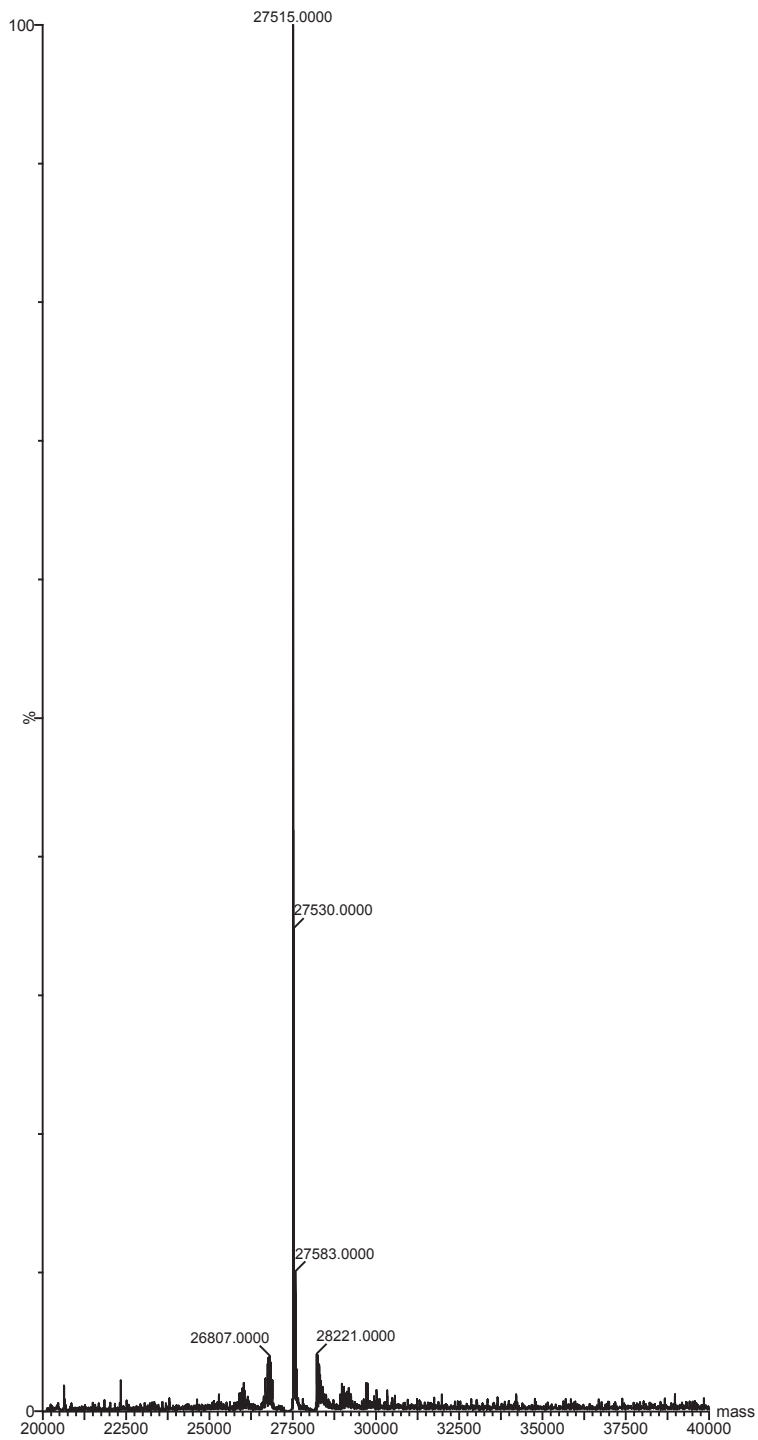
AA/ncAA: L-his

Expected mass: 27515

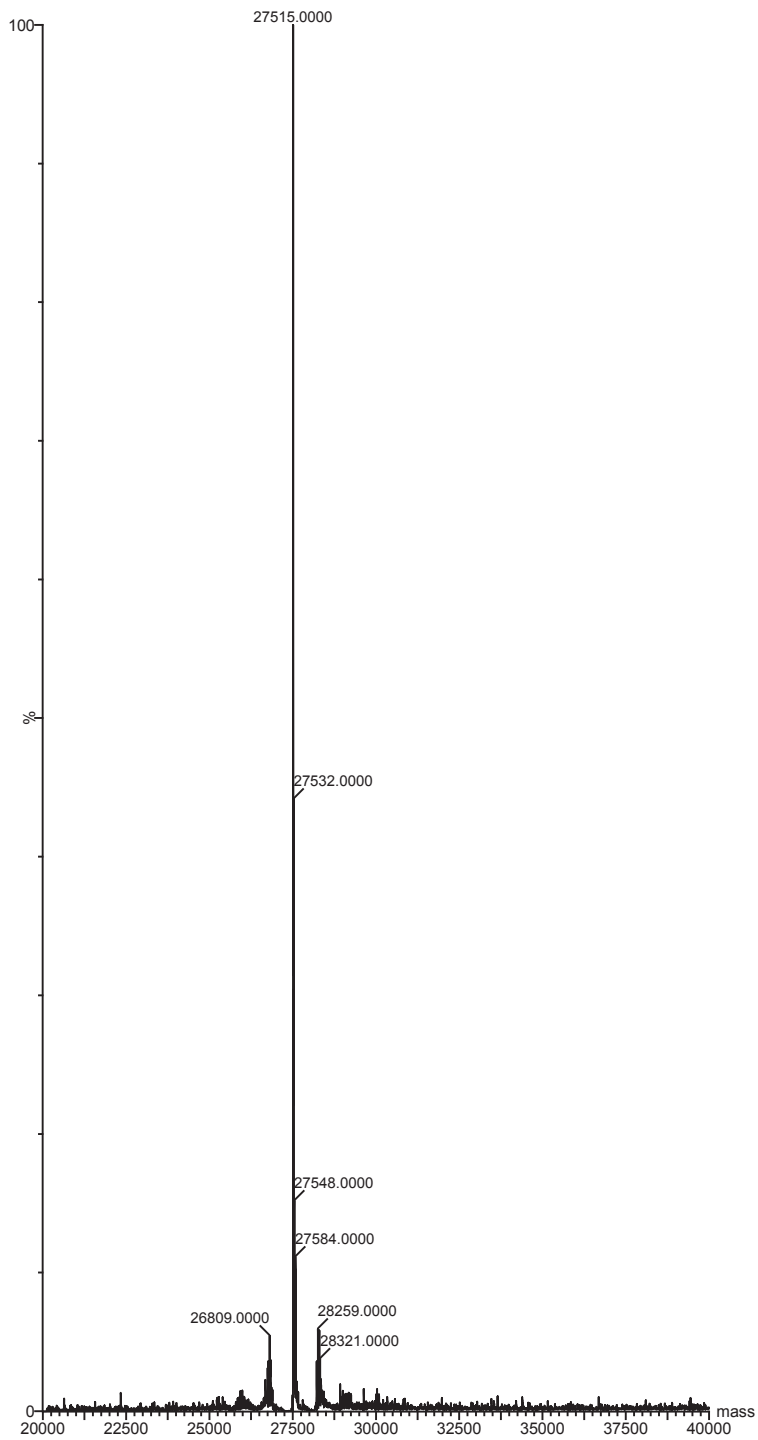
Observed mass: 27529



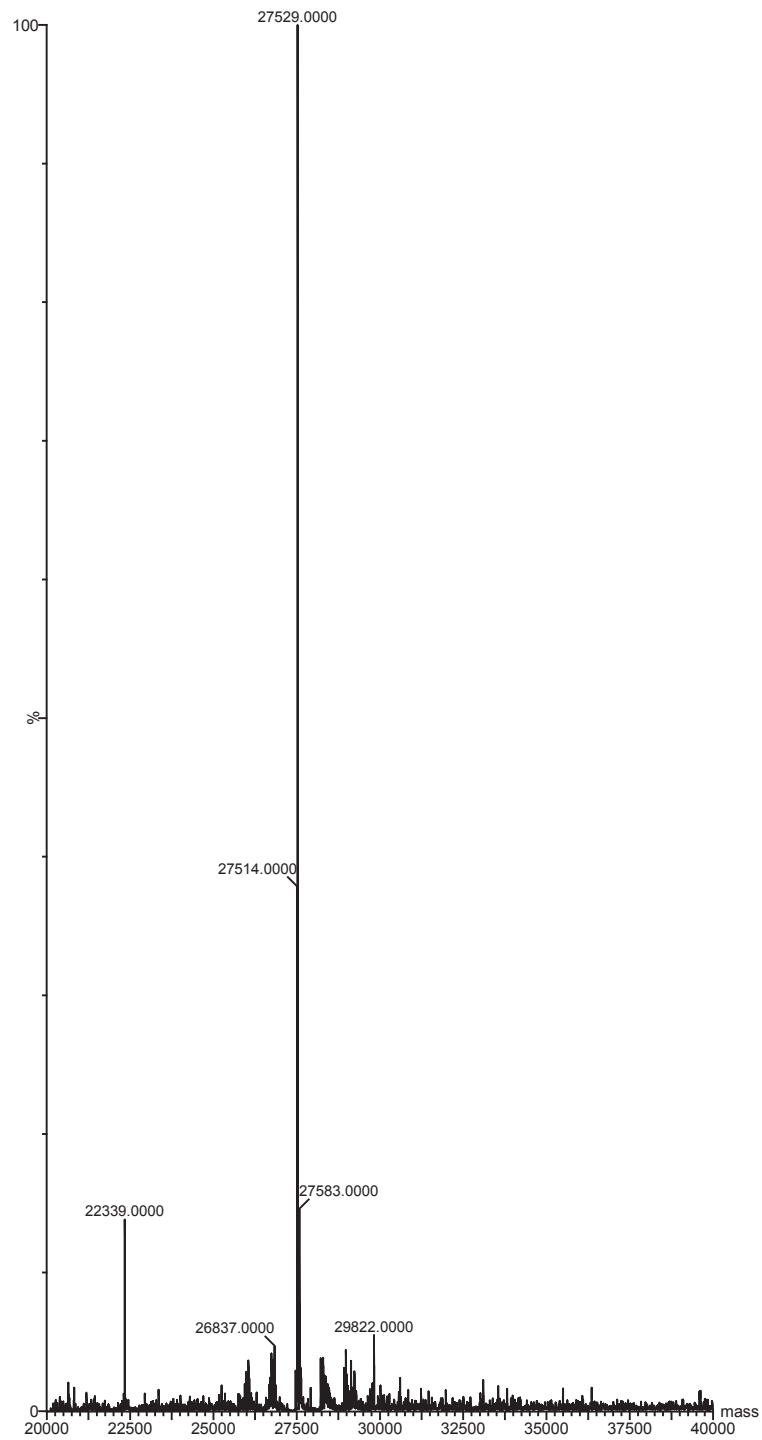
Condition: Top 5%
Codon: AGGA
AA/ncAA: L-his
Expected mass: 27515
Observed mass: 27515



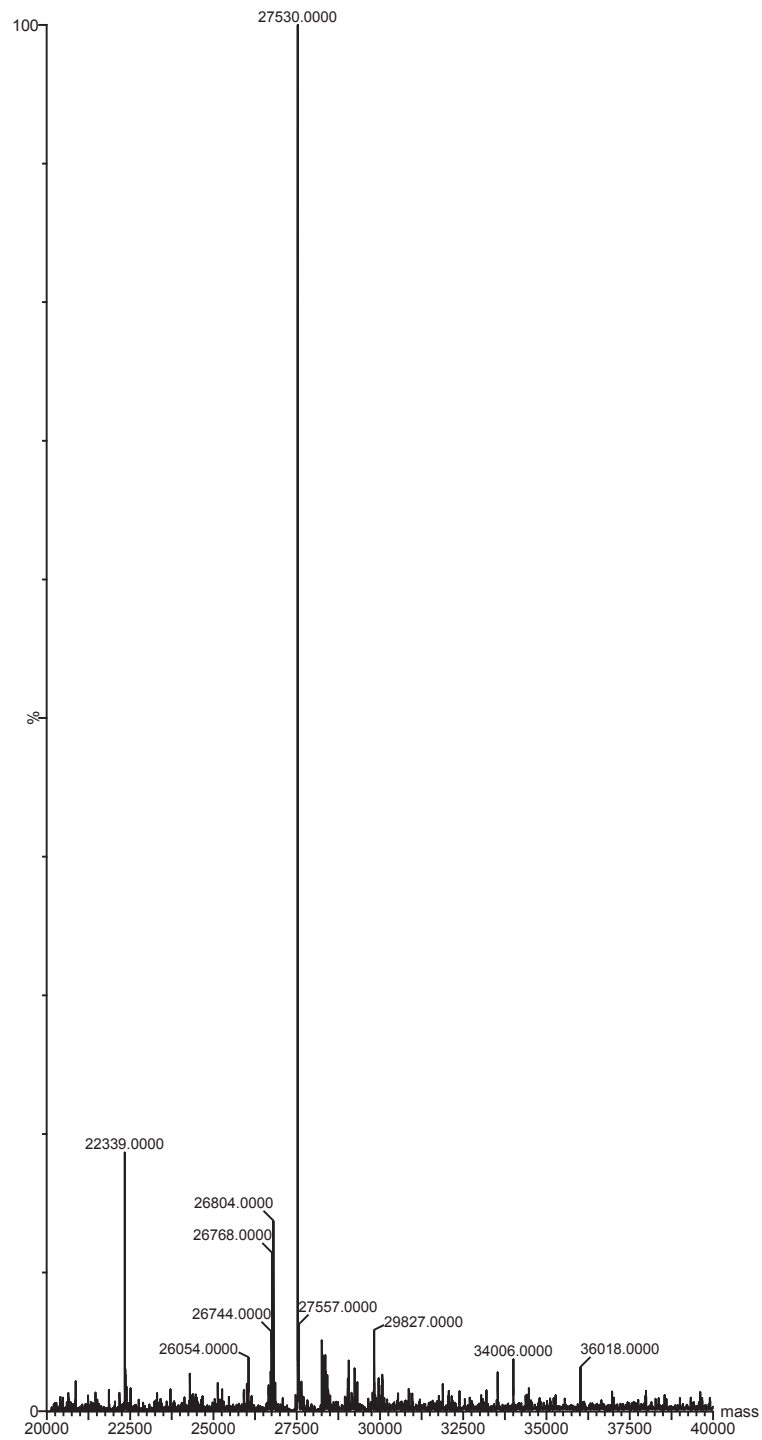
Condition: Top 1%
Codon: AGGA
AA/ncAA: L-his
Expected mass: 27515
Observed mass: 27515



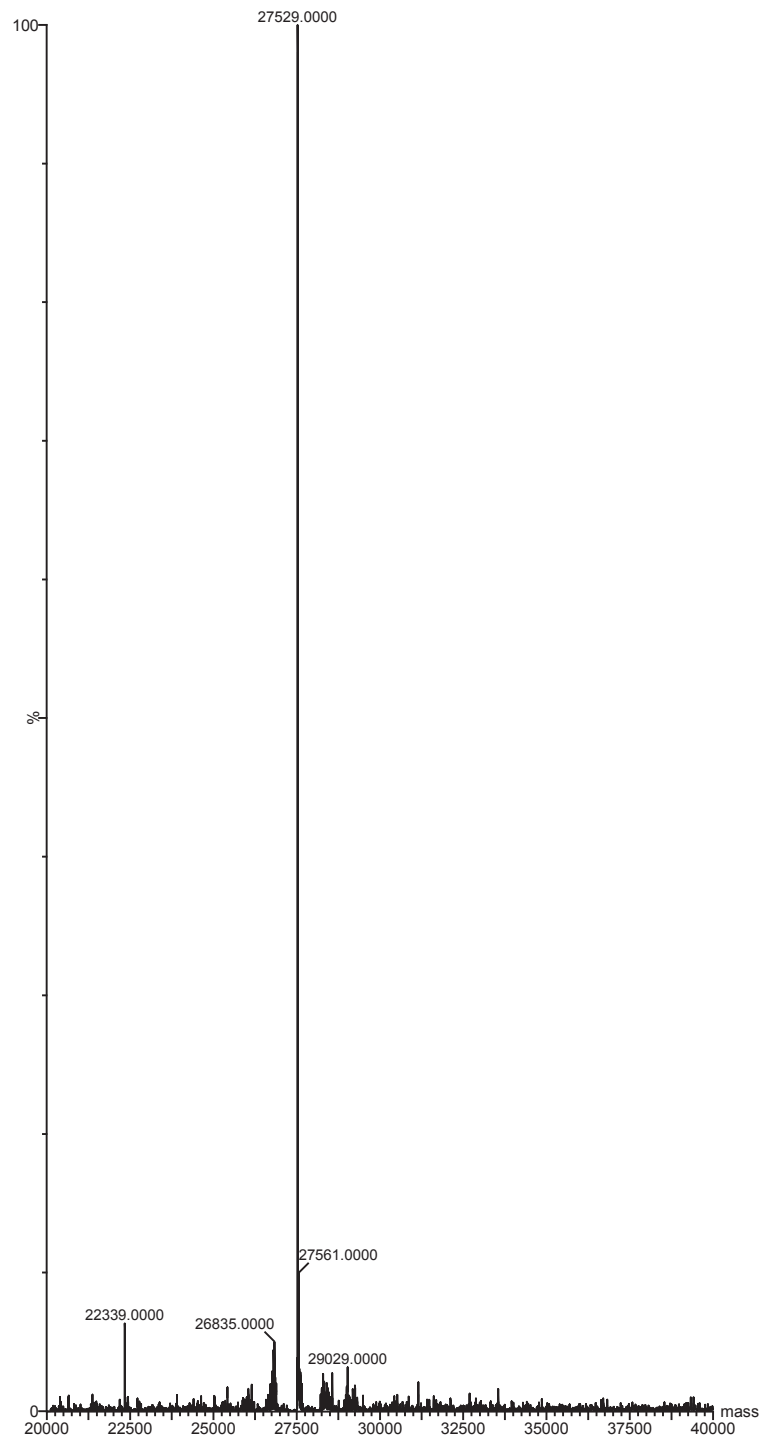
Condition: Library
Codon: AGGA
AA/ncAA: 4-iodo-L-phe
Expected mass: 27515
Observed mass: 27529



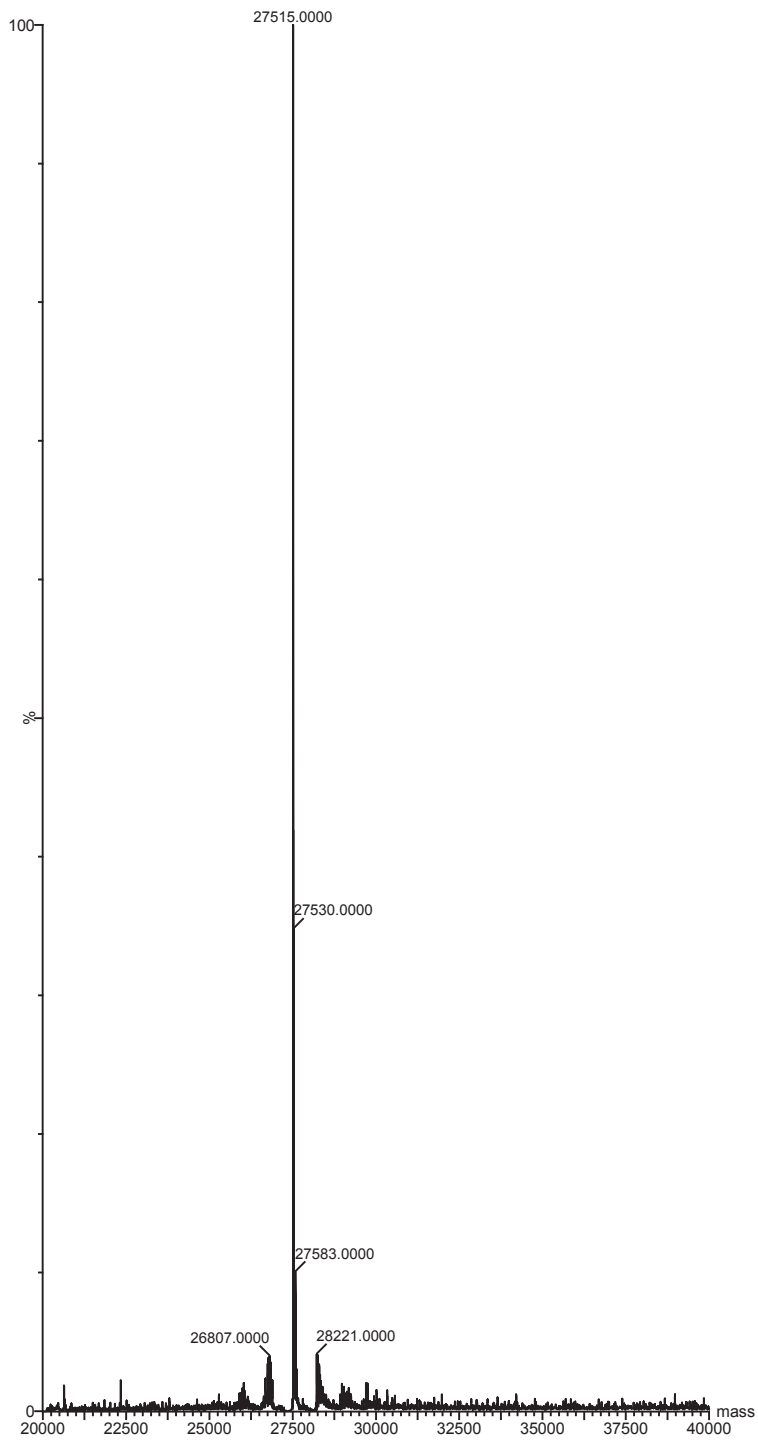
Condition: Bottom 1%
Codon: AGGA
AA/ncAA: 4-iodo-L-phe
Expected mass: 27515
Observed mass: 27530



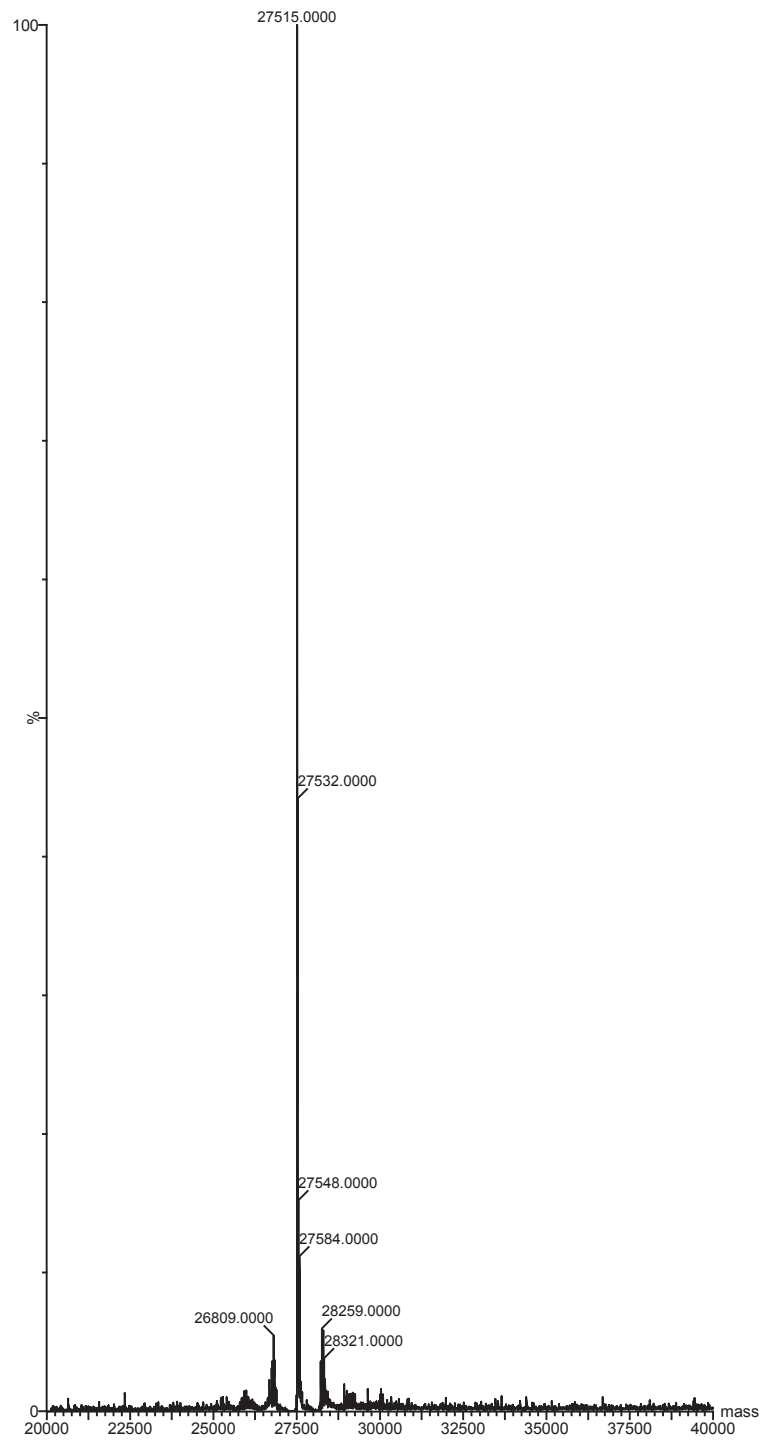
Condition: Bottom 5%
Codon: AGGA
AA/ncAA: 4-iodo-L-phe
Expected mass: 27515
Observed mass: 27529



Condition: Top 5%
Codon: AGGA
AA/ncAA: 4-iodo-L-phe
Expected mass: 27515
Observed mass: 27515



Condition: Top 1%
Codon: AGGA
AA/ncAA: 4-iodo-L-phe
Expected mass: 27515
Observed mass: 27515

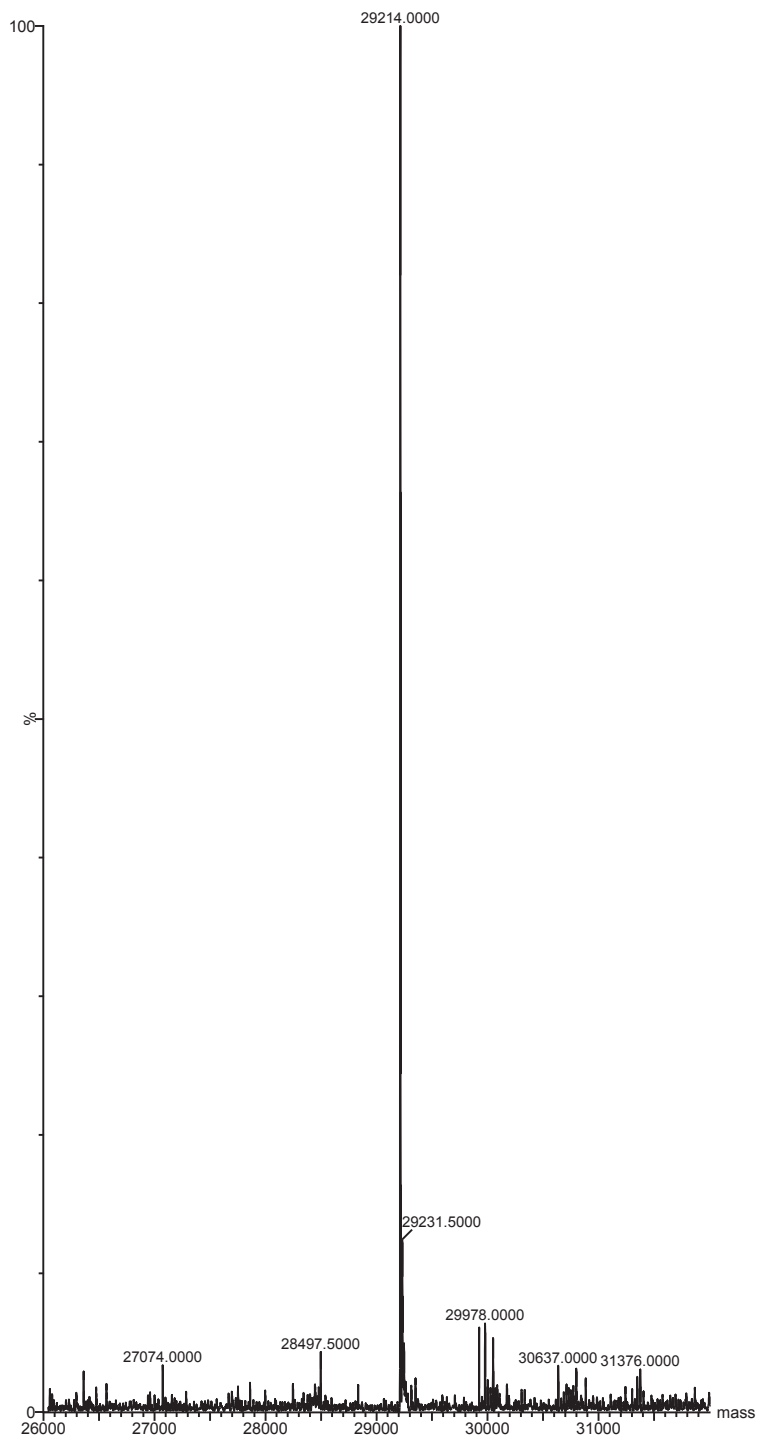


Supplementary Data 2 | Mass Spectrometry Data for Supplementary Figure 11.

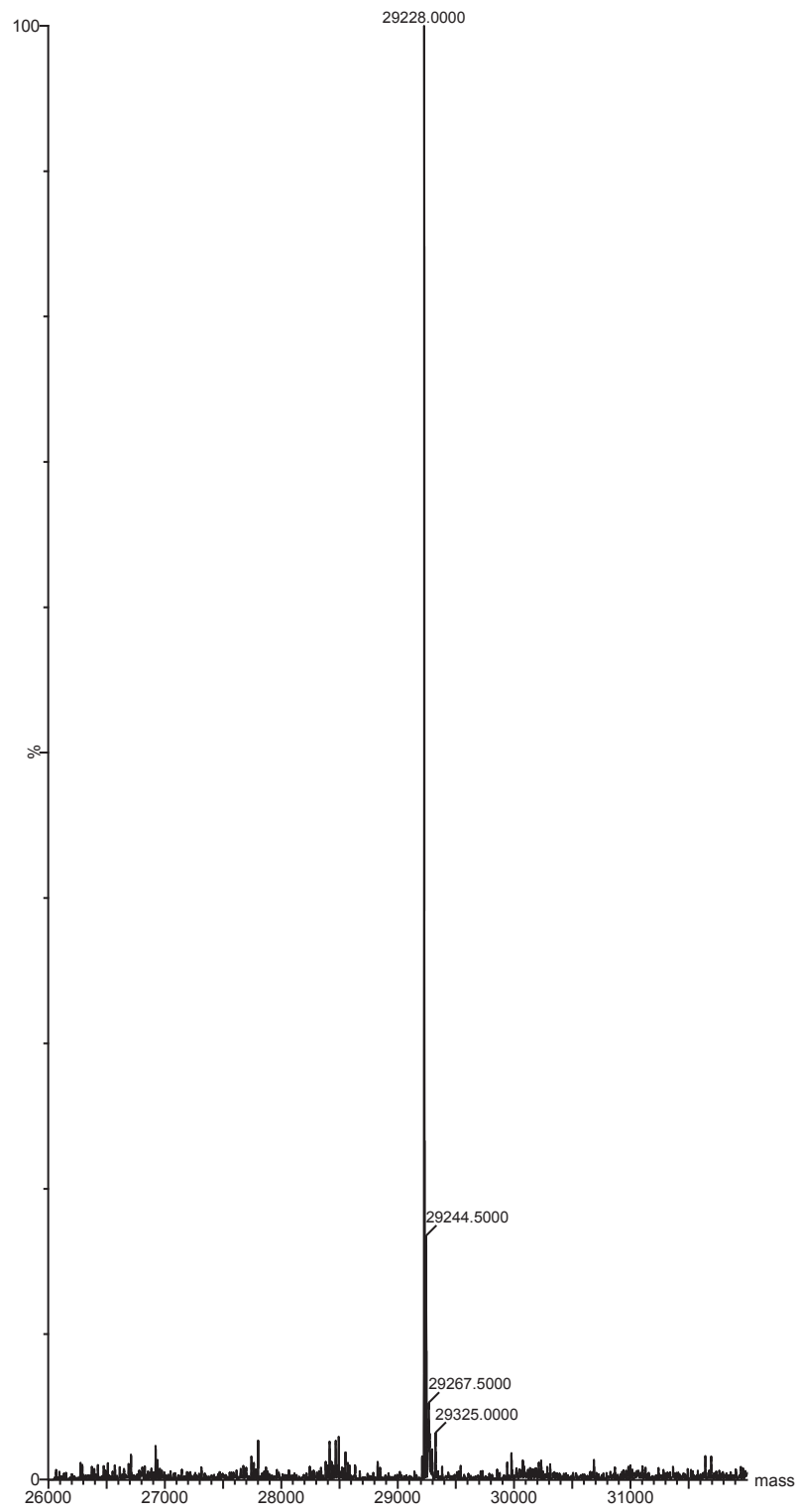
WT cMyc-sfGFP-His6

Expected mass: 29213.65

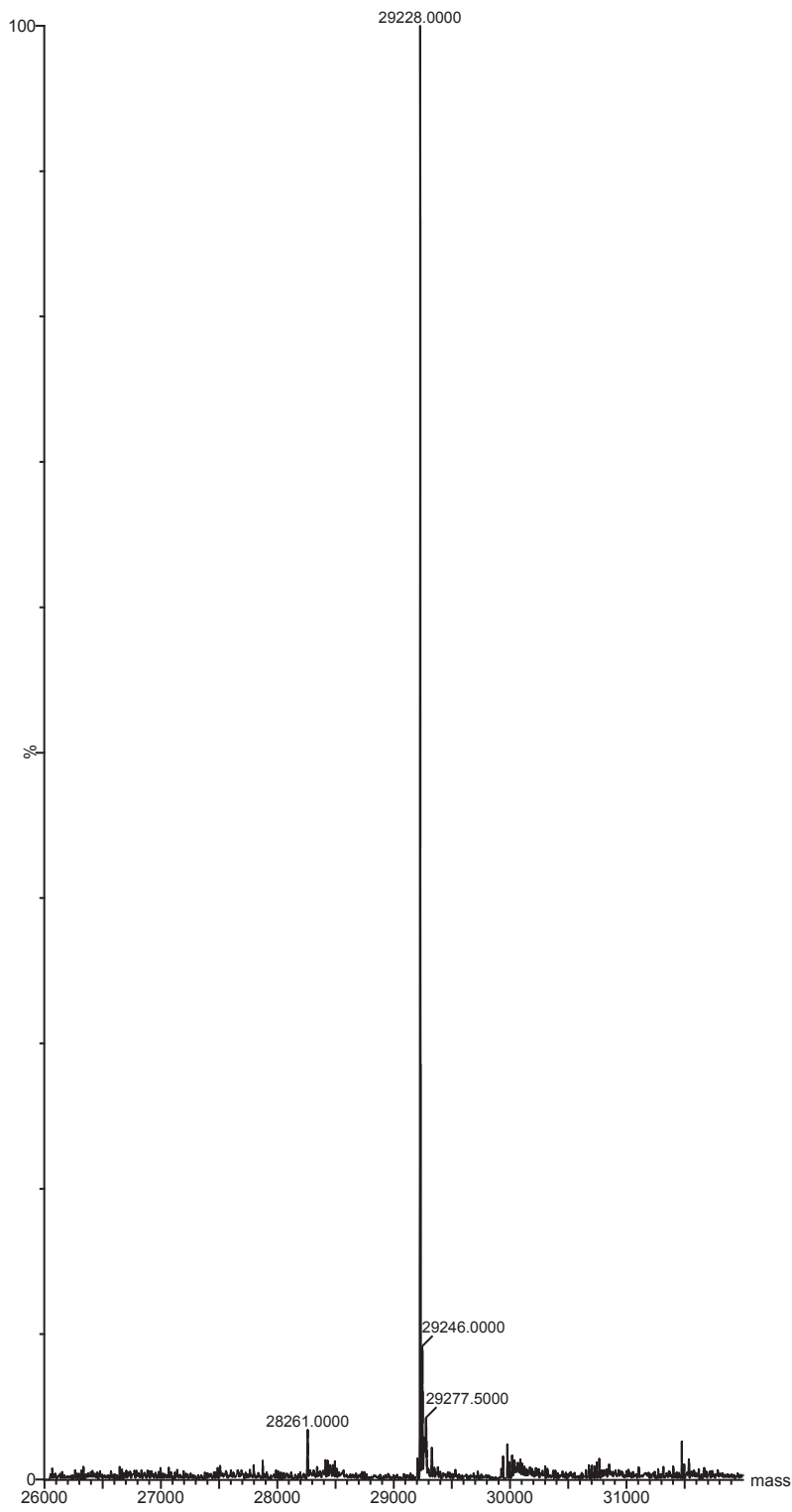
Observed mass: 29214.00



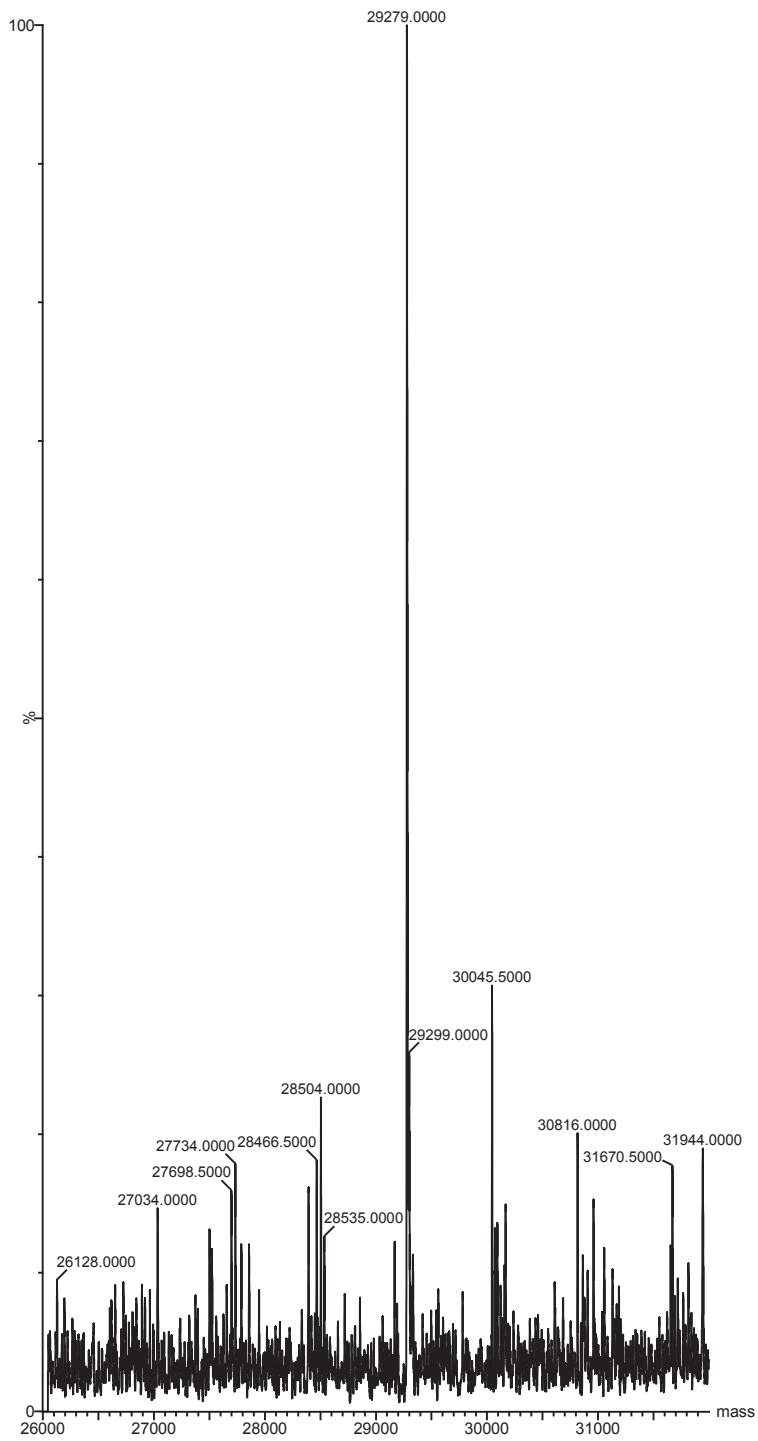
WT cMyc-sfGFP-Y151AGGA-His6
Int tRNAPylAGGA (start) 3-methoxy-L-phe
Expected mass: 29227.67
Observed mass: 29228.00



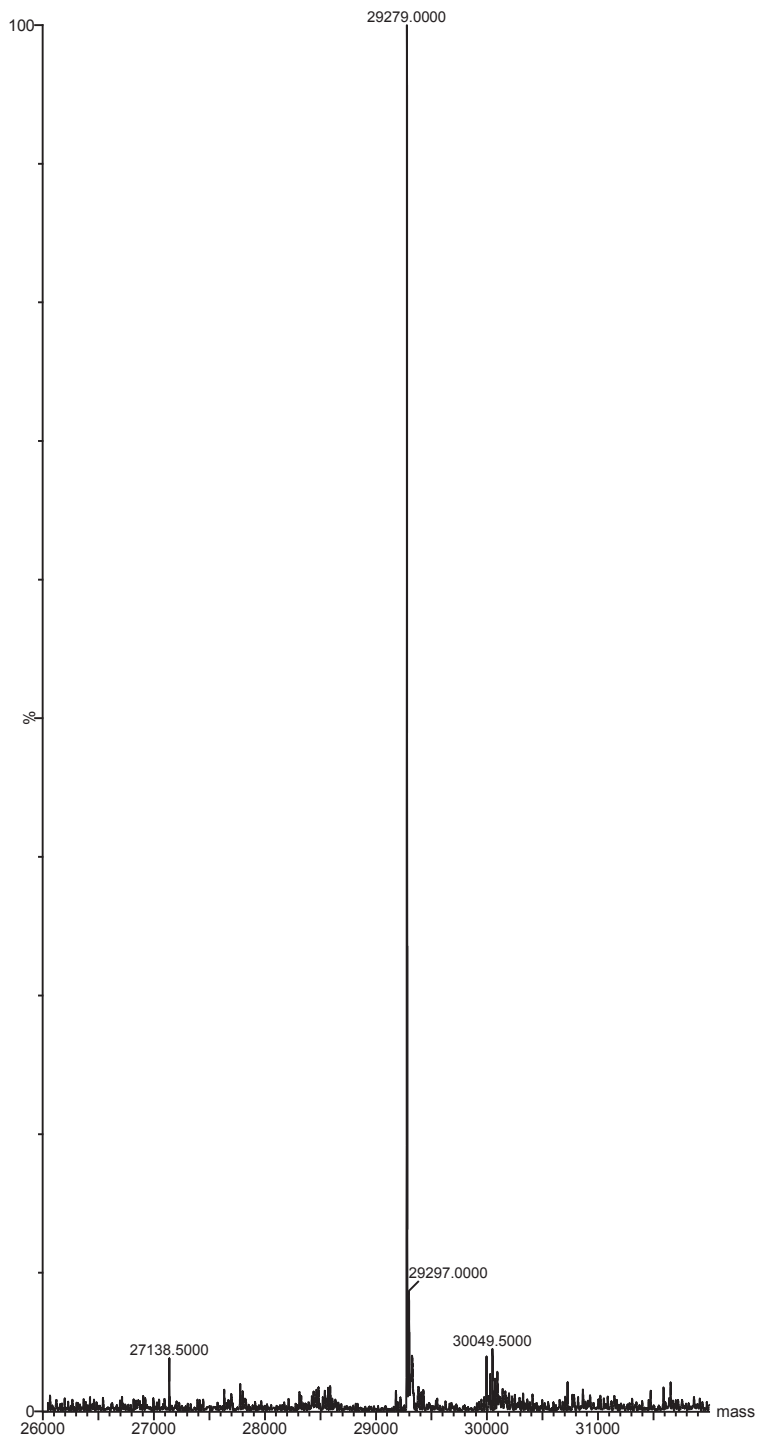
WT cMyc-sfGFP-Y151AGGA-His6
Int tRNAPylAGGA (evo) 3-methoxy-L-phe
Expected mass: 29227.67
Observed mass: 29228.00



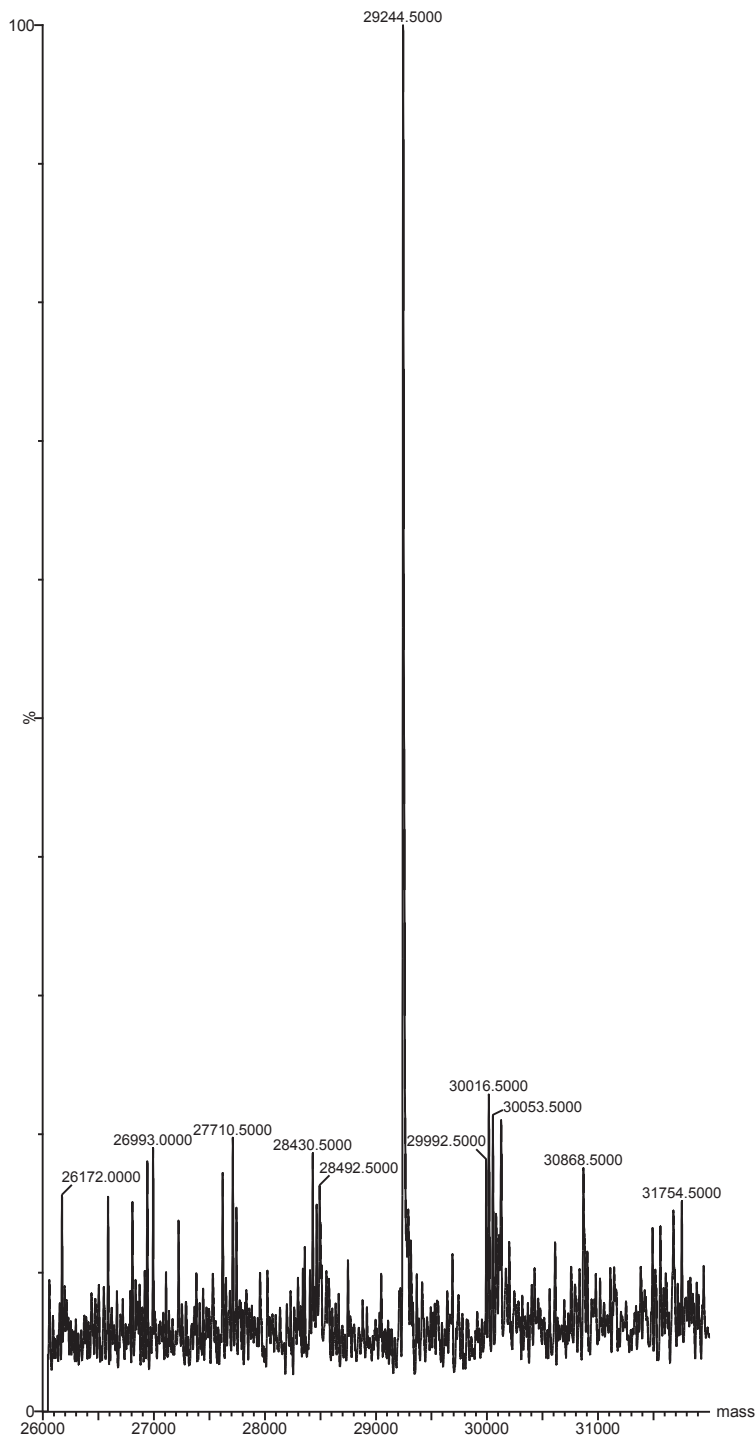
WT cMyc-sfGFP-Y151UAGA-His6
Spe tRNAPyIUAGA (start) N6-Boc-L-lys
Expected mass: 29278.73
Observed mass: 29279.00



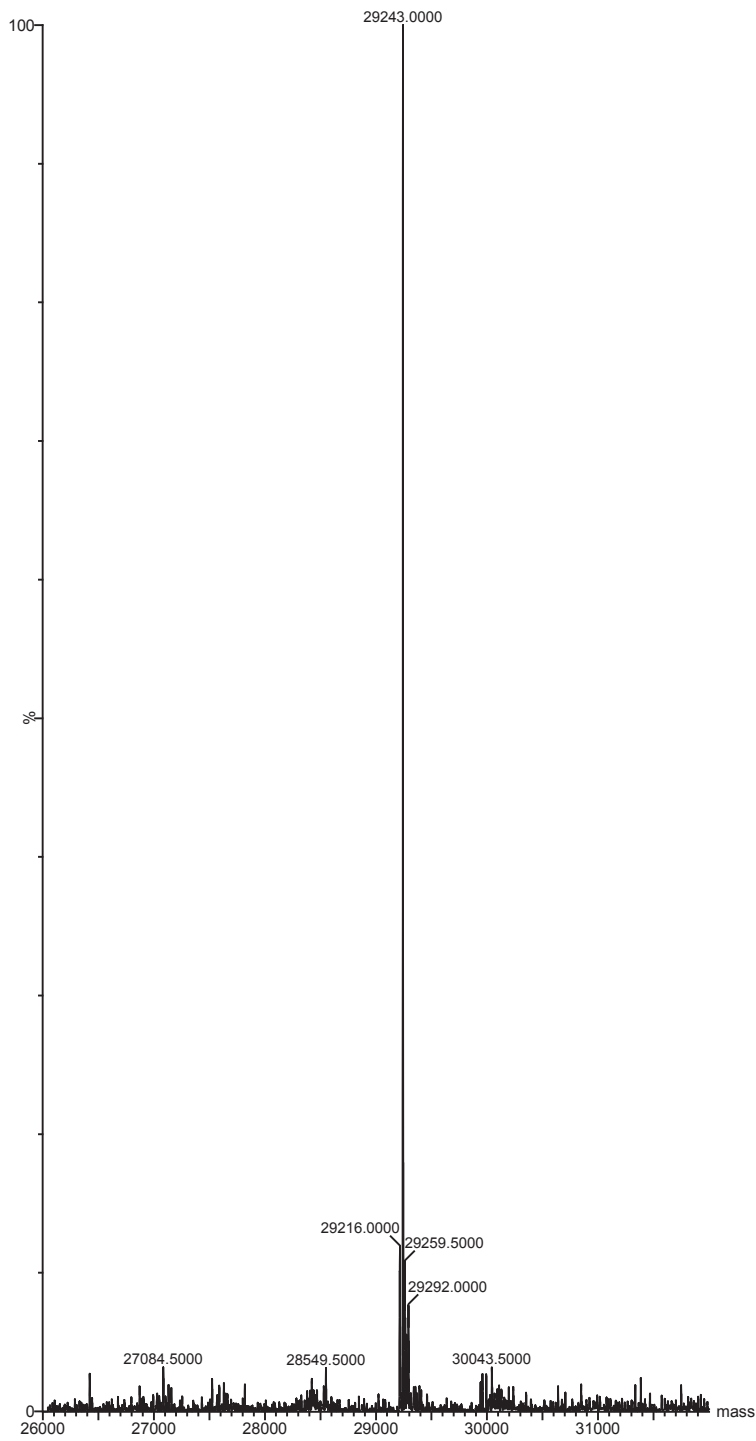
WT cMyc-sfGFP-Y151UAGA-His6
Spe tRNAPyIUAGA (evo) N6-Boc-L-lys
Expected mass: 29278.73
Observed mass: 29279.00



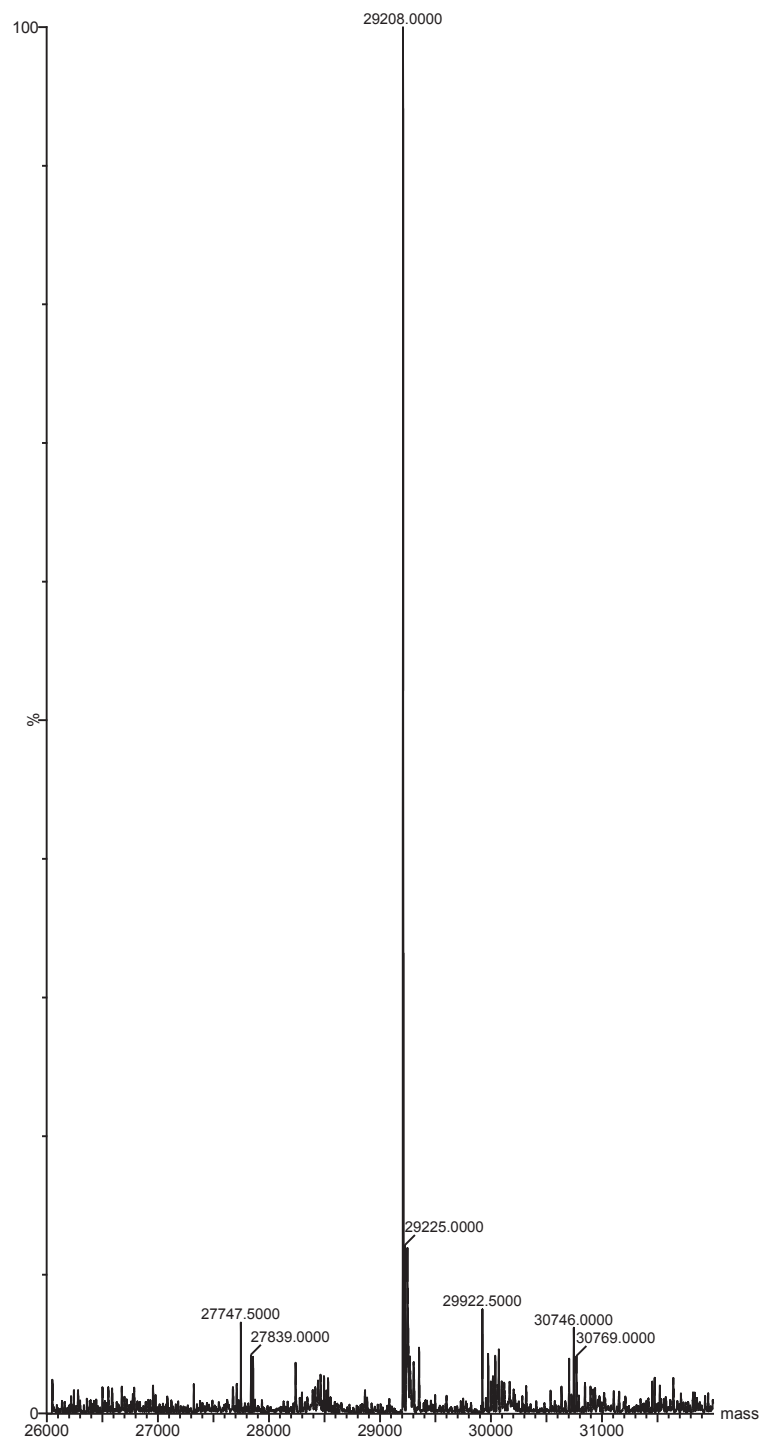
WT cMyc-sfGFP-Y151AUAG-His6
Ma tRNAPylAUAG (start) 3-nitro-L-phe
Expected mass: 29242.64
Observed mass: 29244.50



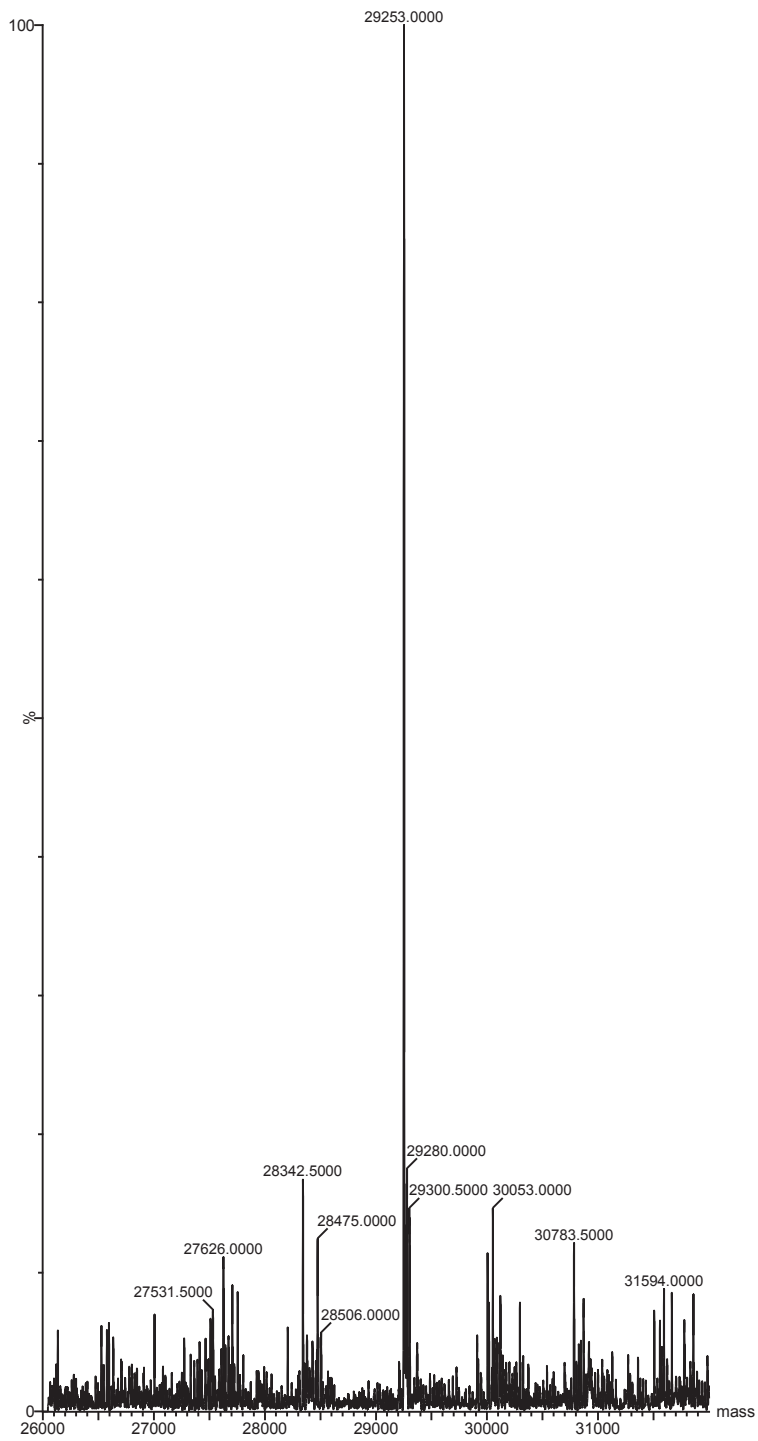
WT cMyc-sfGFP-Y151AUAG-His6
Ma tRNAPylAUAG (evo) 3-nitro-L-phe
Expected mass: 29242.64
Observed mass: 29243.00



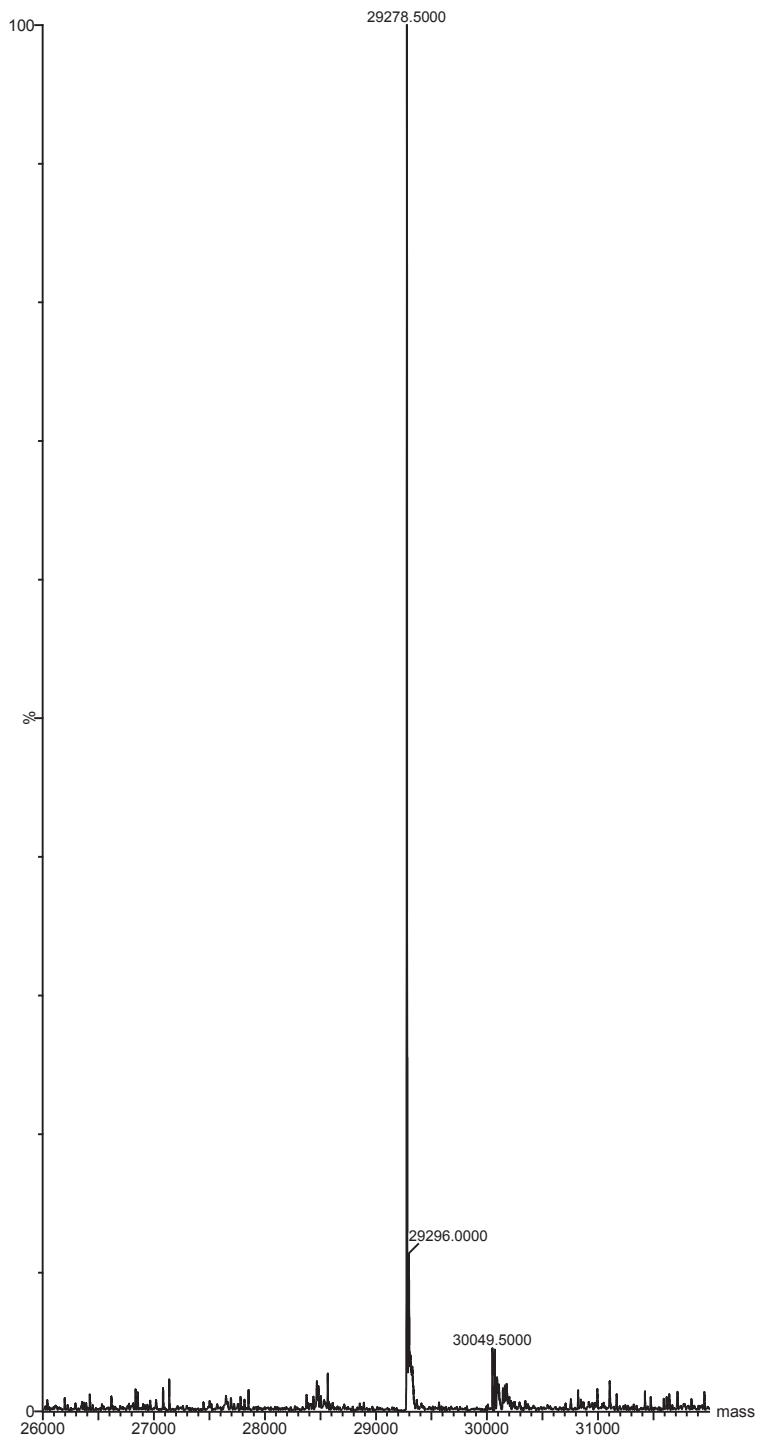
WT cMyc-sfGFP-Y151CGGA-His6
Sc tRNA^{Trp}CGGA (start) 5-hydroxy-L-trp
Expected mass: 29252.66
Observed mass: 29208.00



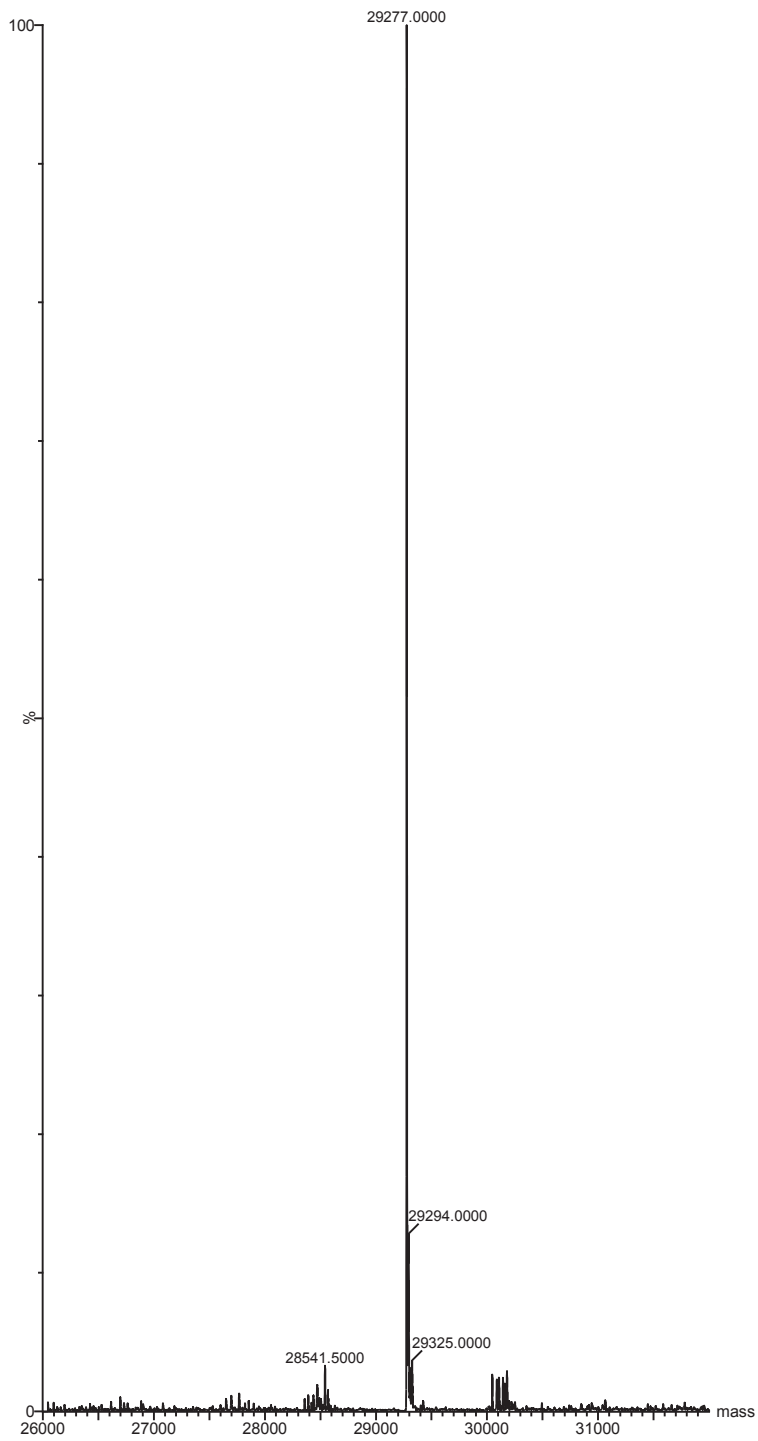
WT cMyc-sfGFP-Y151CGGA-His6
Sc tRNA^{Trp}CGGA (evo) 5-hydroxy-L-trp
Expected mass: 29252.66
Observed mass: 29253.00



WT cMyc-sfGFP-Y151CUAG-His6
Af tRNA^{Tyr}CUAG (start) 4-bromo-L-phe
Expected mass: 29275.56
Observed mass: 29278.50



WT cMyc-sfGFP-Y151CUAG-His6
Af tRNA^{Tyr}CUAG (evo) 4-bromo-L-phe
Expected mass: 29275.56
Observed mass: 29277.00



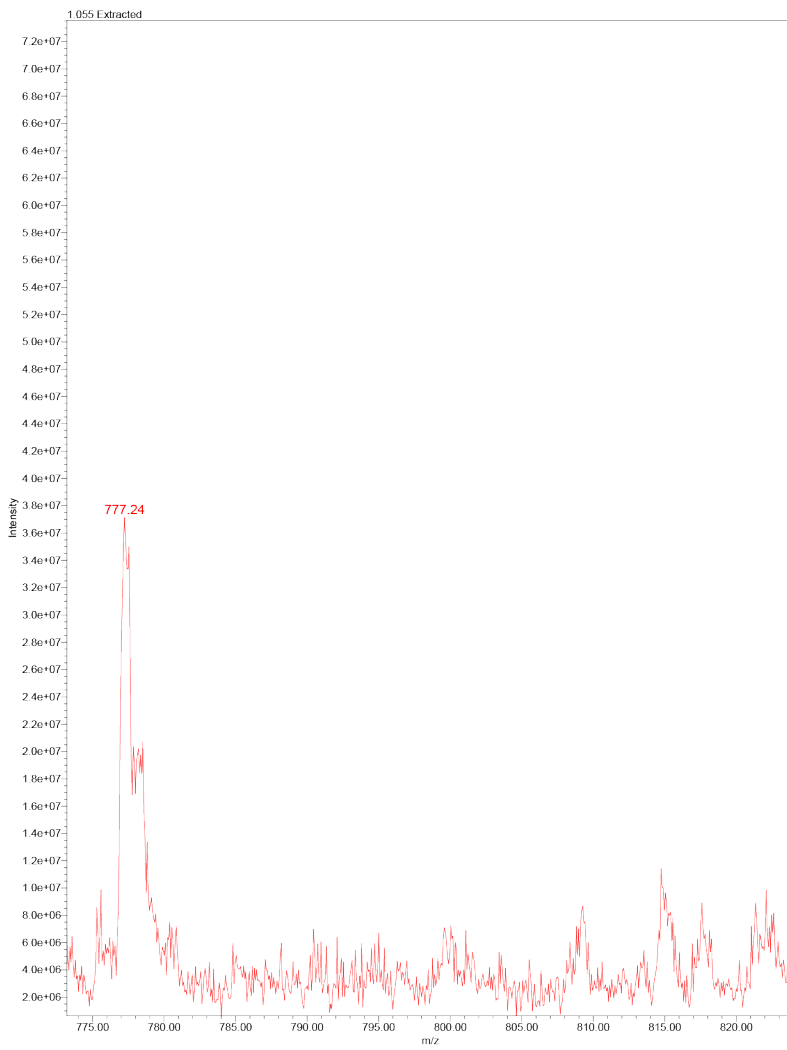
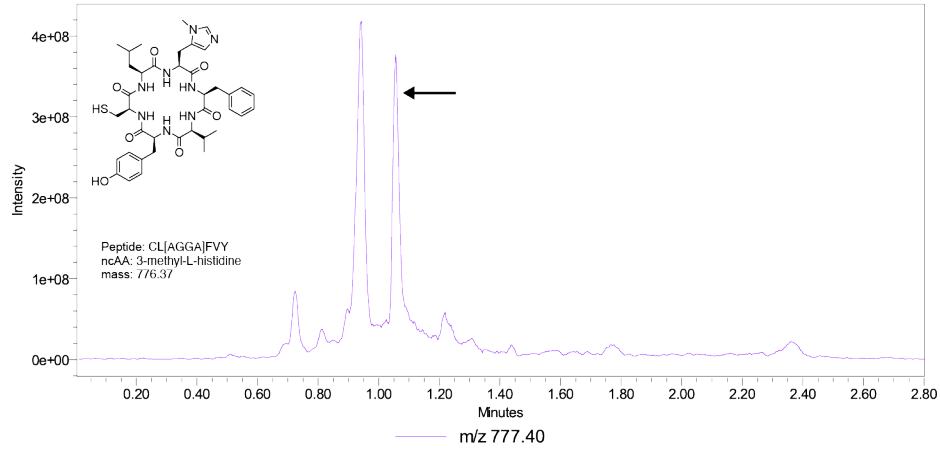
Supplementary Data 3 | Mass Spectrometry Data for Main Text Figure 5.

In ED data file	Macrocycle	ncAA(s)	Mass	Mass+H
1	C[AGGA]LFVY	3-methyl-L-histidine	776.37	777.37
2	CL[AGGA]FVY	3-methyl-L-histidine	776.37	777.37
3	CLL[AGGA]VY	3-methyl-L-histidine	742.38	743.38
4	CLLF[AGGA]Y	3-methyl-L-histidine	790.38	791.38
5	CLLFV[AGGA]	3-methyl-L-histidine	726.94	727.94
6	C(AGGA)LFVY	3-bromo-L-phenylalanine	850.27	851.27
7	CL(AGGA)FVY	3-bromo-L-phenylalanine	850.27	851.27
8	CLLFV(AGGA)	3-bromo-L-phenylalanine	800.29	801.29
9	C(AGGA)LFVY	3-chloro-L-phenylalanine	806.32	807.32
10	CL(AGGA)FVY	3-chloro-L-phenylalanine	806.32	807.32
11	CLLFV(AGGA)	3-chloro-L-phenylalanine	756.34	757.34
12	C(AGGA)LFVY	3-methoxy-L-phenylalanine	802.37	803.37
13	CL(AGGA)FVY	3-methoxy-L-phenylalanine	802.37	803.37
14	CLLFV(AGGA)	3-methoxy-L-phenylalanine	752.39	753.39
15	C(AGGA)LFVY	3-cyano-L-phenylalanine	797.36	798.36
16	CL(AGGA)FVY	3-cyano-L-phenylalanine	797.36	798.36
17	CLLFV(AGGA)	3-cyano-L-phenylalanine	747.38	748.38
18	C(AGGA)LFVY	3-pyridyl-L-alanine	773.36	774.36
19	CL(AGGA)FVY	3-pyridyl-L-alanine	773.36	774.36
20	CLLFV(AGGA)	3-pyridyl-L-alanine	723.38	724.38
21	CL[CUAG]FVY	4-iodo-L-phenylalanine	898.26	899.26
22	CLL[CUAG]VY	4-iodo-L-phenylalanine	864.27	865.27
23	CLLFV[CUAG]	4-iodo-L-phenylalanine	848.28	849.28
24	CL[CUAG]FVY	4-bromo-L-phenylalanine	850.27	851.27
25	C[UAGA]LFVY	2-chloro-Cbz-L-lysine	921.39	922.39
26	CLLF[UAGA]Y	2-chloro-Cbz-L-lysine	935.4	936.4
27	CLLFV[UAGA]	2-chloro-Cbz-L-lysine	871.41	872.41
28	CL[UAGA]FVY	Cbz-L-lysine	887.43	888.43
29	C[AUAG]LFVY	3-nitro-L-phenylalanine	817.35	818.35
30	CL[AUAG]FVY	3-nitro-L-phenylalanine	817.35	818.35
31	CLL[AUAG]VY	3-nitro-L-phenylalanine	783.36	784.36
32	CLLF[AUAG]Y	3-nitro-L-phenylalanine	831.36	832.36
33	CLLFV[AUAG]	3-nitro-L-phenylalanine	767.37	768.37
34	CLL[AUAG]VY	4-benzoyl-L-phenylalanine	842.4	843.4
35	CLLF[AUAG]Y	4-benzoyl-L-phenylalanine	890.4	891.4
36	CL[AUAG]FVY	4-nitro-L-phenylalanine	817.35	818.35
37	CLL[AUAG]VY	4-nitro-L-phenylalanine	783.36	784.36
38	CLLF[AUAG]Y	4-nitro-L-phenylalanine	831.36	832.36

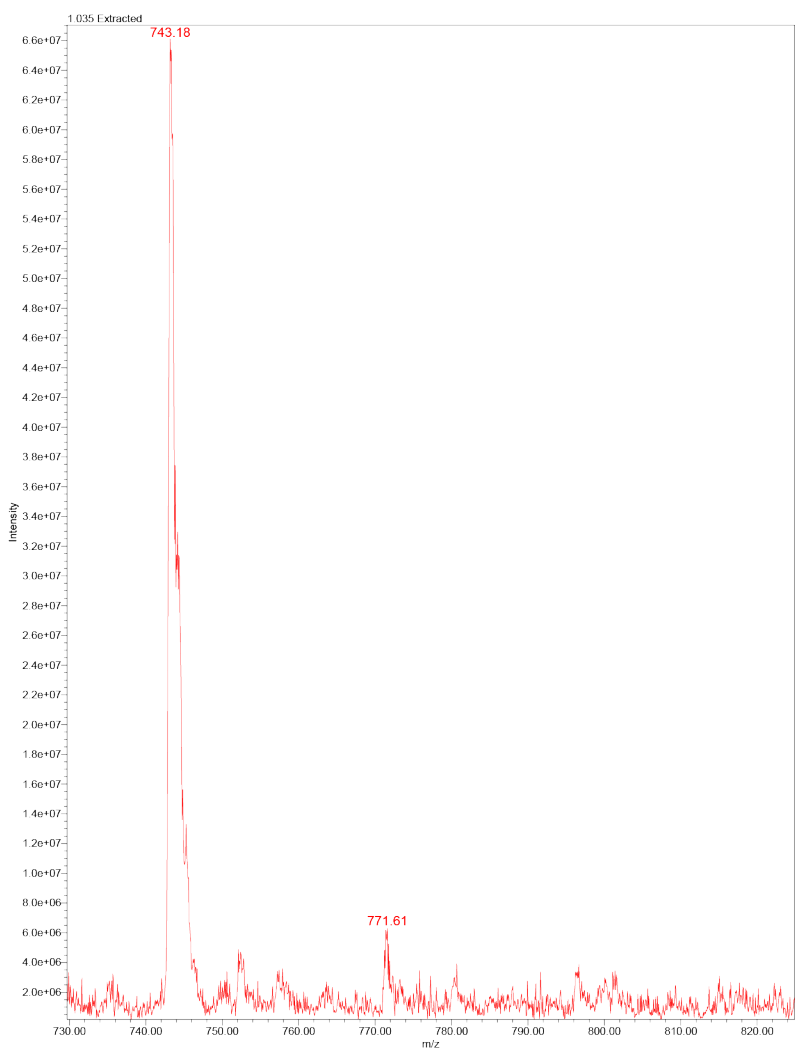
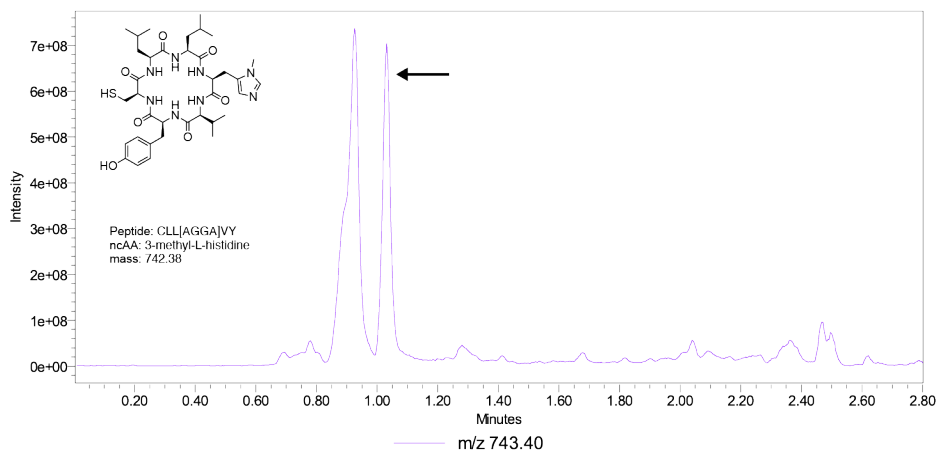
39	CL[AUAG]FVY	4-bromo-L-phenylalanine	850.27	851.27
40	CL[AUAG]FVY	4-acetyl-L-phenylalanine	814.37	815.37
41	C[CGGA]LFVY	3-(1-naphthyl)-L-alanine	822.38	823.38
42	CL[CGGA]FVY	3-(1-naphthyl)-L-alanine	822.38	823.38
43	CLL[CGGA]VY	3-(1-naphthyl)-L-alanine	788.39	789.39
44	C[CGGA]LFVY	1-methyl-L-tryptophan	825.39	826.39
45	CL[CGGA]FVY	1-methyl-L-tryptophan	825.39	826.39
46	CLLFV[CGGA]	1-methyl-L-tryptophan	775.41	776.41
47	CLL[CGGA]VY	1-methyl-L-tryptophan	791.4	792.4
48	CLL[CGGA]VY	6-methyl-L-tryptophan	791.4	792.4
49	CL[CGGA]FVY	3-benzothienyl-Ala	828.33	829.33
50	CLL[CGGA]VY	3-benzothienyl-Ala	794.35	795.35

Products highlighted in white text appear in main text **Figure 5**.

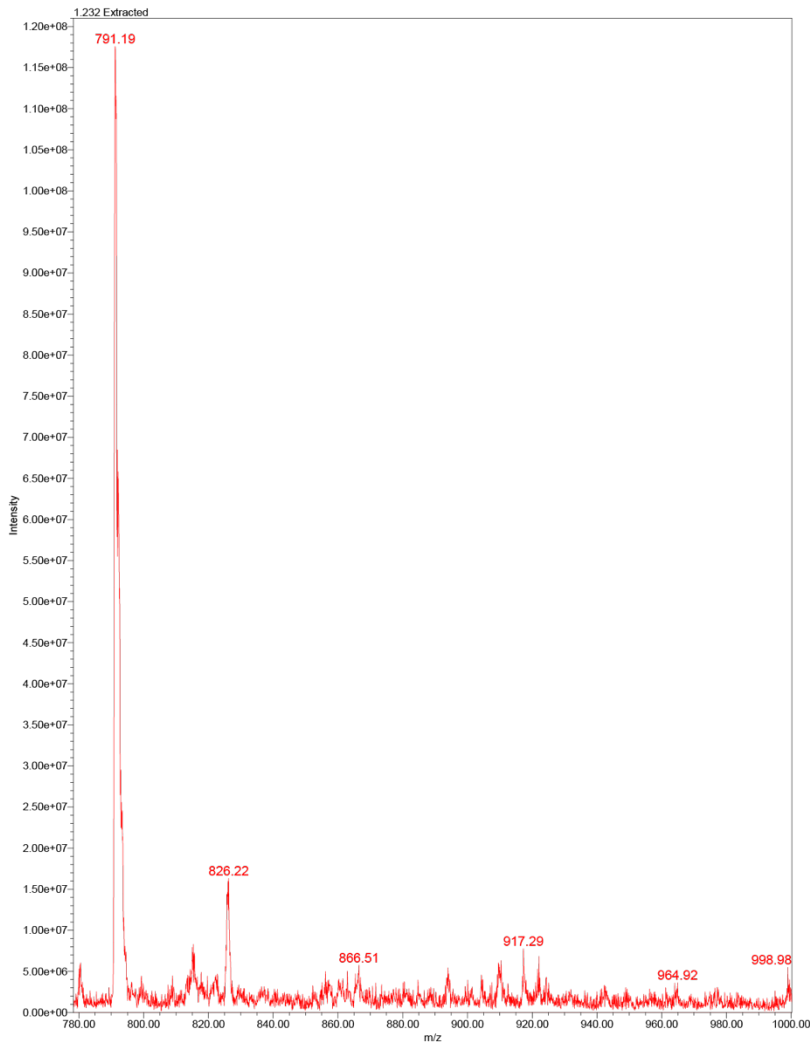
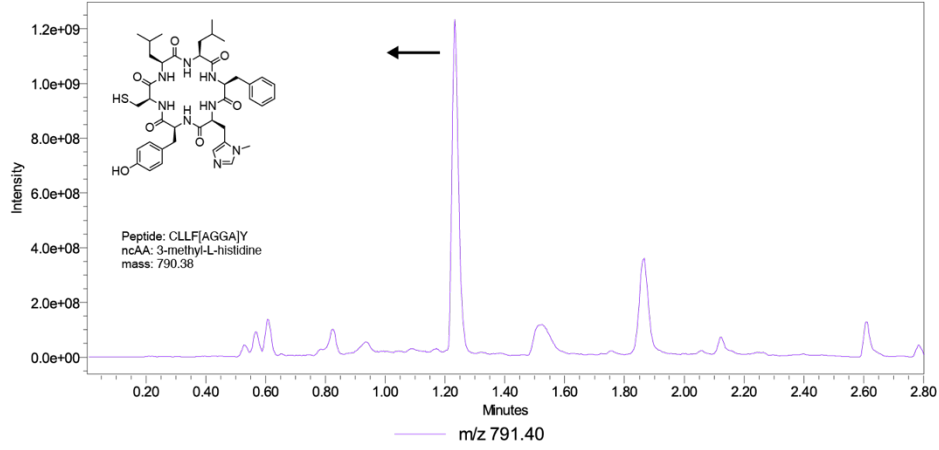
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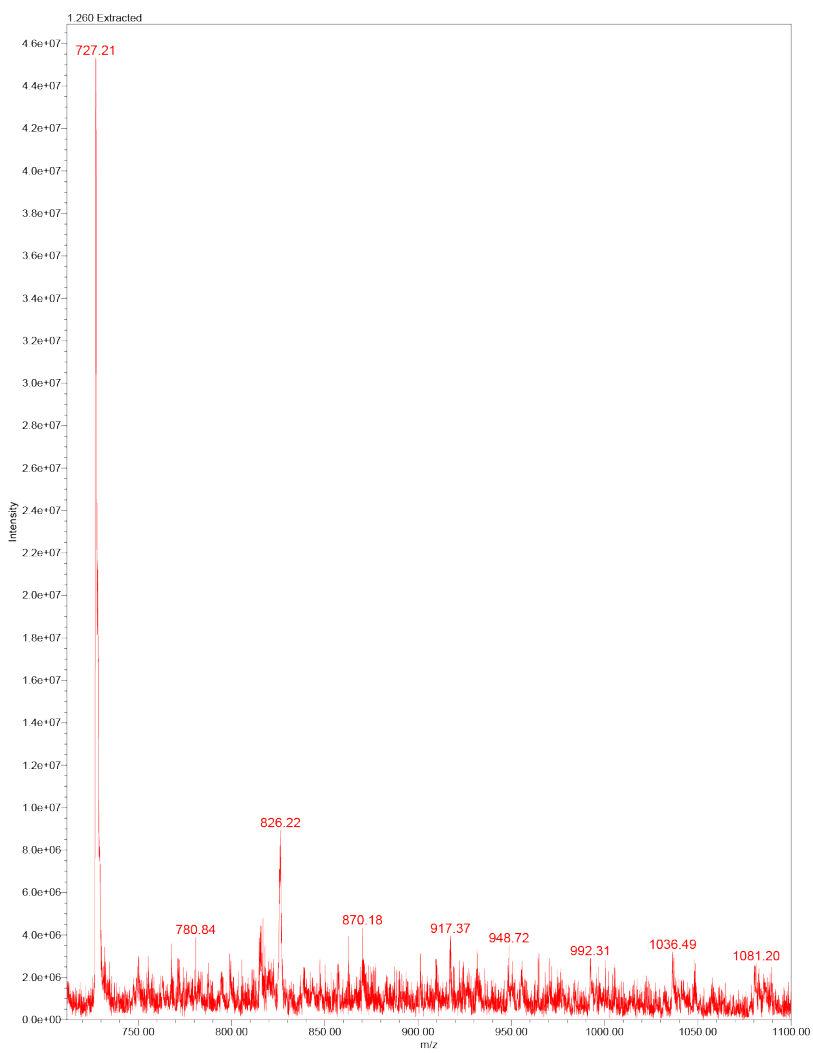
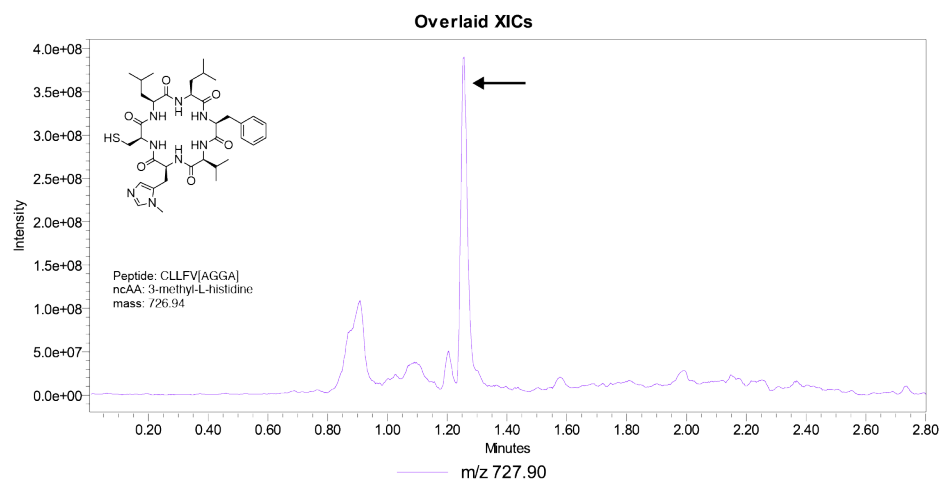


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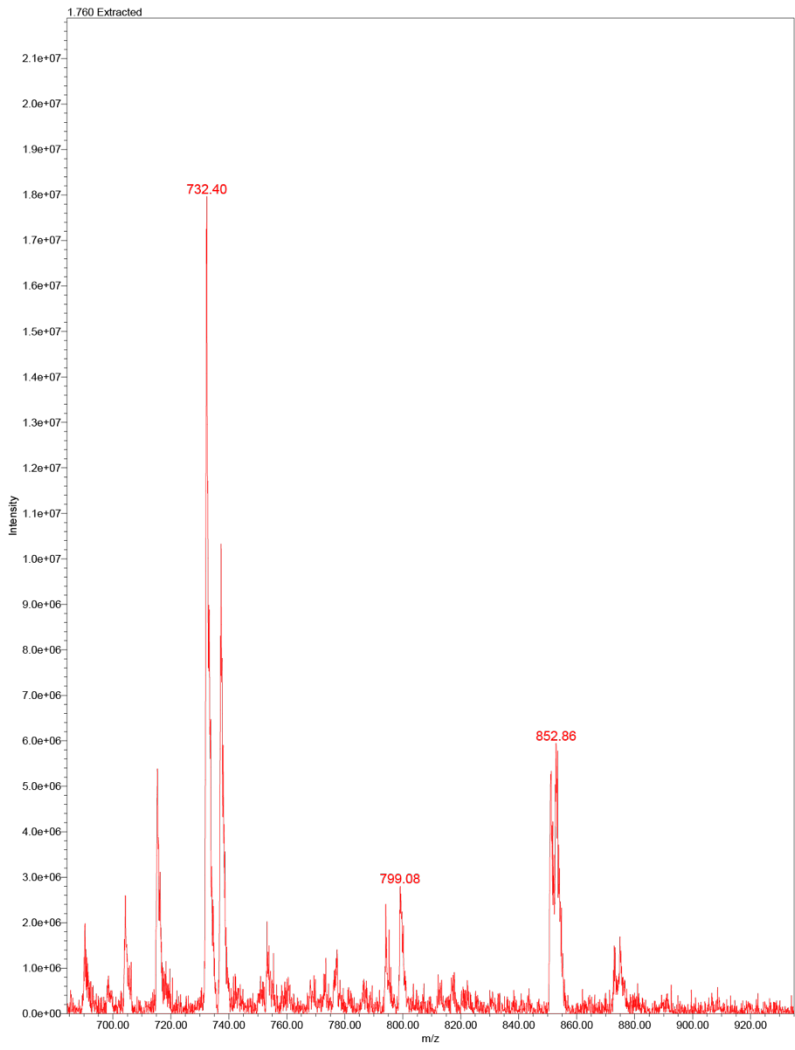
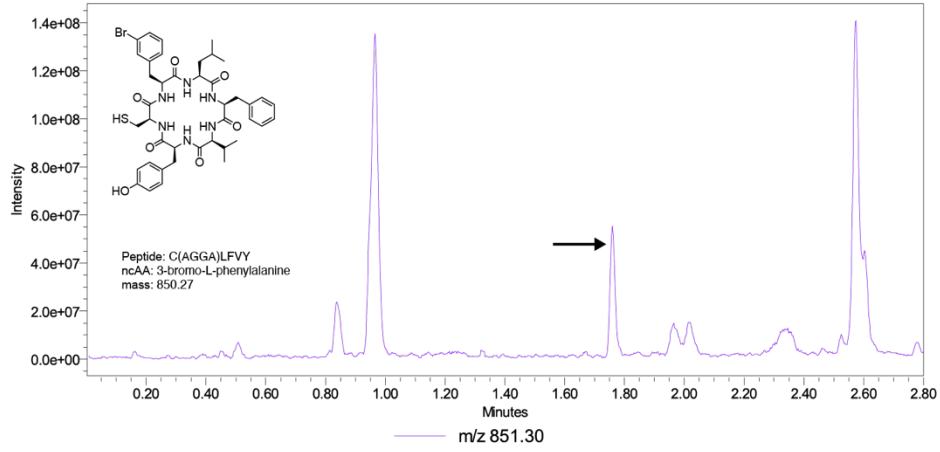


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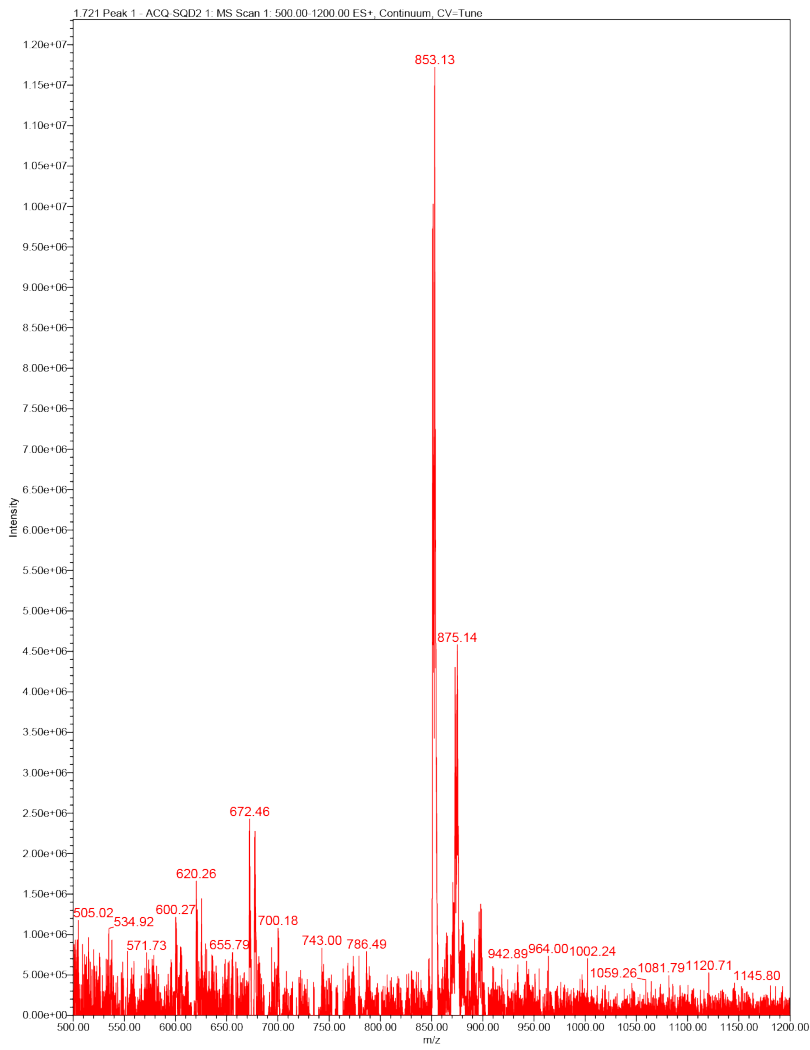
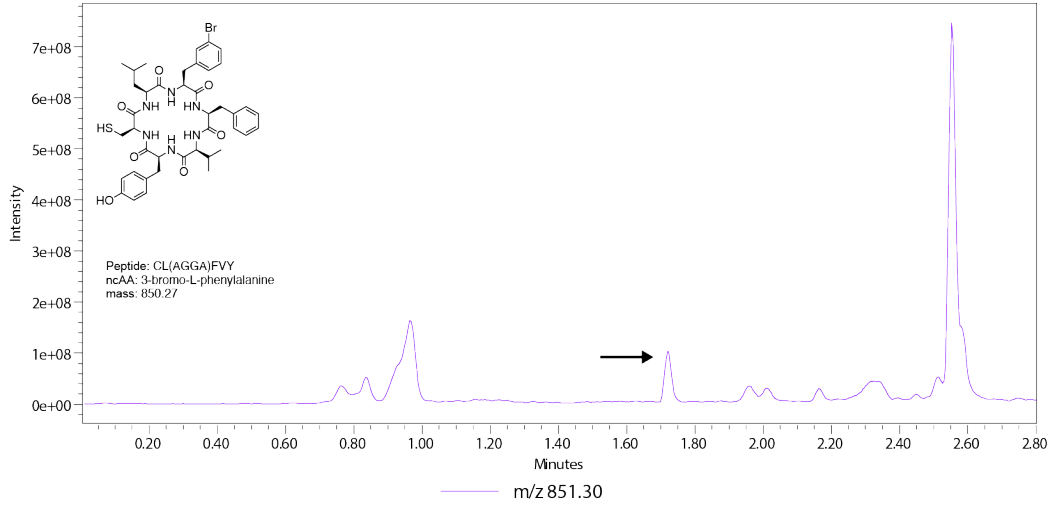




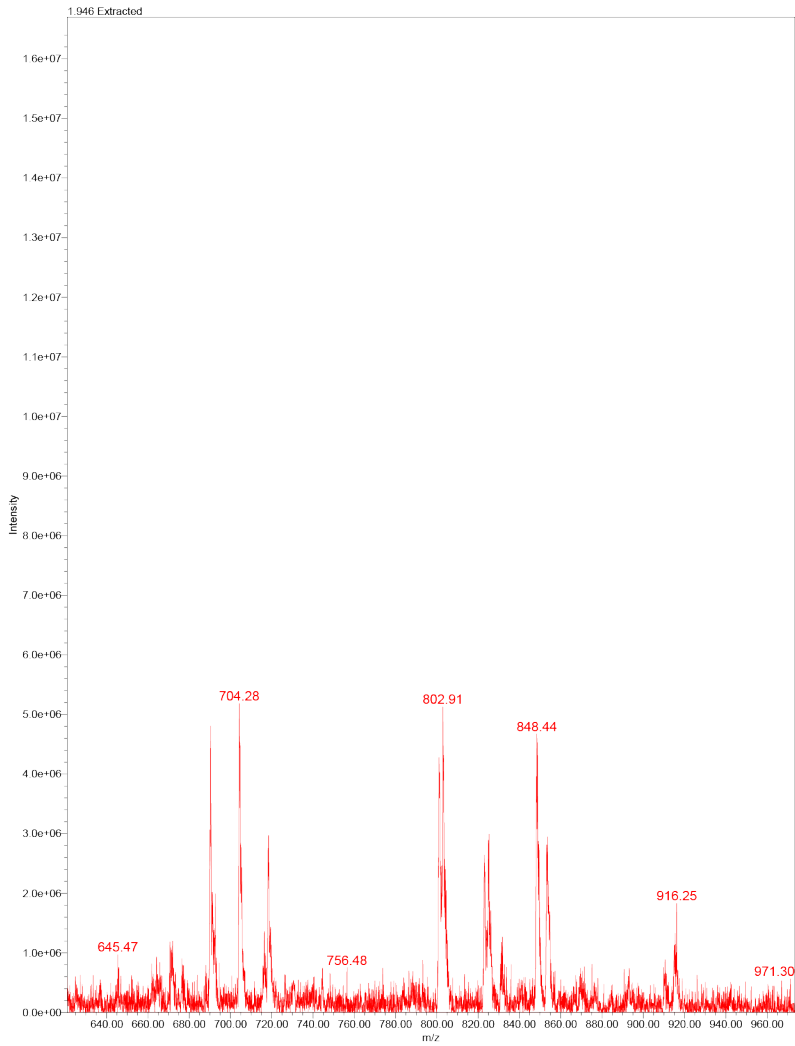
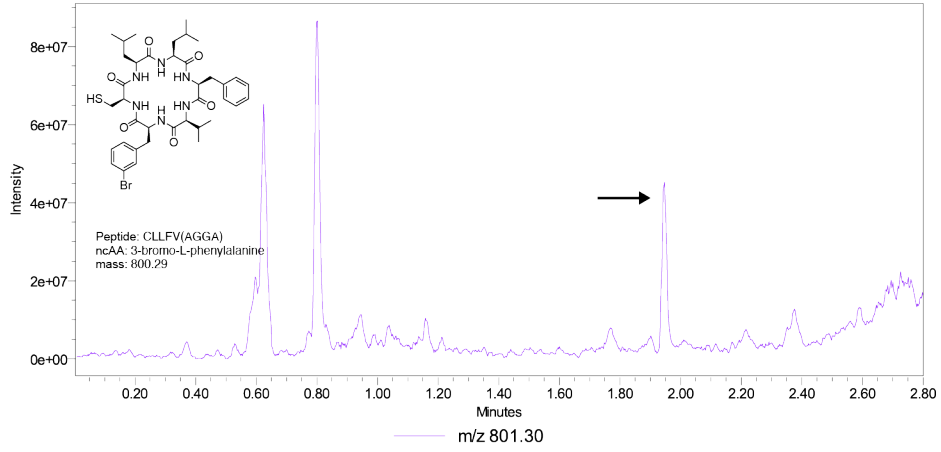
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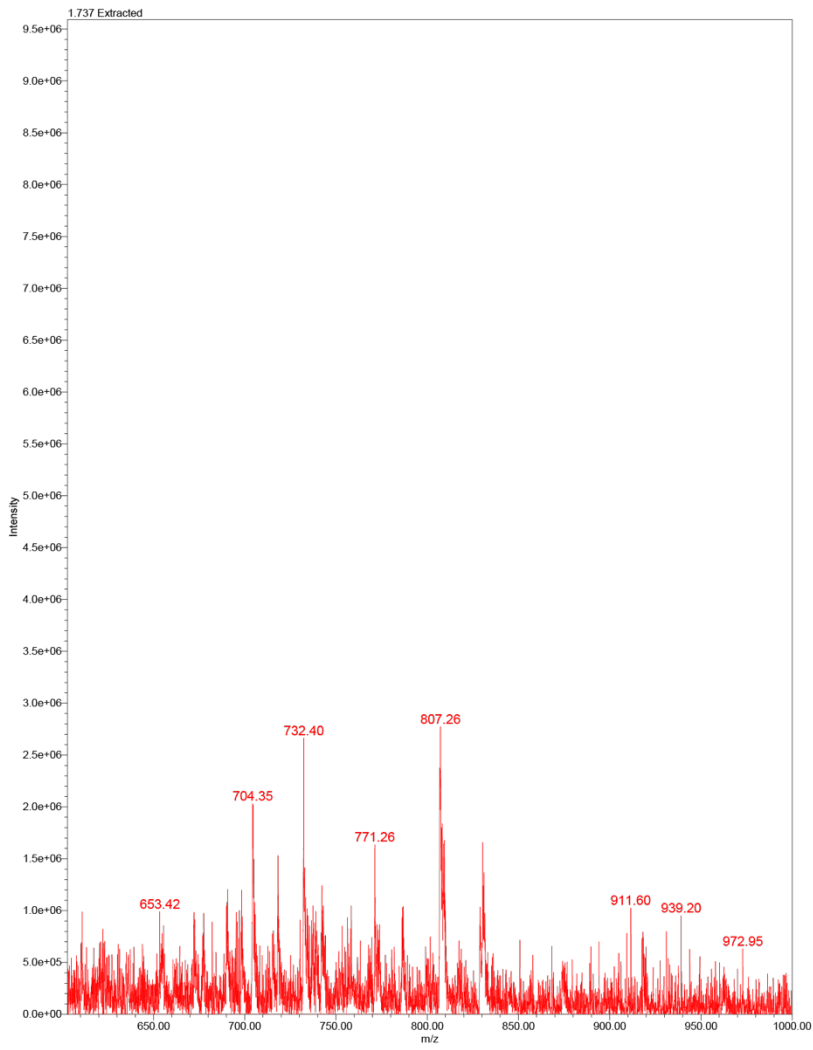
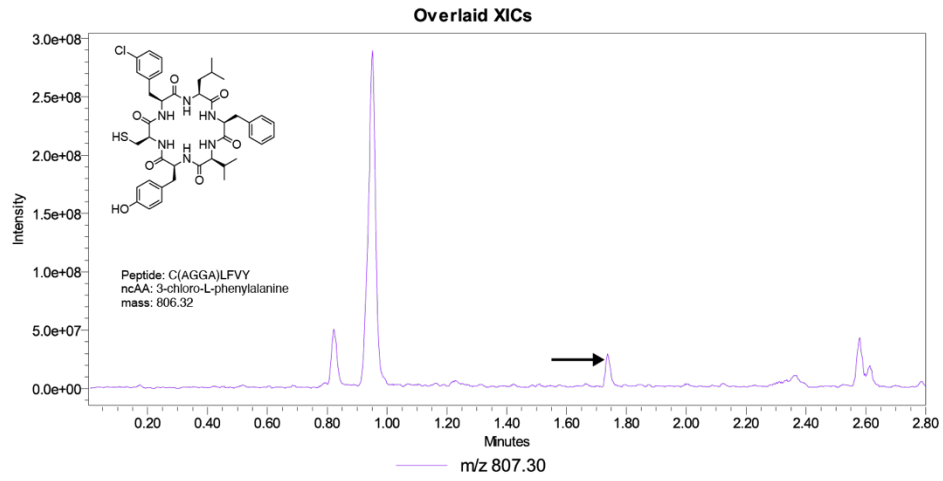


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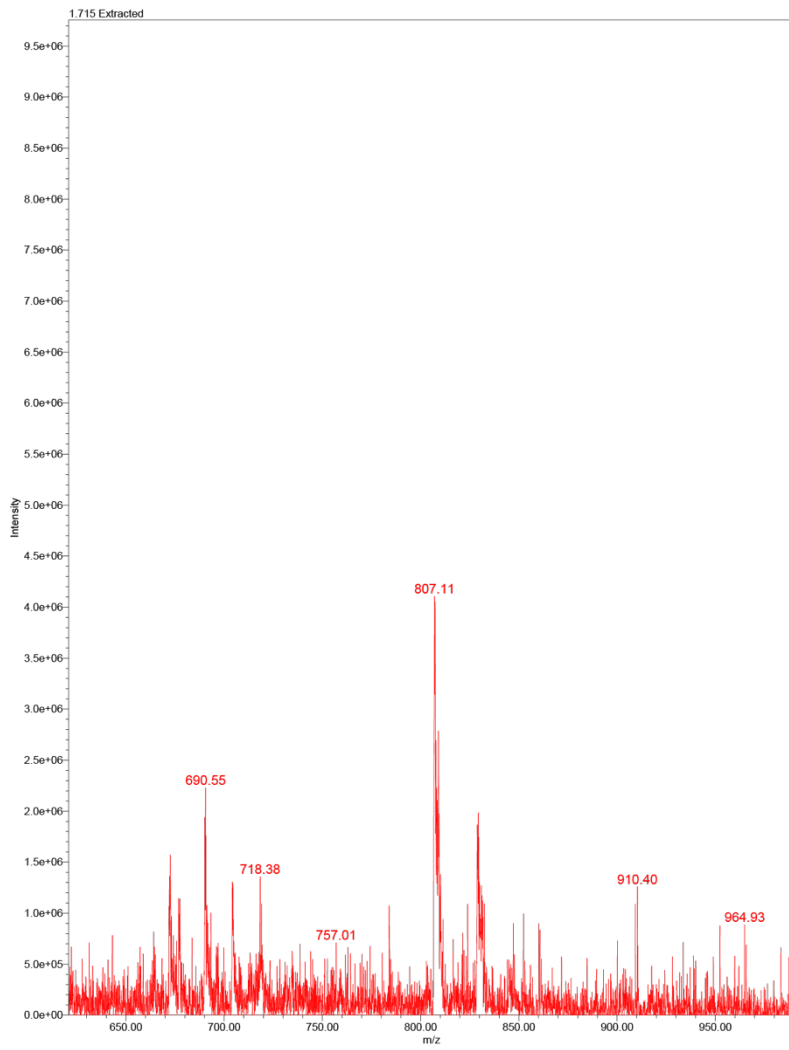
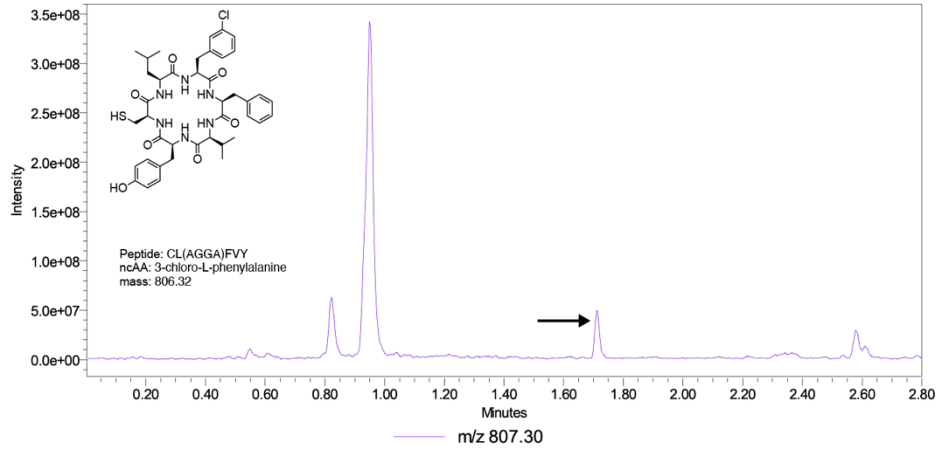


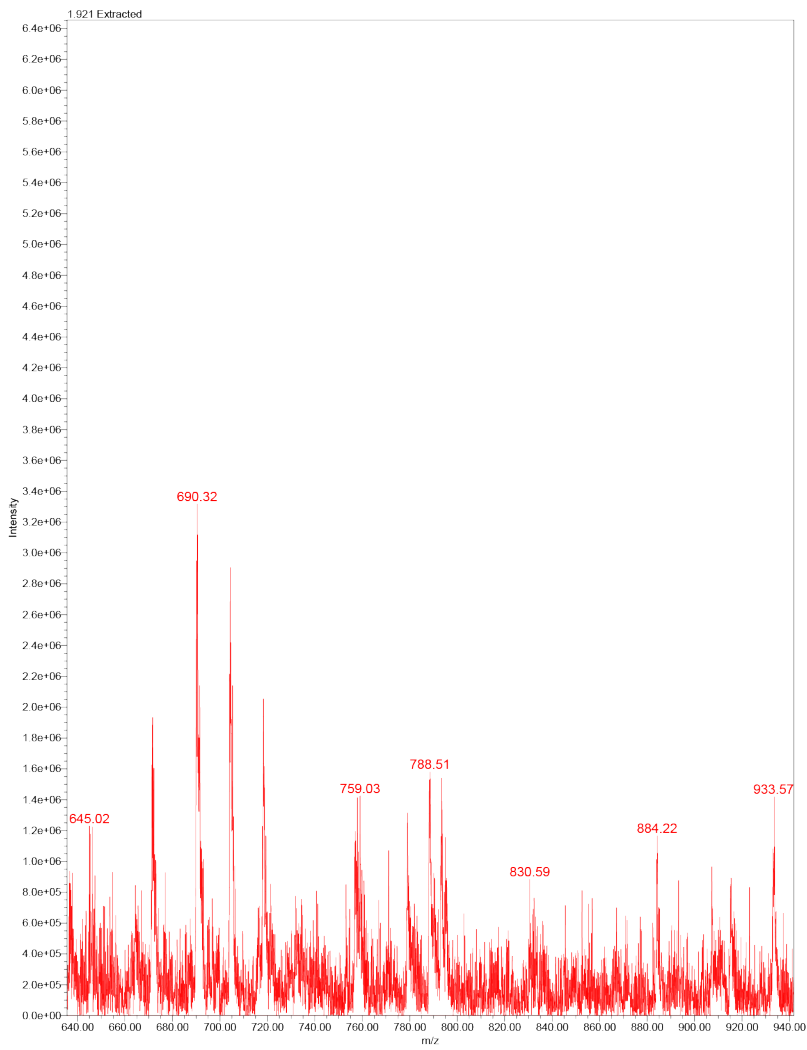
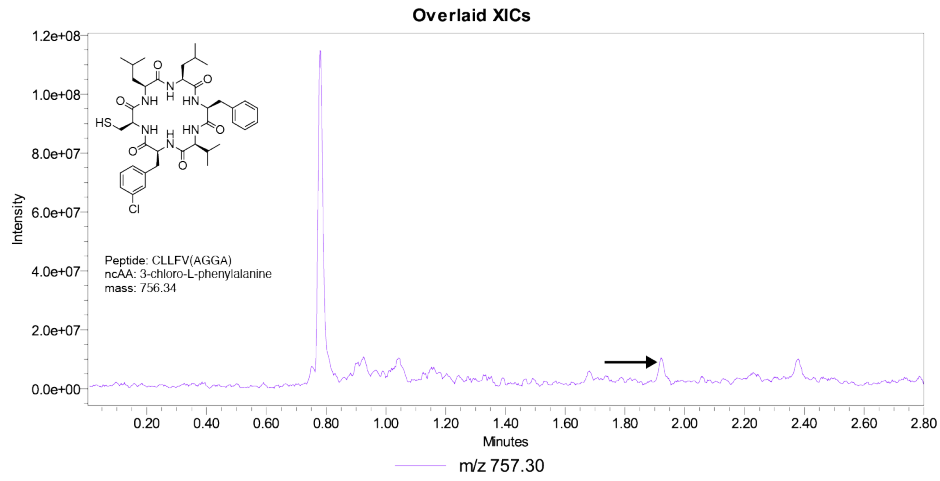
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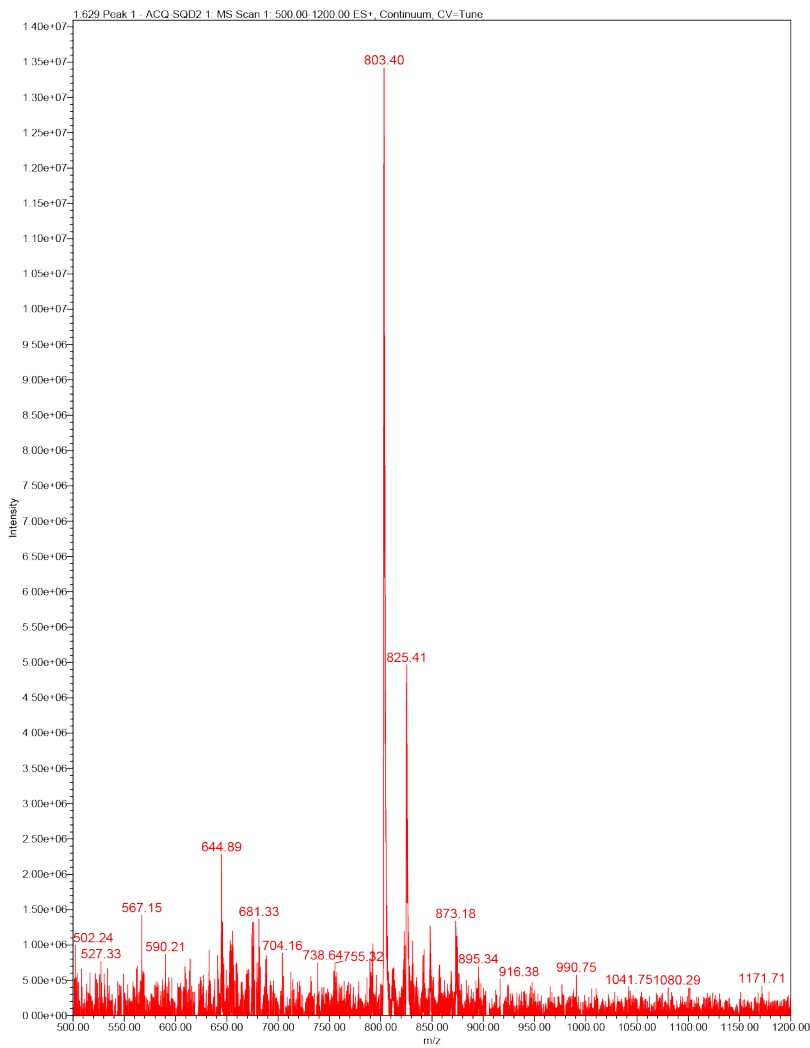
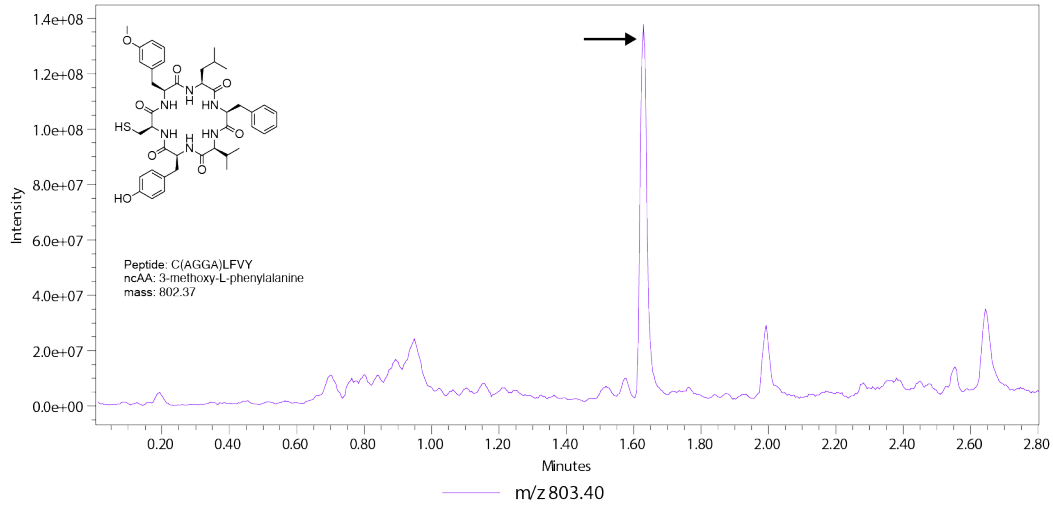


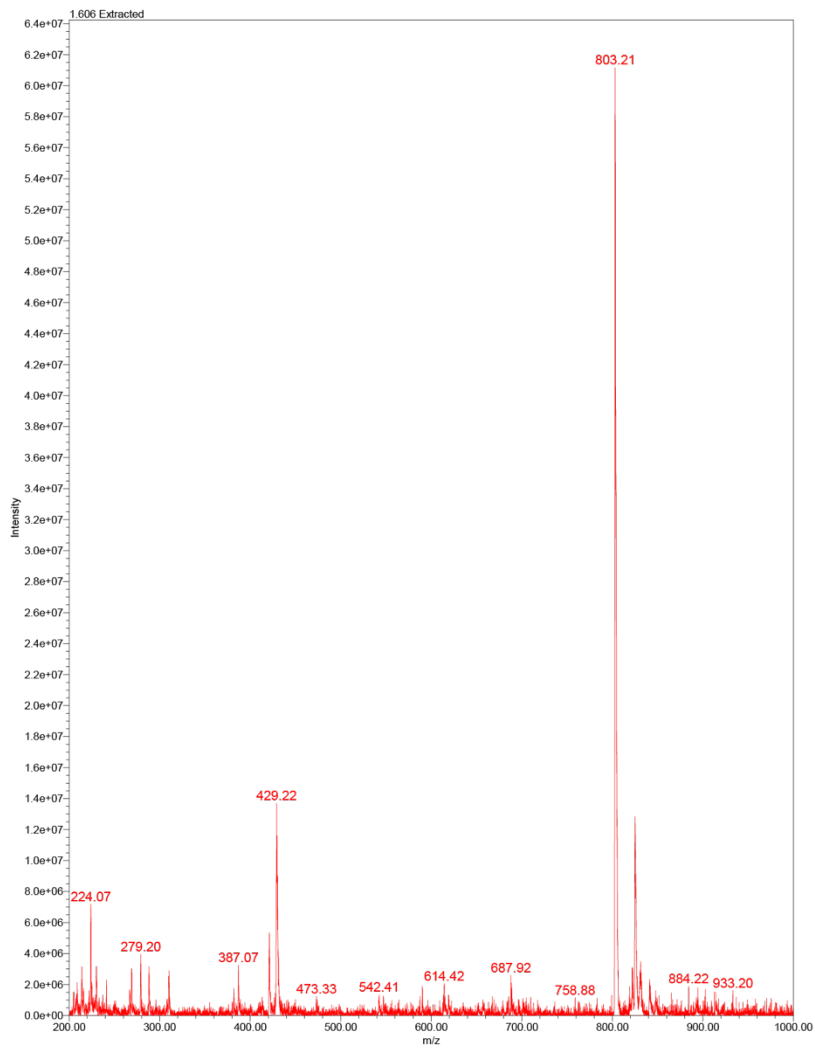
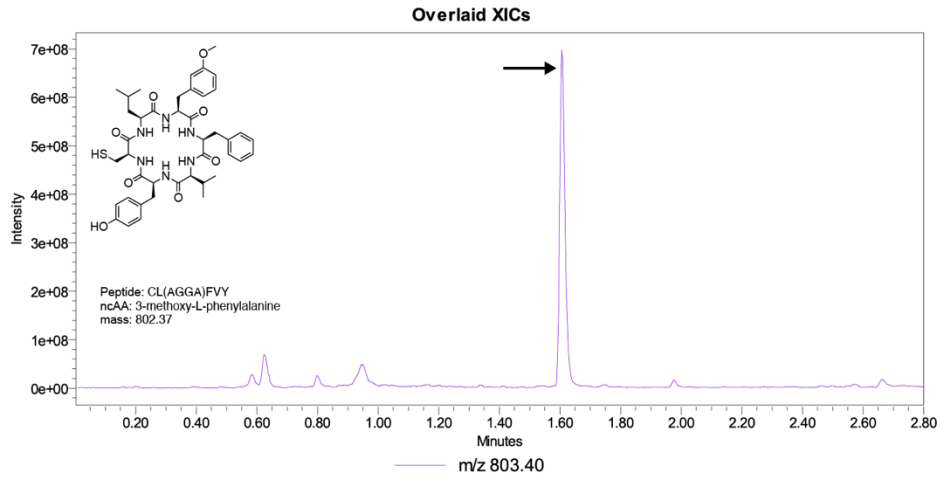
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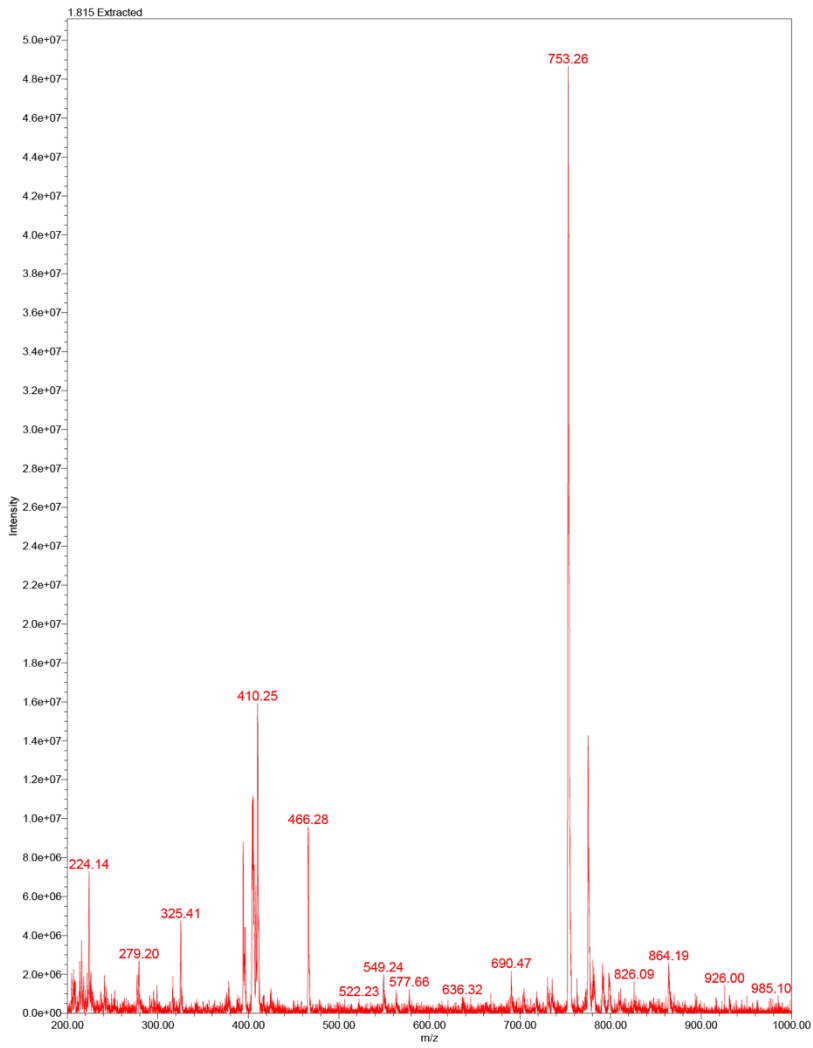
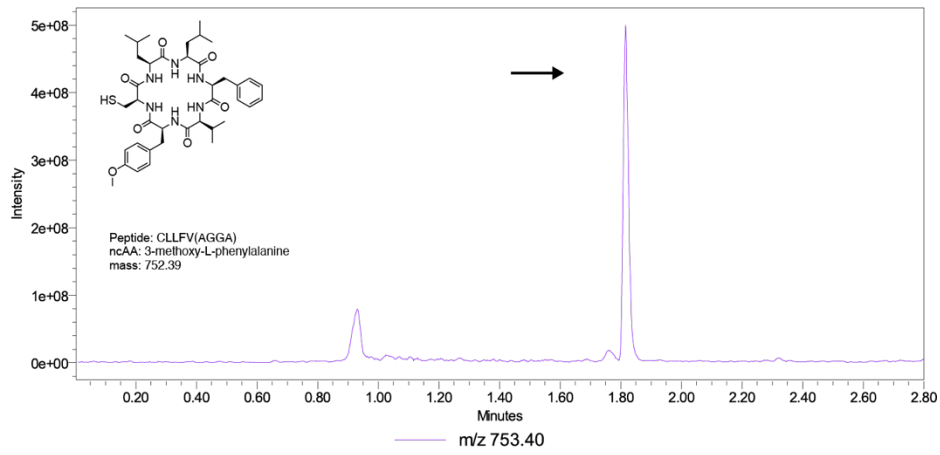


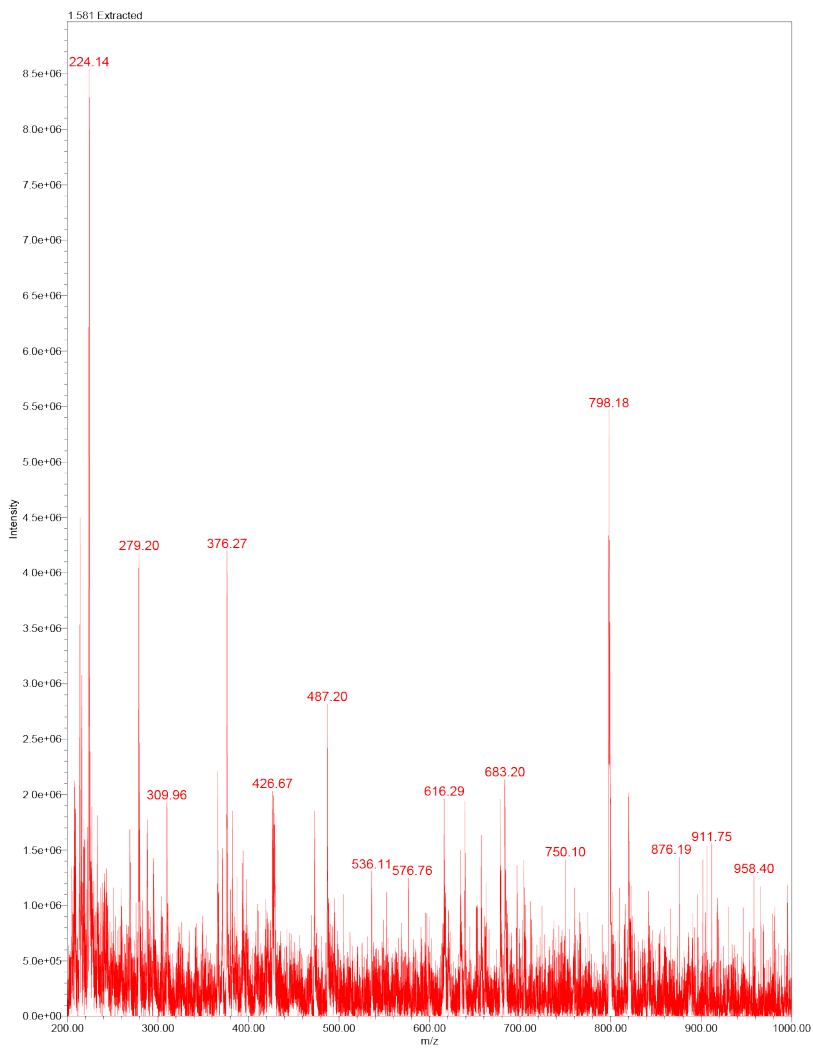
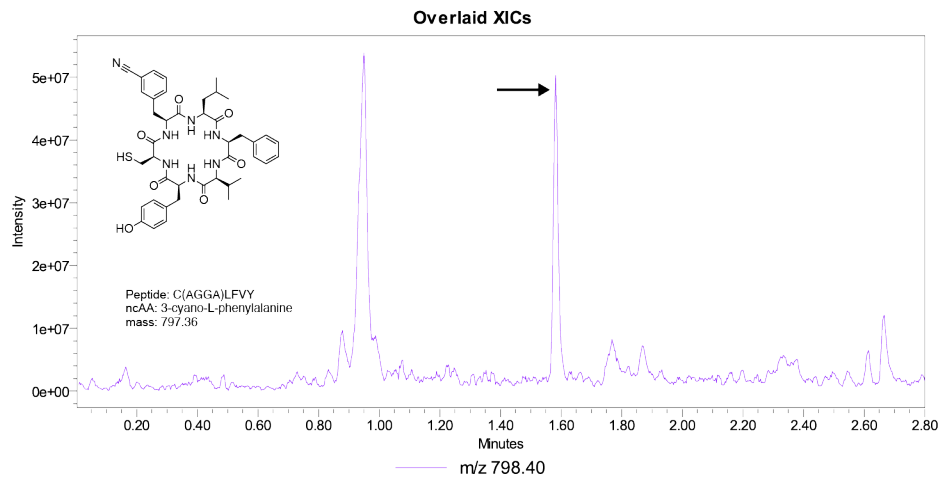


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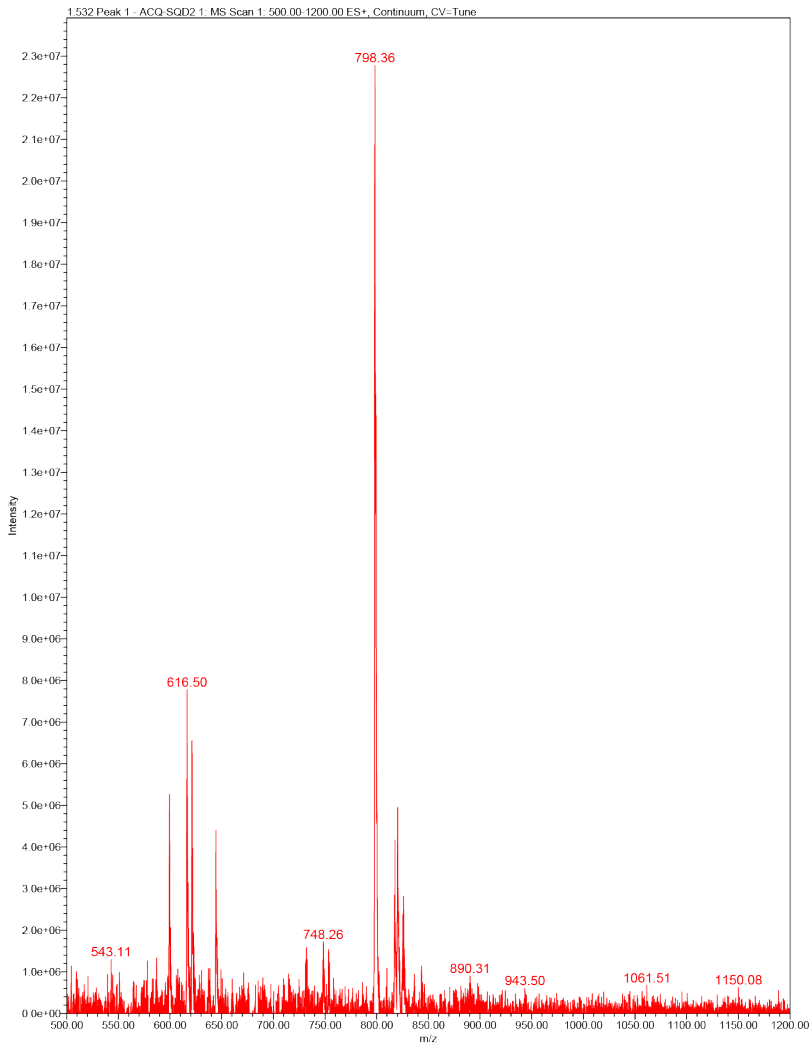
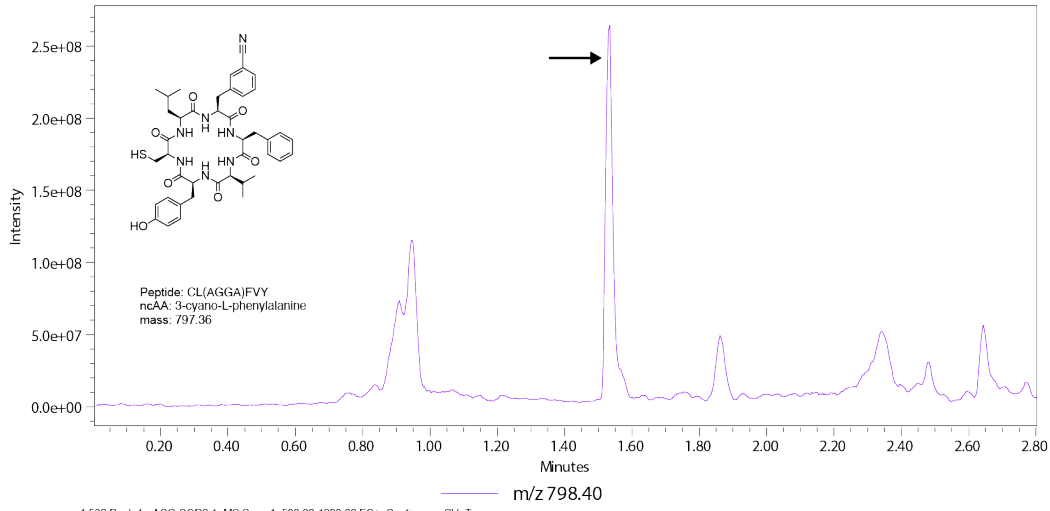




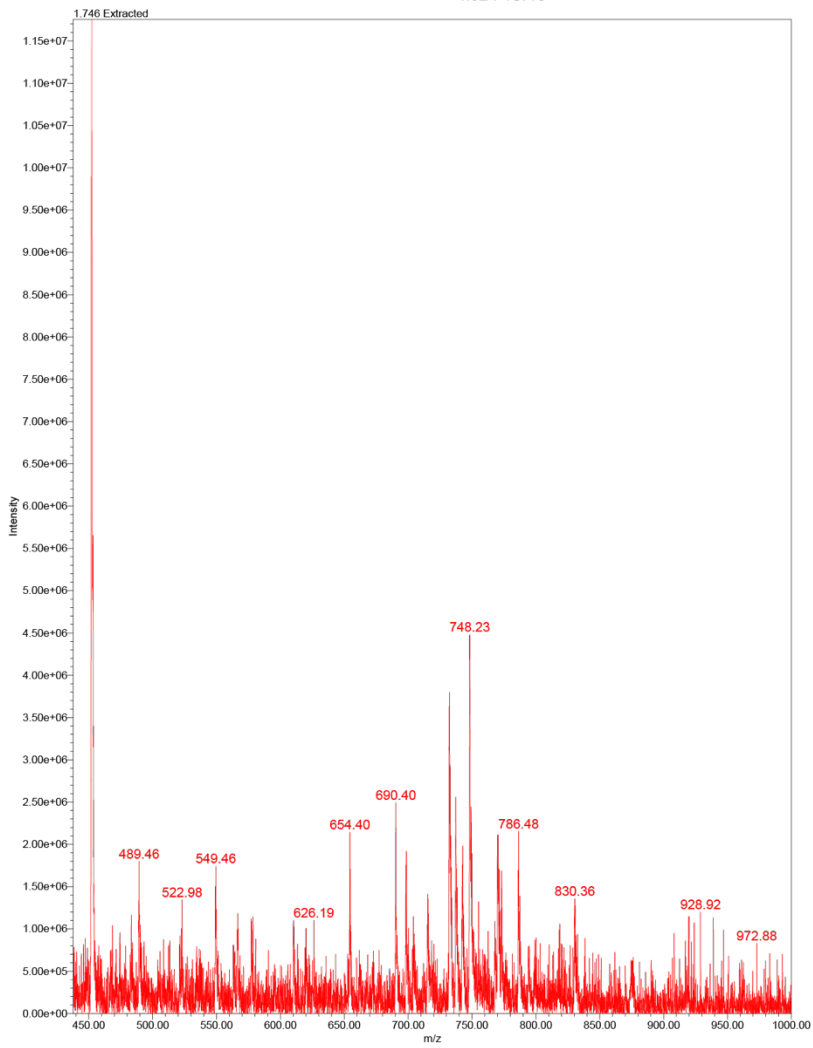
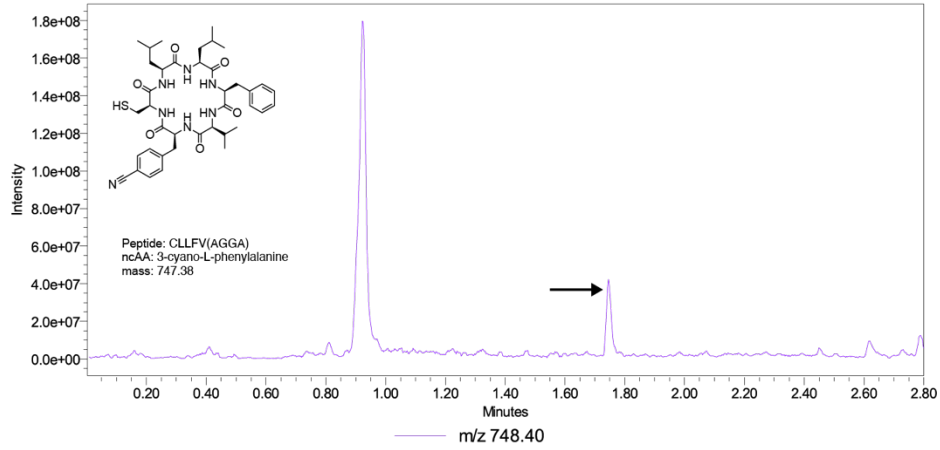




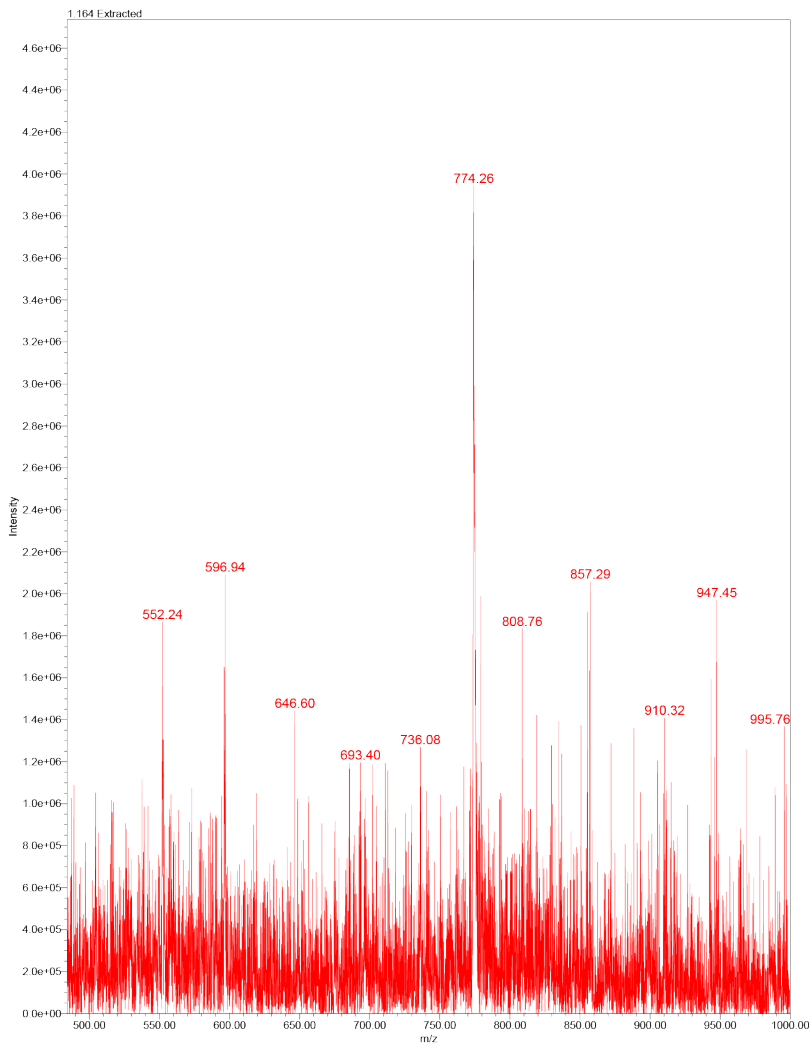
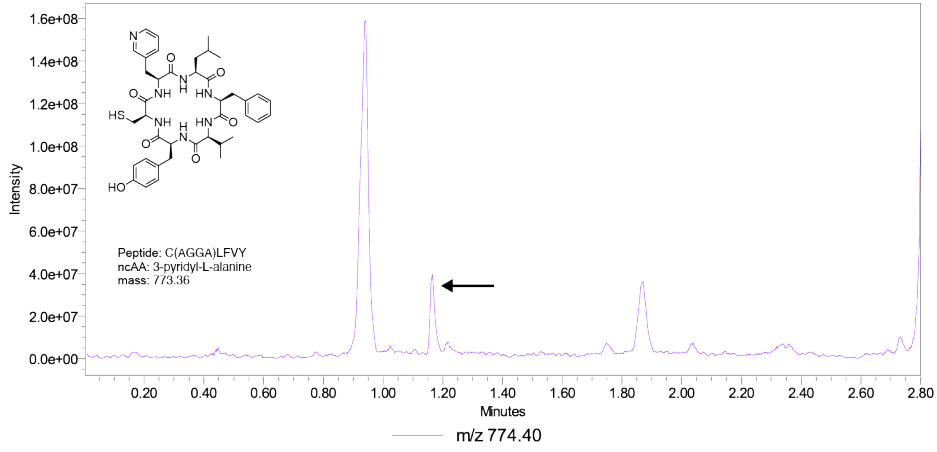
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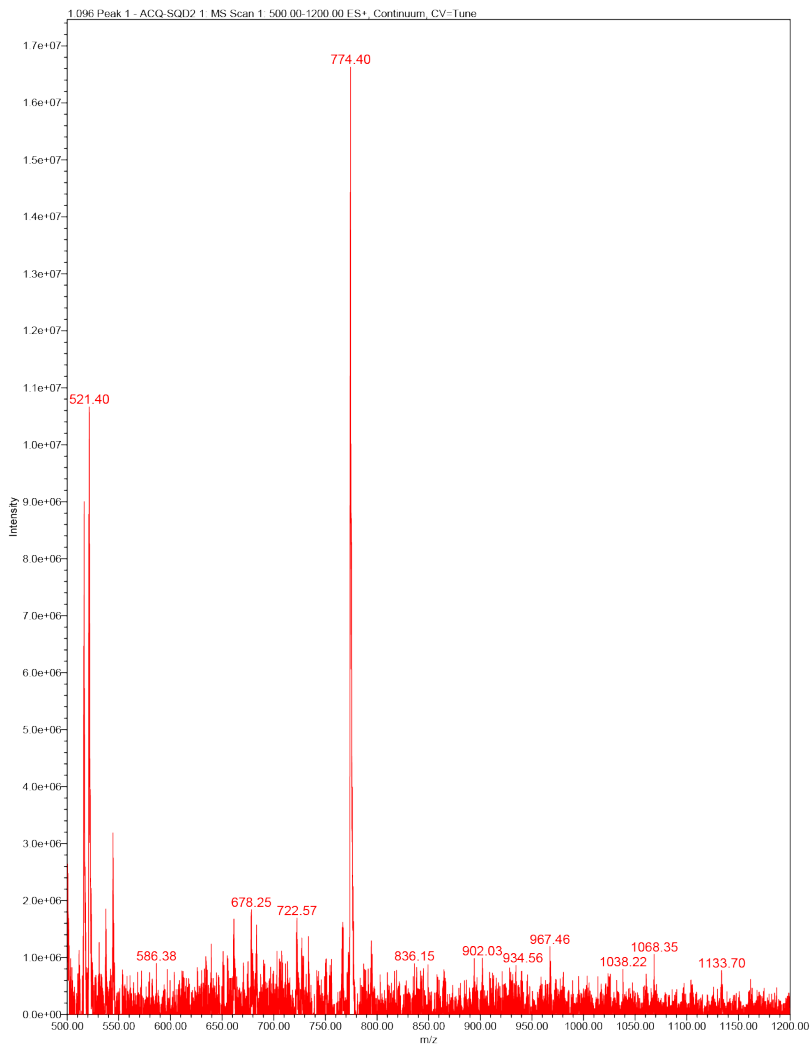
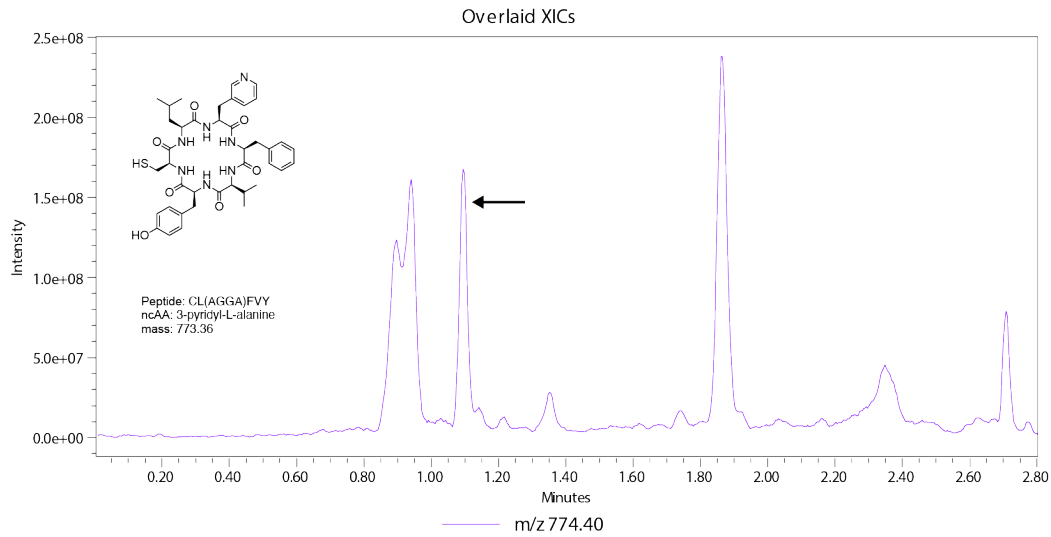


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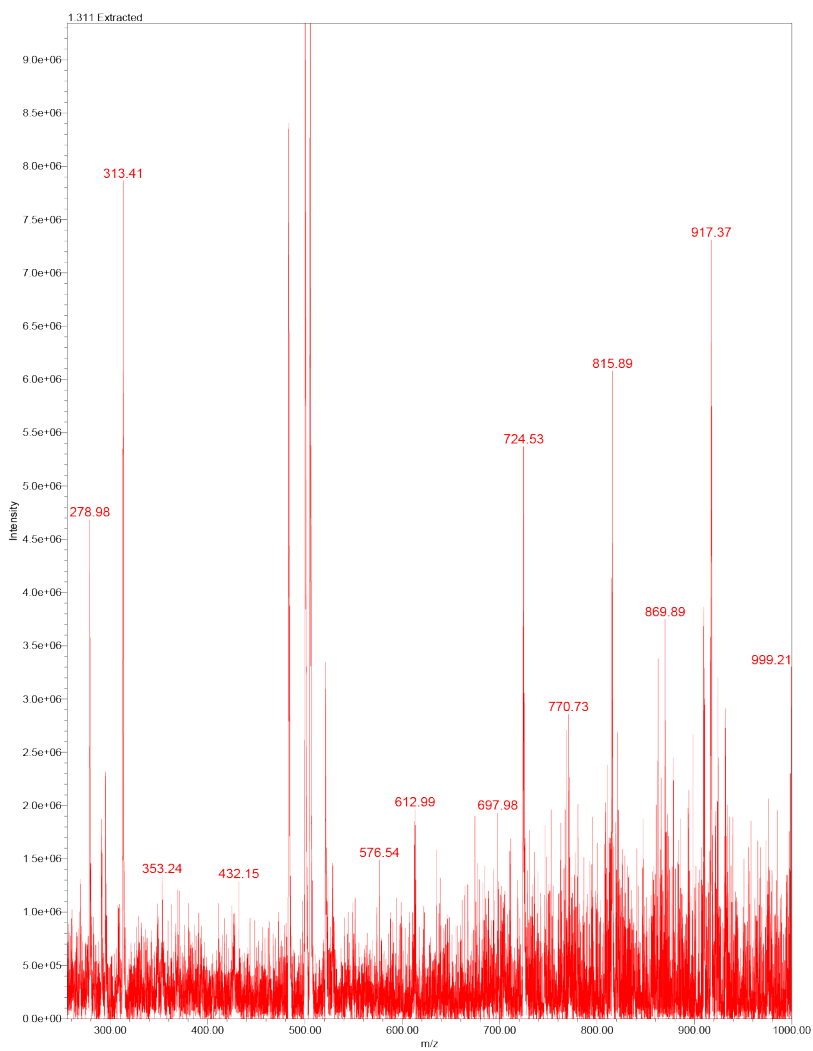
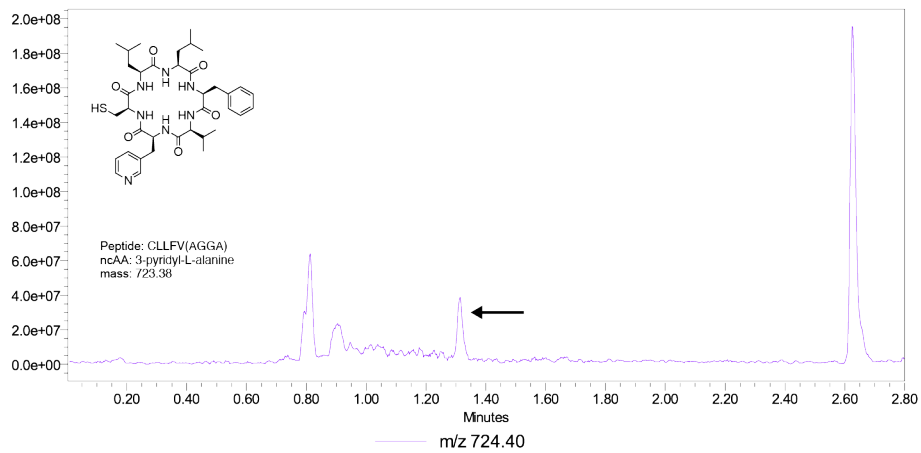


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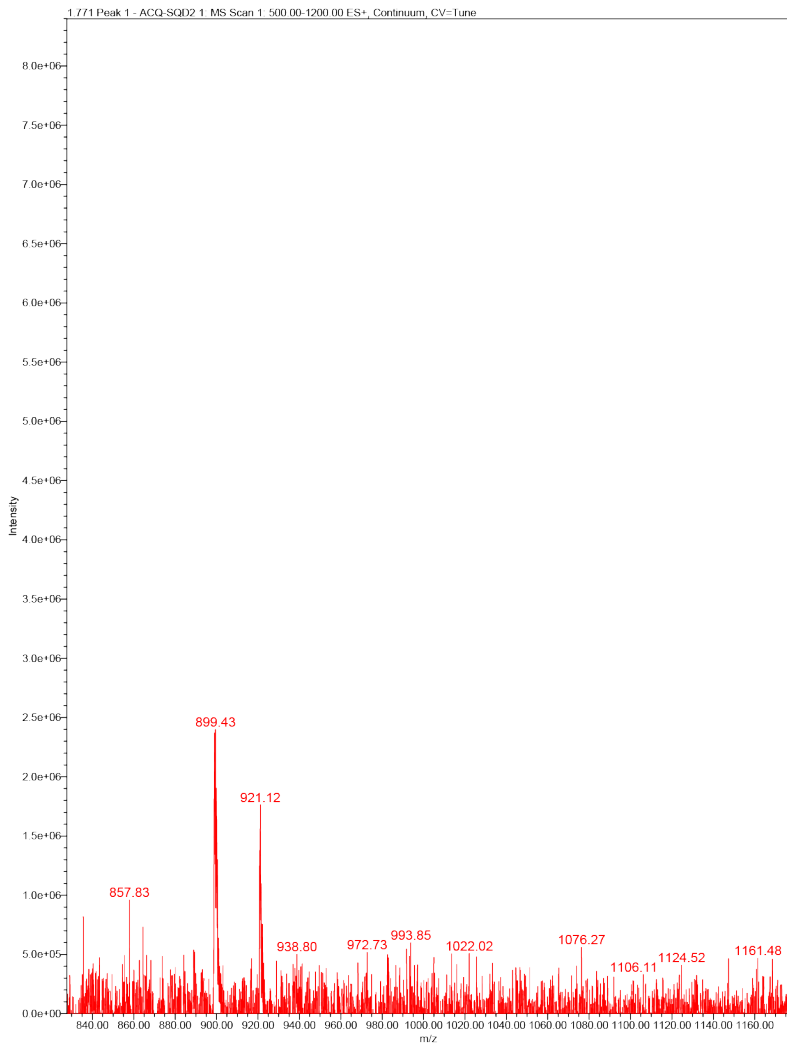
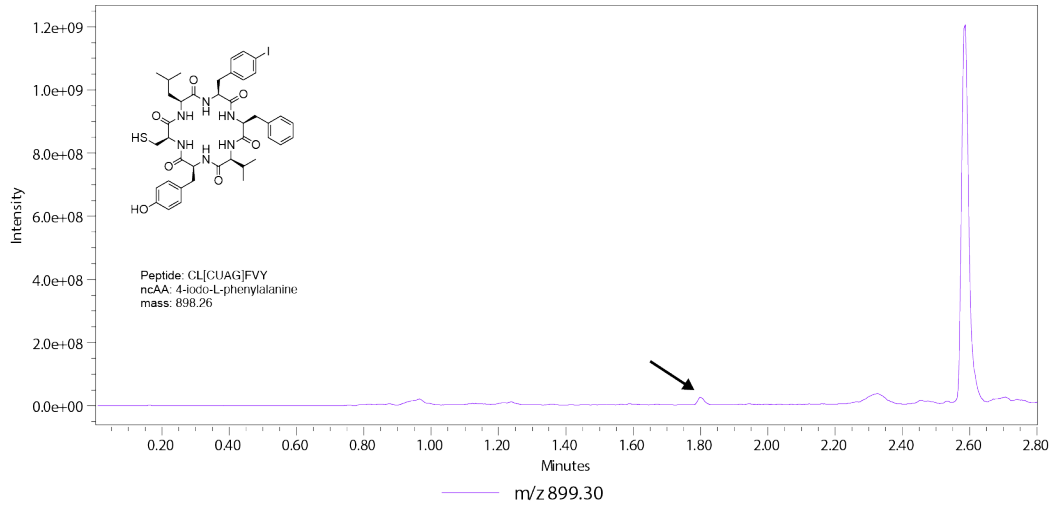




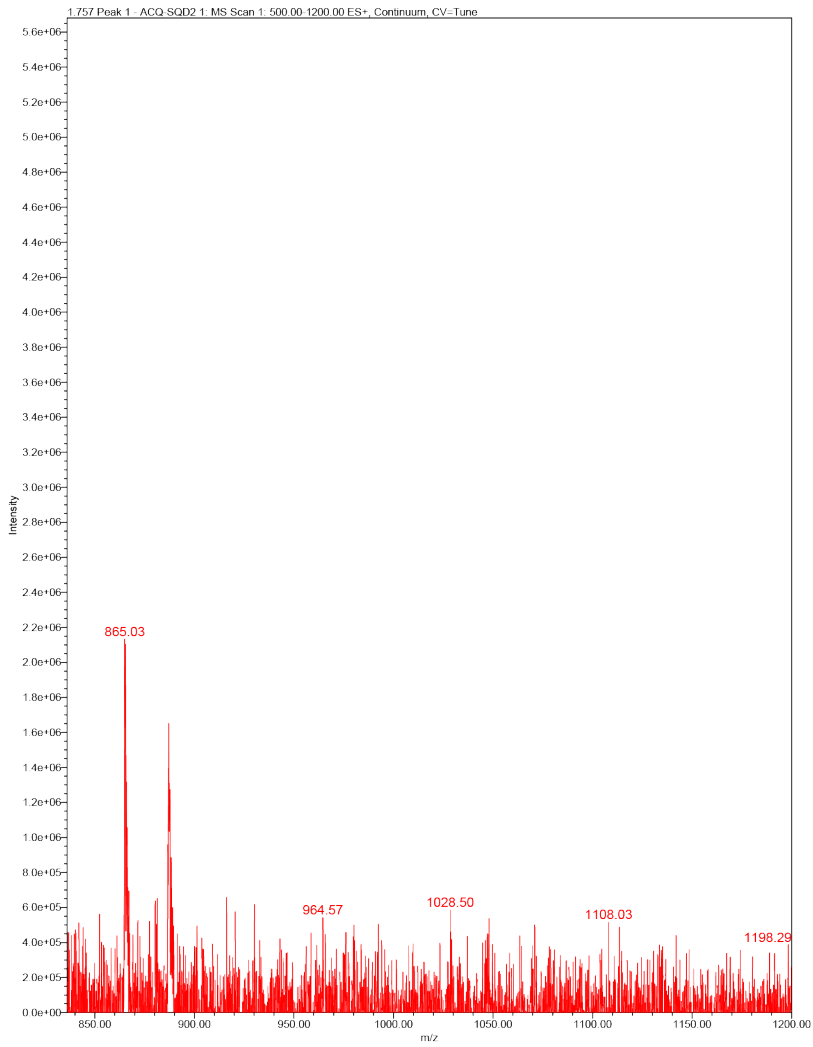
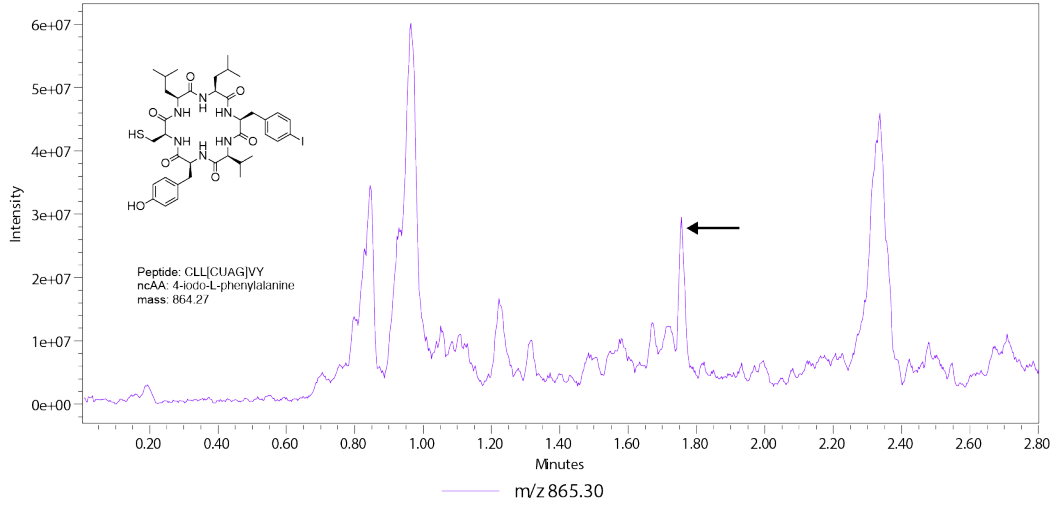
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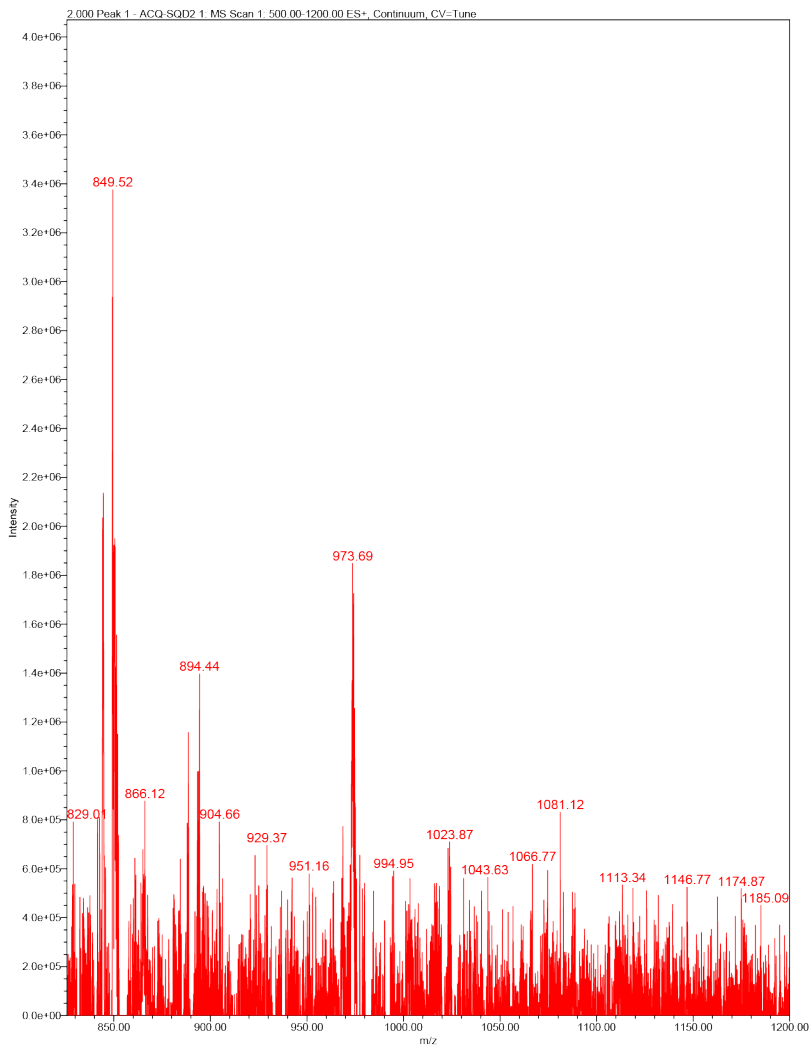
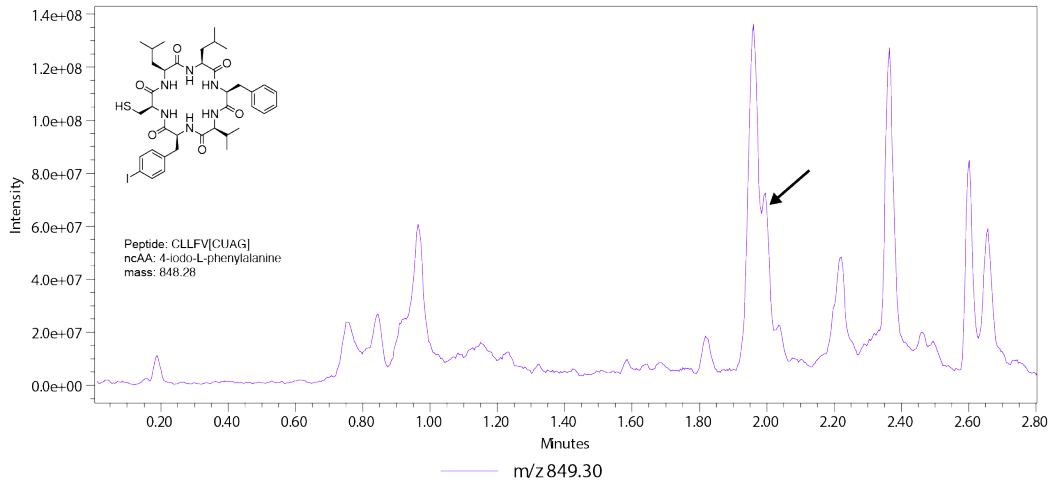
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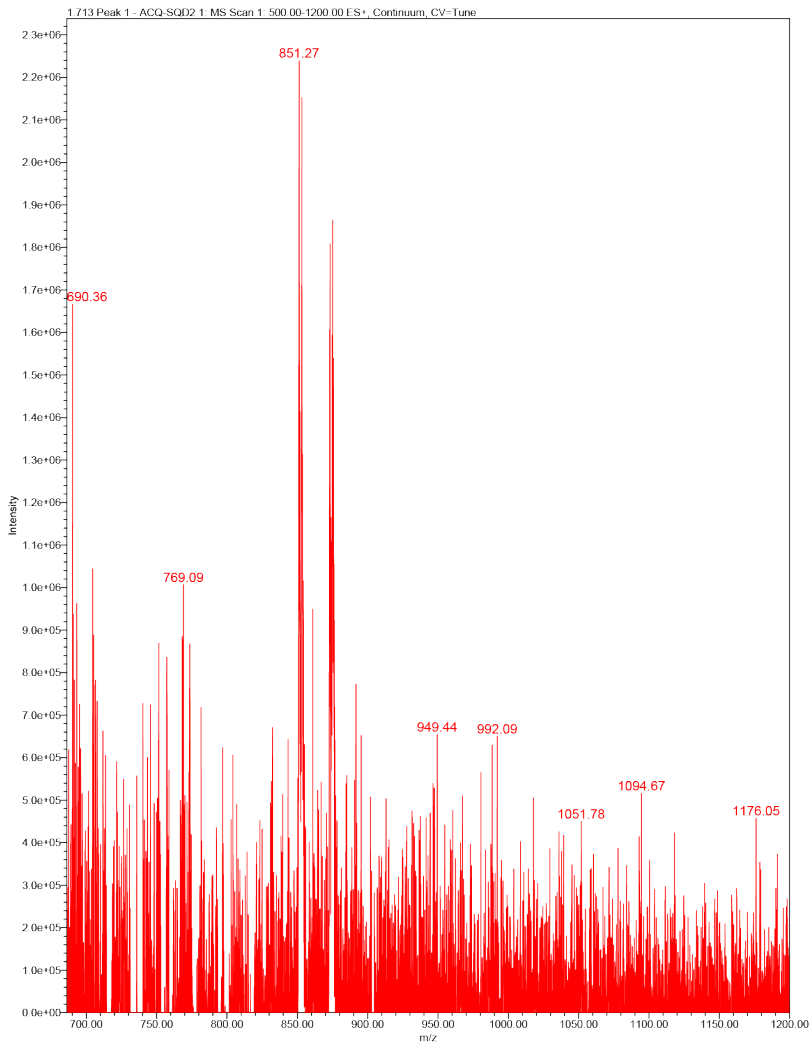
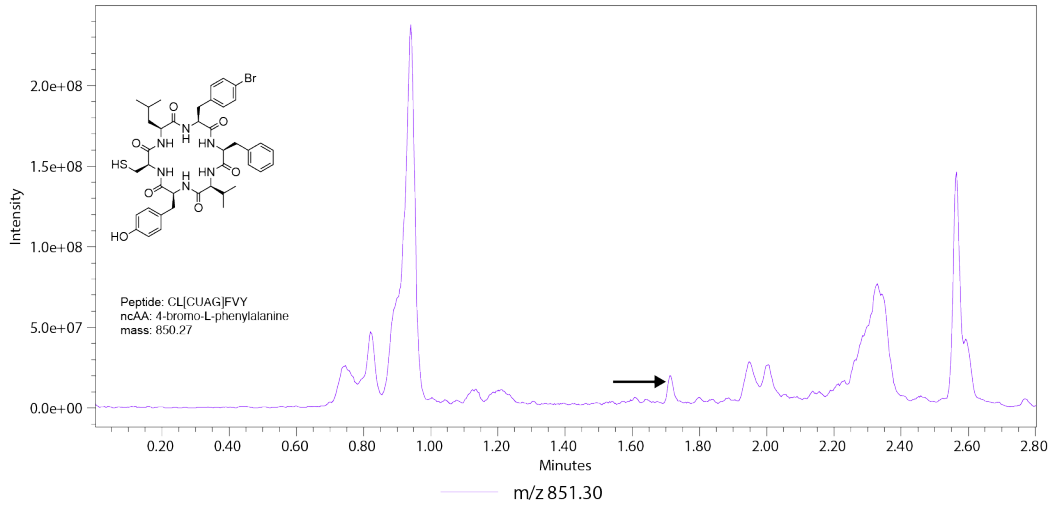
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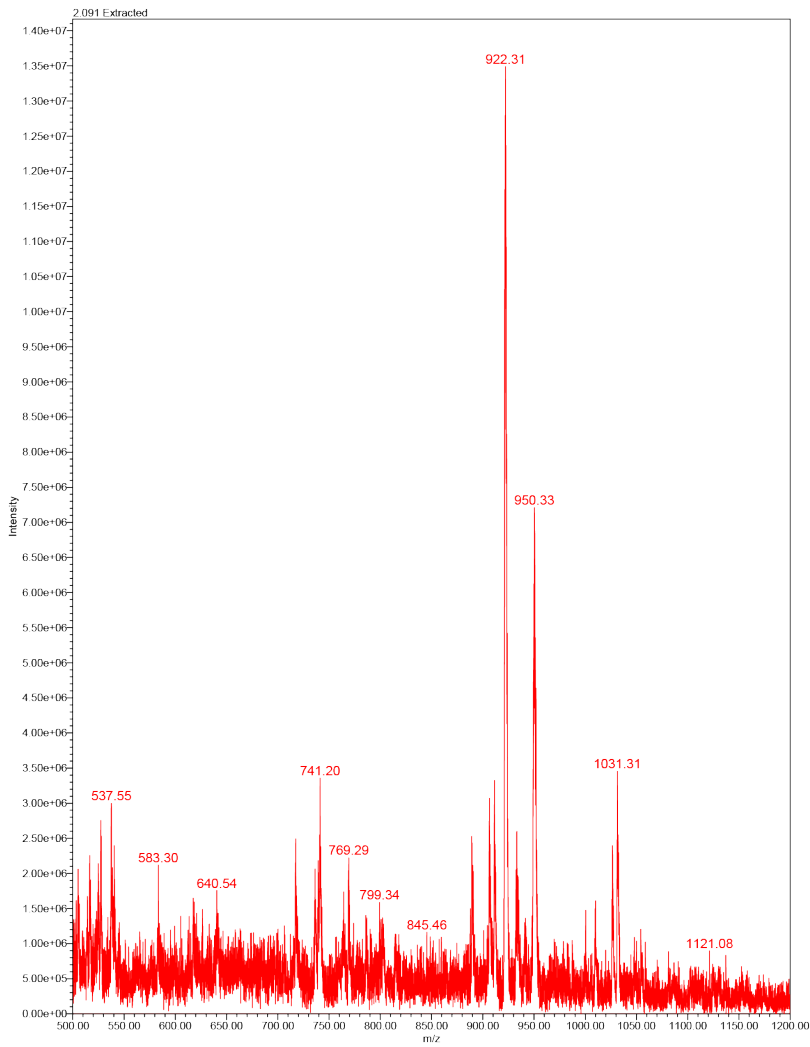
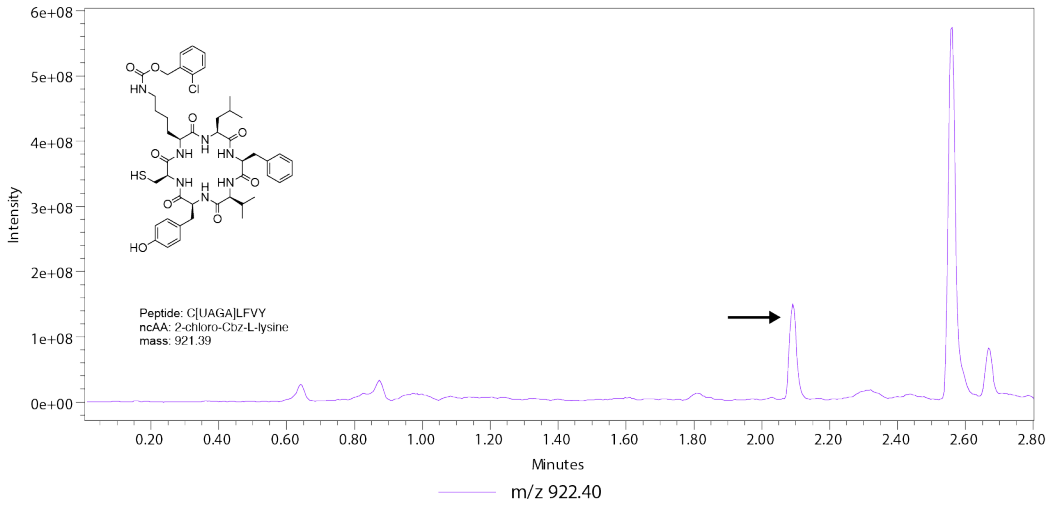
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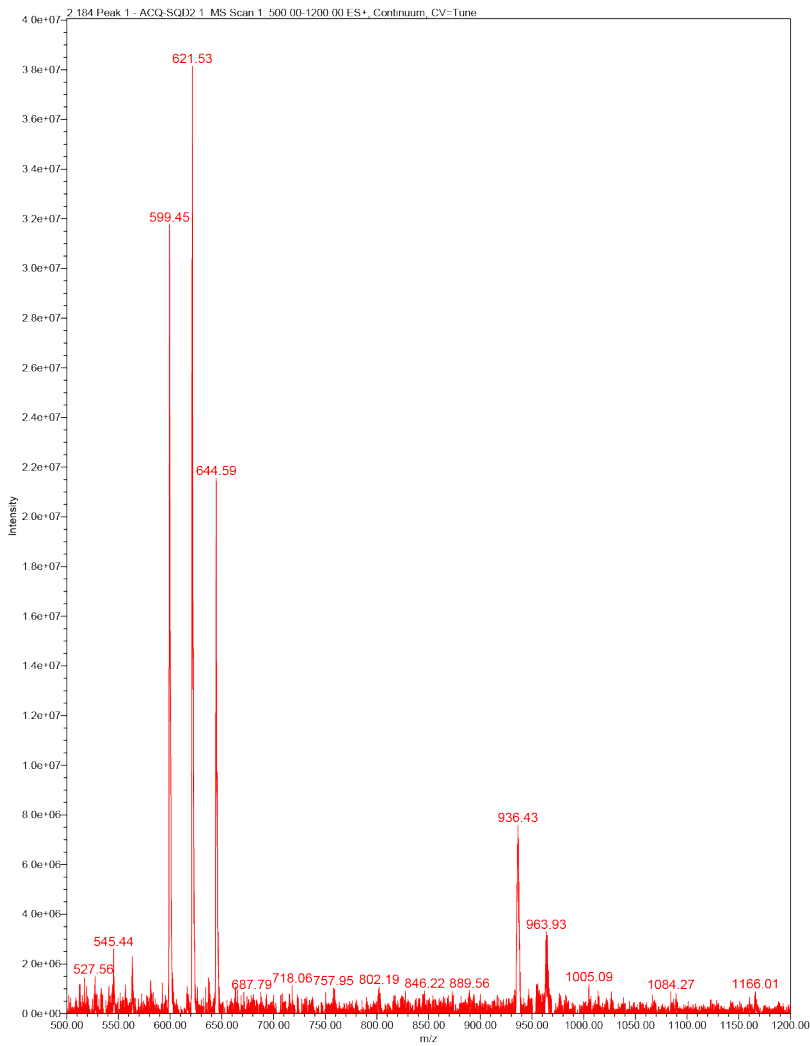
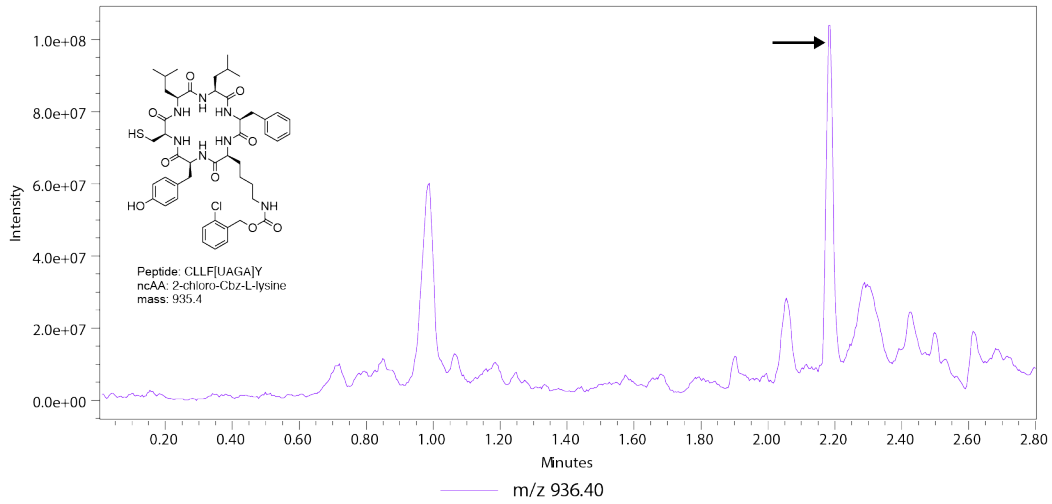
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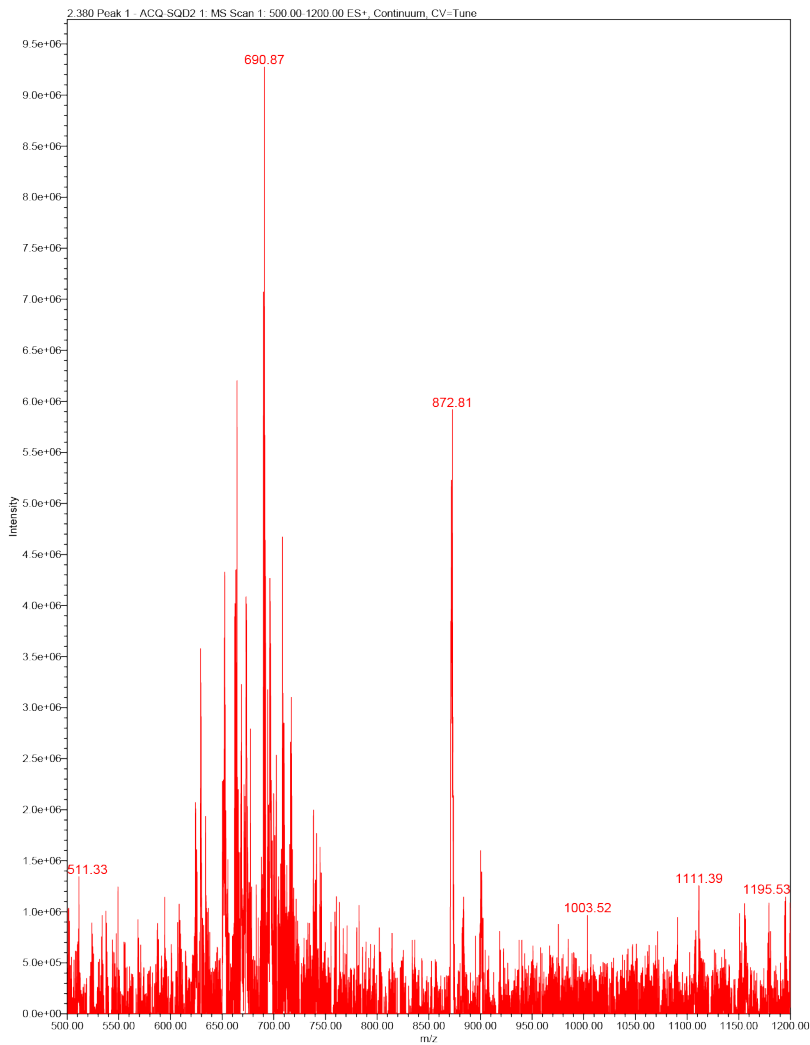
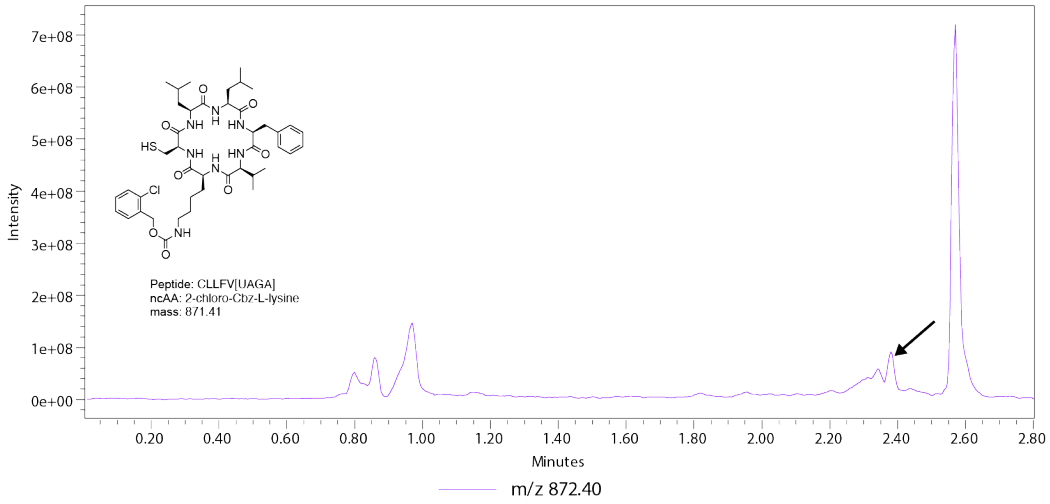
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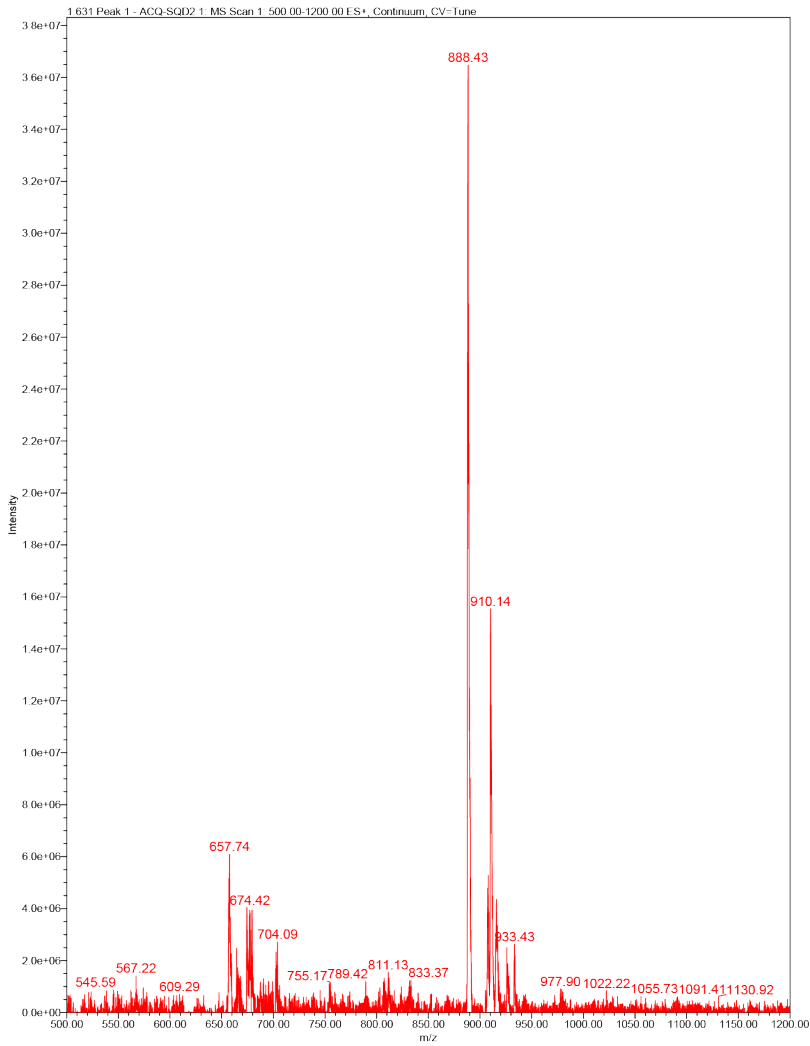
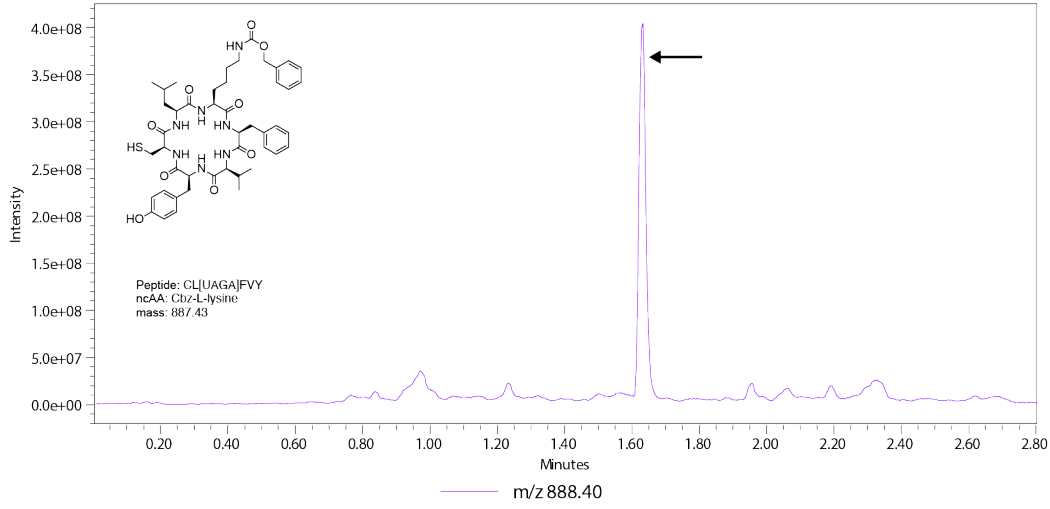
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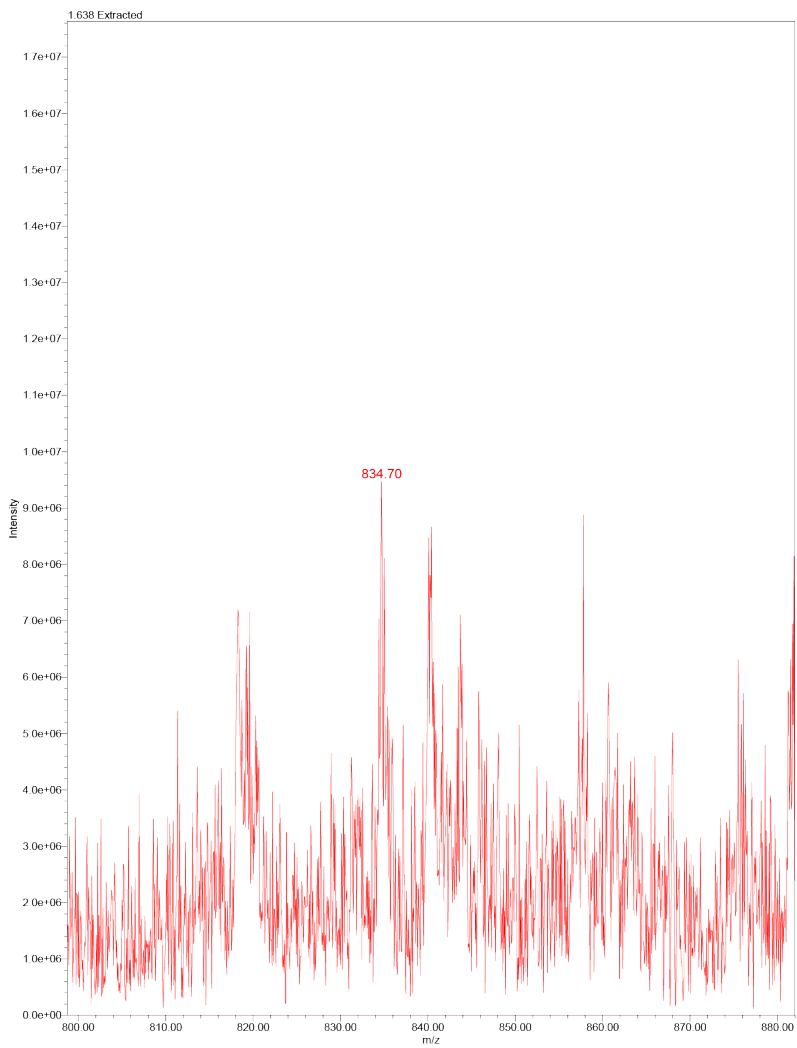
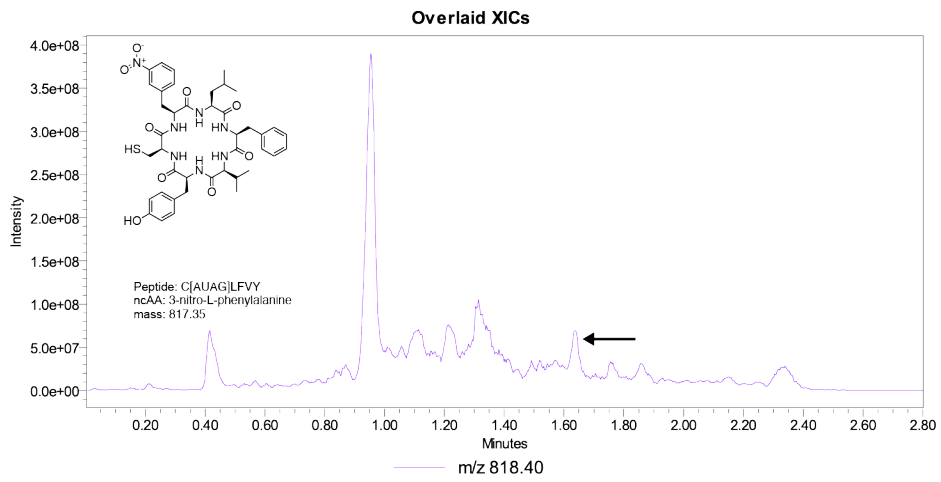


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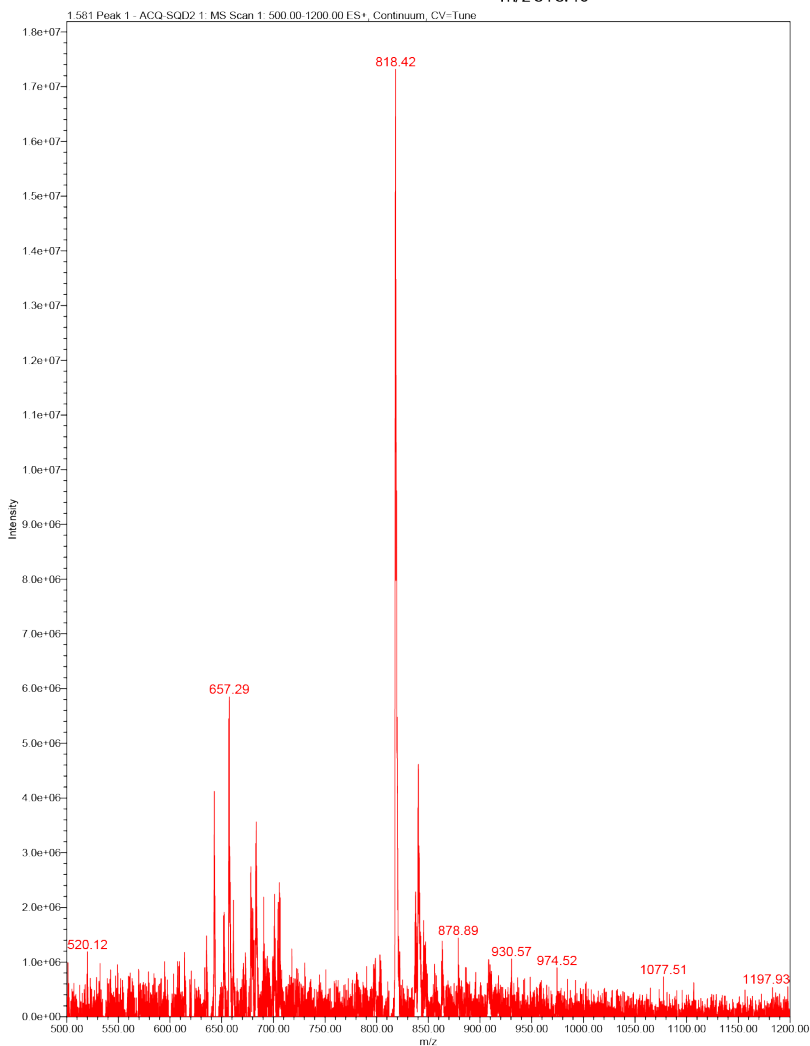
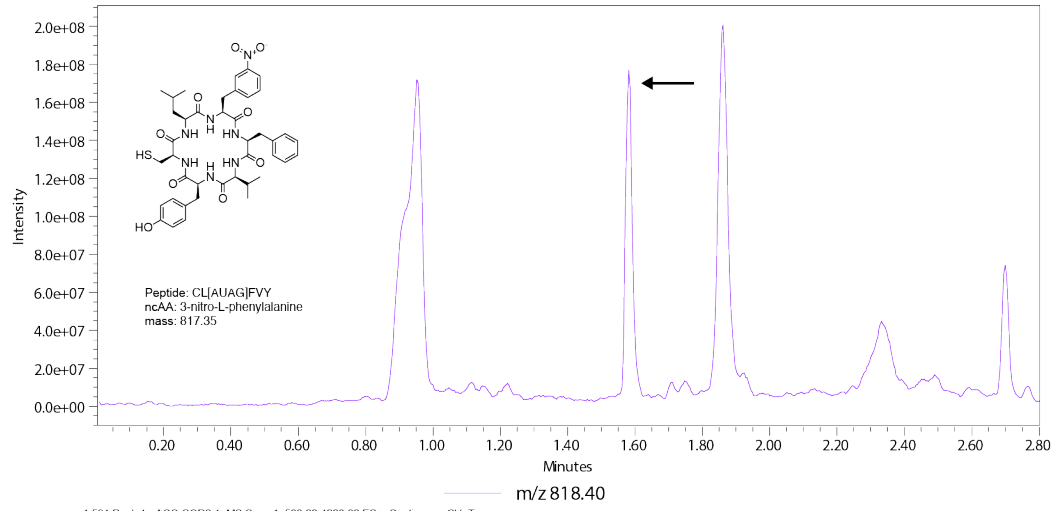


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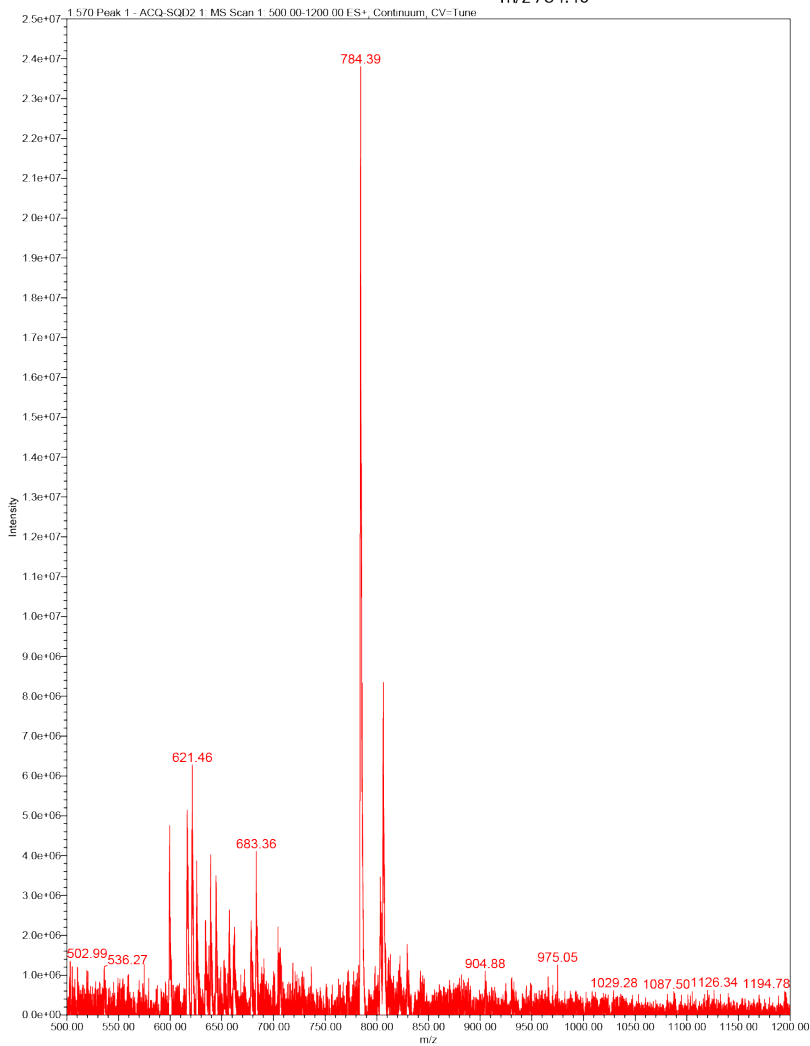
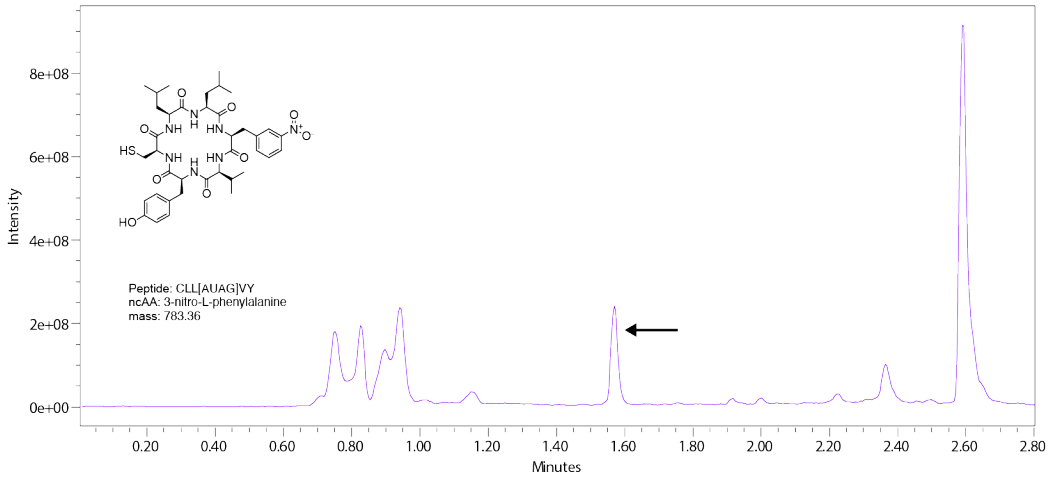


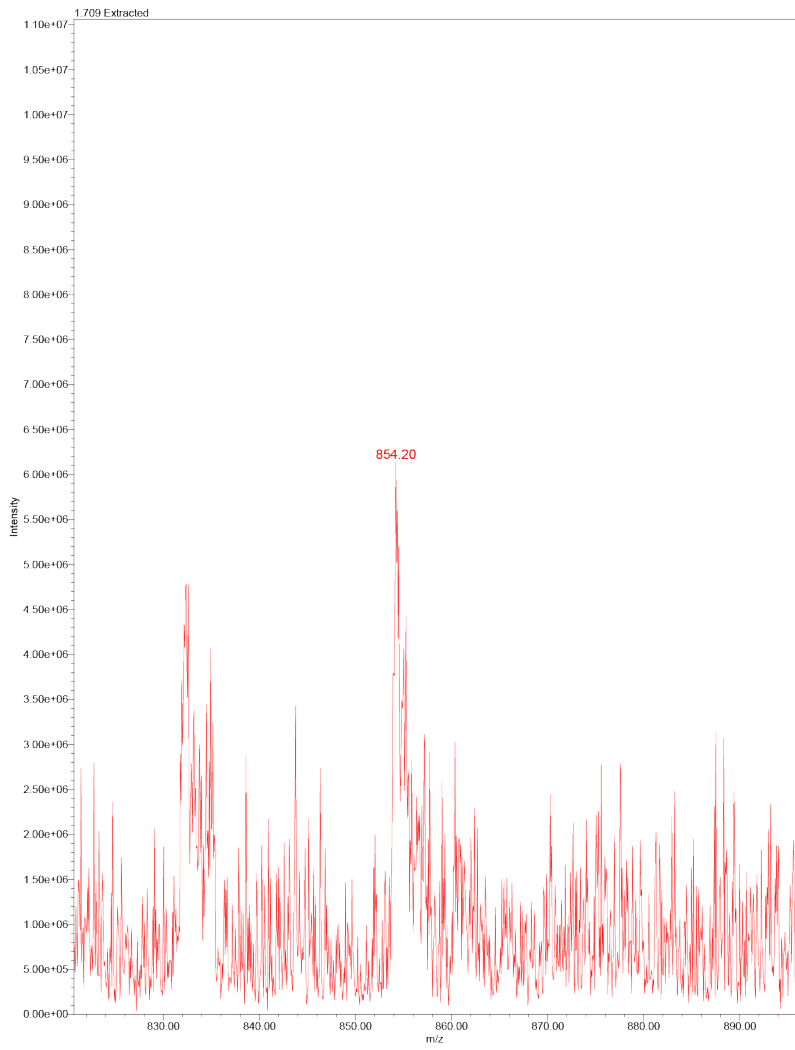
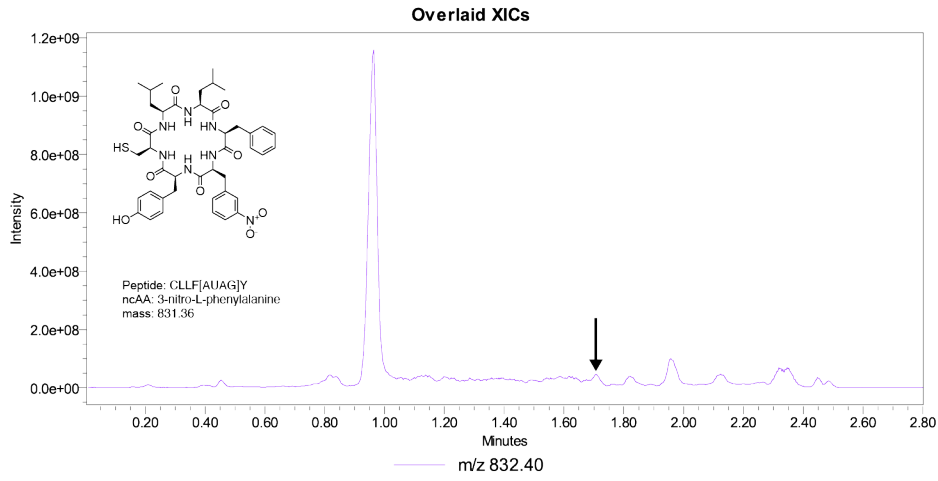


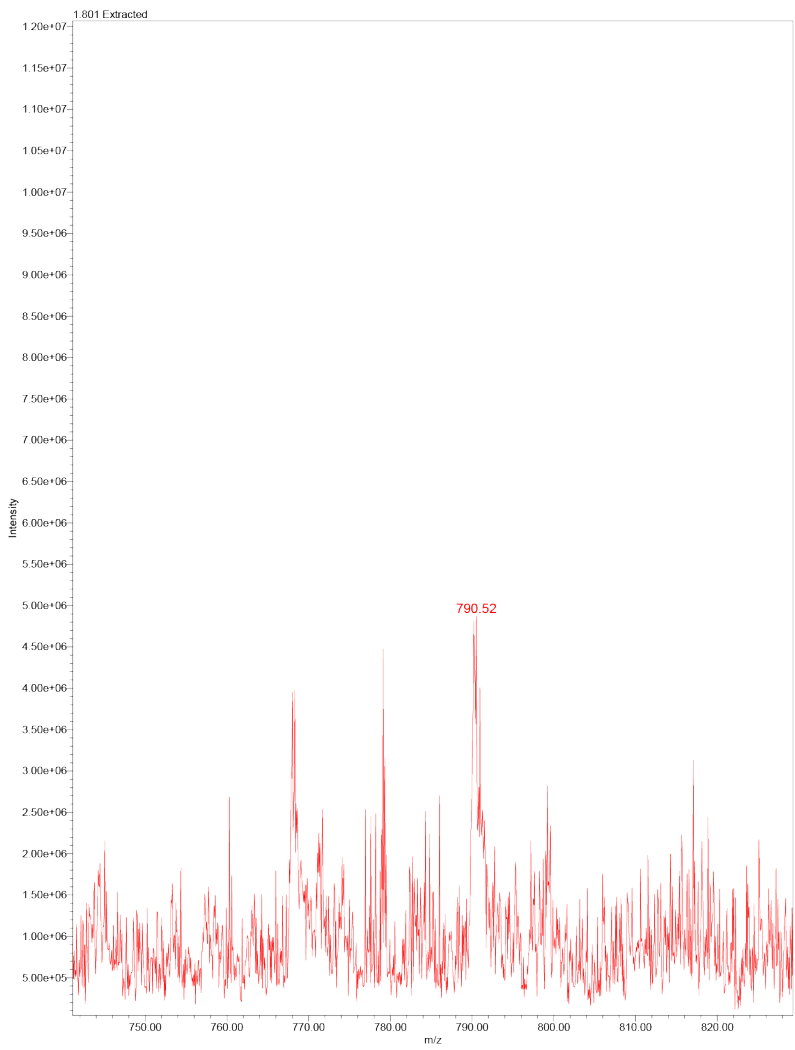
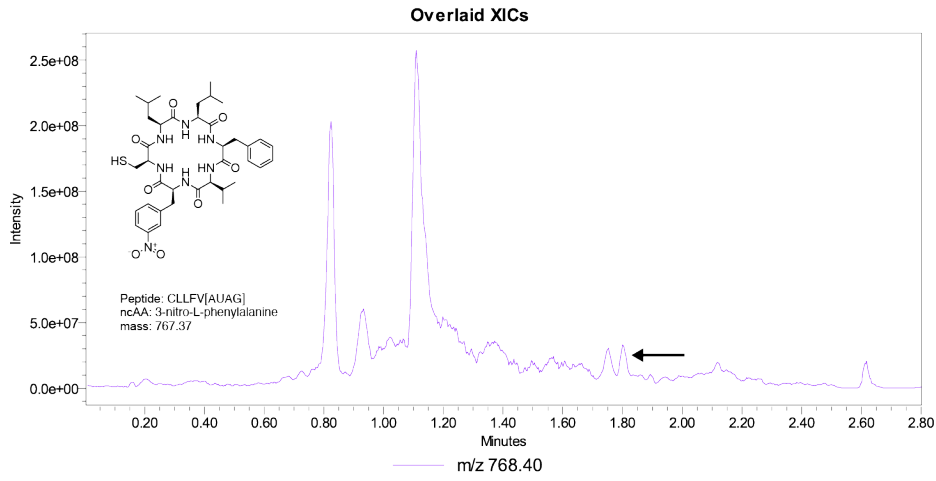
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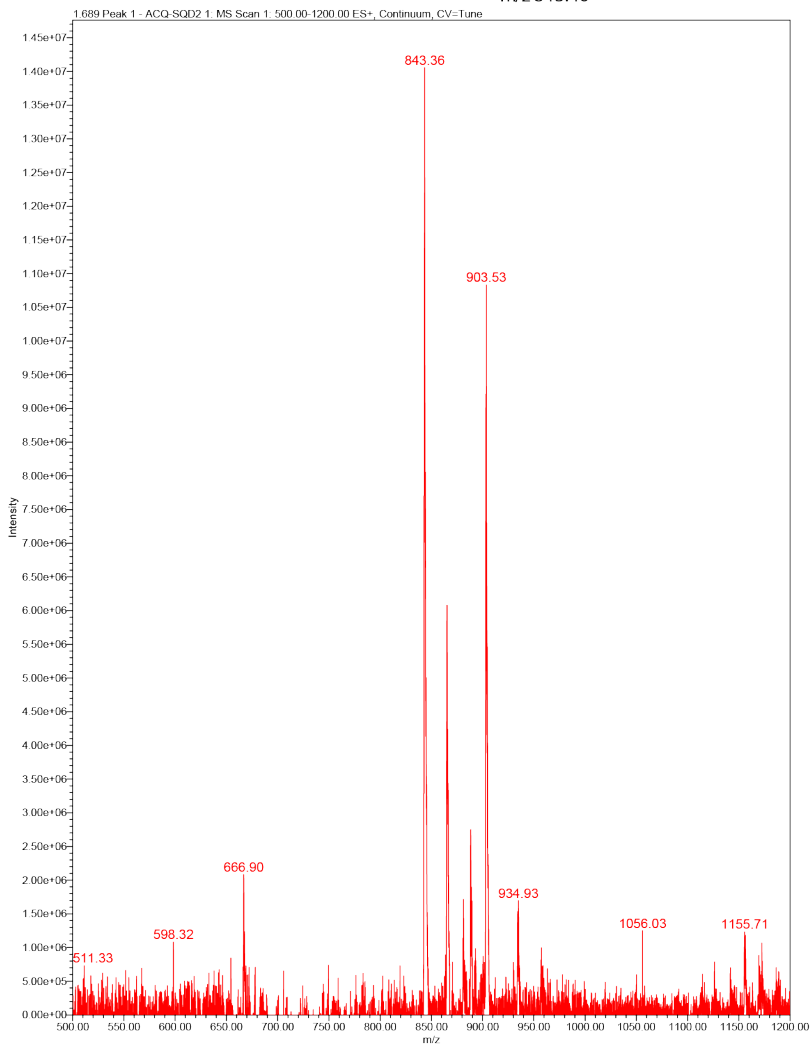
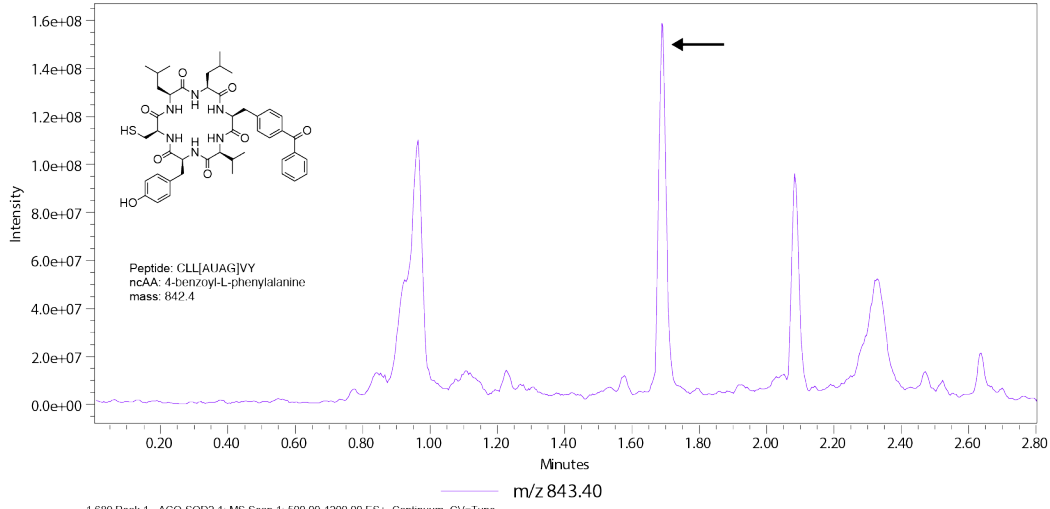
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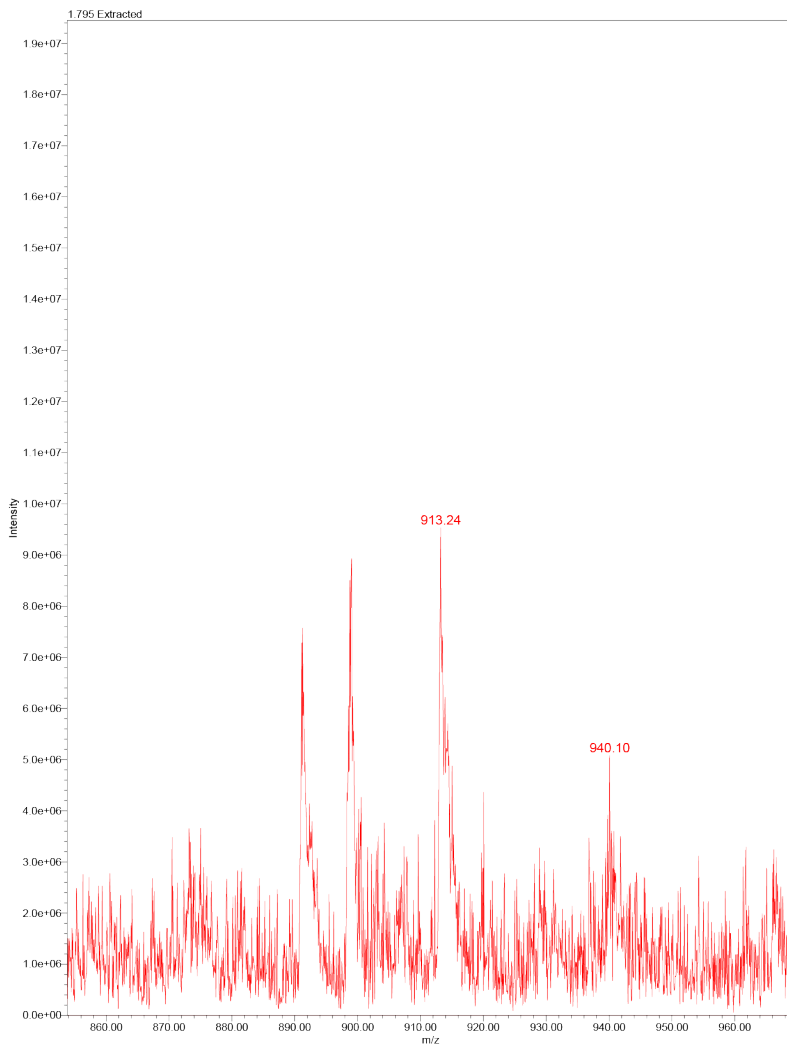
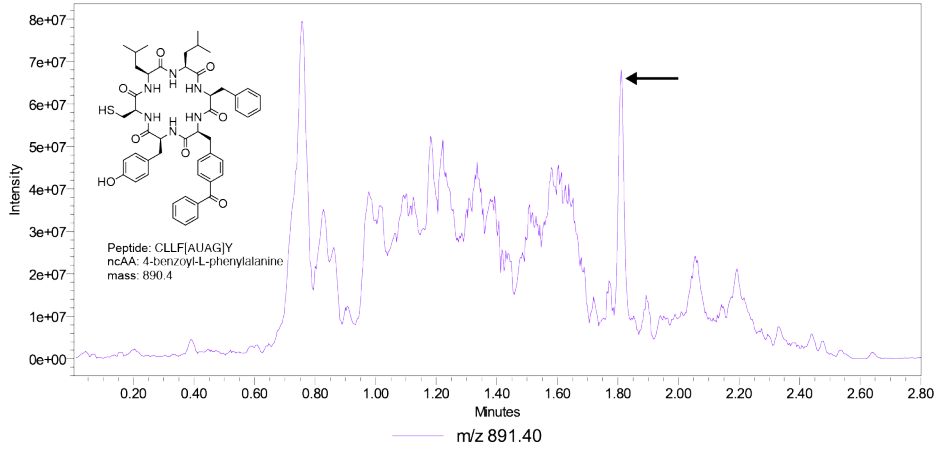




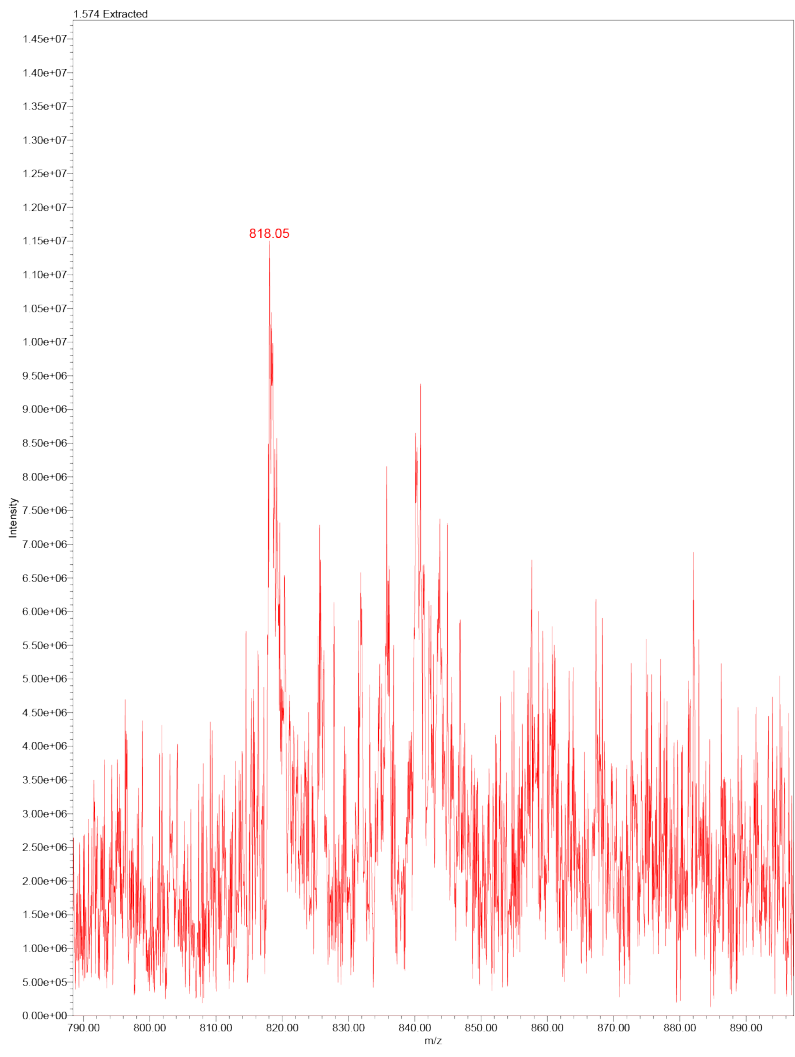
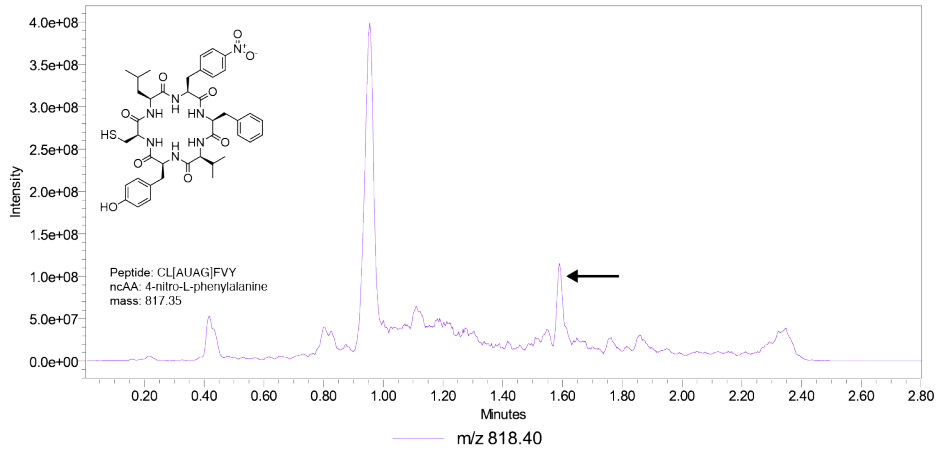
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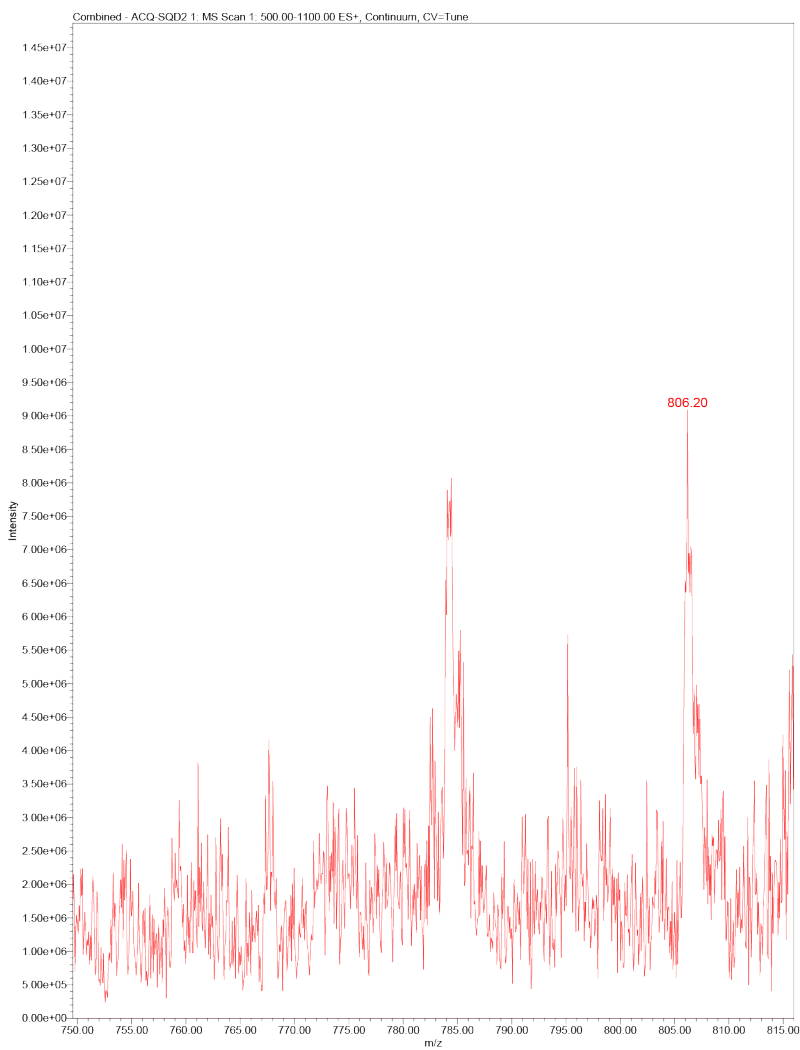
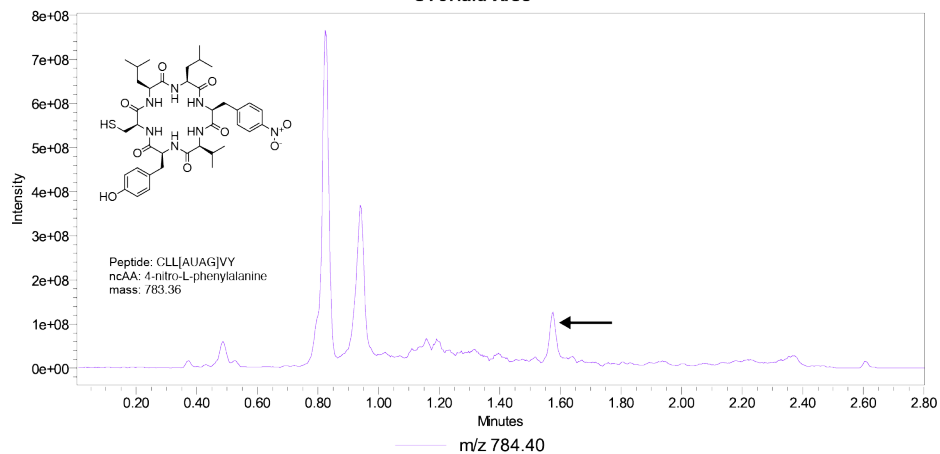
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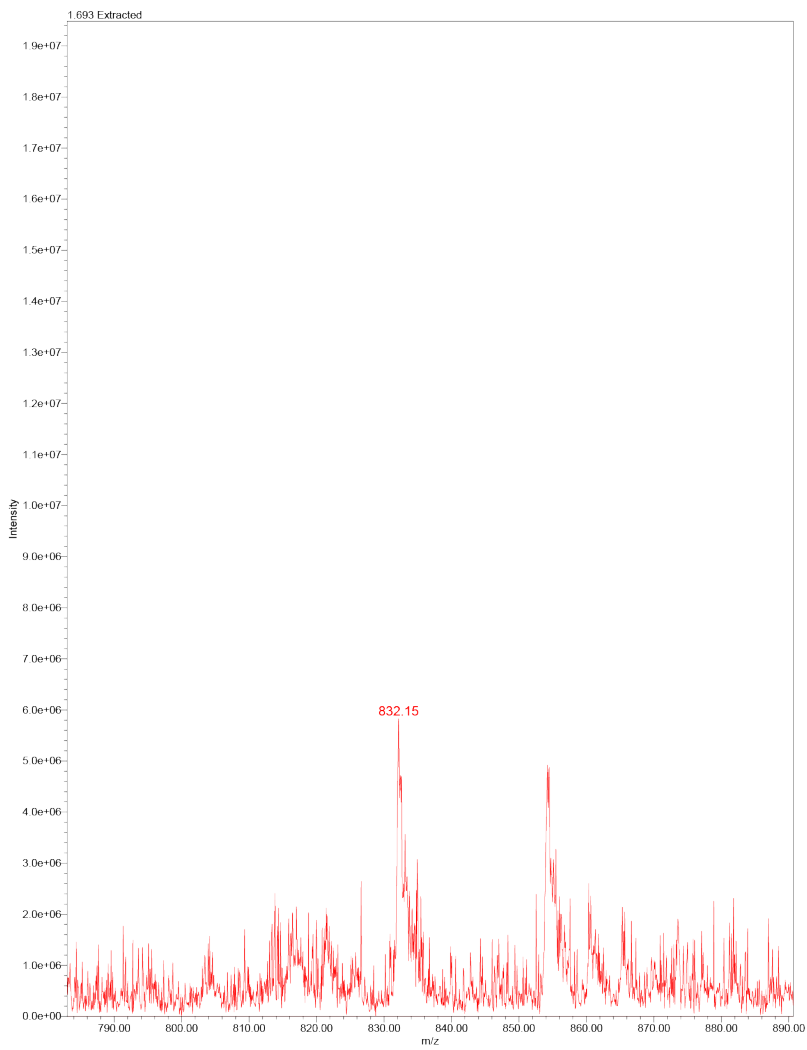
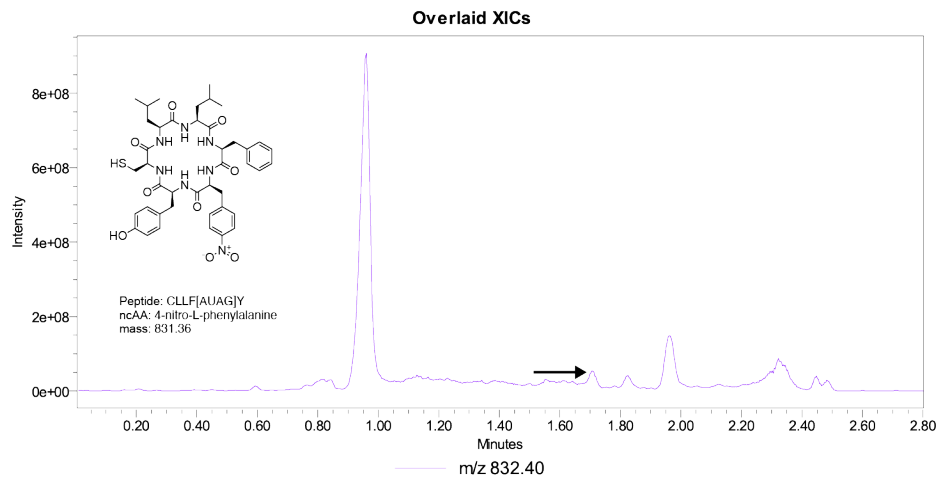


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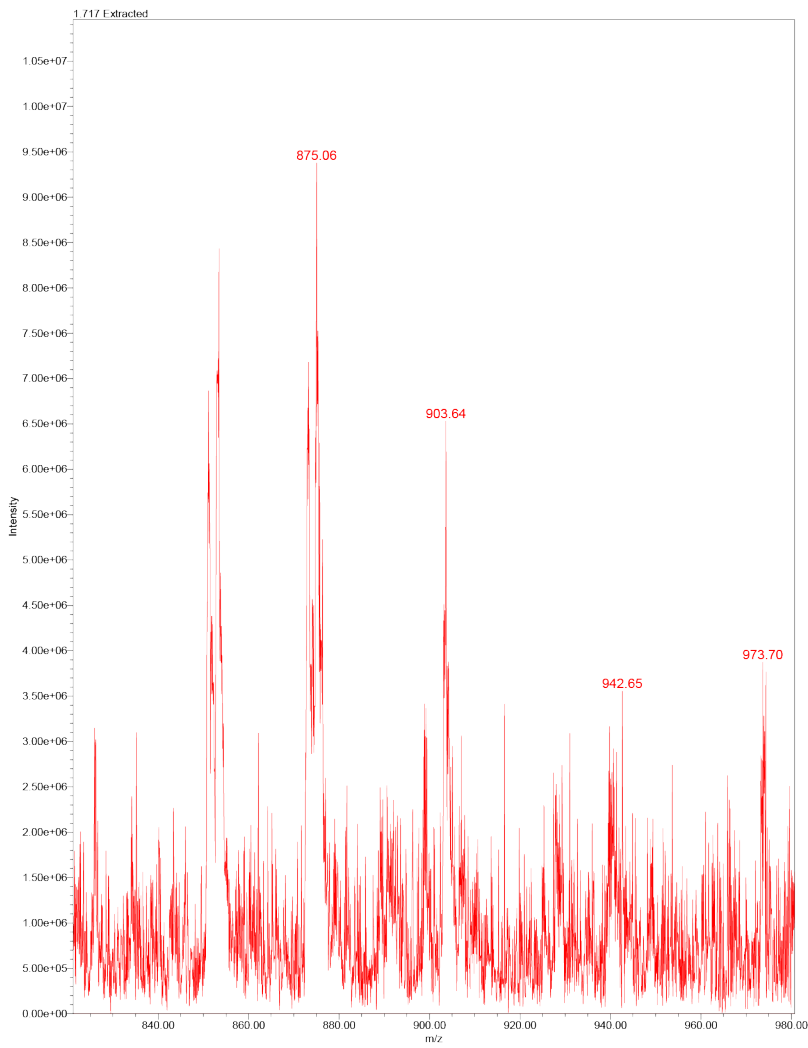
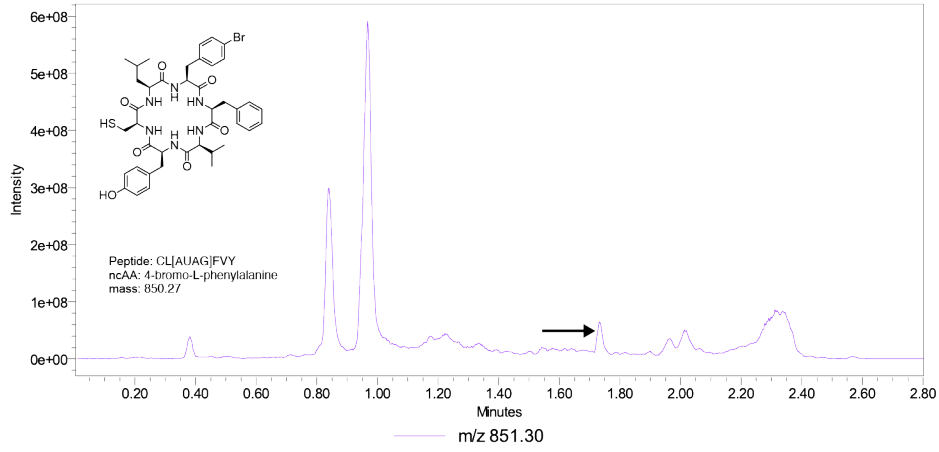


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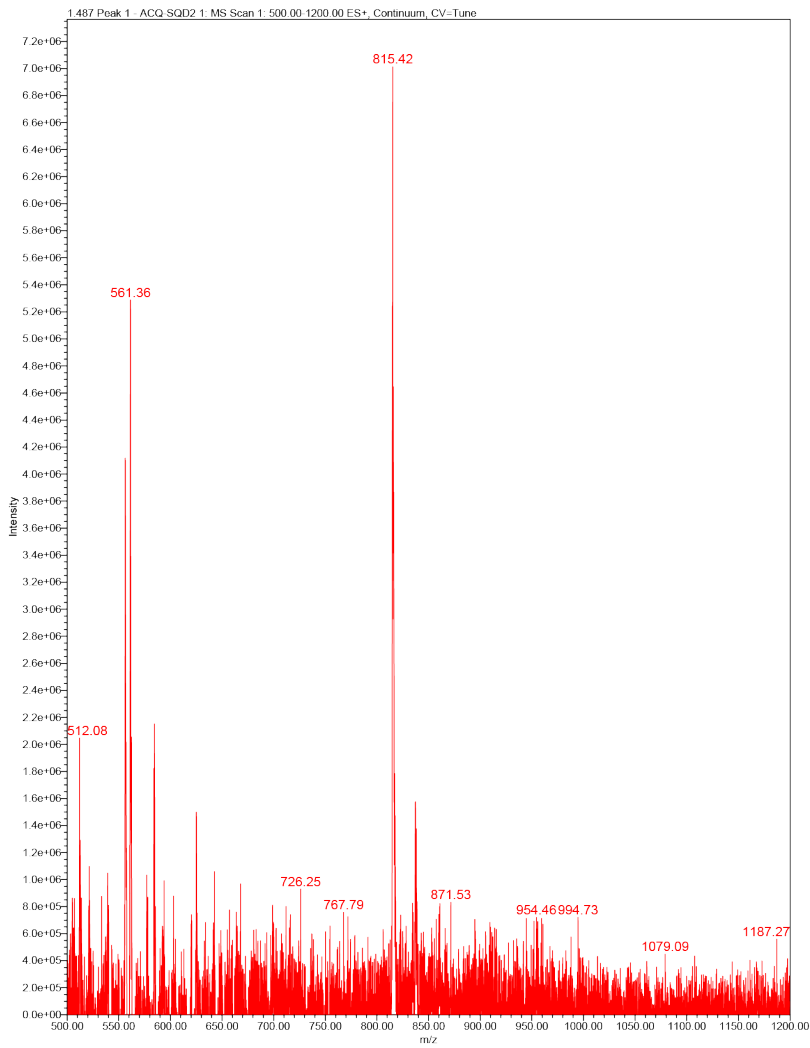
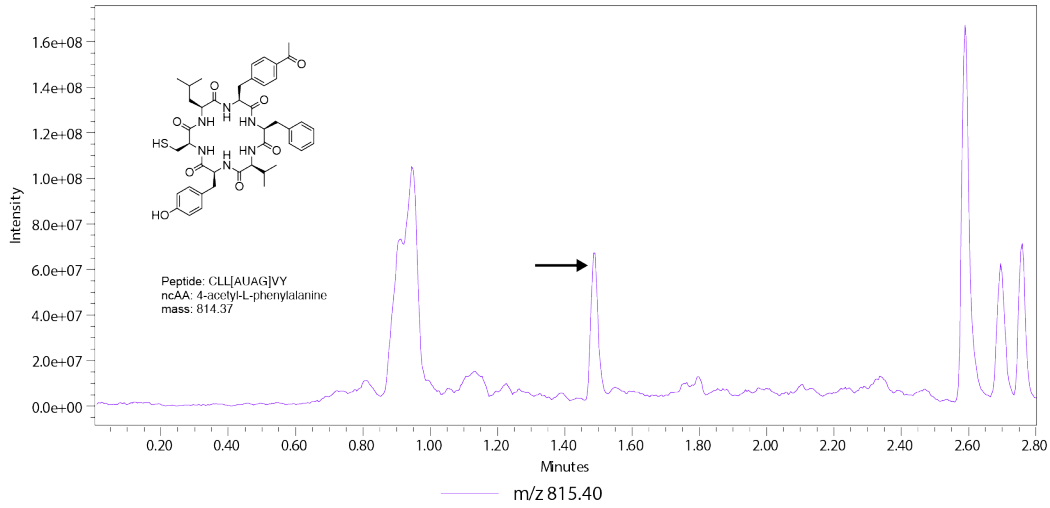


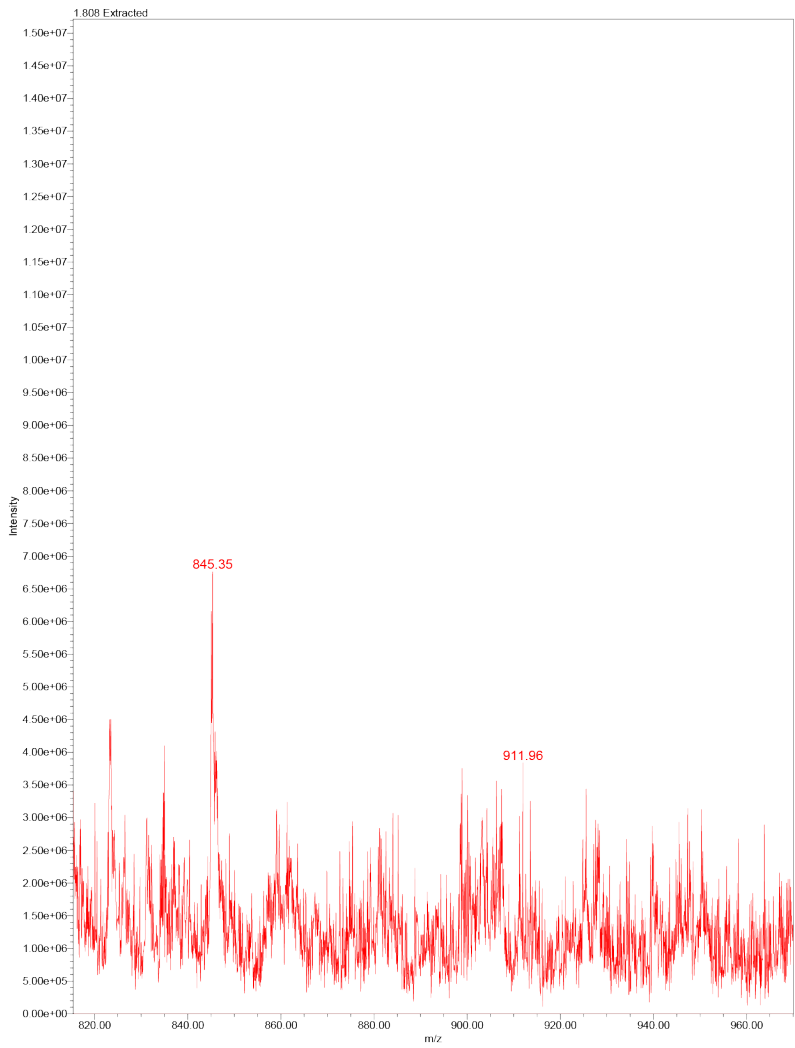
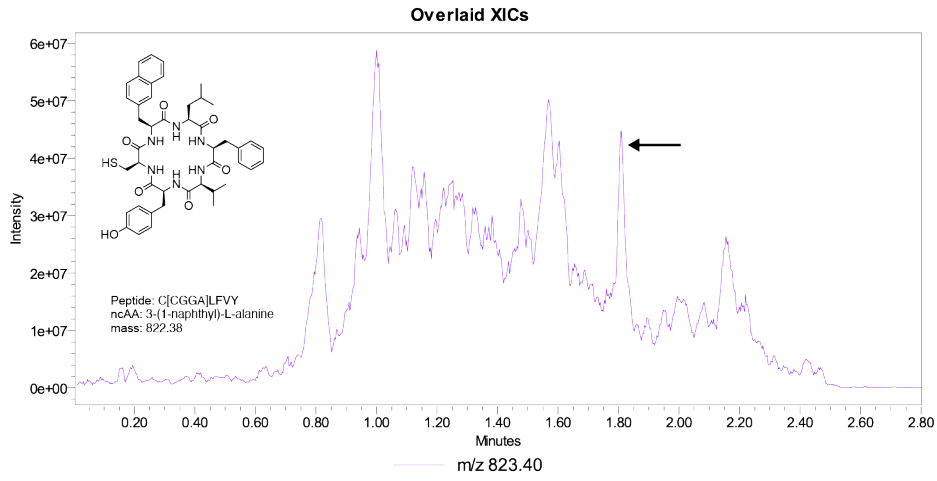


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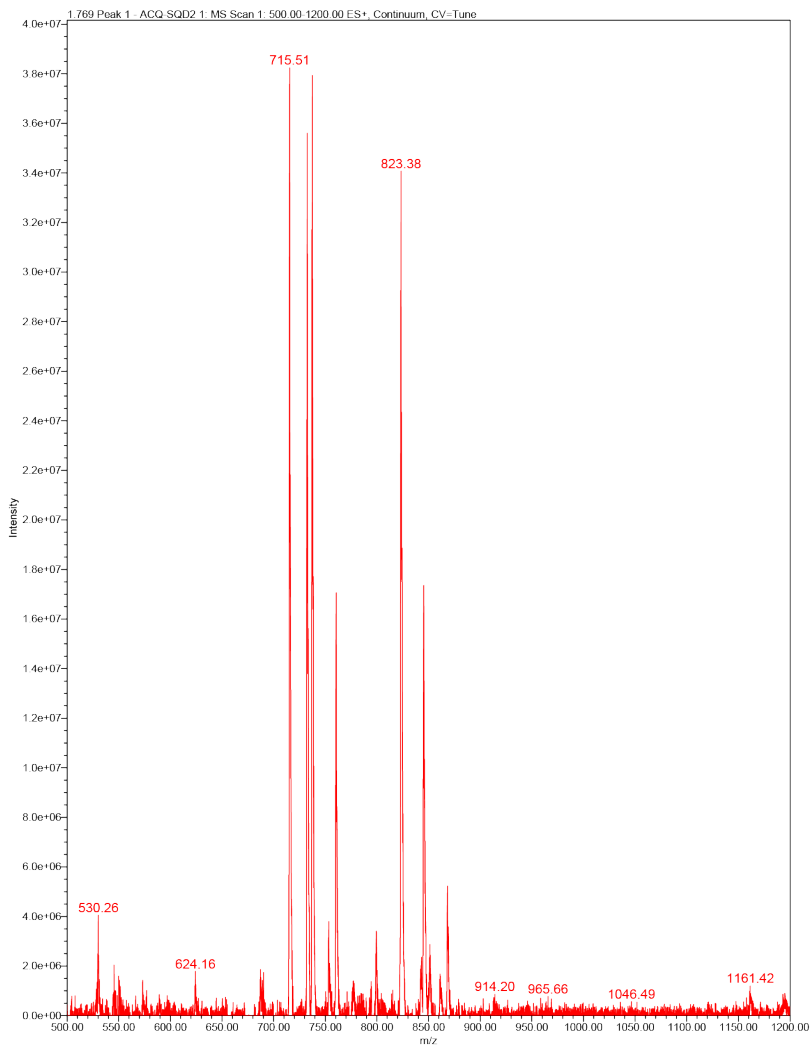
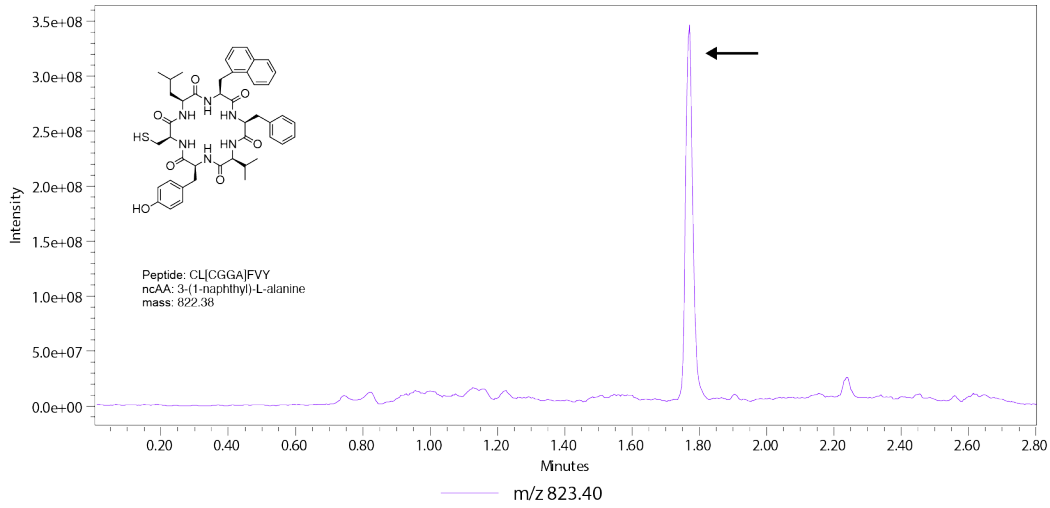


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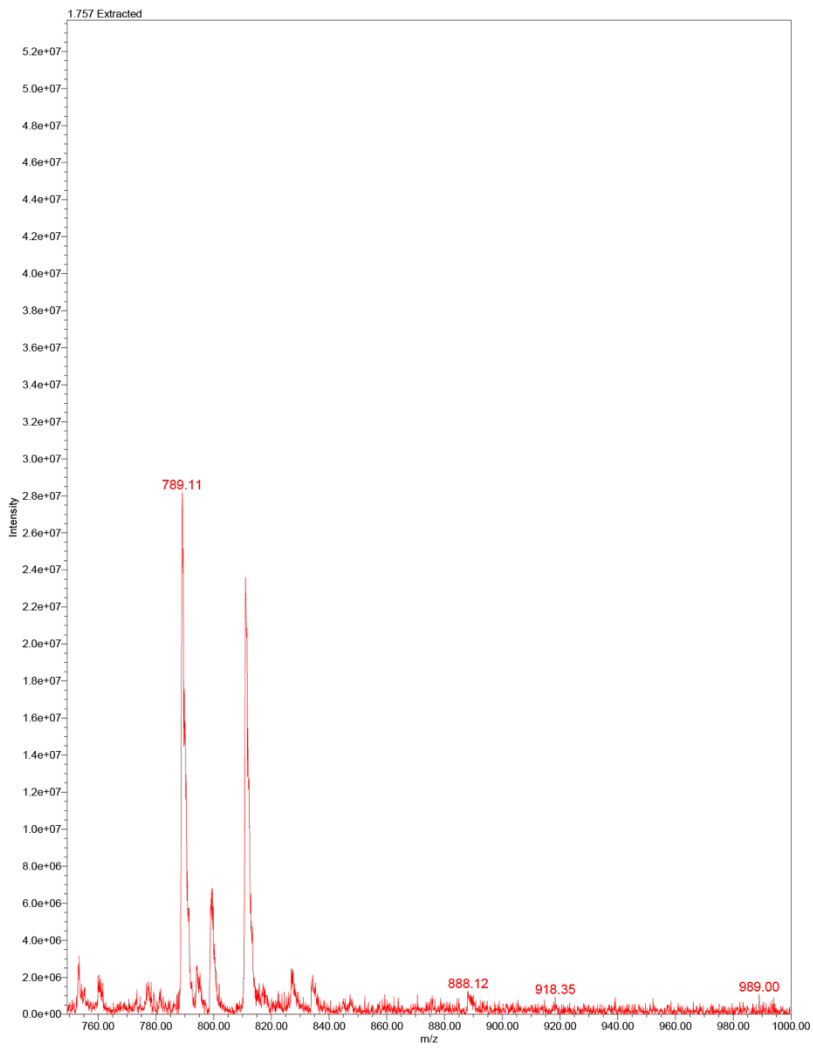
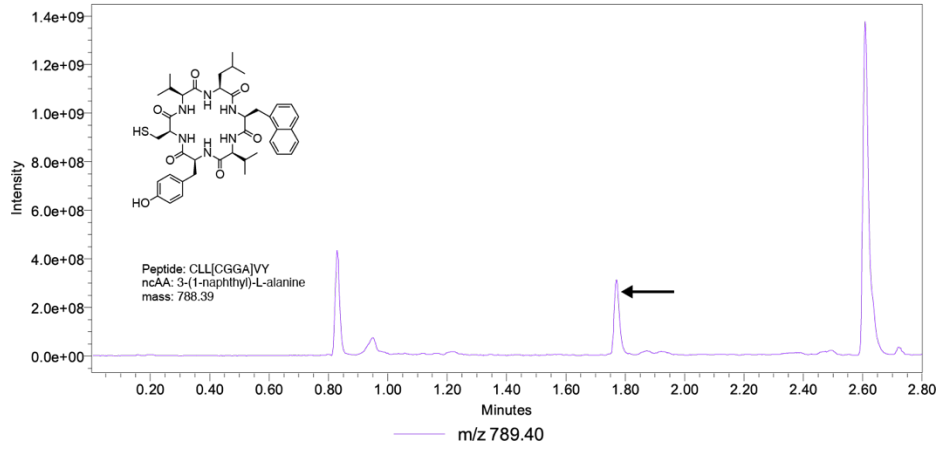


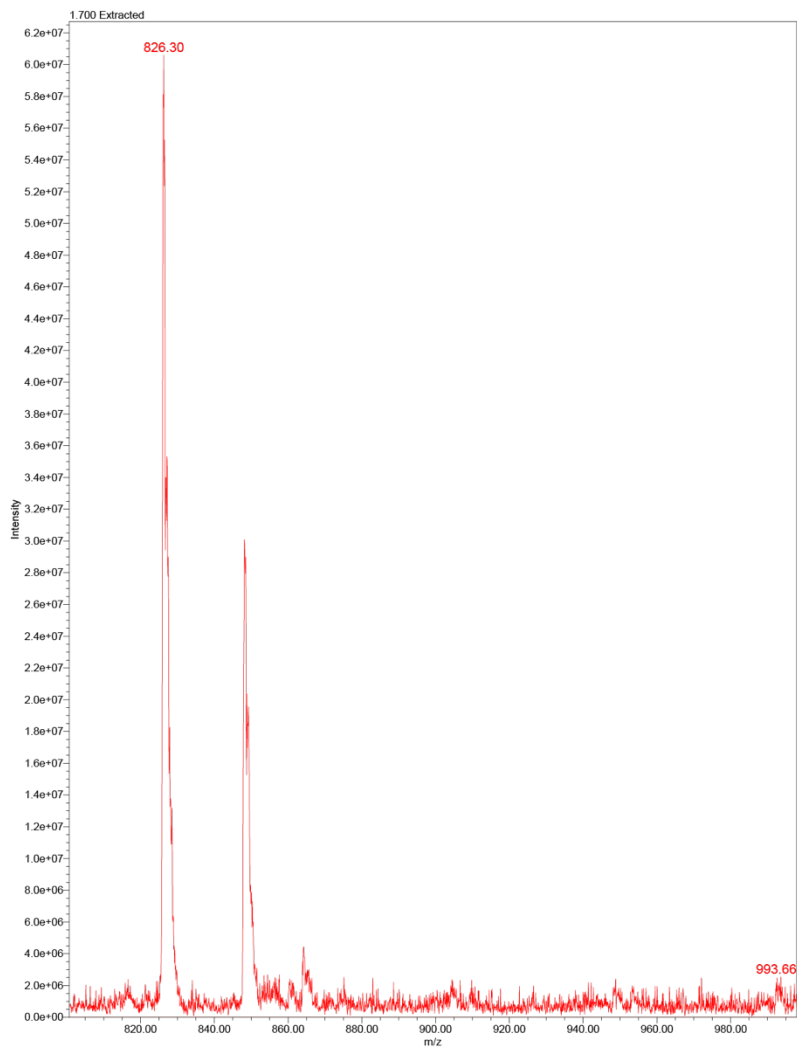
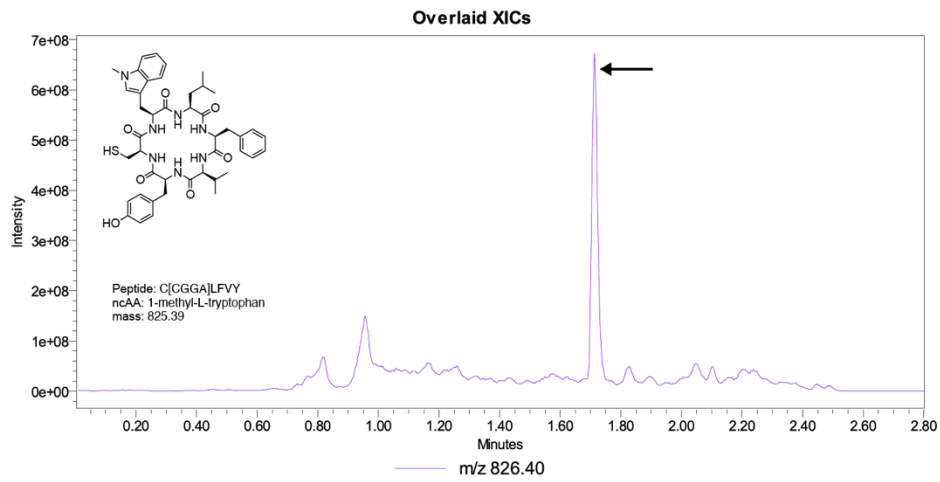


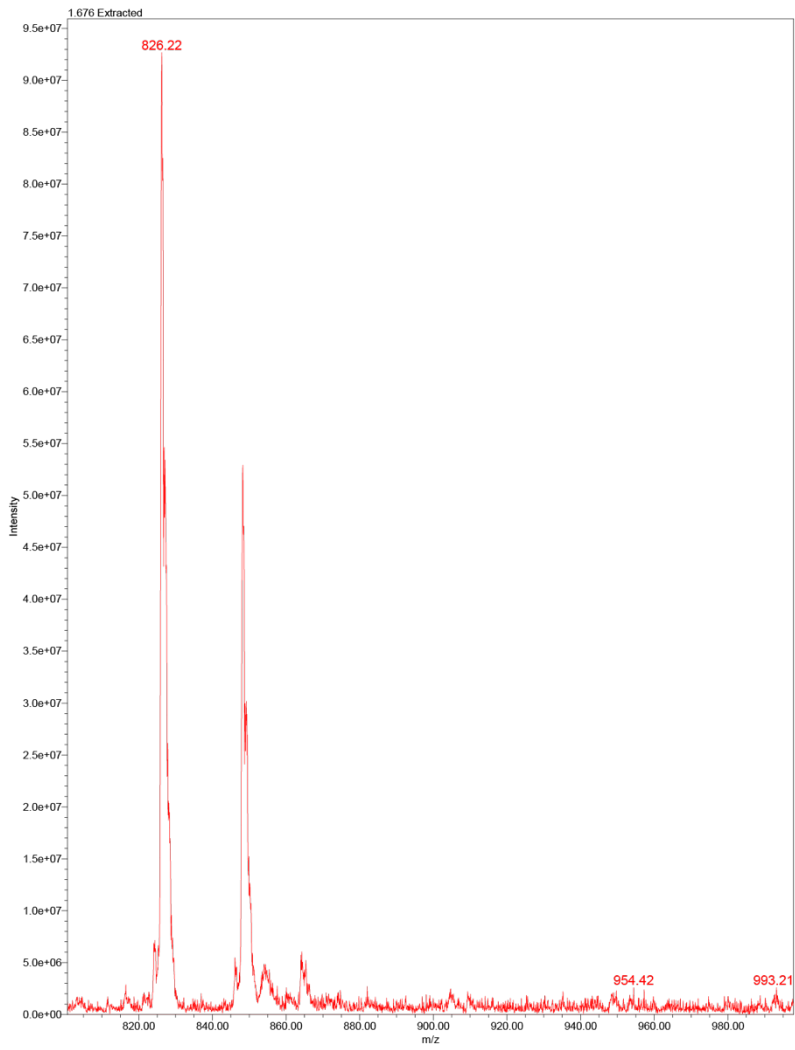
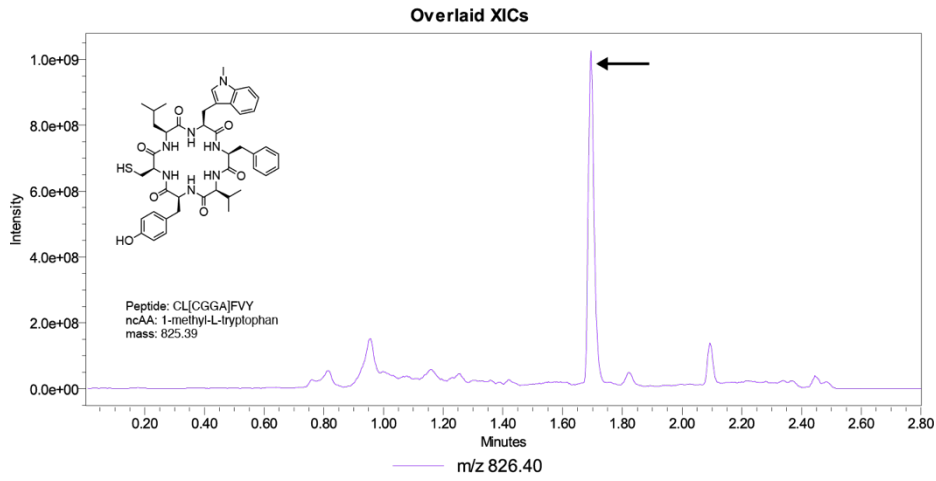
Overlaid XICs



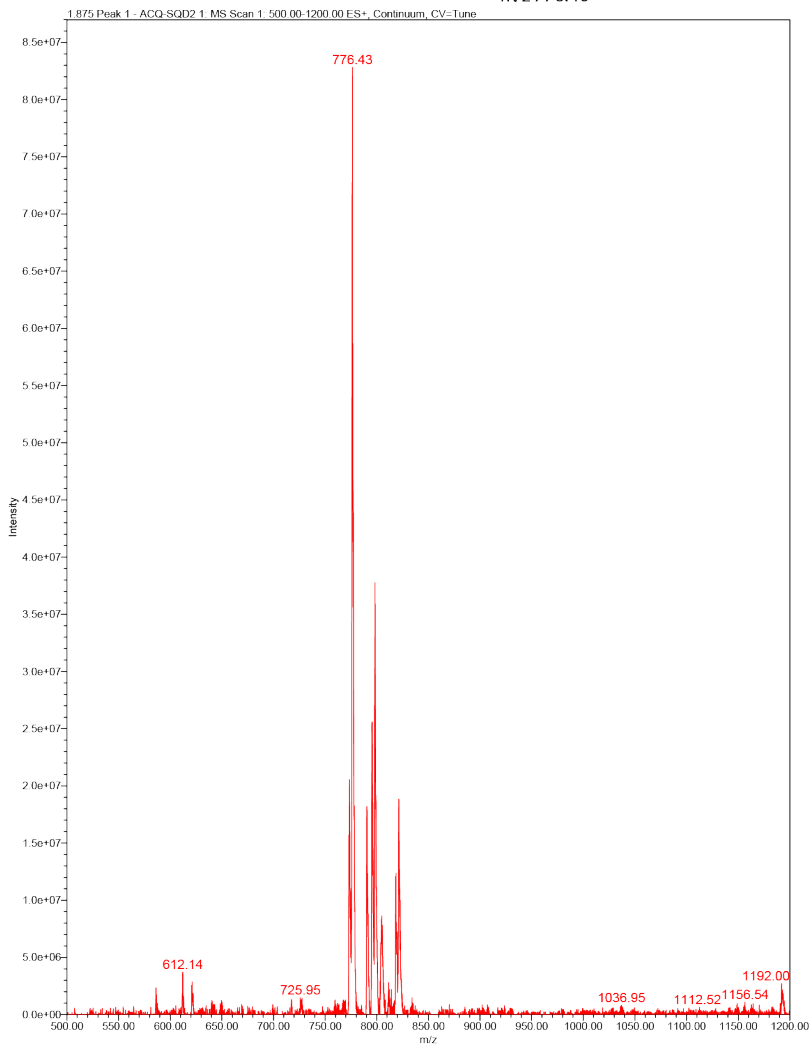
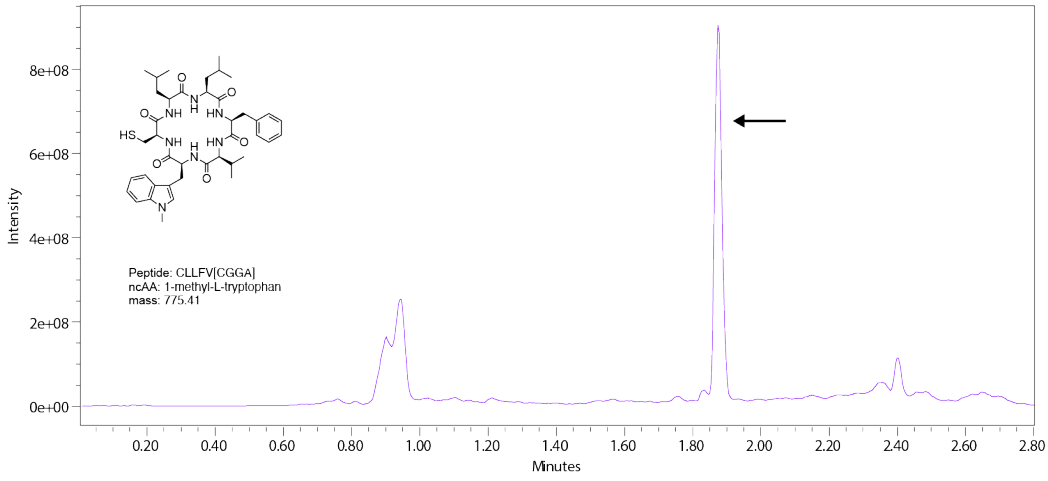
Overlaid XICs



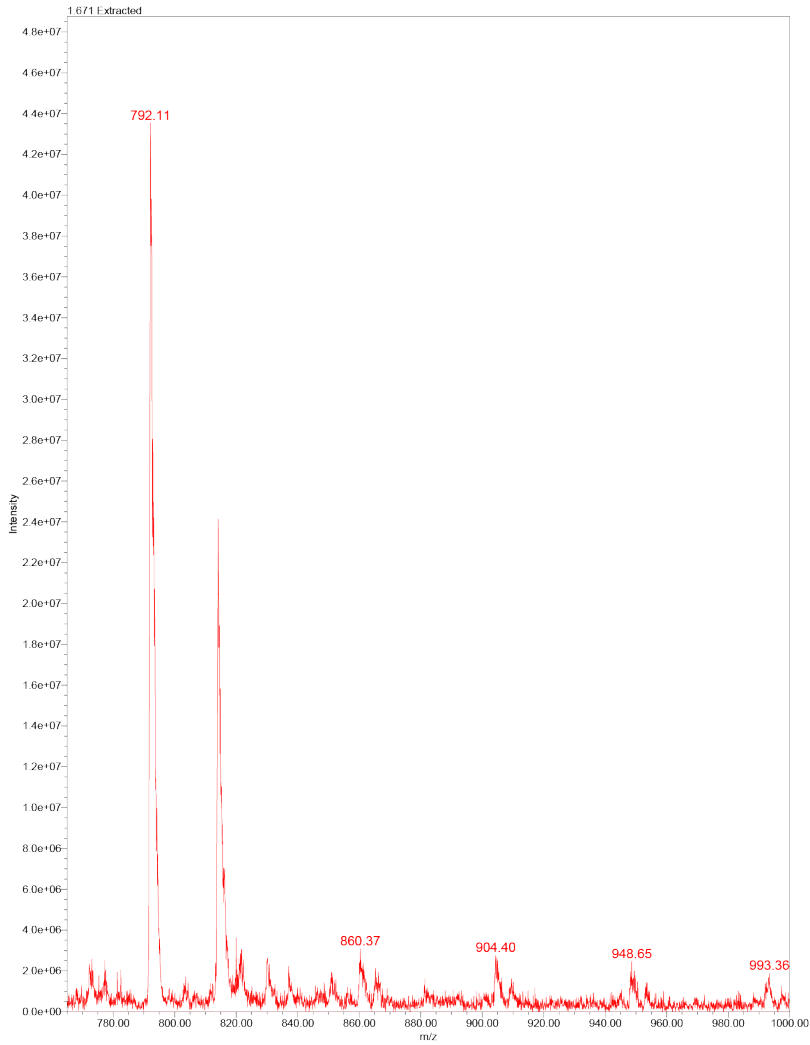
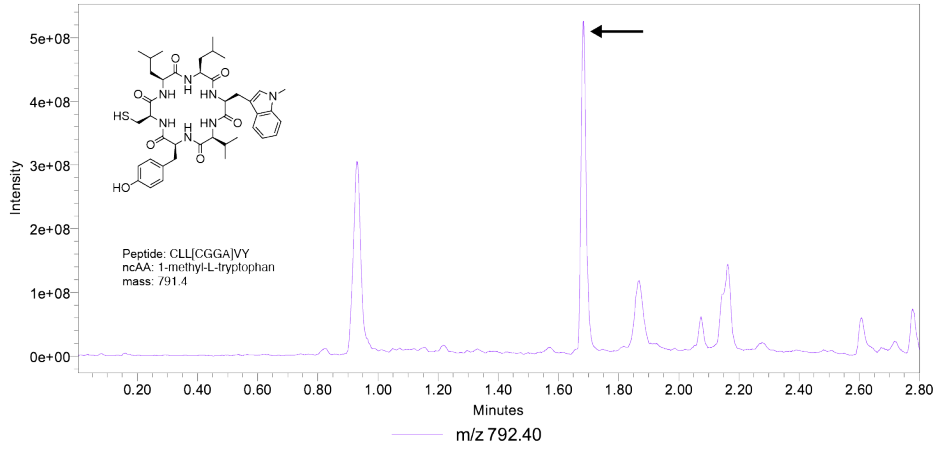




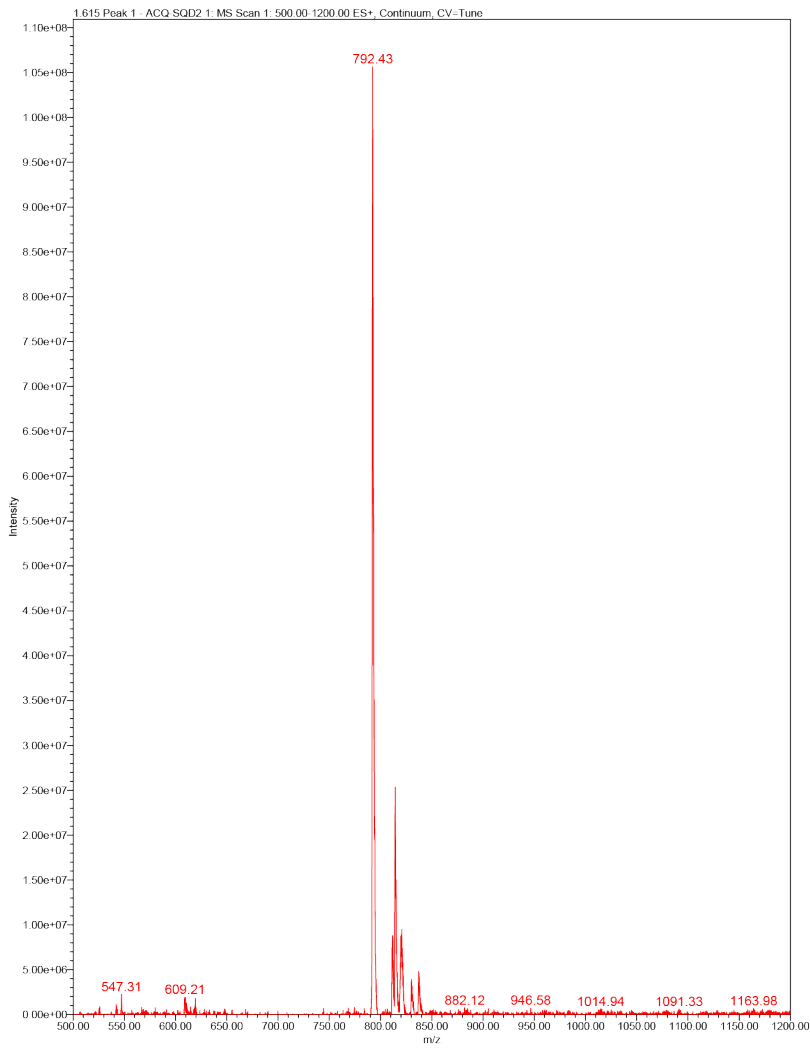
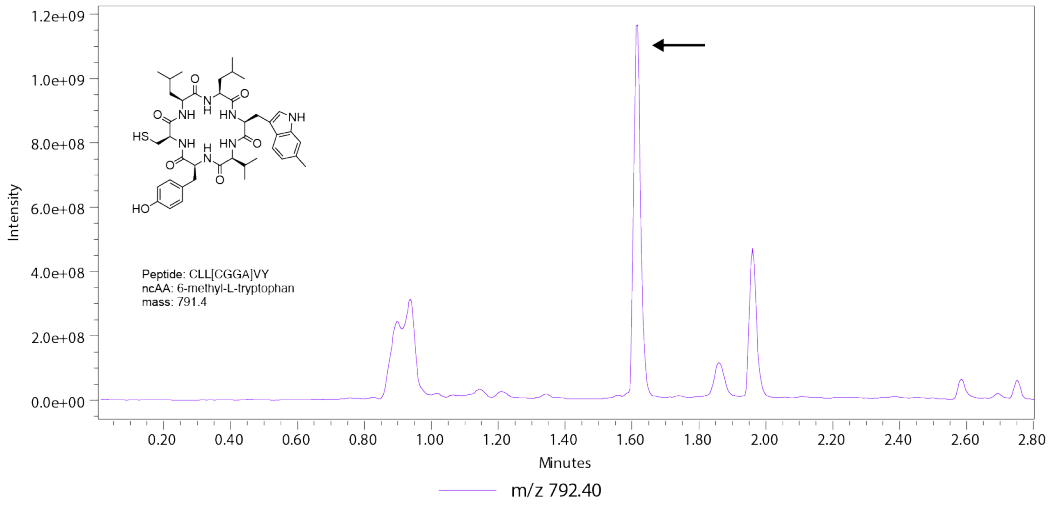
Overlaid XICs



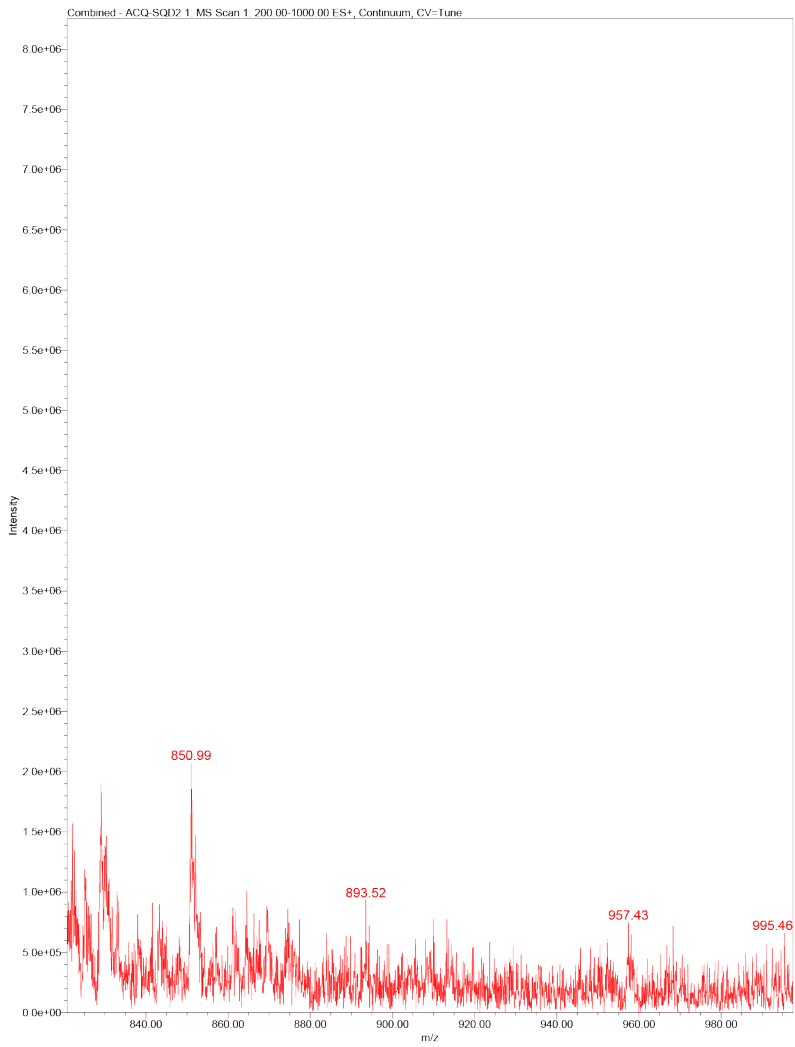
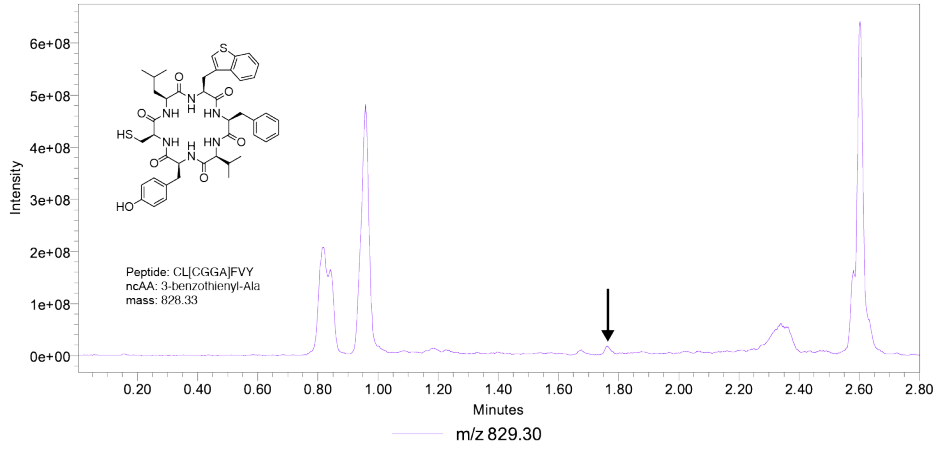
Overlaid XICs

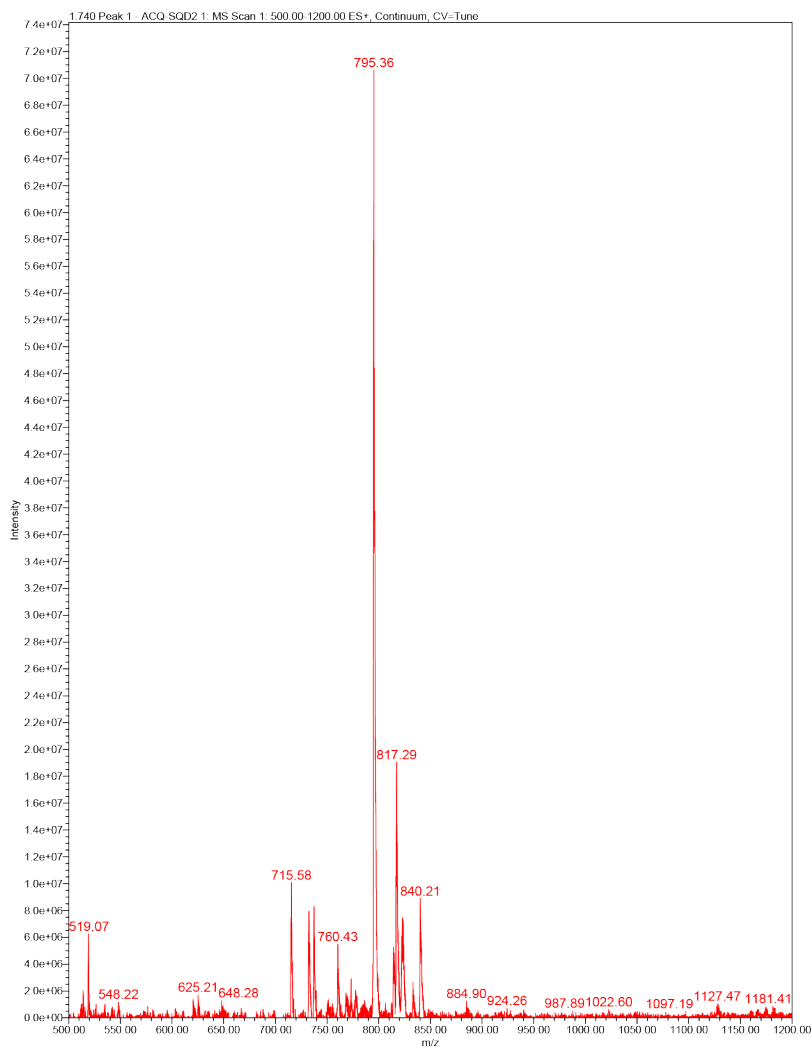
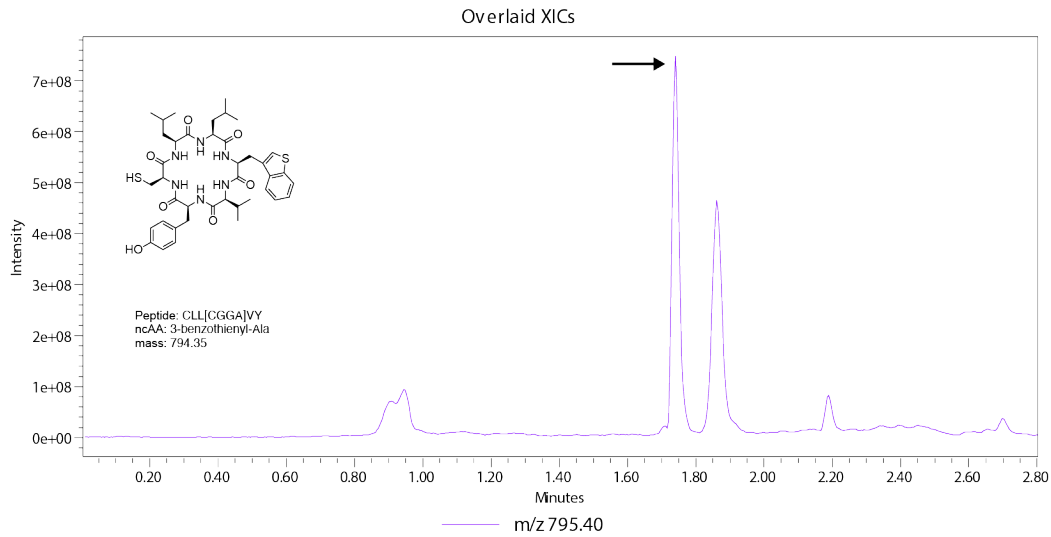


Overlaid XICs



Overlaid XICs





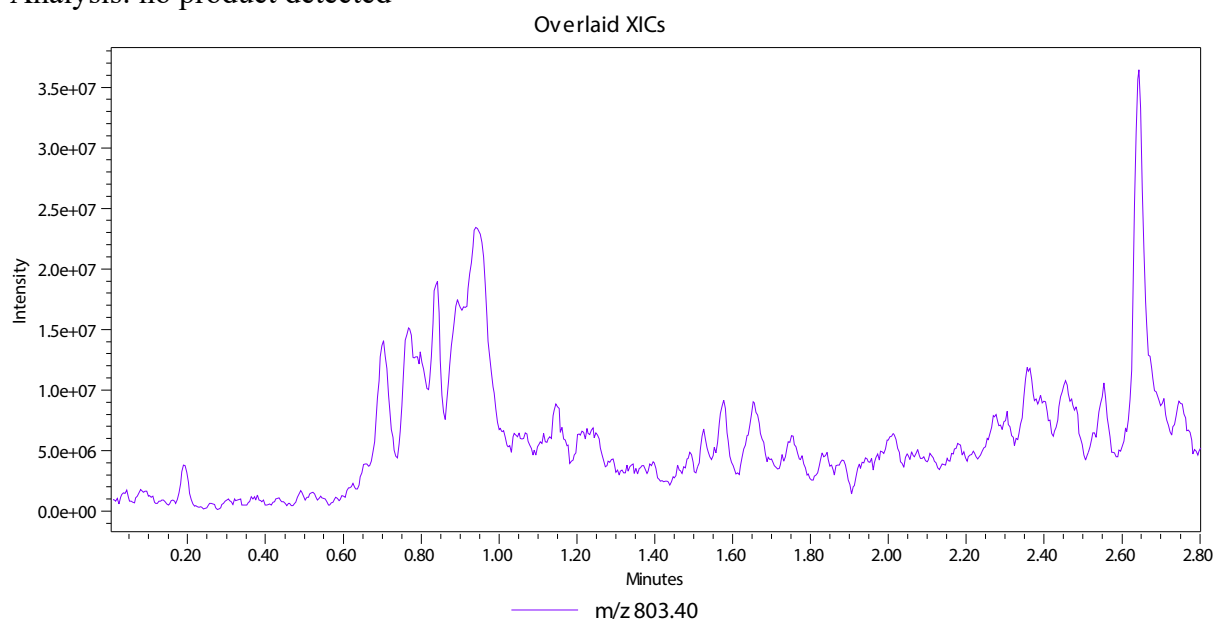
Additional Controls from Main Text Figure 5.

Below are XICs from (C1A mutants run + ncAA) and (– ncAA) controls. XICs correspond to those seen in Main Text Figure 5. The (– ncAA) conditions are shown as overlapping traces where all relevant m/z (these may differ based on ncAA used in positive condition) are searched against the single plasmid condition.

AGGA decoding controls:

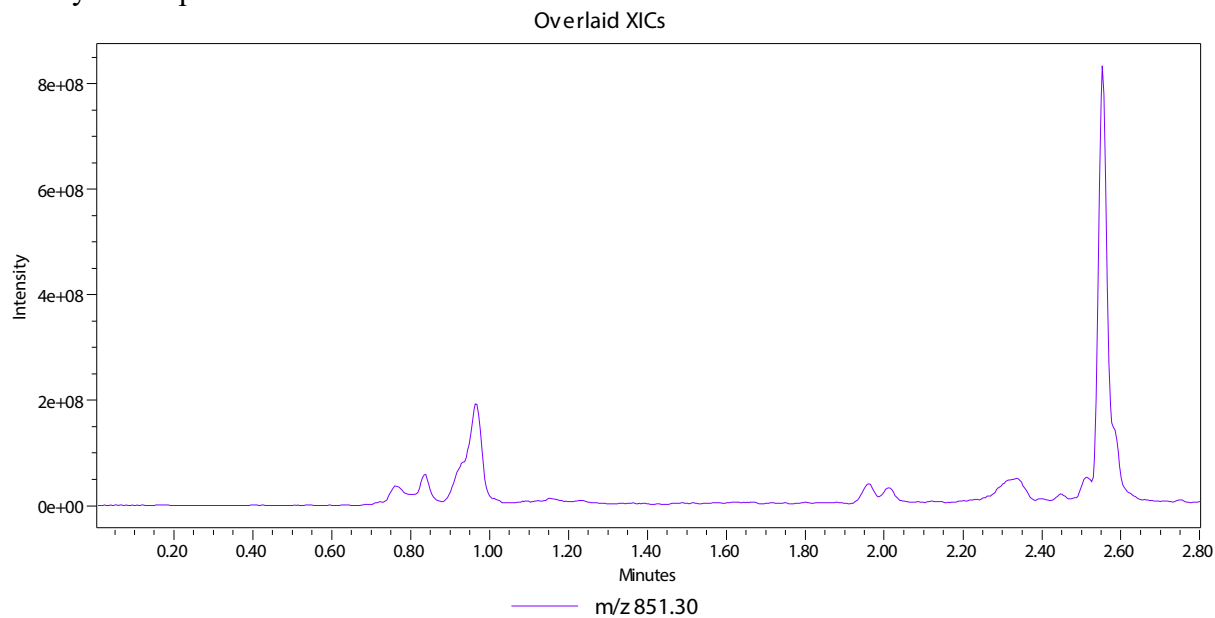
Macrocycle: C(AGGA)LFVY (C1A mutant)

Analysis: no product detected



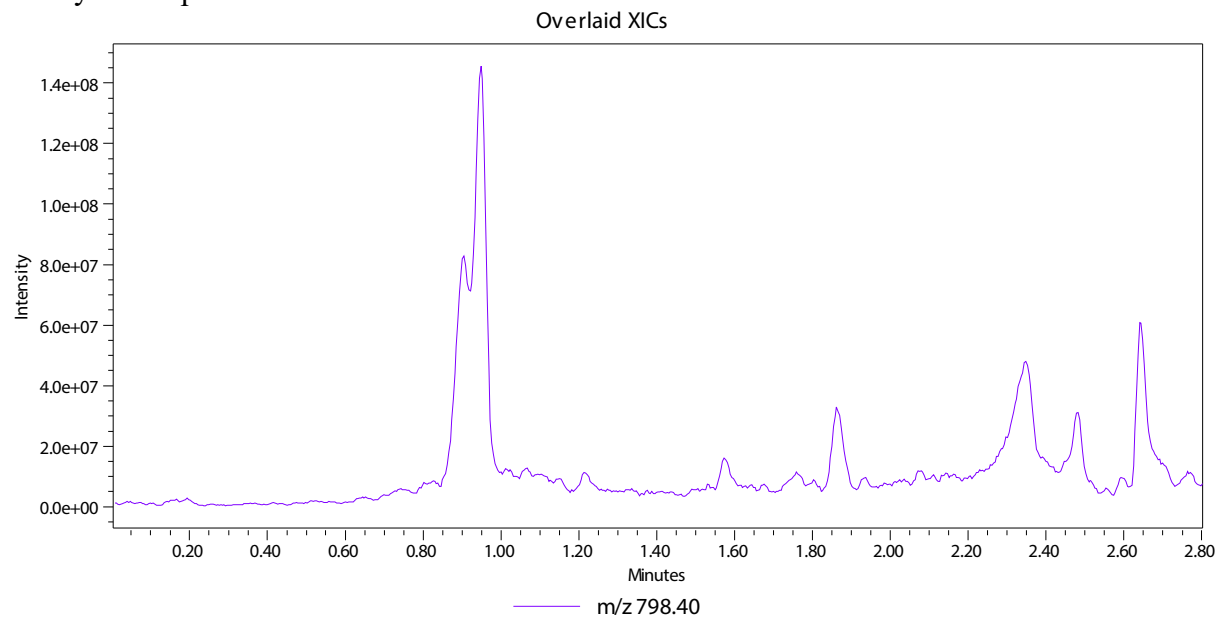
Macrocycle: CL(AGGA)FVY (C1A mutant)

Analysis: no product detected



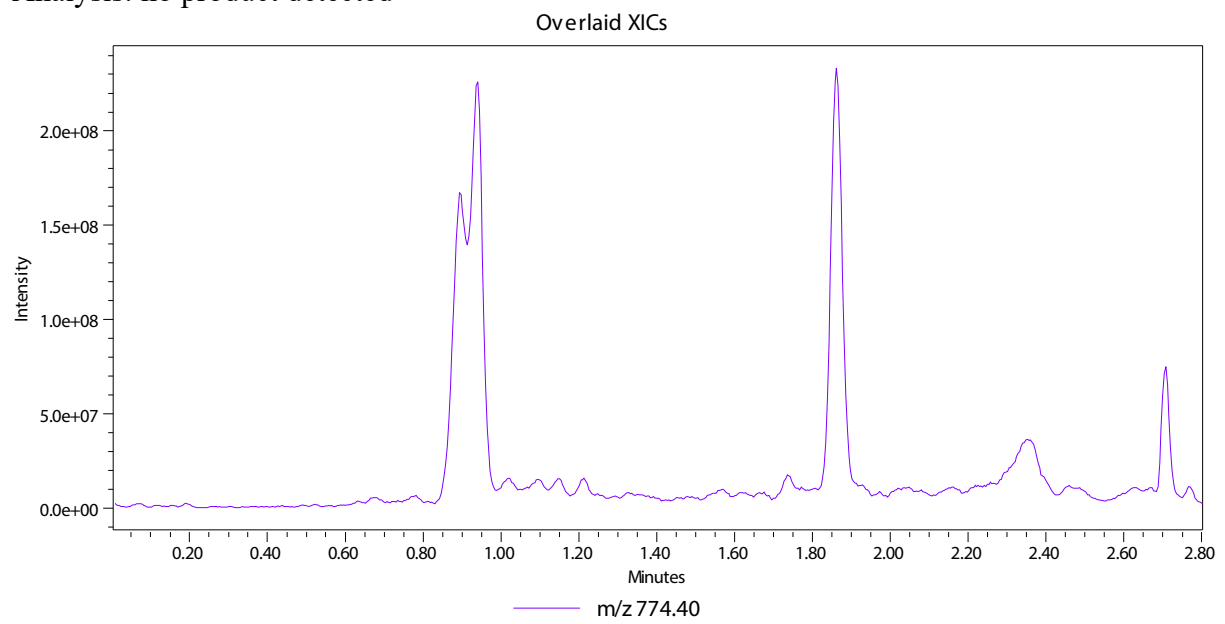
Macrocycle: CL(AGGA)FVY (C1A mutant)

Analysis: no product detected

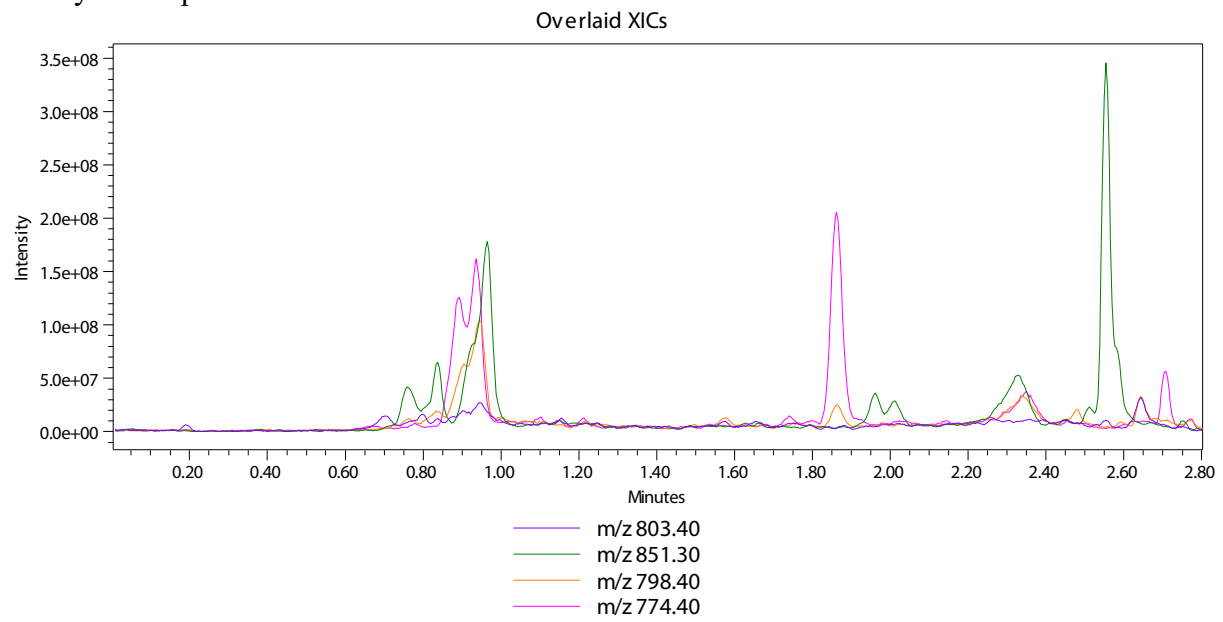


Macrocycle: CL(AGGA)FVY (C1A mutant)

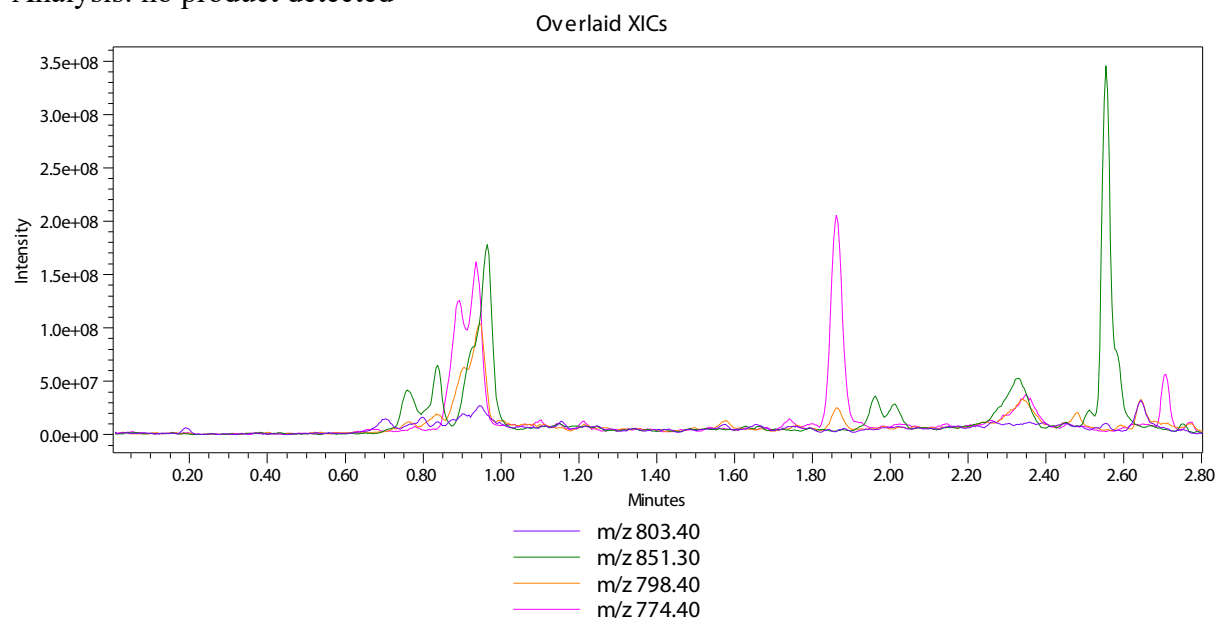
Analysis: no product detected



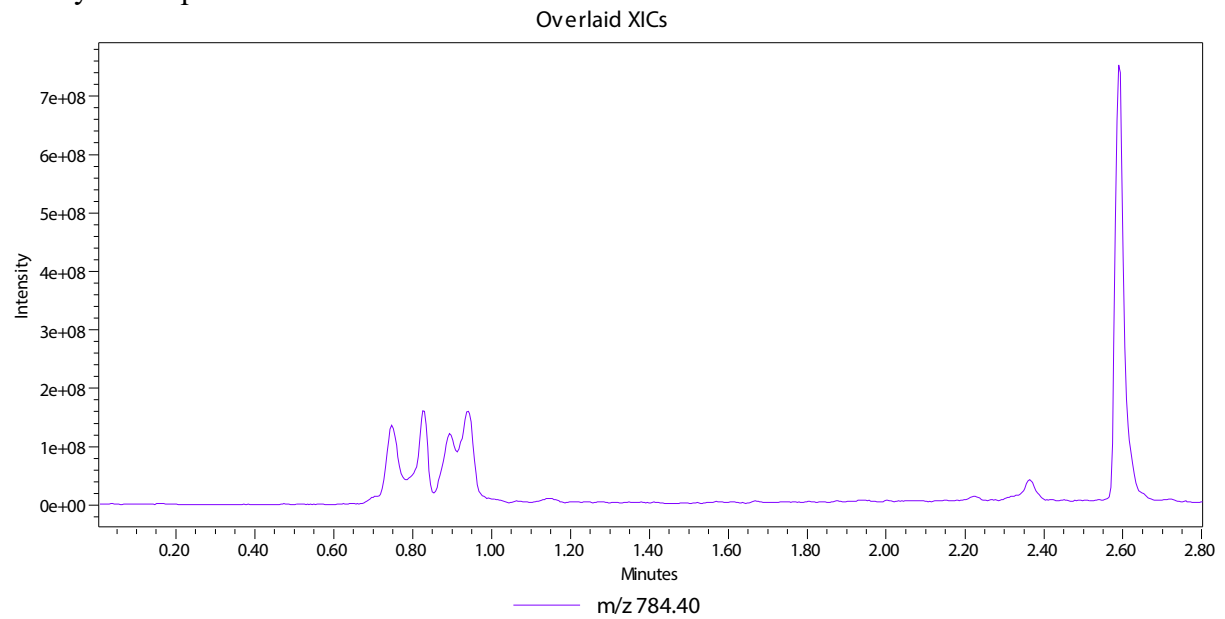
Macrocycle: C(AGGA)LFVY (- ncAA)
Analysis: no product detected



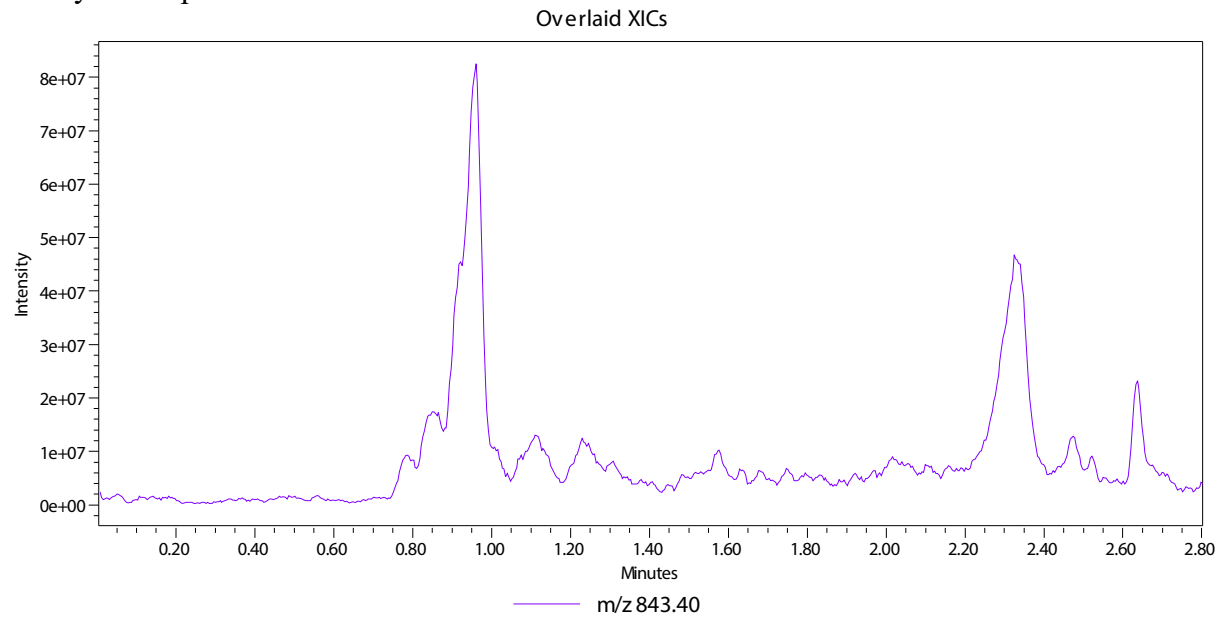
Macrocycle: CL(AGGA)FVY (- ncAA)
Analysis: no product detected



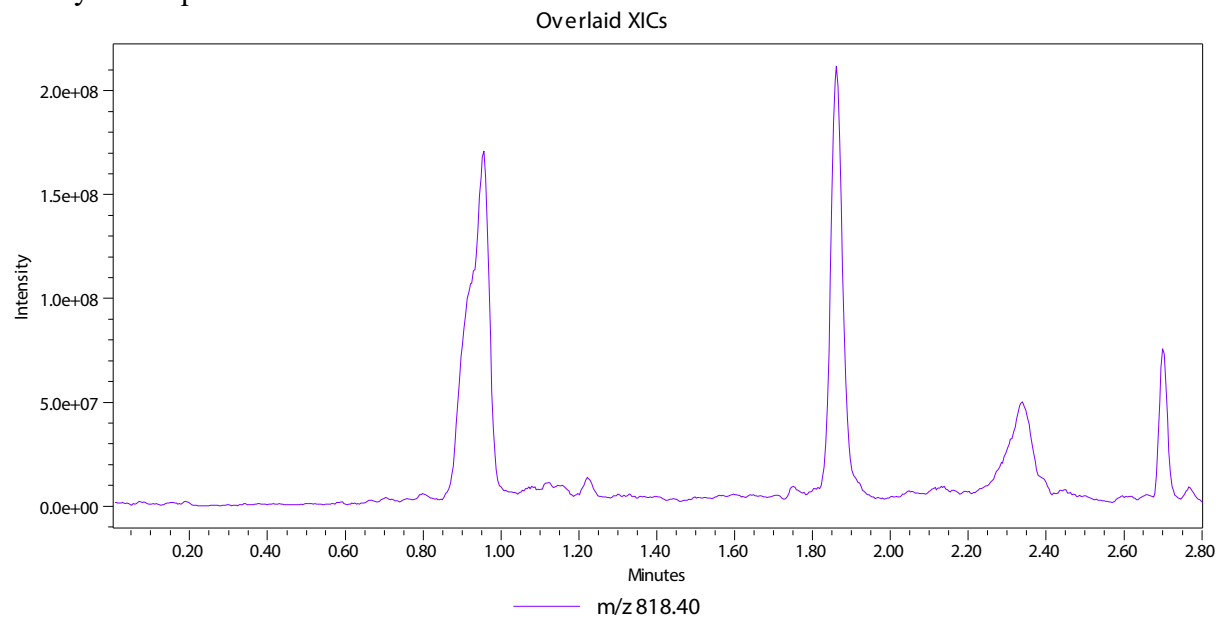
AUAG decoding controls:
Macrocycle: CLL(AUAG)VY (C1A mutant)
Analysis: no product detected



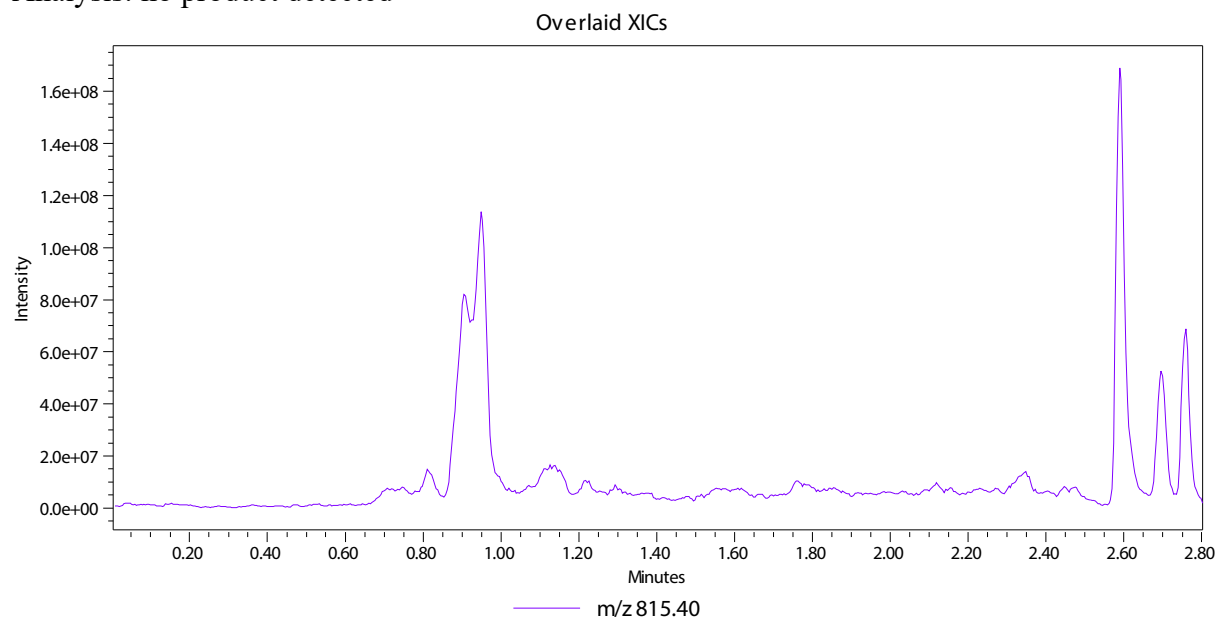
Macrocycle: CLL(AUAG)VY (C1A mutant)
Analysis: no product detected



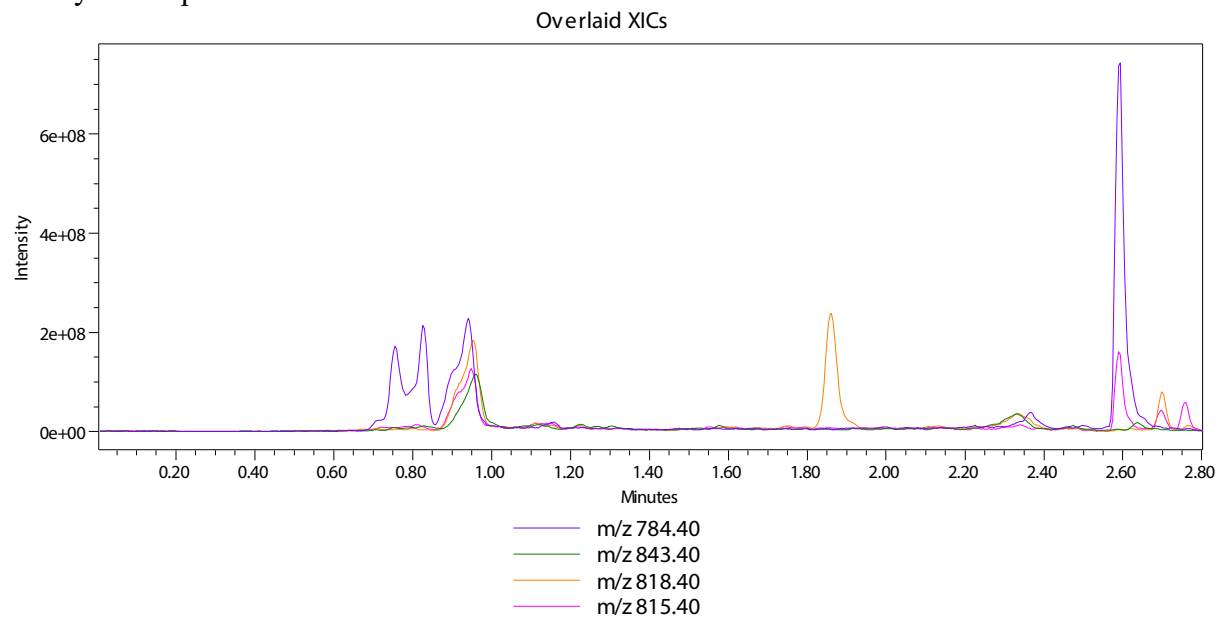
Macrocycle: CL(AUAG)FVY (C1A mutant)
Analysis: no product detected



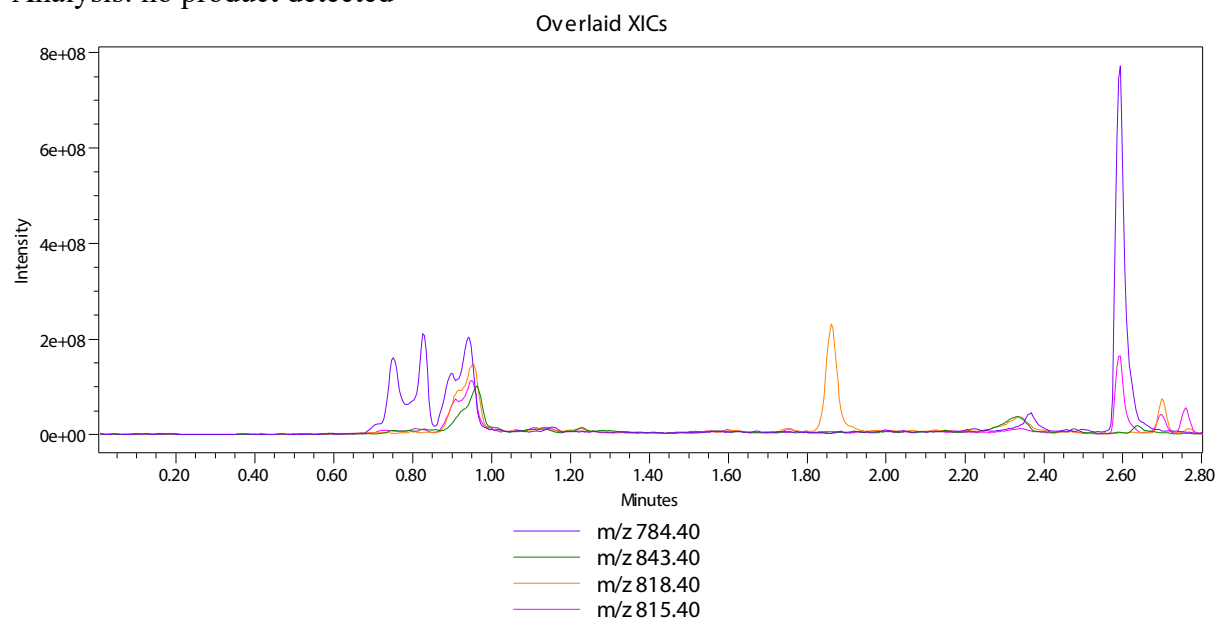
Macrocycle: CL(AUAG)FVY (C1A mutant)
Analysis: no product detected



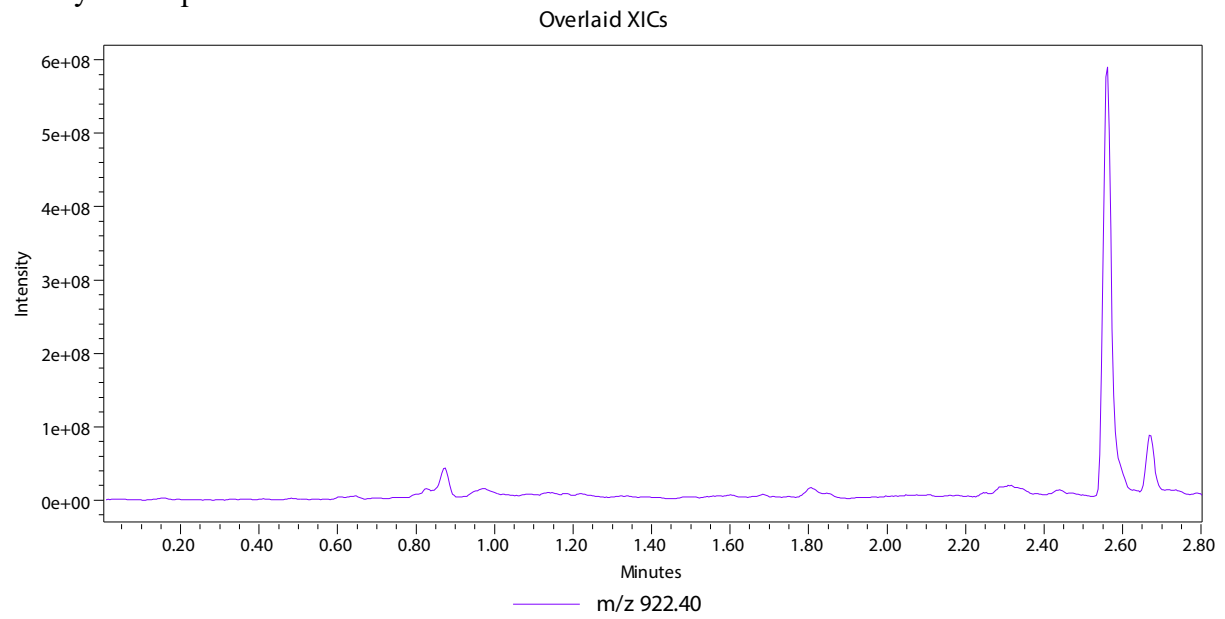
Macrocycle: CLL(AUAG)VY (- ncAA)
Analysis: no product detected



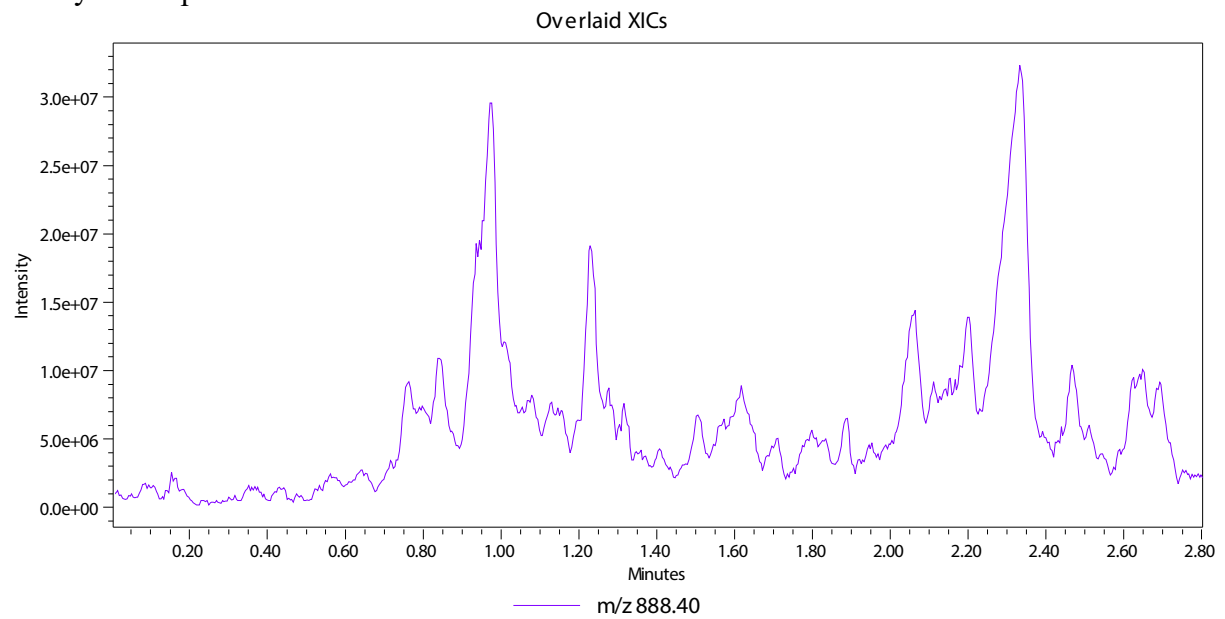
Macrocycle: CL(AUAG)FVY (- ncAA)
Analysis: no product detected



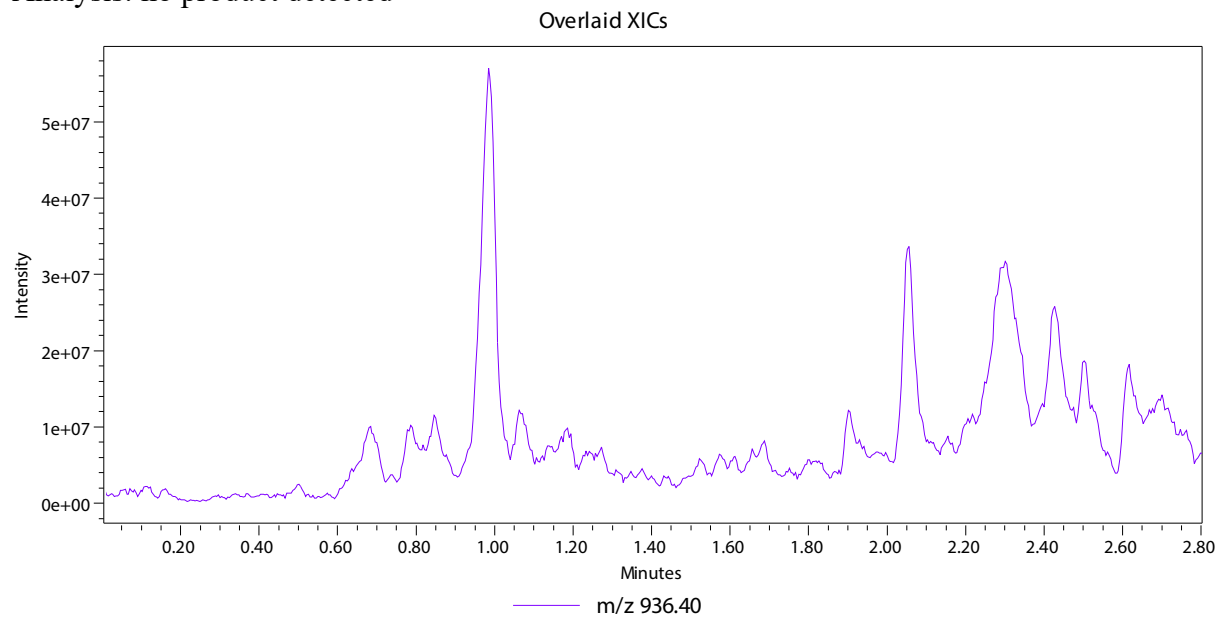
UAGA decoding controls:
Macrocycle: C(UAGA)LFVY (C1A mutant)
Analysis: no product detected



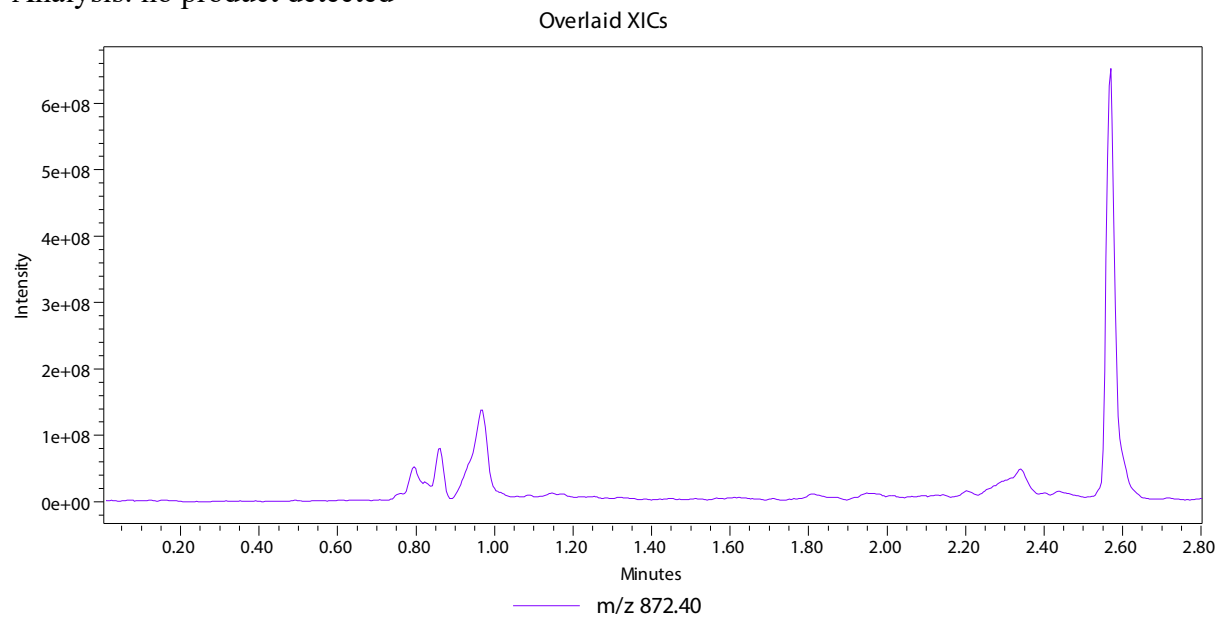
Macrocycle: CL(UAGA)FVY (C1A mutant)
Analysis: no product detected



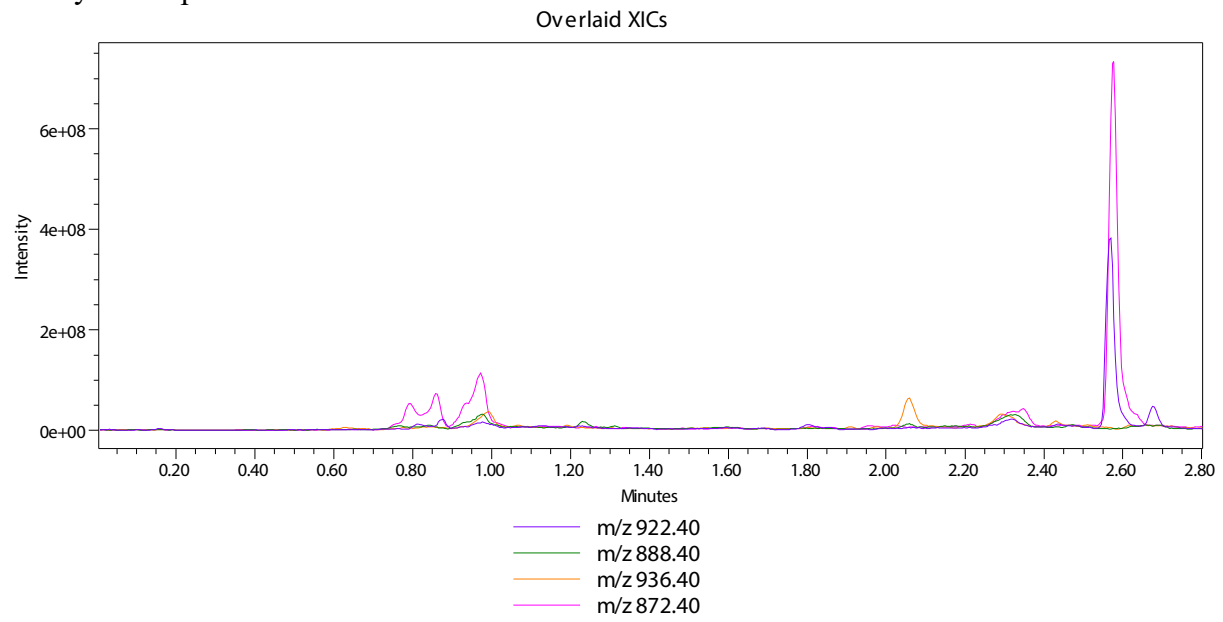
Macrocycle: CLLF(UAGA)Y (C1A mutant)
Analysis: no product detected



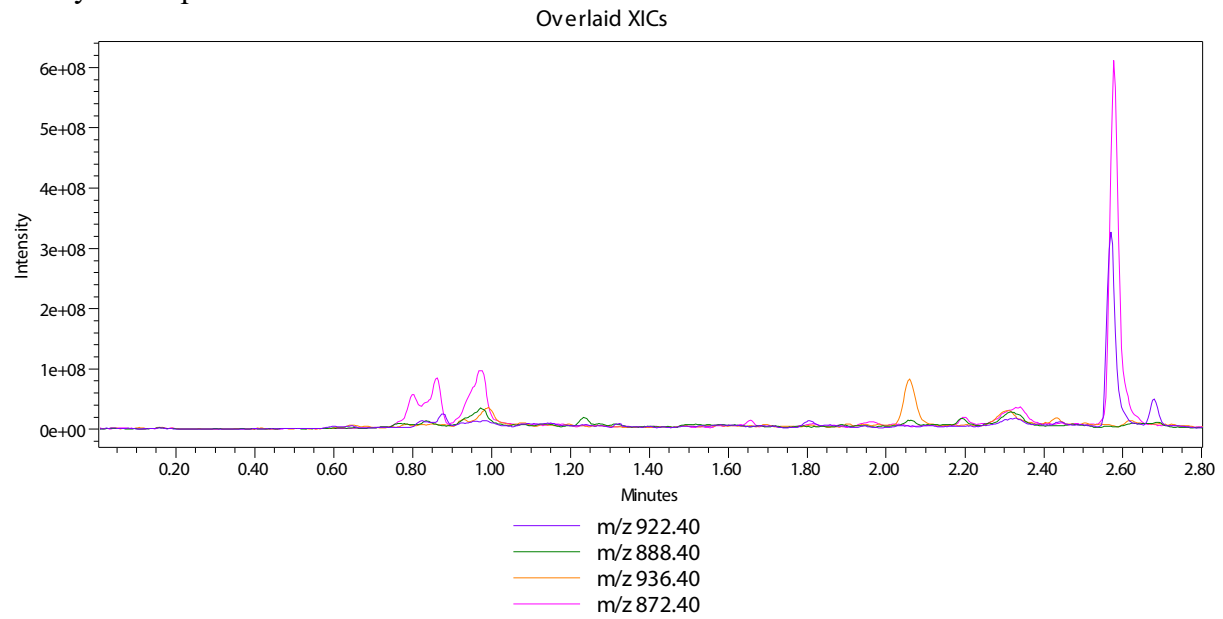
Macrocycle: CLLFV(UAGA) (C1A mutant)
Analysis: no product detected



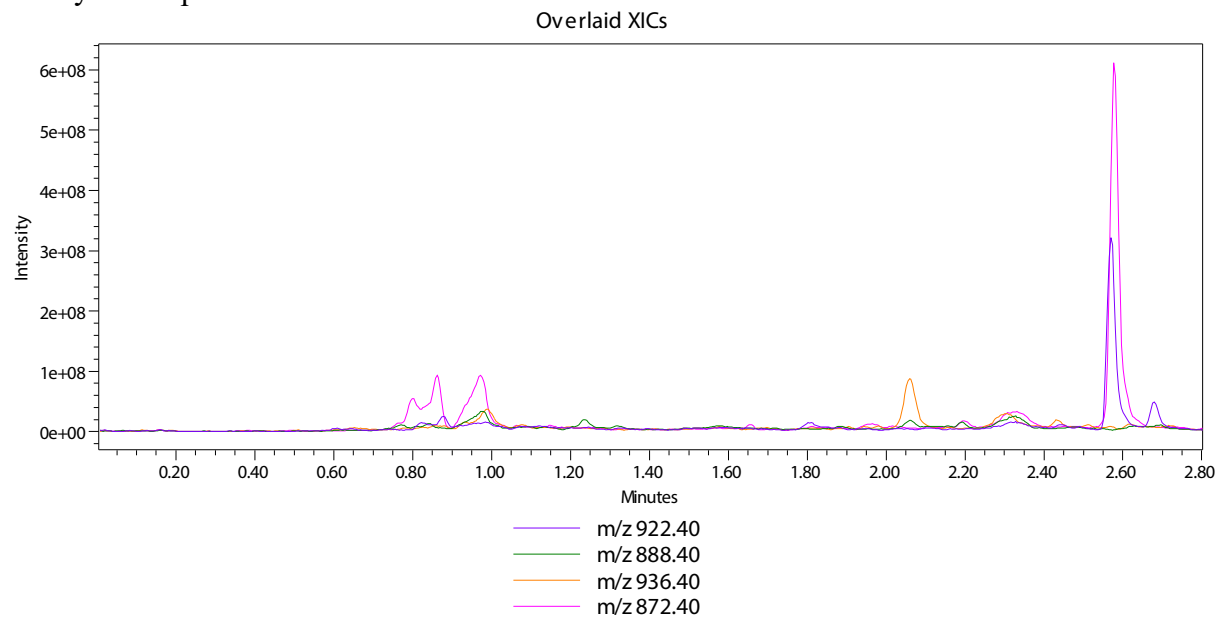
Macrocycle: C(UAGA)LFVY (- ncAA)
Analysis: no product detected



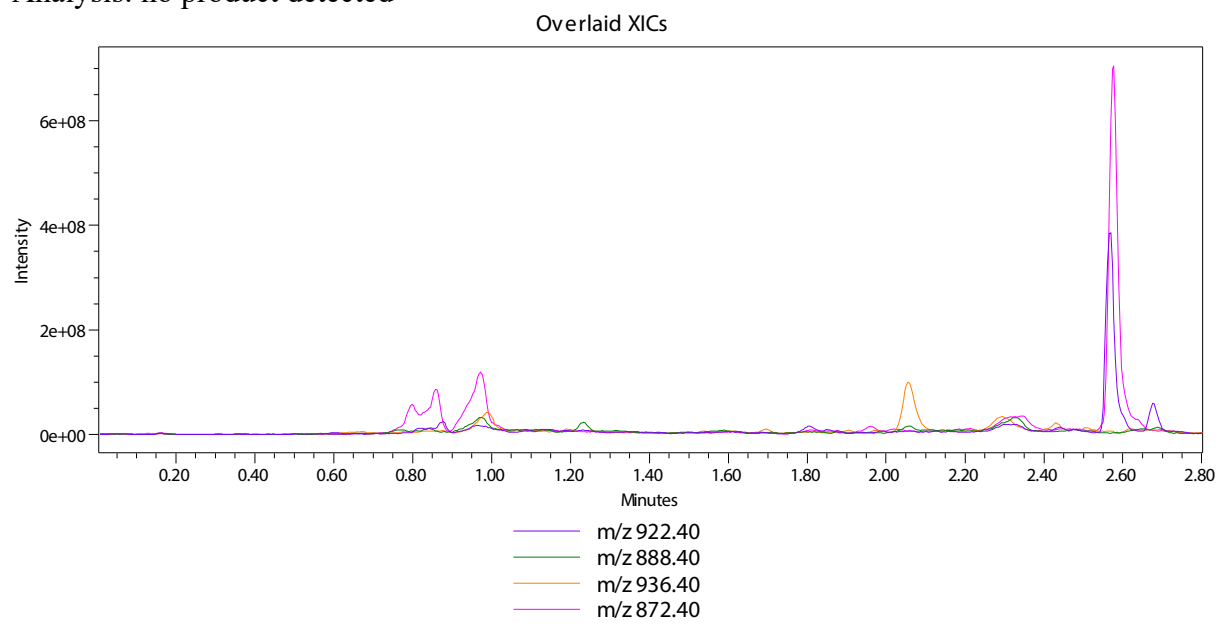
Macrocycle: CL(UAGA)FVY (- ncAA)
Analysis: no product detected



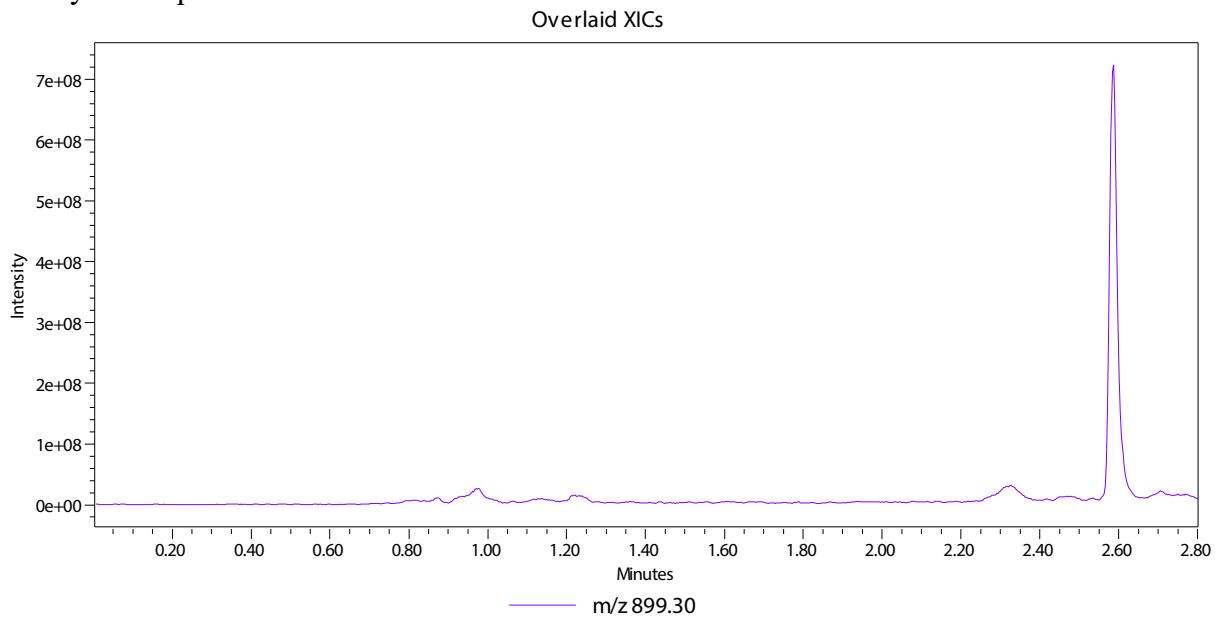
Macrocycle: CLLF(UAGA)Y (- ncAA)
Analysis: no product detected



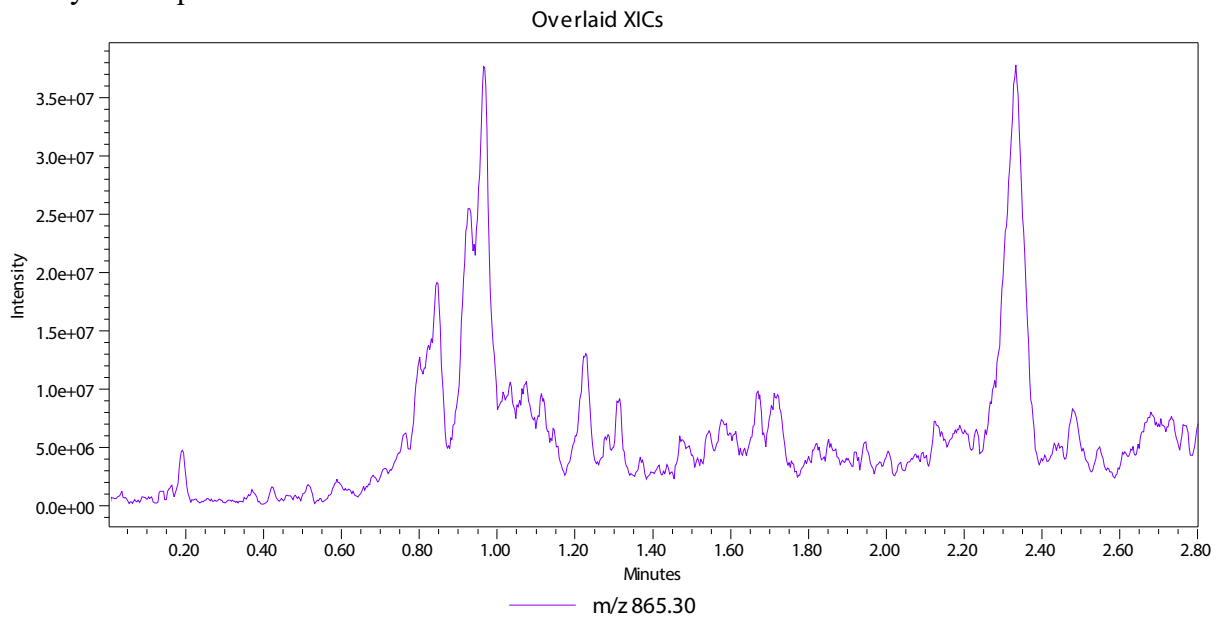
Macrocycle: CLLFV(UAGA) (- ncAA)
Analysis: no product detected



CUAG decoding controls:
Macrocycle: CL(CUAG)FVY (C1A mutant)
Analysis: no product detected

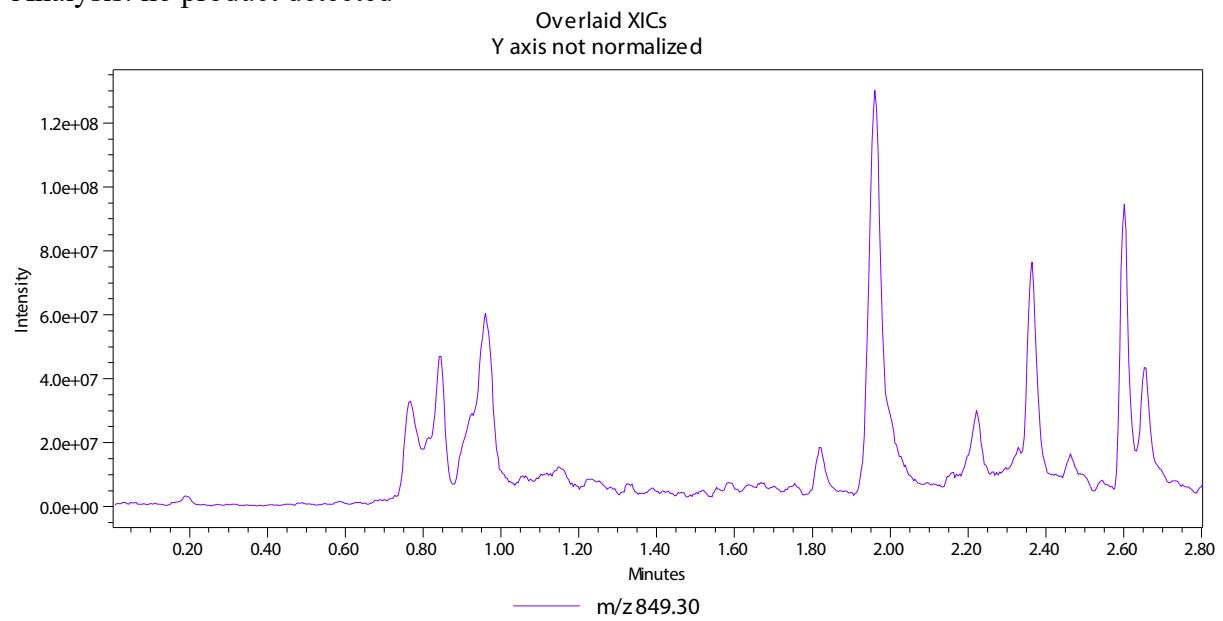


Macrocycle: CLL(CUAG)VY (C1A mutant)
Analysis: no product detected



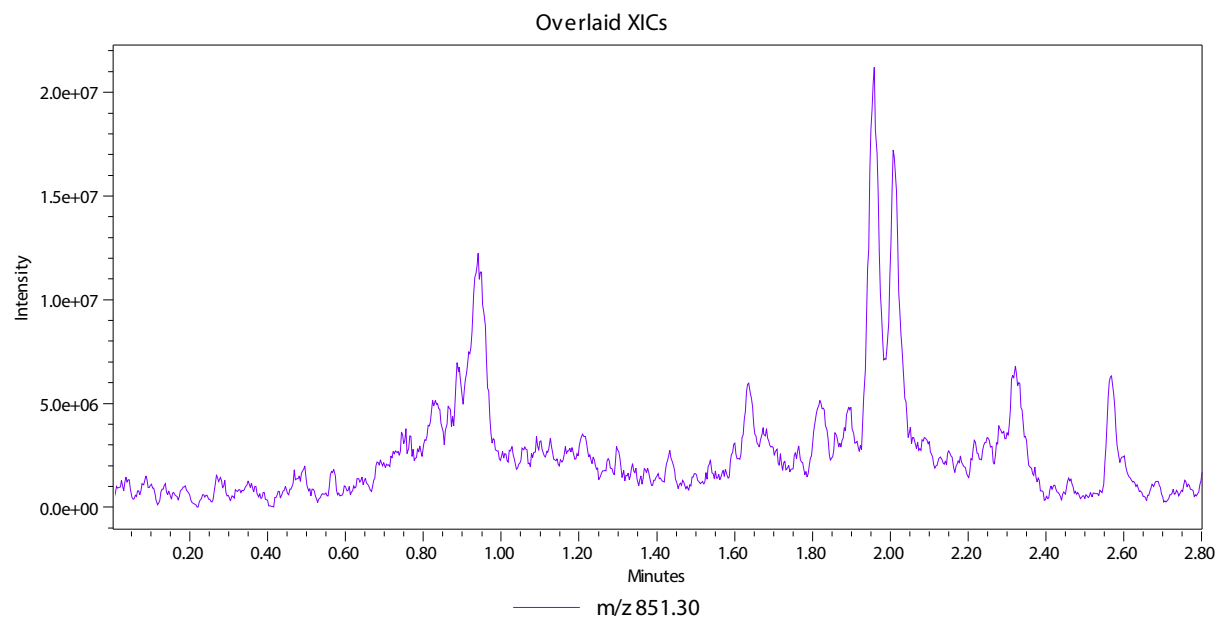
Macrocycle: CLLFV(CUAG) (C1A mutant)

Analysis: no product detected

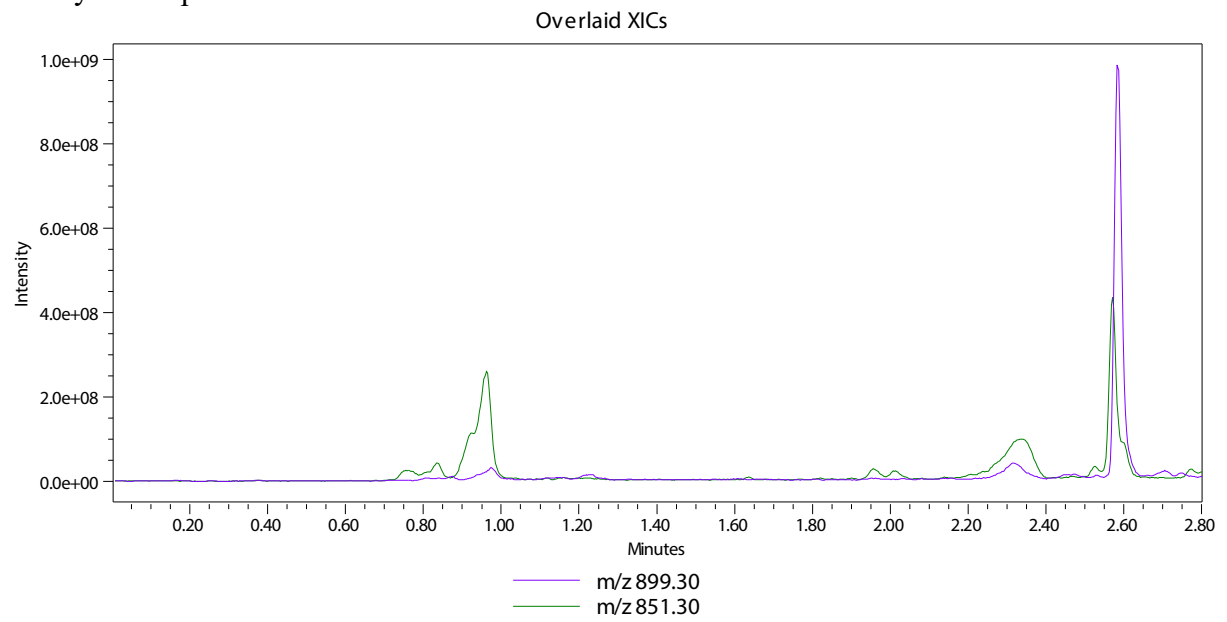


Macrocycle: CL(CUAG)FVY (C1A mutant)

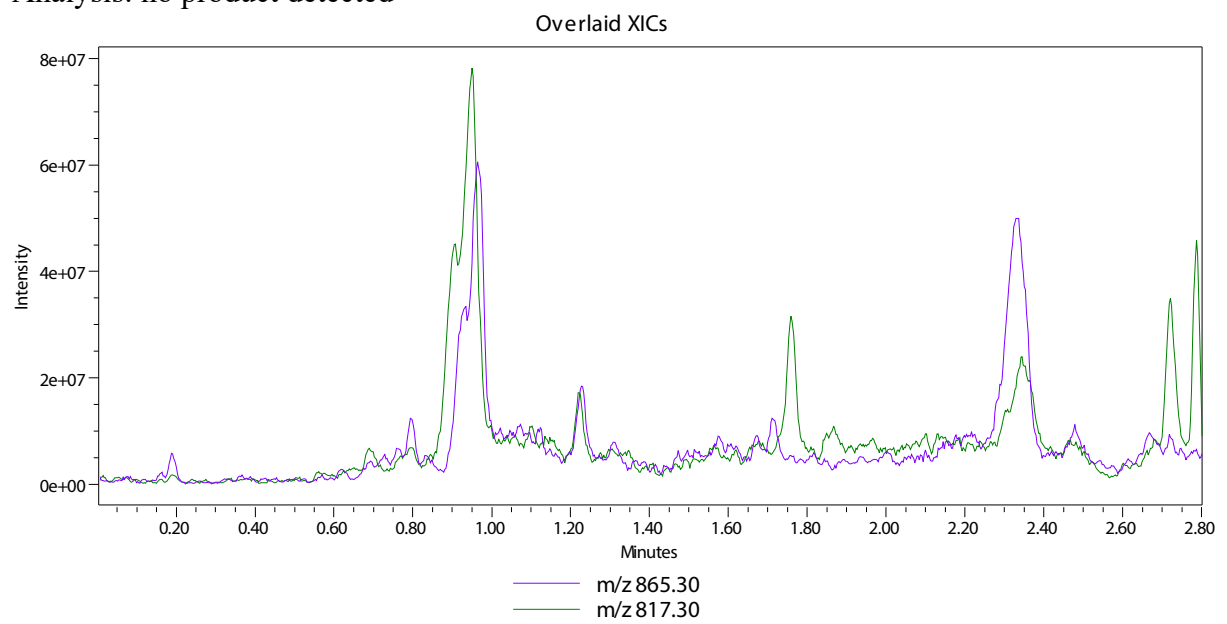
Analysis: no product detected



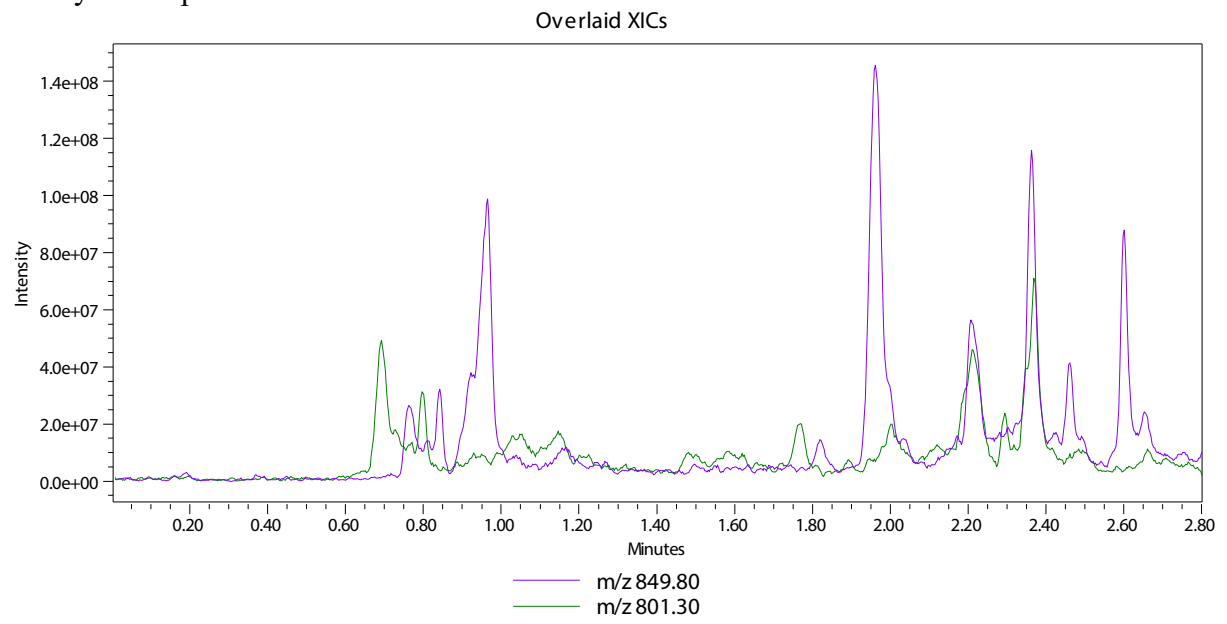
Macrocycle: CL(CUAG)FVY (- ncAA)
Analysis: no product detected



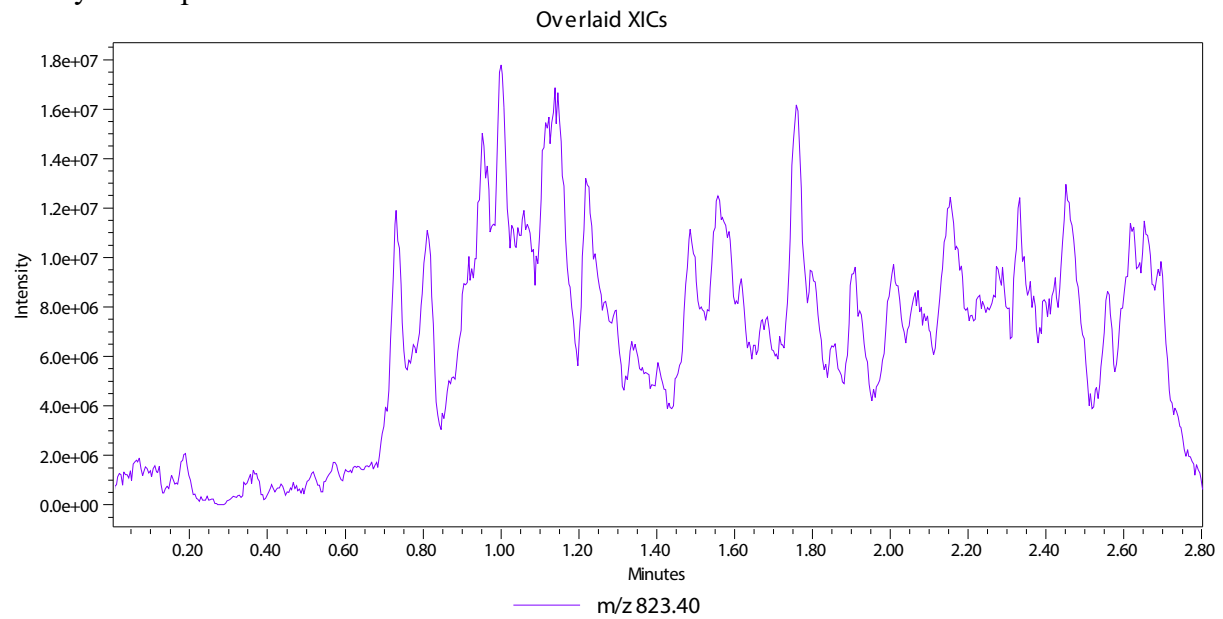
Macrocycle: CLL(CUAG)VY (- ncAA)
Analysis: no product detected



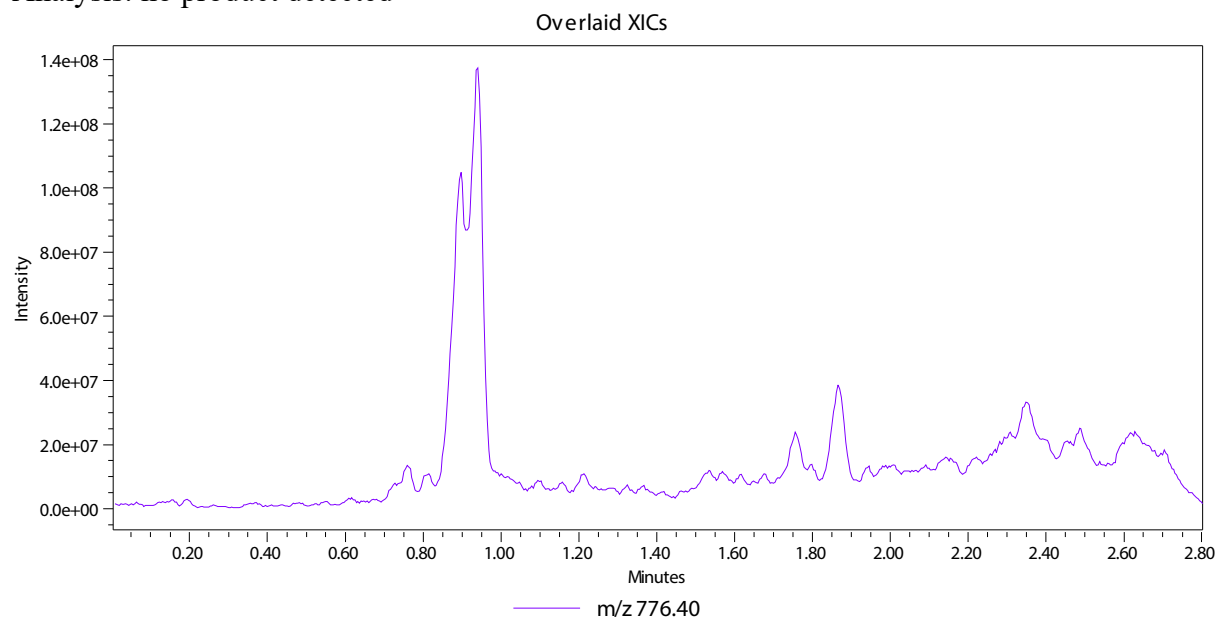
Macrocycle: CLLFV(CUAG) (- ncAA)
Analysis: no product detected



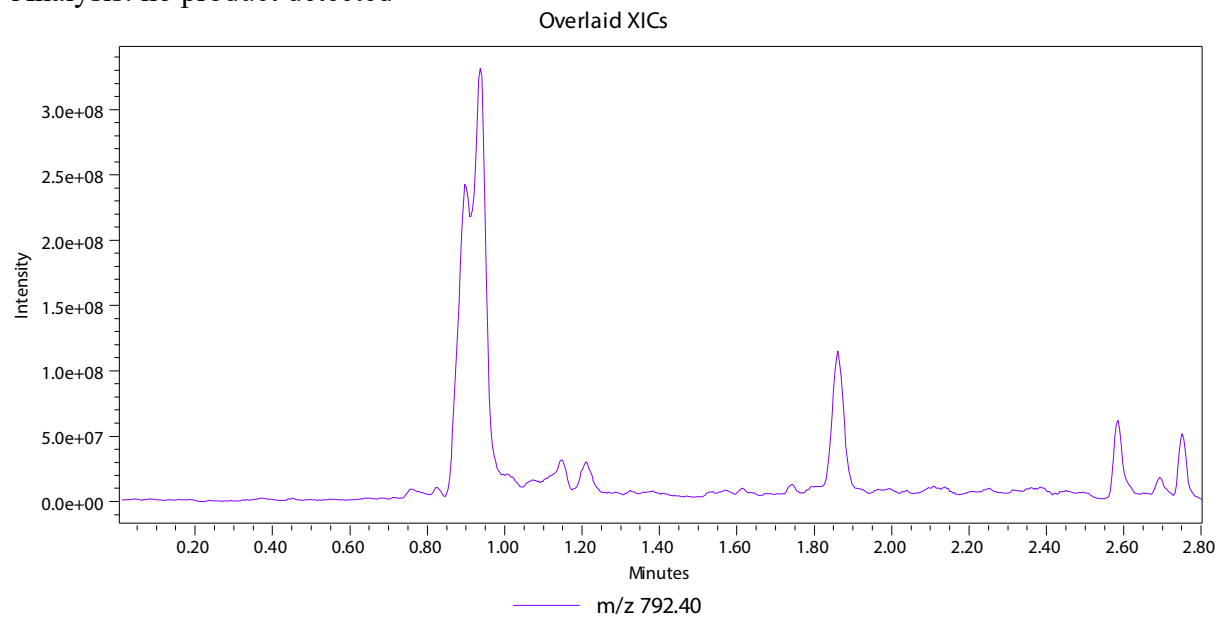
CGGA decoding controls:
Macrocycle: CL(CUAG)FVY (C1A mutant)
Analysis: no product detected



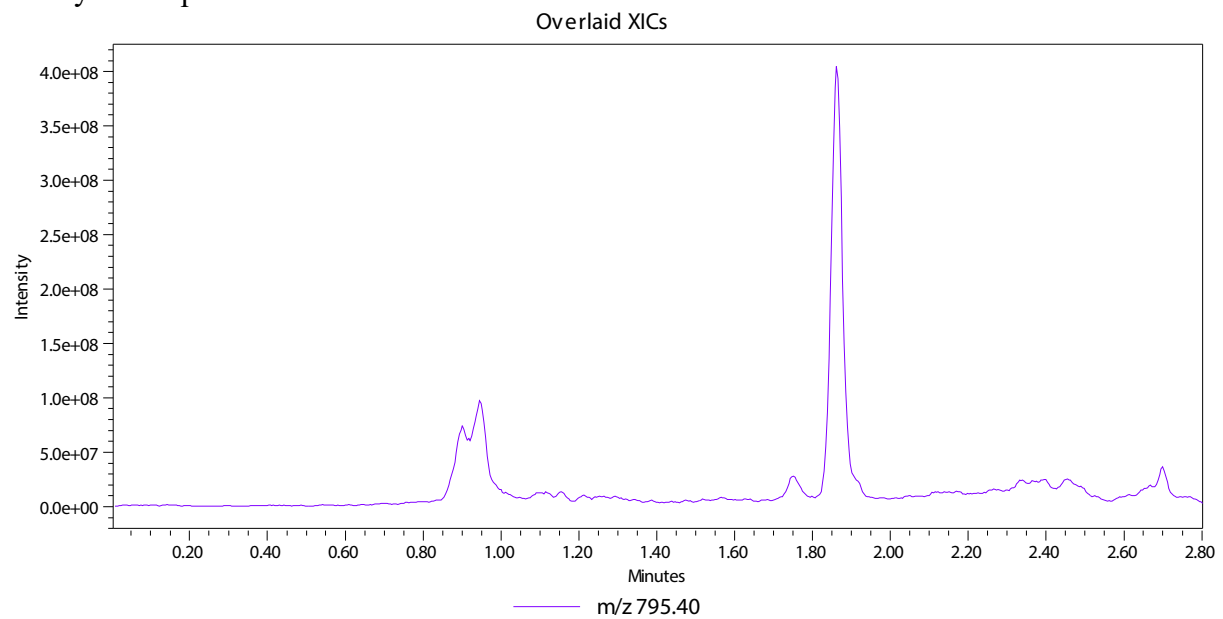
Macrocycle: CLLFV(CUAG) (C1A mutant)
Analysis: no product detected



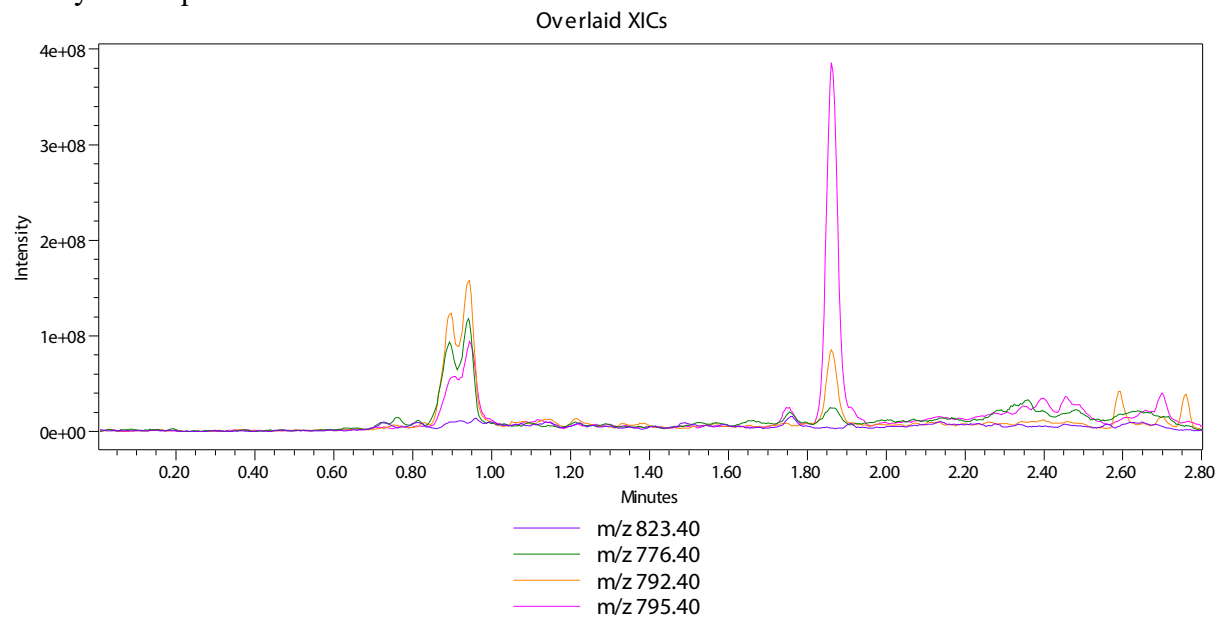
Macrocycle: CLL(CUAG)VY (C1A mutant)
Analysis: no product detected



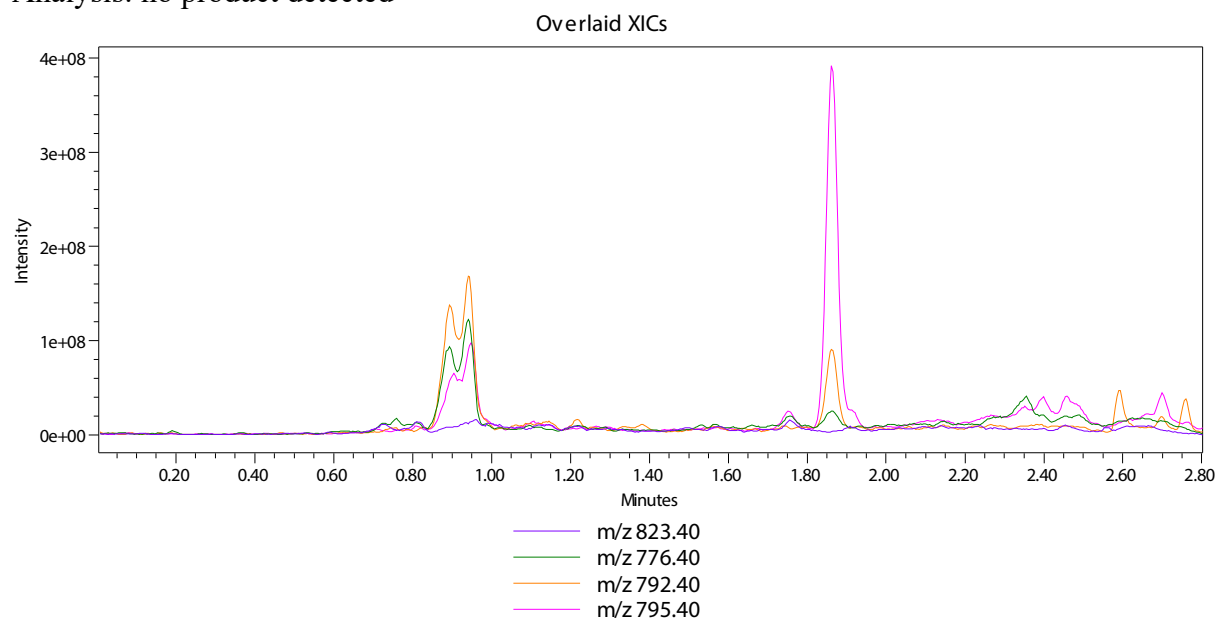
Macrocycle: CLL(CUAG)VY (C1A mutant)
Analysis: no product detected



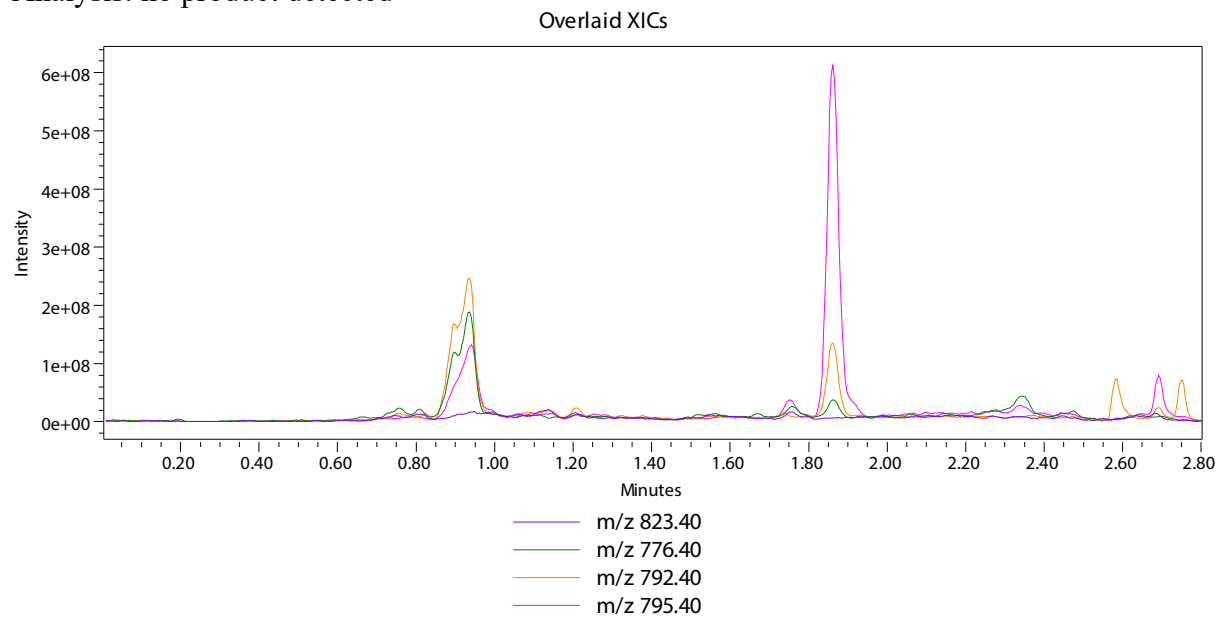
Macrocycle: CL(CUAG)FVY (- ncAA)
Analysis: no product detected



Macrocycle: CLLFV(CUAG) (- ncAA)
Analysis: no product detected



Macrocycle: CLL(CUAG)VY (- ncAA)
Analysis: no product detected



Supplementary Data 4 | Mass Spectrometry Data for Extended Data Figure 10.

TARGET MASS ANALYSIS

Sample Set Name:	G1_AAA0292_96_AlexP	Acq. Method Set: G1_AAA0292 Processing Method: G1_AAA0292
Date Acquired:	4/26/2024 4:55:27 PM PDT, 4/26/2024 4:59:08 PM PDT, 4/26/2024 5:02:50 PM PDT,	
Date Processed:	4/26/2024 4:58:33 PM PDT, 4/26/2024 5:02:15 PM PDT, 4/26/2024 5:05:57 PM PDT,	

TARGET MASS ANALYSIS

Sample Name:
Vial:

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

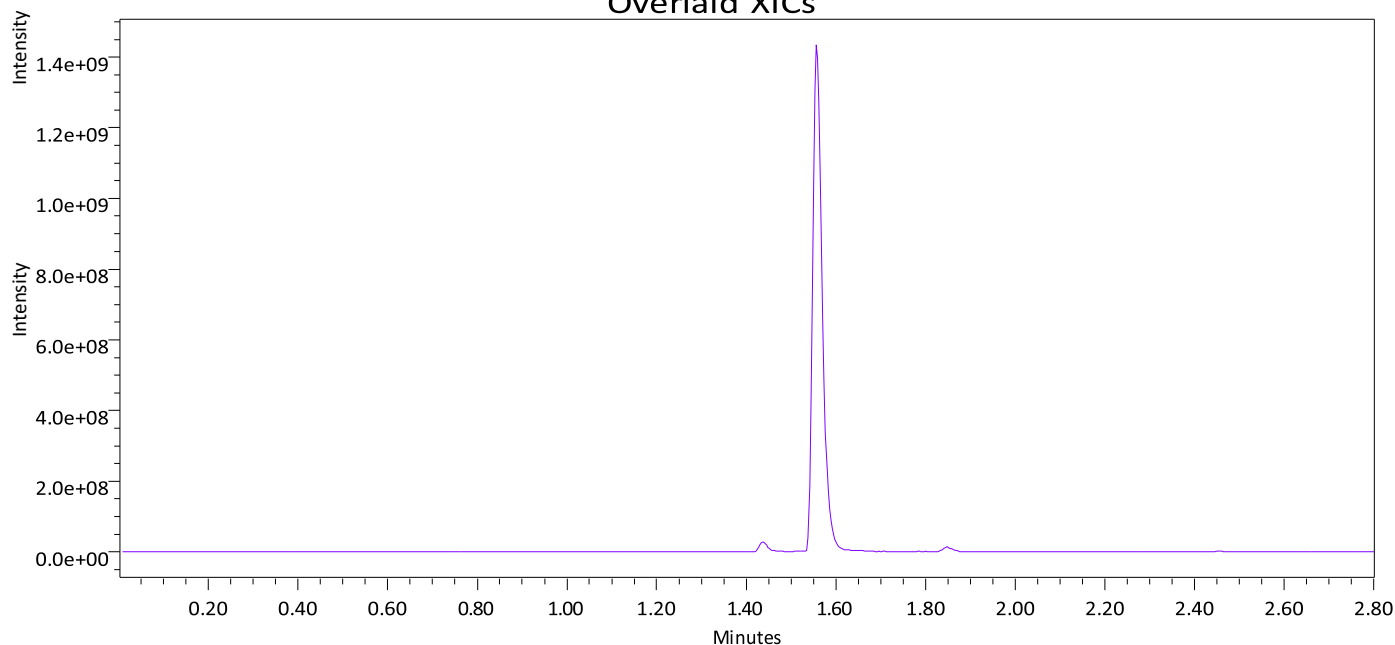
TARGET MASS ANALYSIS

Sample Name:
Vial 2:A,1

Acq. Method Set: G1 AAA0292
Processing Method:G1_AAA0292

Date Acquired: 4/26/2024 4:55:27 PM PDT
Date Processed: 4/26/2024 4:58:33 PM PDT

Overlaid XICs



A1
2:A,1

4/26/2024 4:59:08 PM PDT
4/26/2024 5:02:15 PM PDT

Overlaid XICs

TARGET MASS ANALYSIS

Sample Name:

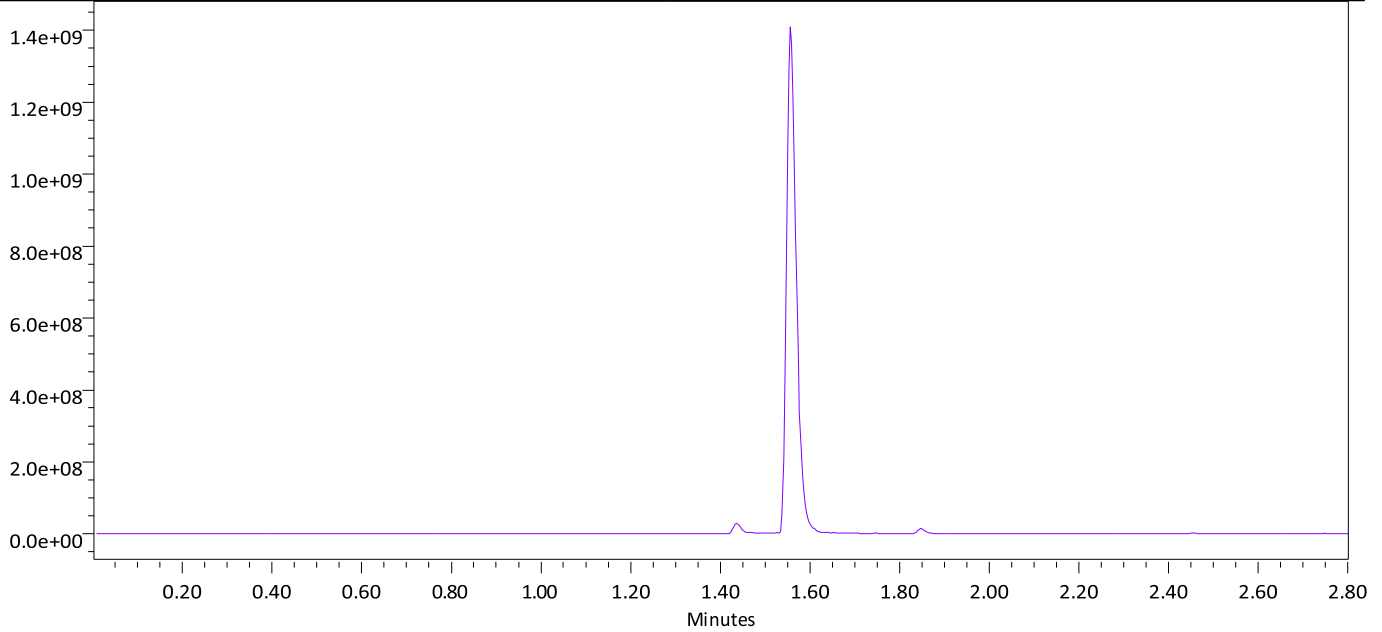
Acq. Method Set: G1 AAA0292

Vial:

Processing Method: G1 AAA0292

Date Acquired:

Date Processed:



m/z 784.40

TARGET MASS ANALYSIS

Sample Name:
Vial:

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

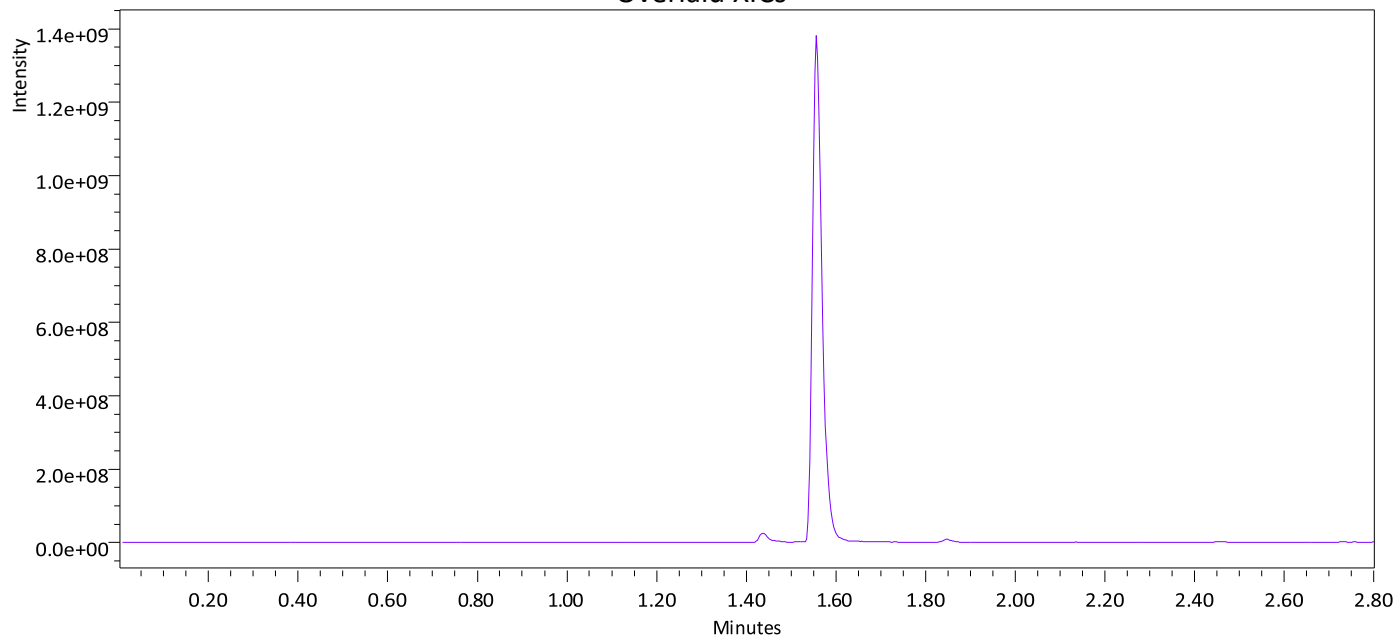
Date Acquired:
Date Processed:

A1
2:A,1

4/26/2024 5:02:50 PM PDT

4/26/2024 5:05:57 PM PDT

Overlaid XICs



m/z 784.40

4/26/2024 5:06:32 PM PDT

4/26/2024 5:09:38 PM PDT

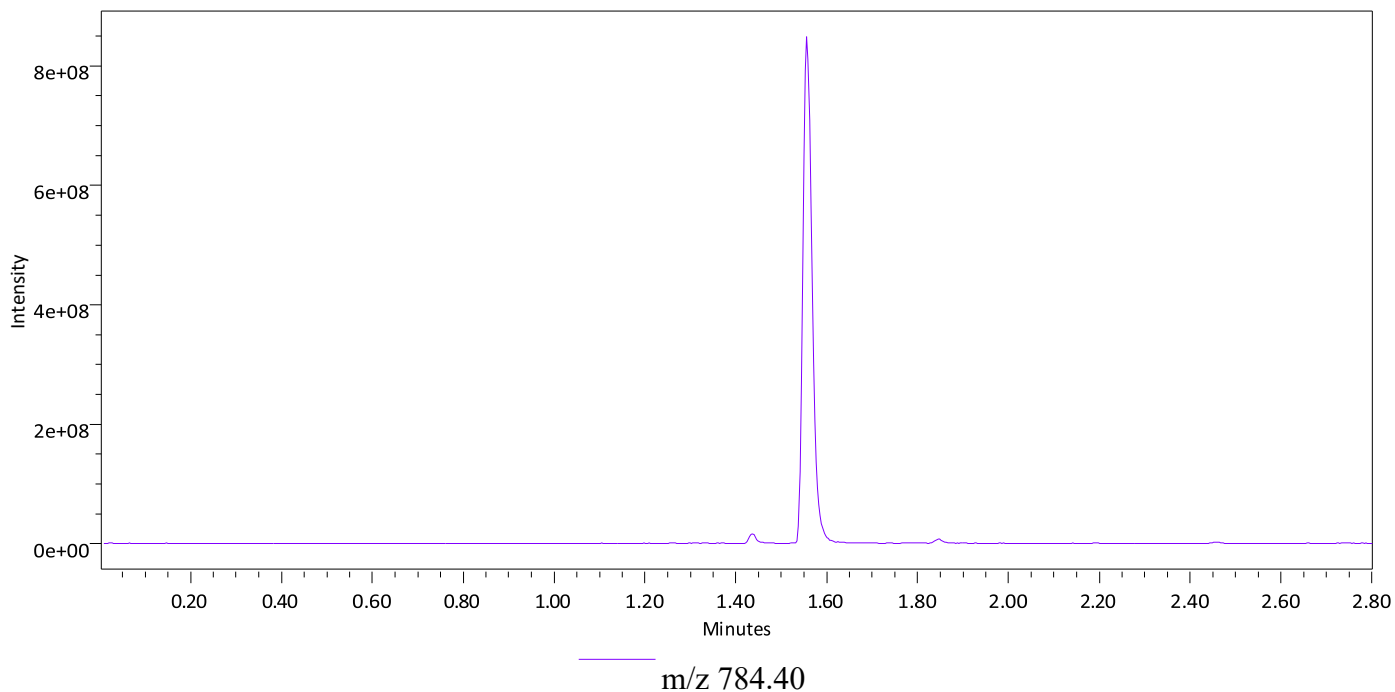
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A2
Vial: 2:A,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 5:10:14 PM PDT
4/26/2024 5:13:20 PM PDT

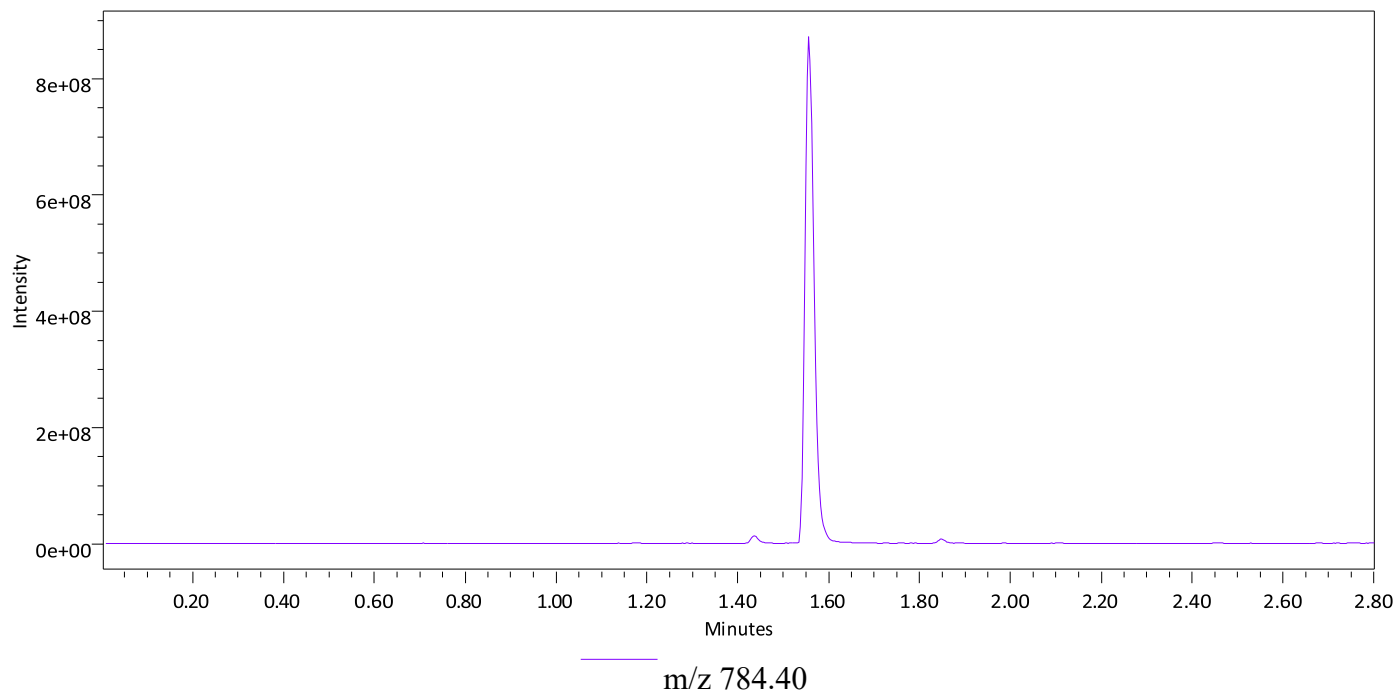
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A2
Vial: 2:A,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 5:13:55 PM PDT
4/26/2024 5:17:05 PM PDT

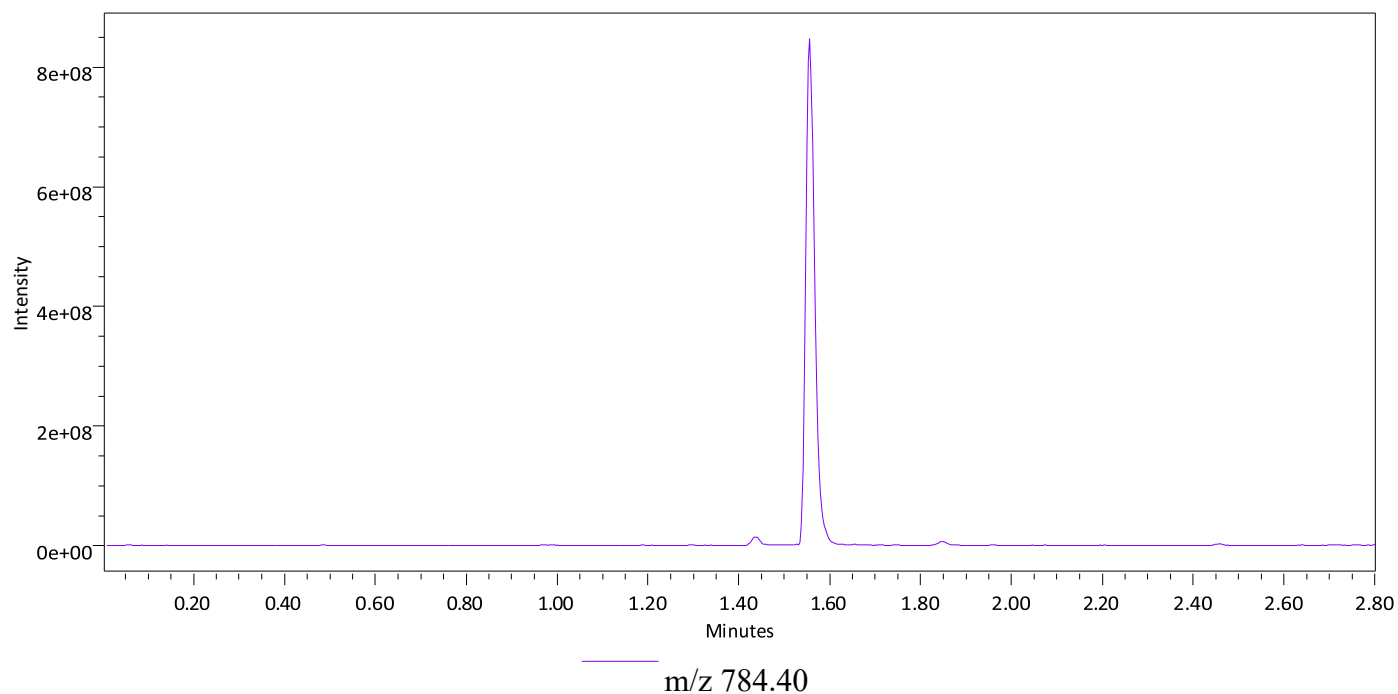
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A2
Vial: 2:A,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: A3
Vial: 2:A,3

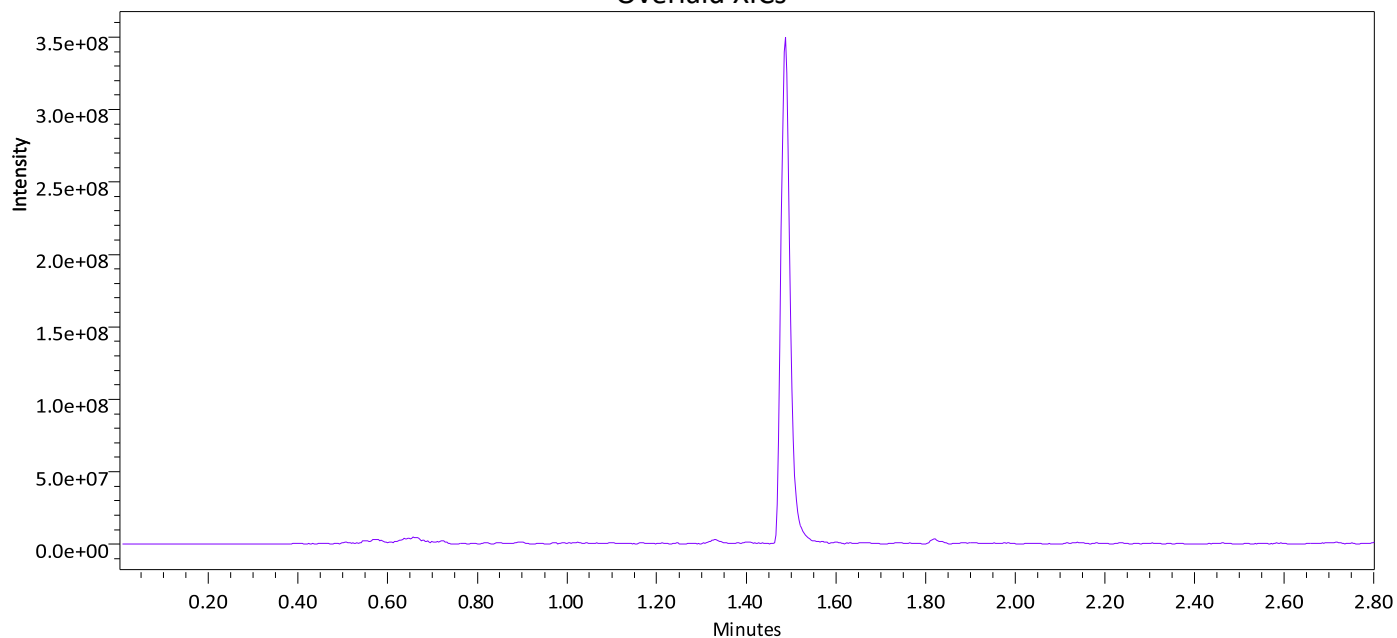
Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 5:17:41 PM PDT

4/26/2024 5:20:46 PM PDT

Overlaid XICs



m/z 784.40

4/26/2024 5:21:21 PM PDT

4/26/2024 5:24:27 PM PDT

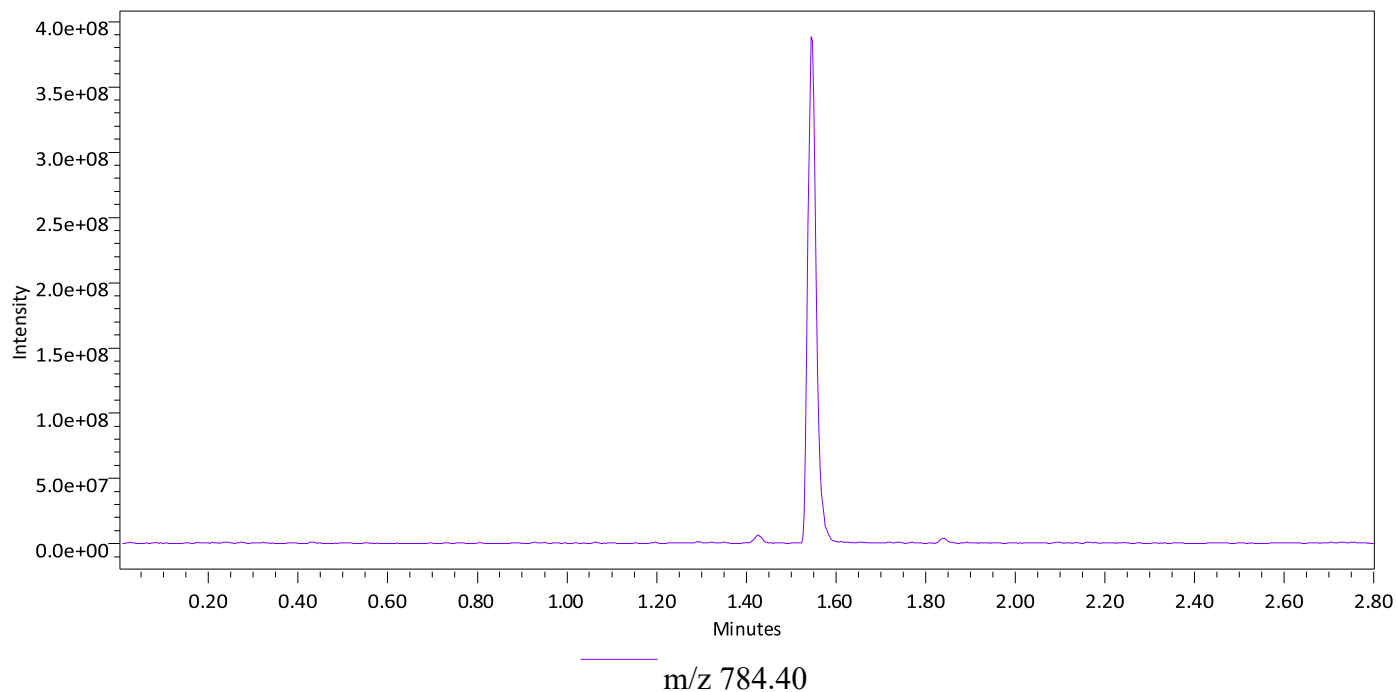
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A3
Vial: 2:A,3

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 5:25:03 PM PDT
4/26/2024 5:28:11 PM PDT

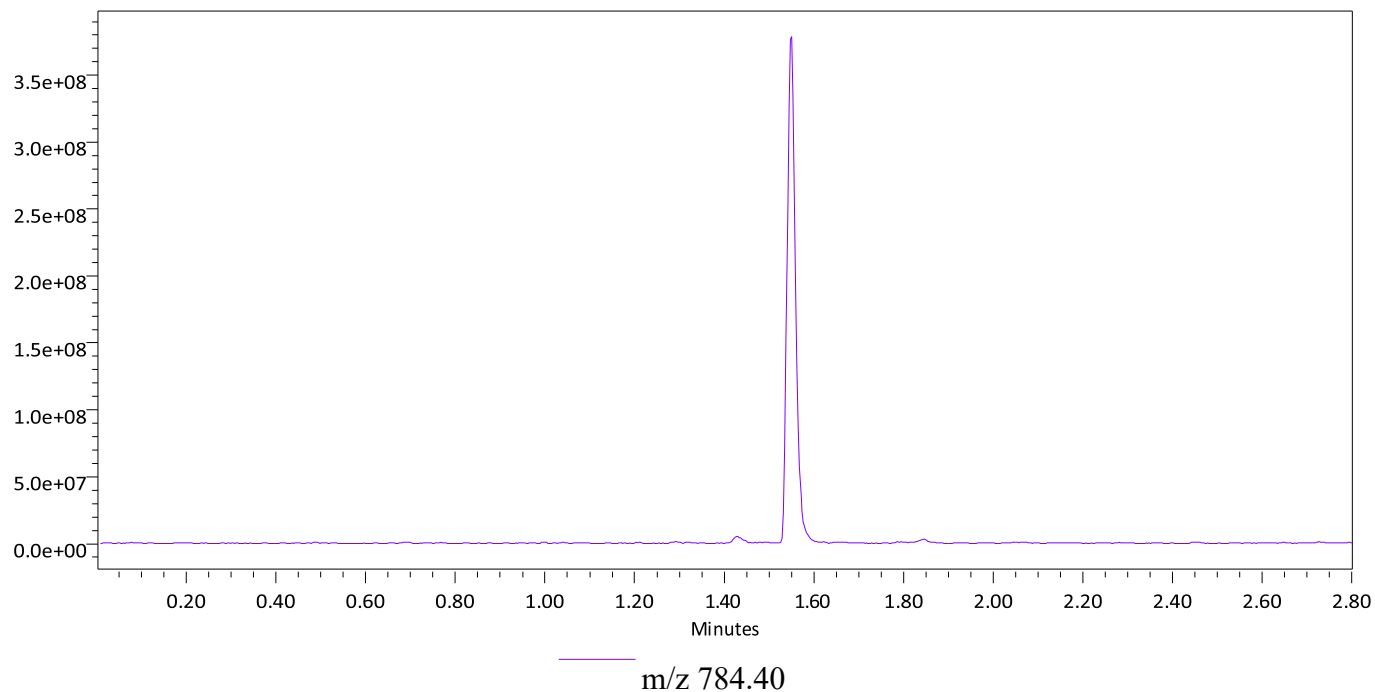
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A3
Vial: 2:A,3

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

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11:14:49 AM US/Pacific

TARGET MASS ANALYSIS

Sample Name: A4
Vial: 2:A,4

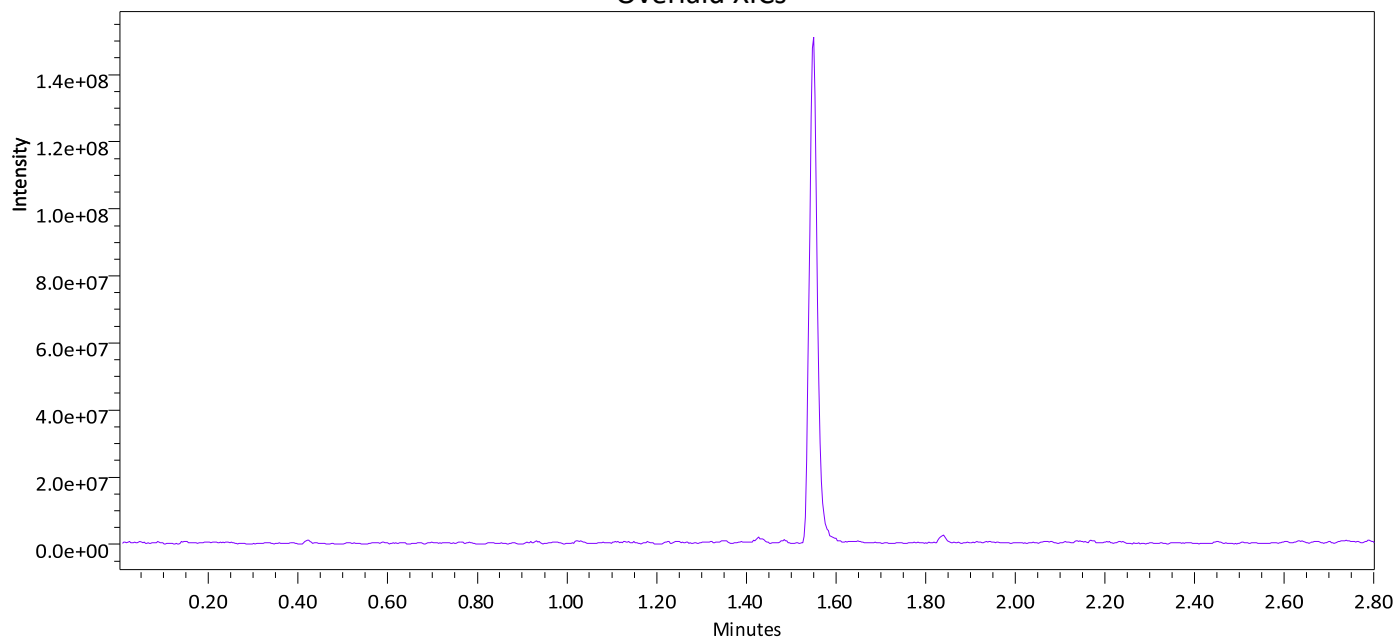
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 5:28:48 PM PDT

4/26/2024 5:31:55 PM PDT

Overlaid XICs



m/z 784.40

4/26/2024 5:32:32 PM PDT

4/26/2024 5:35:36 PM PDT

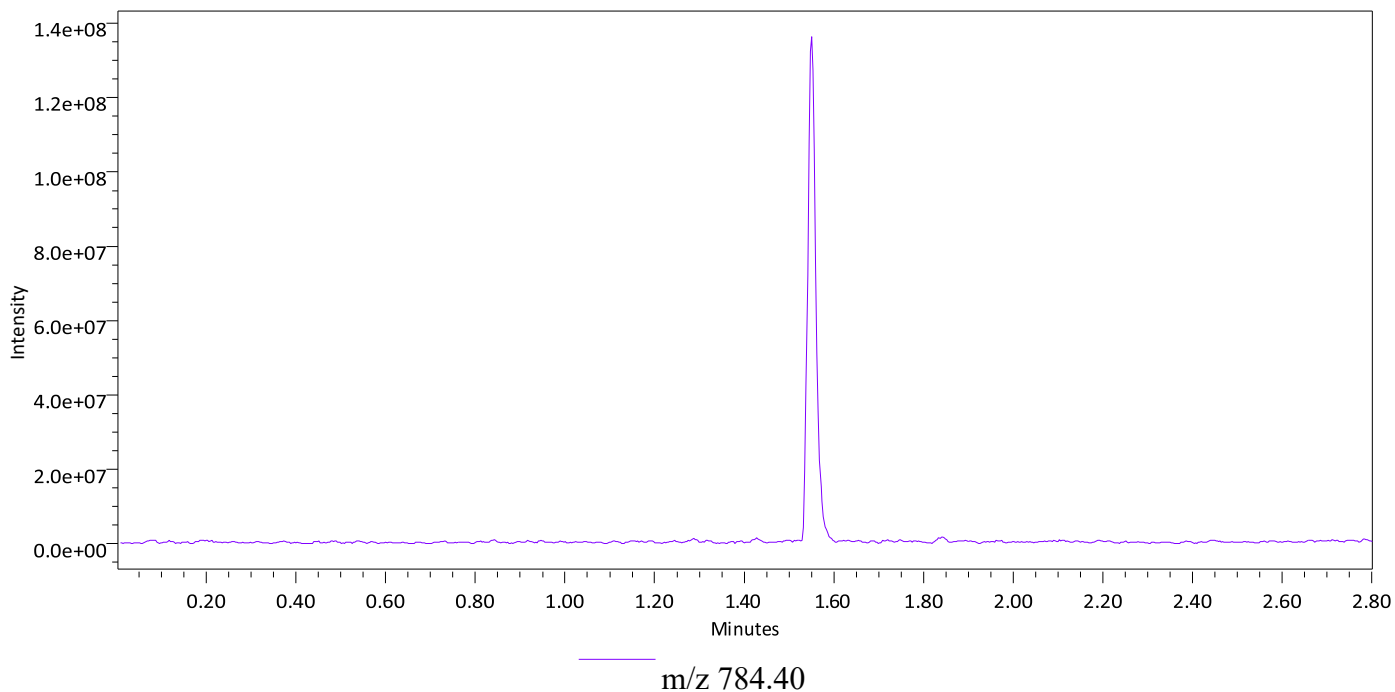
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A4
Vial: 2:A,4

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 5:36:14 PM PDT
4/26/2024 5:39:18 PM PDT

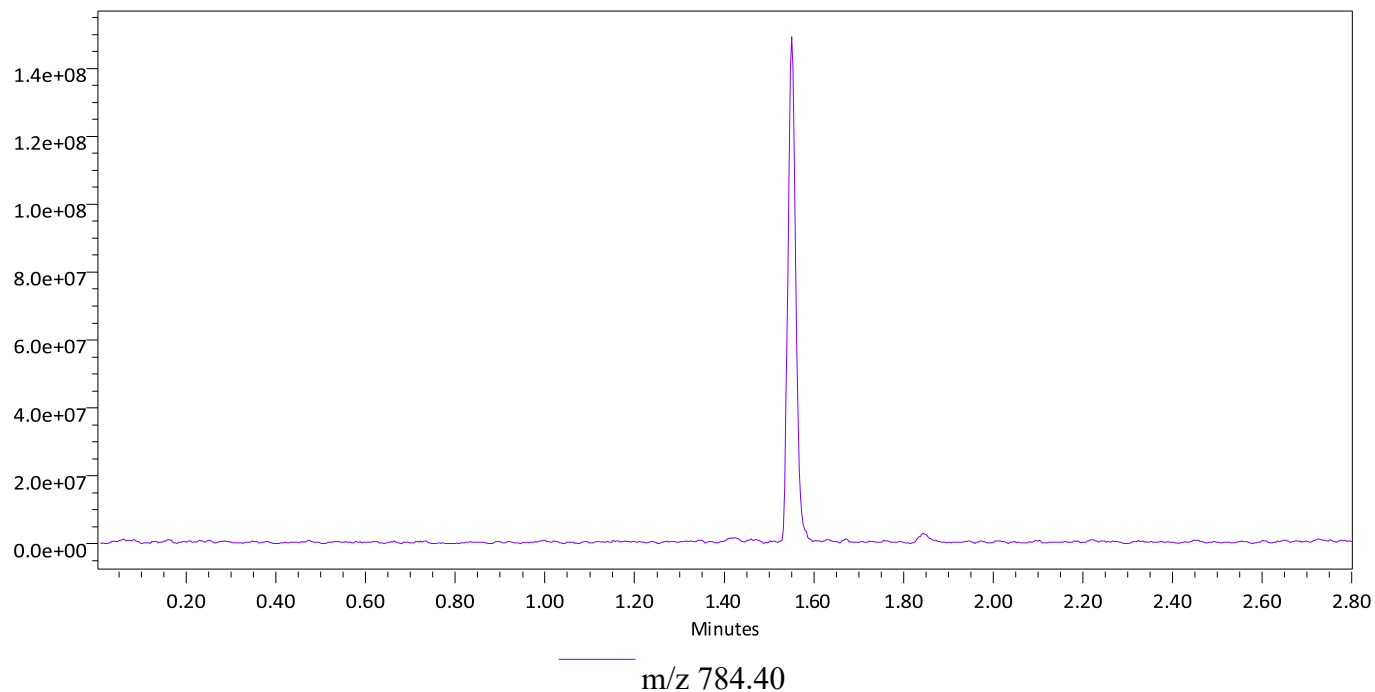
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A4
Vial: 2:A,4

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

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11:14:49 AM US/Pacific

TARGET MASS ANALYSIS

Sample Name: A5
Vial: 2:A,5

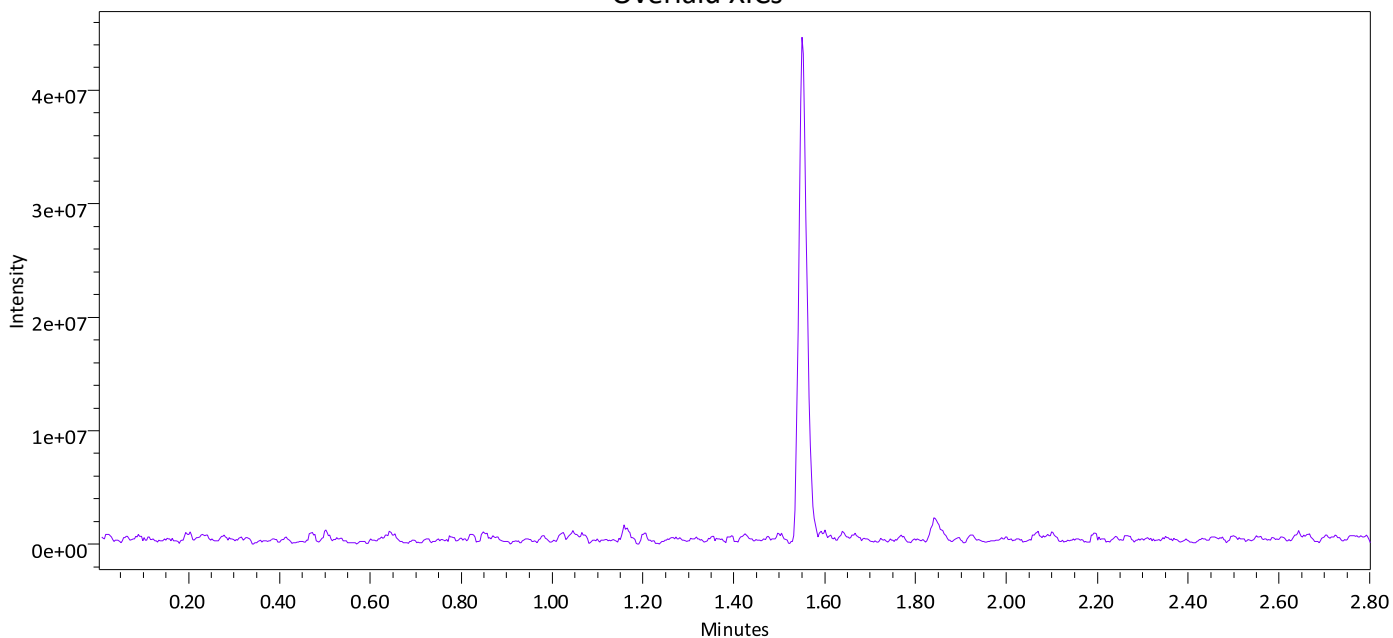
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 5:39:54 PM PDT

4/26/2024 5:42:59 PM PDT

Overlaid XICs



m/z 784.40

4/26/2024 5:43:36 PM PDT

4/26/2024 5:46:45 PM PDT

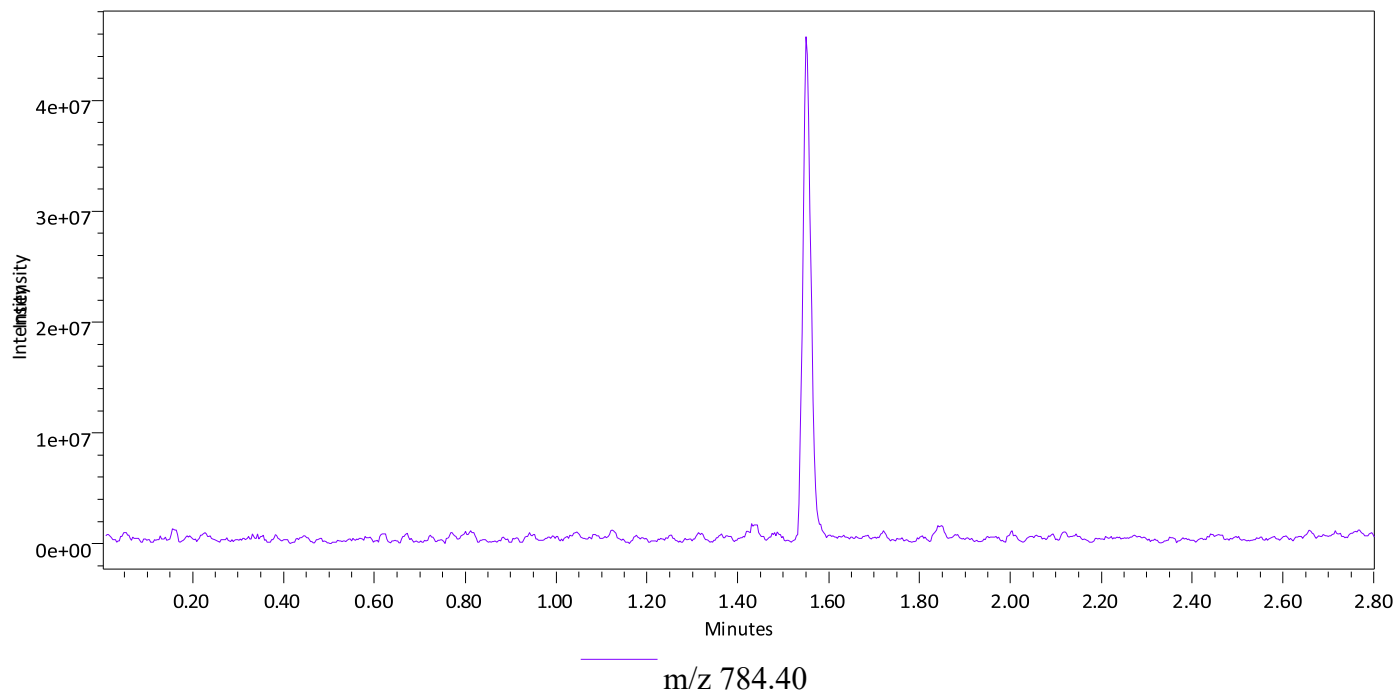
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A5
Vial: 2:A,5

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 5:47:22 PM PDT
4/26/2024 5:50:29 PM PDT

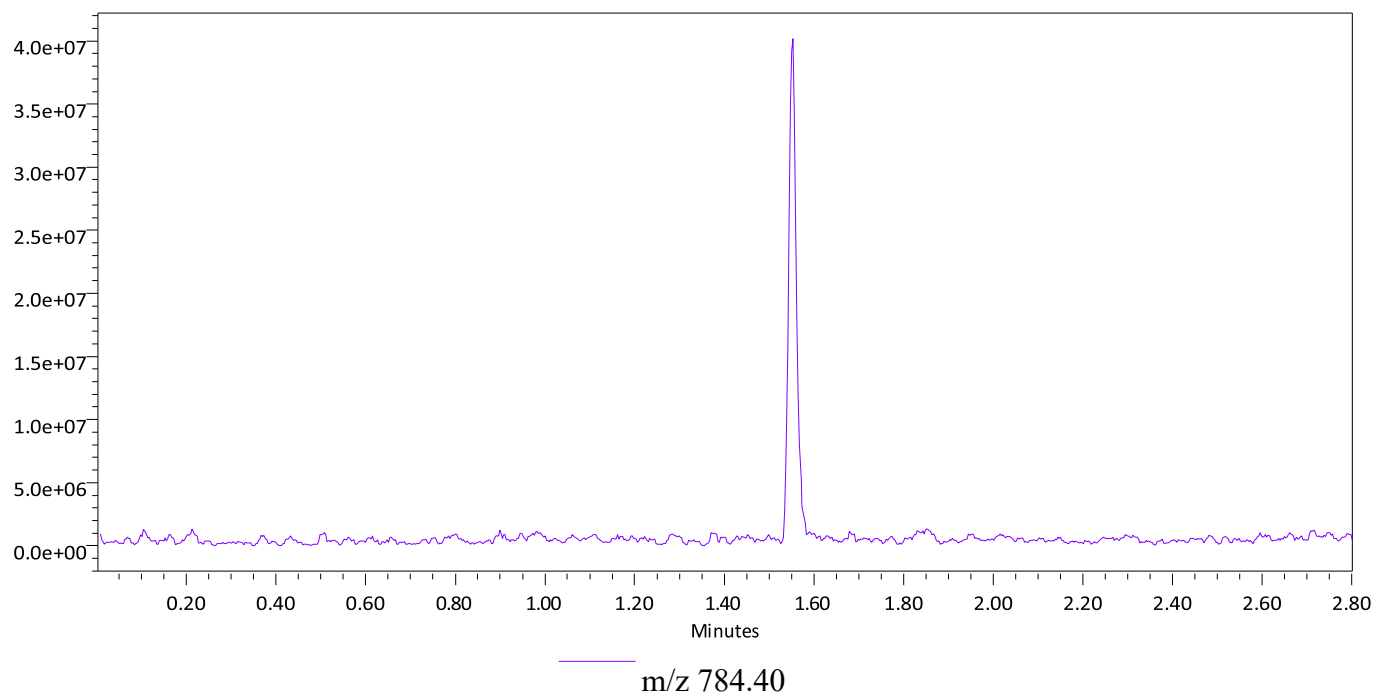
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A5
Vial: 2:A,5

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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TARGET MASS ANALYSIS

Sample Name: A6
Vial: 2:A,6

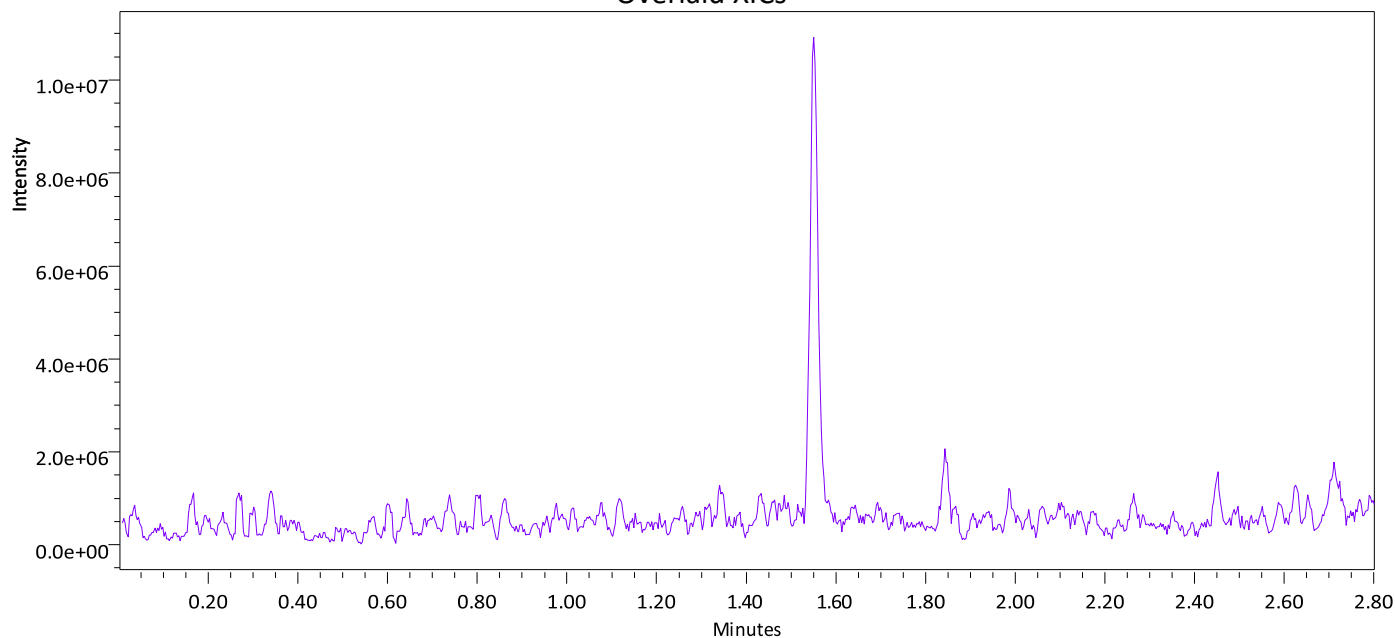
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 5:51:05 PM PDT

4/26/2024 5:54:10 PM PDT

Overlaid XICs



m/z 784.40

4/26/2024 5:54:47 PM PDT

4/26/2024 5:57:52 PM PDT

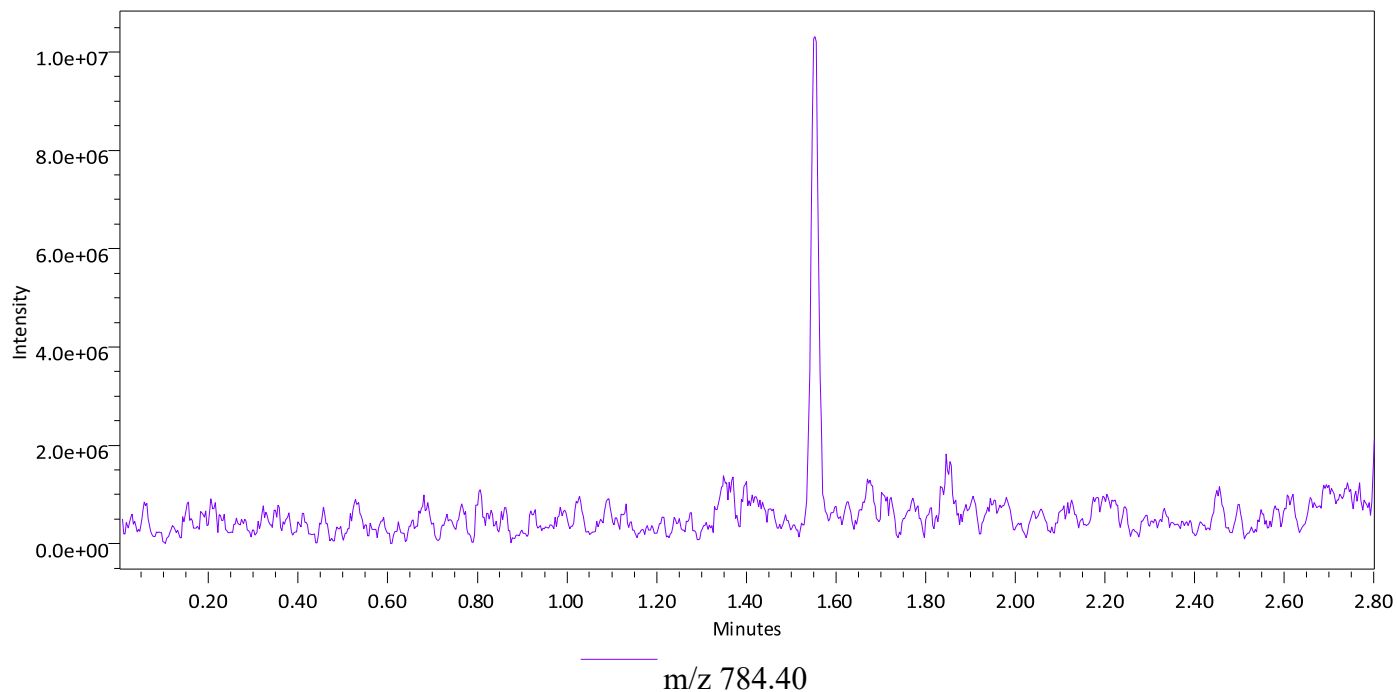
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A6
Vial: 2:A,6

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 5:58:28 PM PDT
4/26/2024 6:01:33 PM PDT

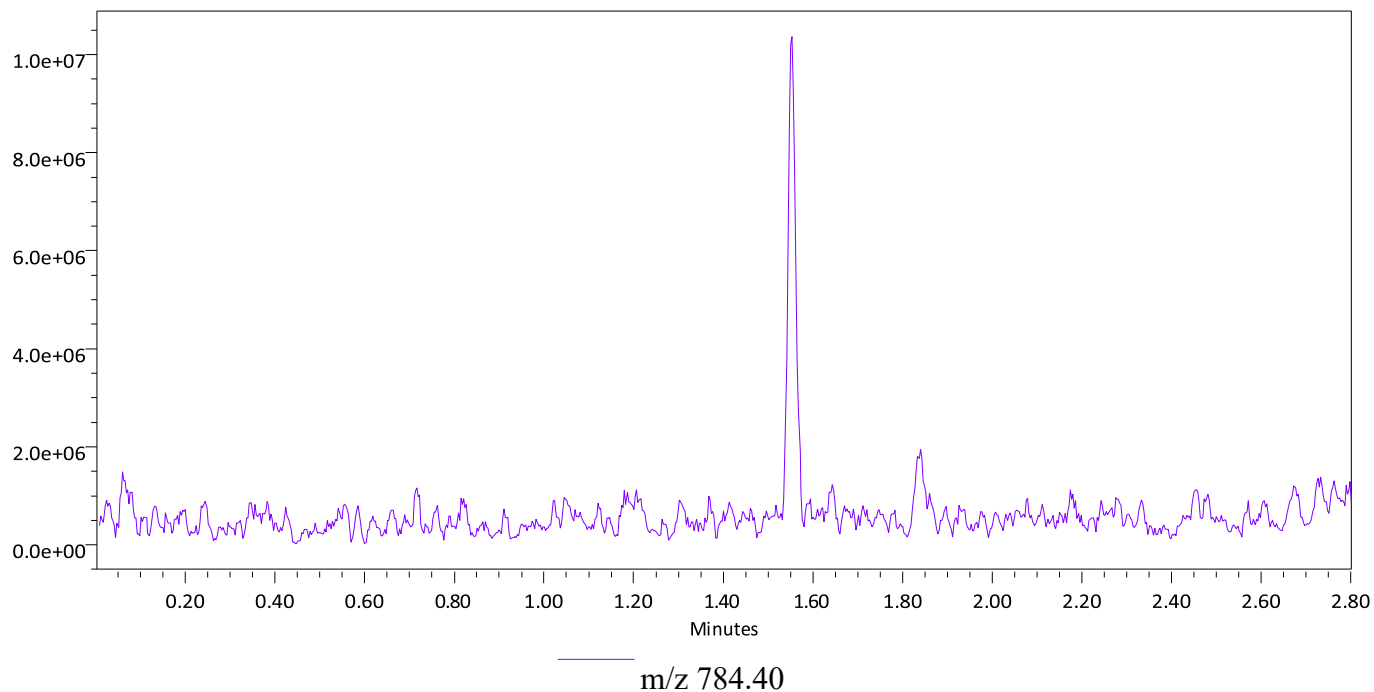
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A6
Vial: 2:A,6

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

TARGET MASS ANALYSIS

Sample Name: A9
Vial: 2:A,9

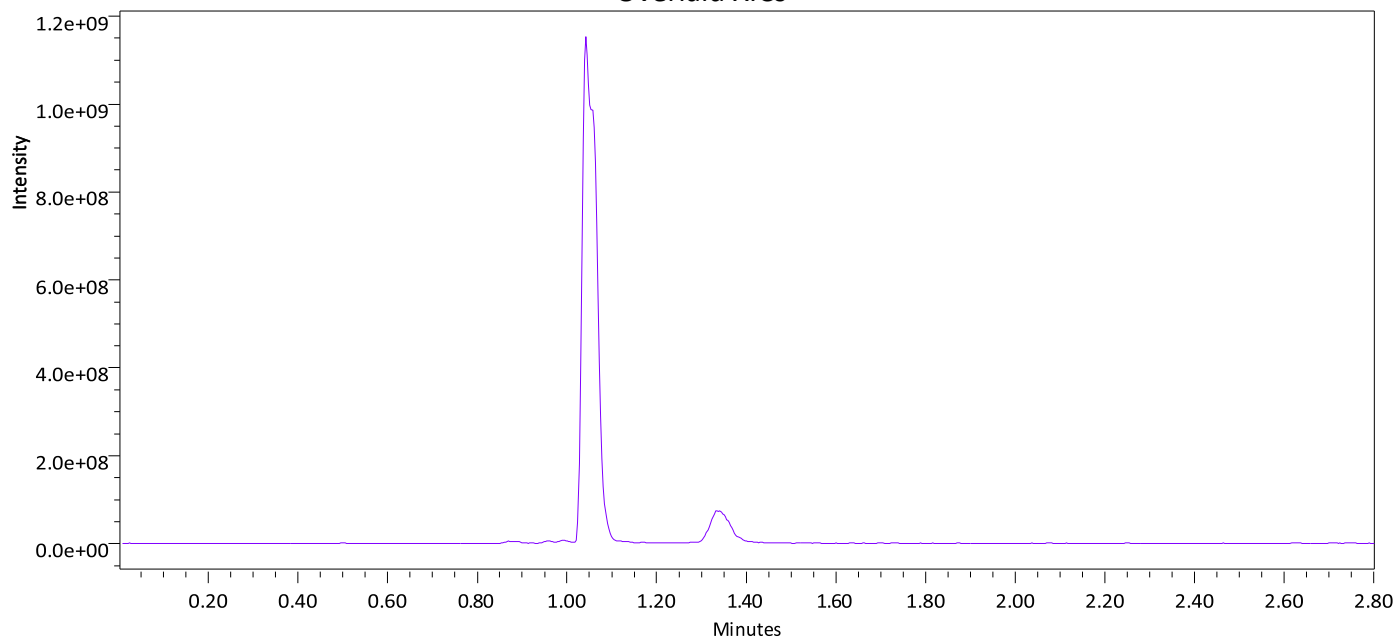
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 6:24:27 PM PDT

4/26/2024 6:27:32 PM PDT

Overlaid XICs



m/z 740.40

4/26/2024 6:28:09 PM PDT

4/26/2024 6:31:14 PM PDT

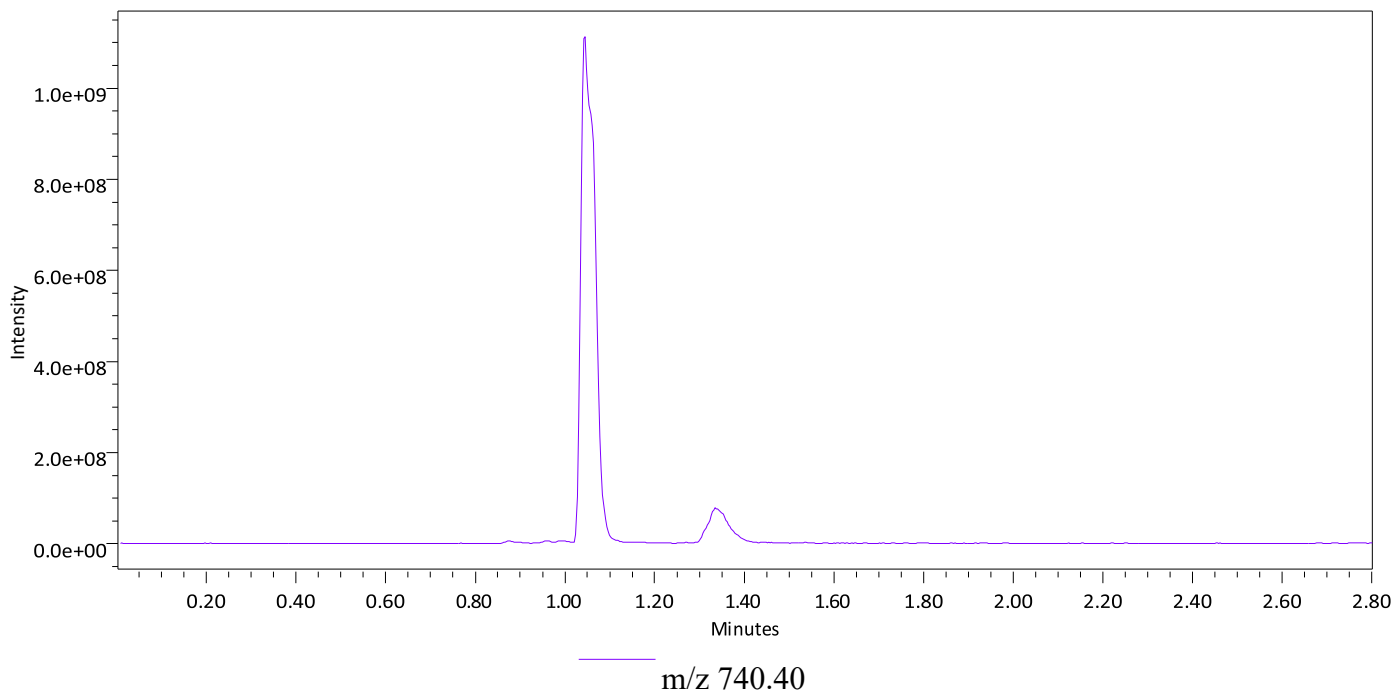
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A9
Vial: 2:A,9

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 6:31:50 PM PDT
4/26/2024 6:34:59 PM PDT

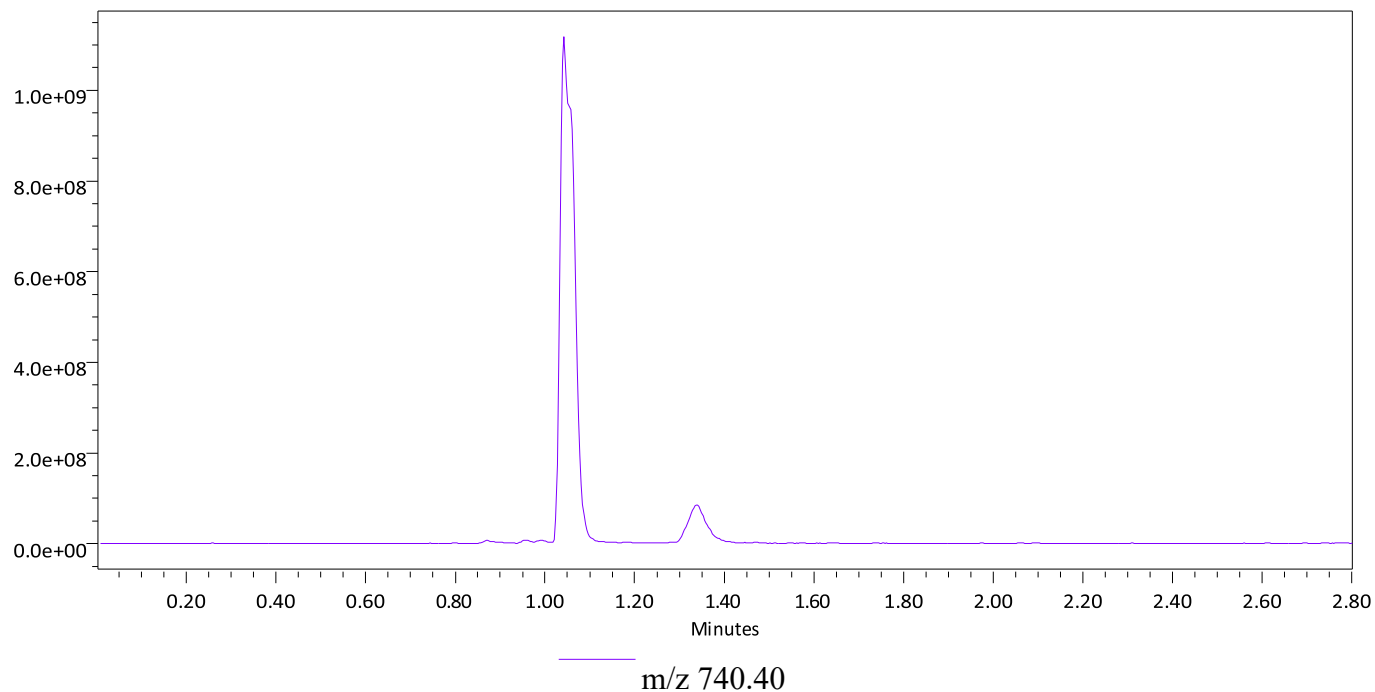
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A9
Vial: 2:A,9

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: A10
Vial: 2:A,10

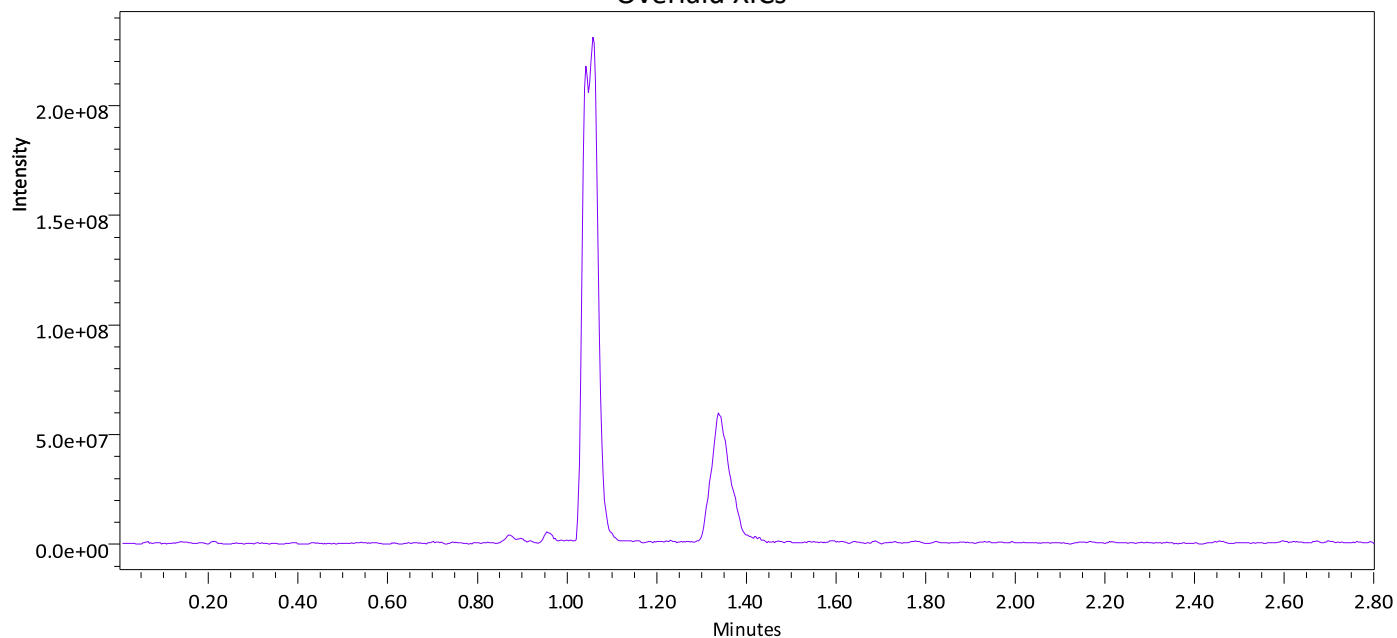
Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 6:35:36 PM PDT

4/26/2024 6:38:42 PM PDT

Overlaid XICs



m/z 740.40

4/26/2024 6:39:19 PM PDT

4/26/2024 6:42:25 PM PDT

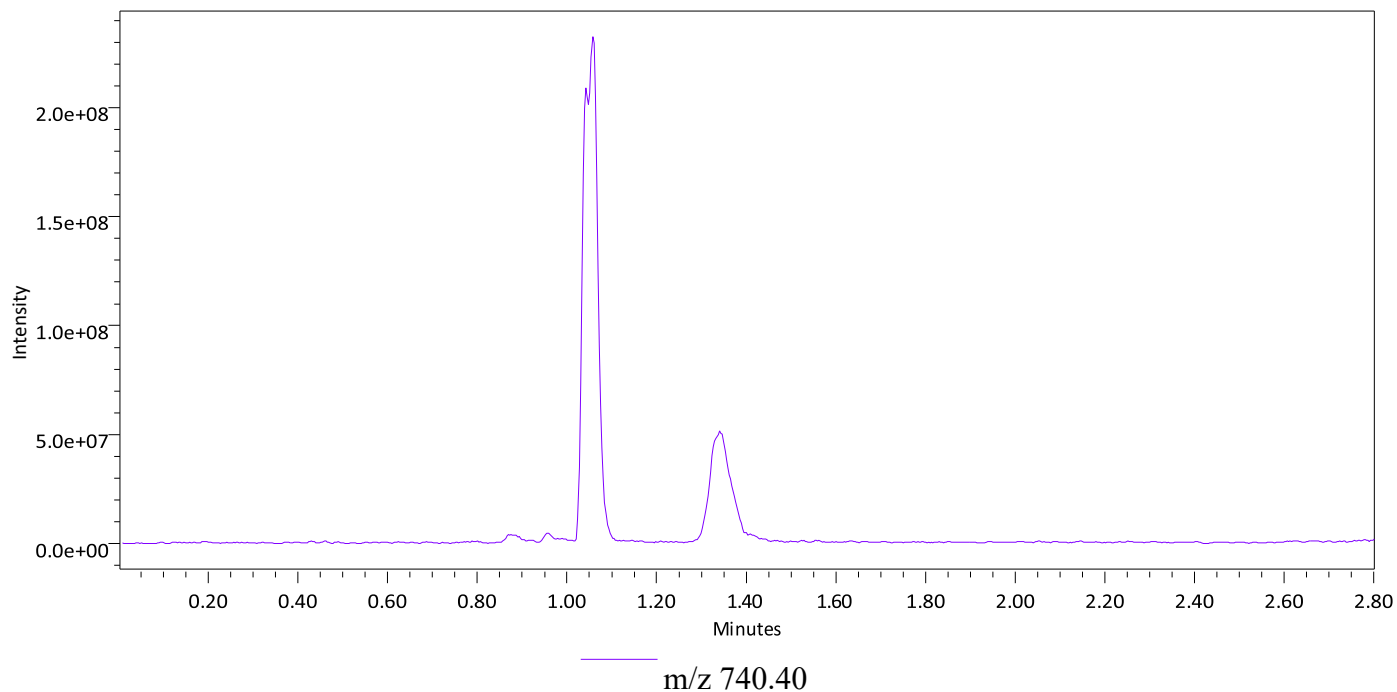
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A10
Vial: 2:A,10

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 6:43:01 PM PDT
4/26/2024 6:46:09 PM PDT

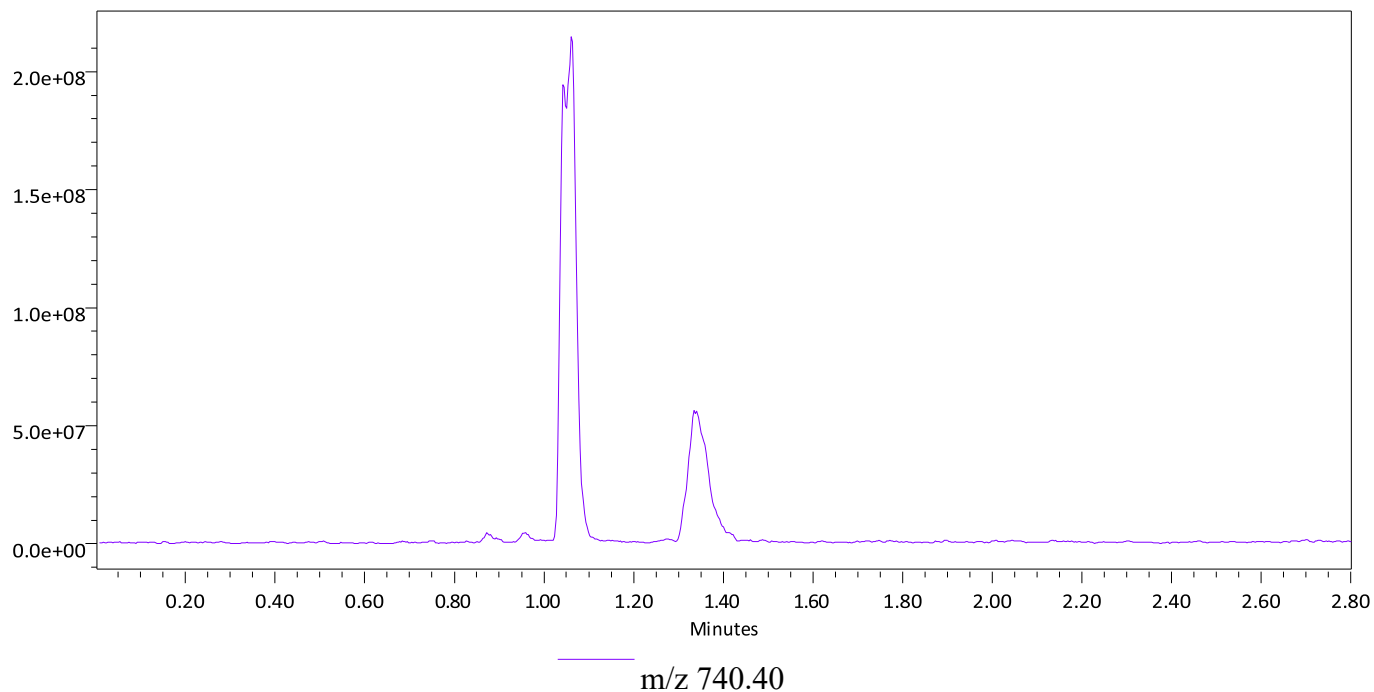
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A10
Vial: 2:A,10

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: A11
Vial: 2:A,11

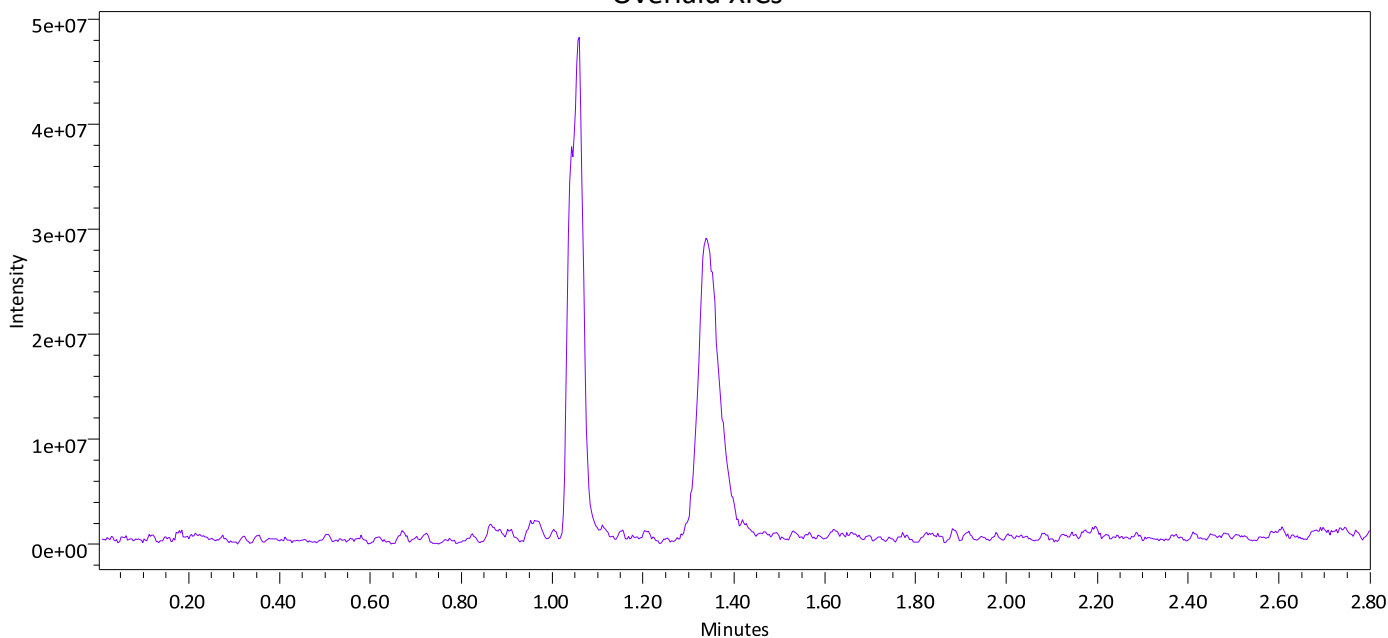
Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 6:46:45 PM PDT

4/26/2024 6:49:51 PM PDT

Overlaid XICs



m/z 740.40

4/26/2024 6:50:28 PM PDT

4/26/2024 6:53:34 PM PDT

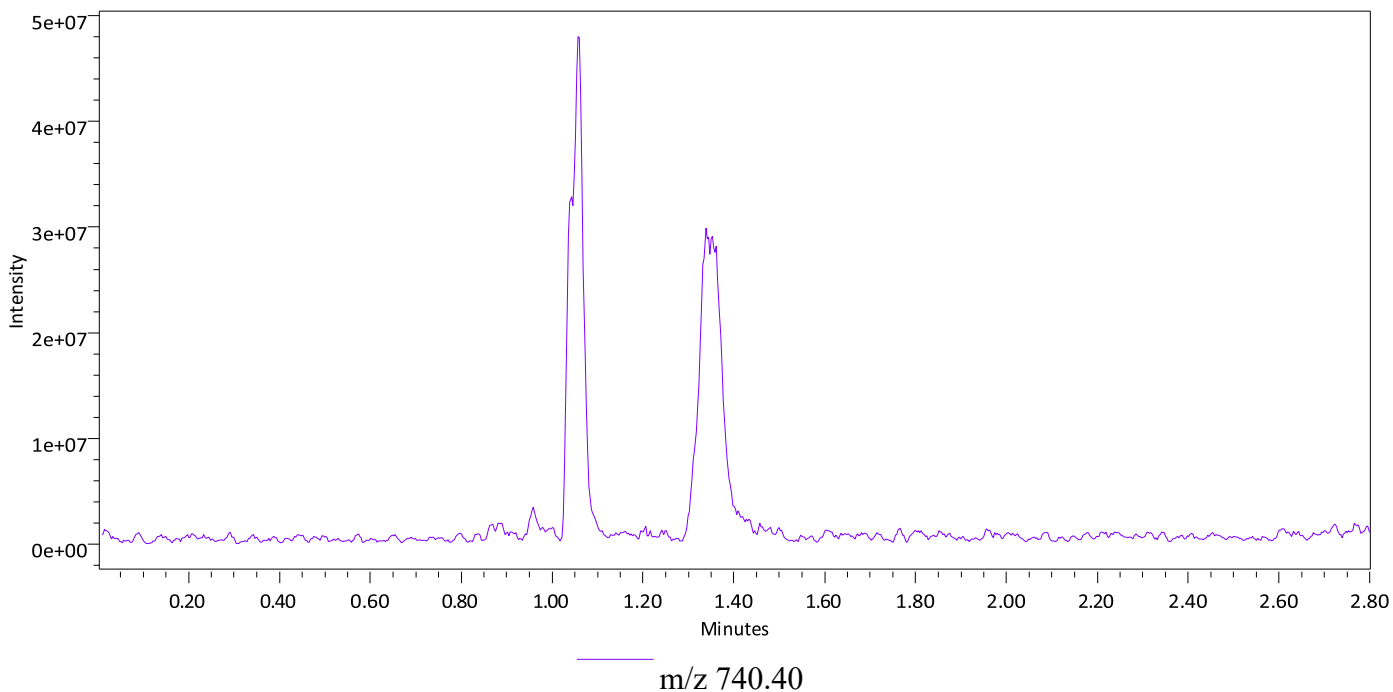
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A11
Vial: 2:A,11

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 6:54:11 PM PDT
4/26/2024 6:57:20 PM PDT

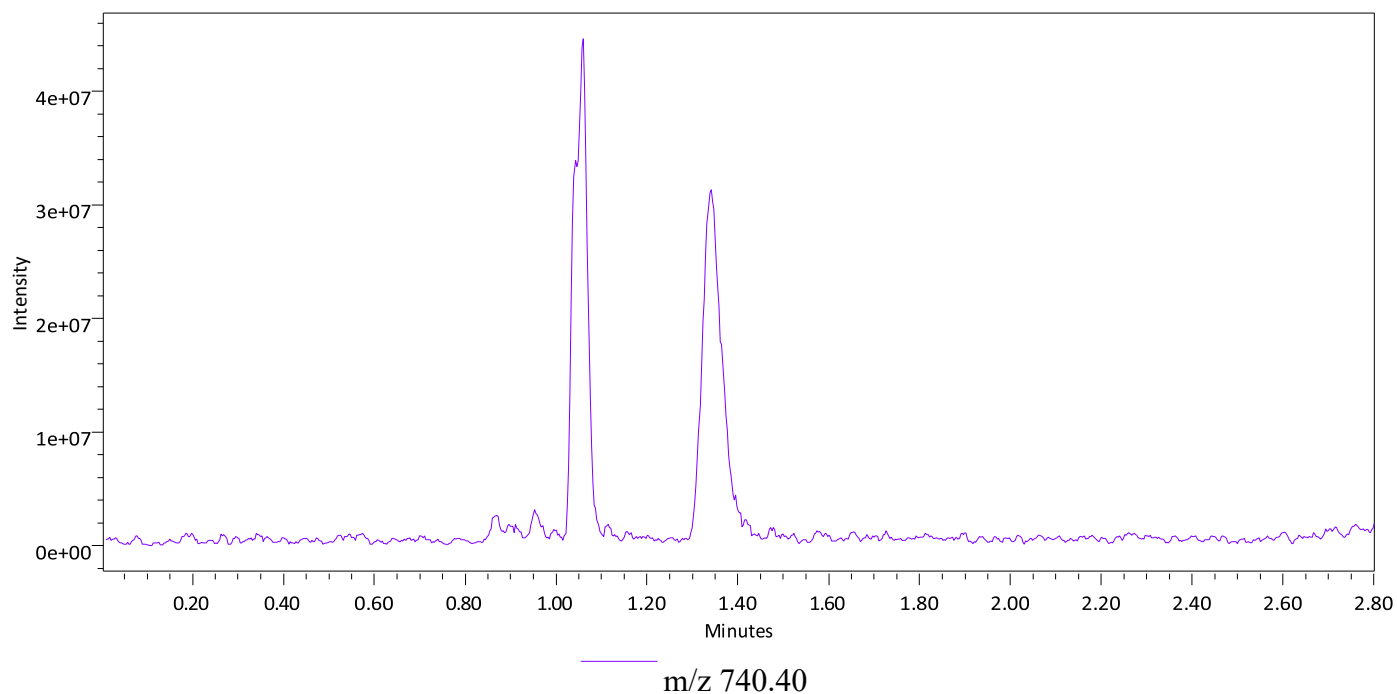
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A11
Vial: 2:A,11

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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TARGET MASS ANALYSIS

Sample Name: A12
Vial: 2:A,12

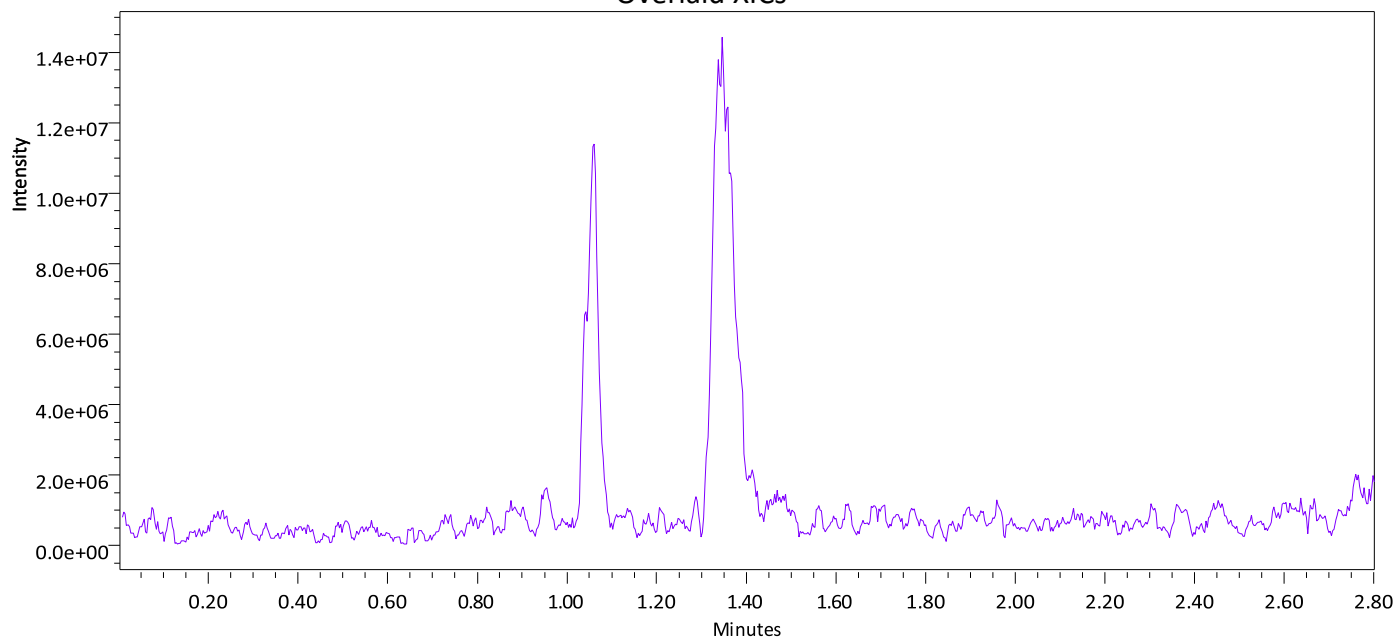
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 6:57:56 PM PDT

4/26/2024 7:01:03 PM PDT

Overlaid XICs



m/z 740.40

4/26/2024 7:01:39 PM PDT

4/26/2024 7:04:49 PM PDT

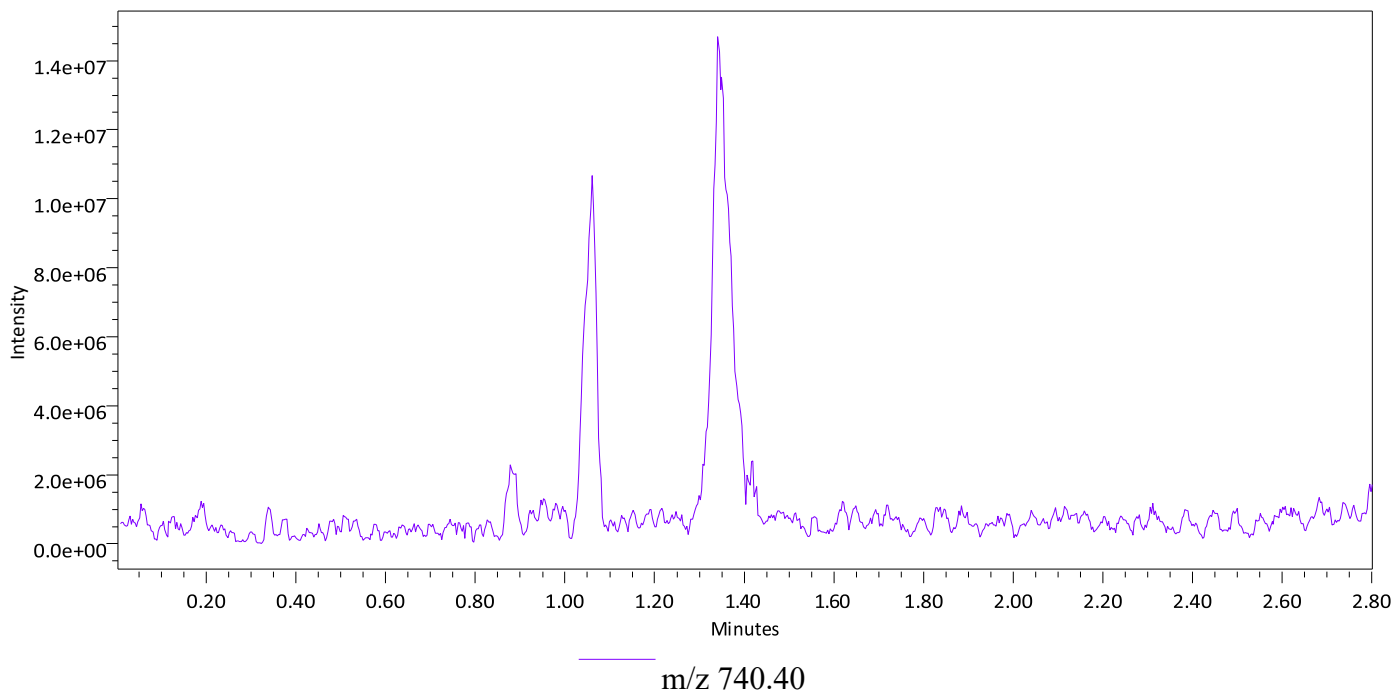
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A12
Vial: 2:A,12

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 7:05:26 PM PDT
4/26/2024 7:08:32 PM PDT

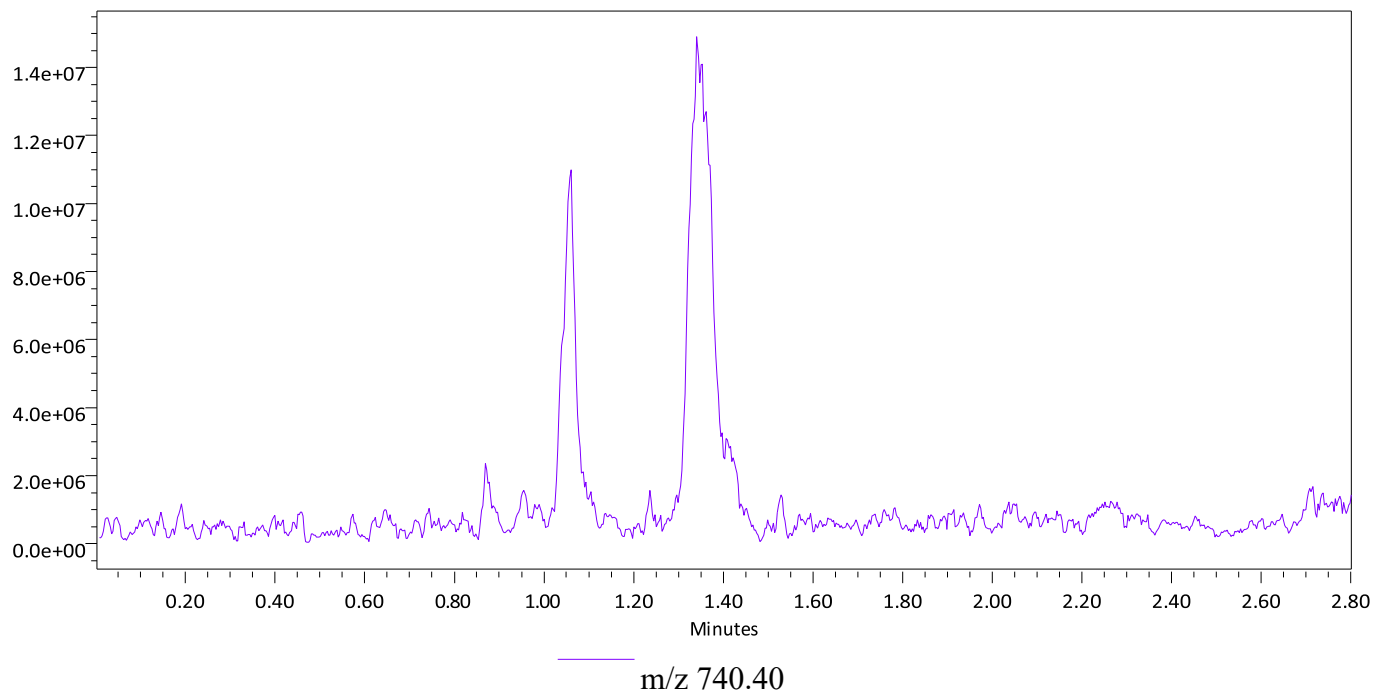
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: A12
Vial: 2:A,12

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: B5
Vial: 2:B,5

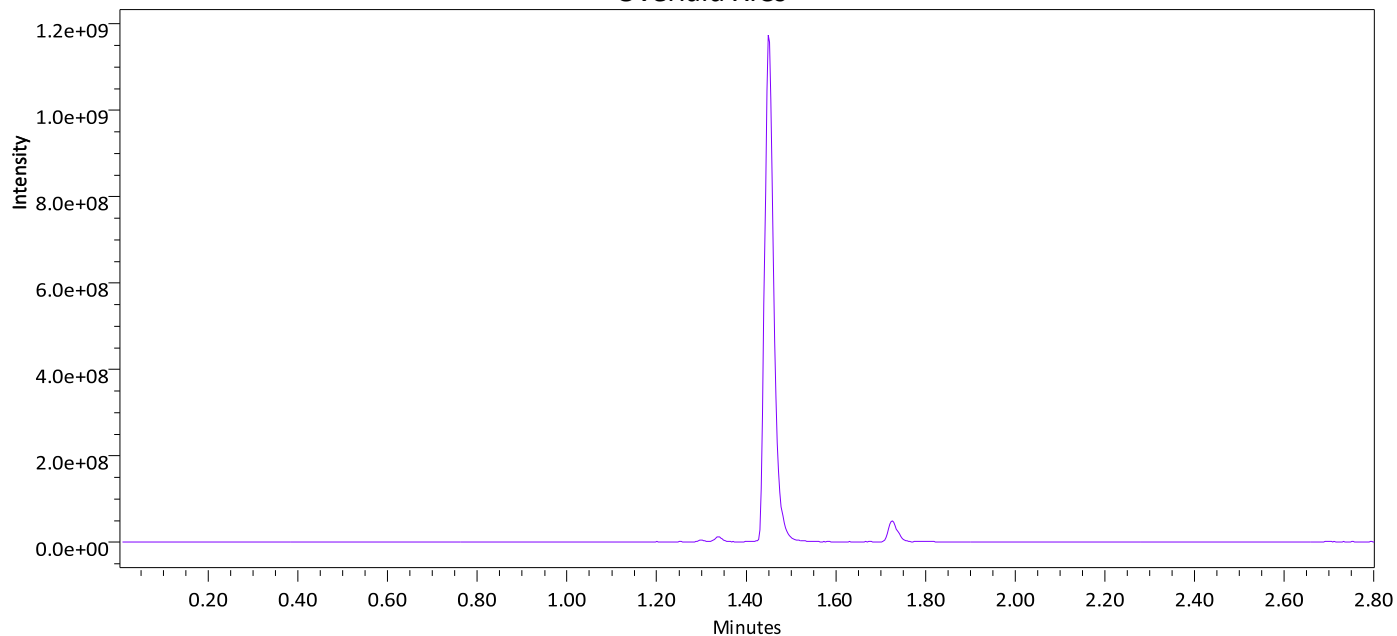
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 7:53:25 PM PDT

4/26/2024 7:56:32 PM PDT

Overlaid XICs



m/z 804.40

4/26/2024 7:57:08 PM PDT

4/26/2024 8:00:15 PM PDT

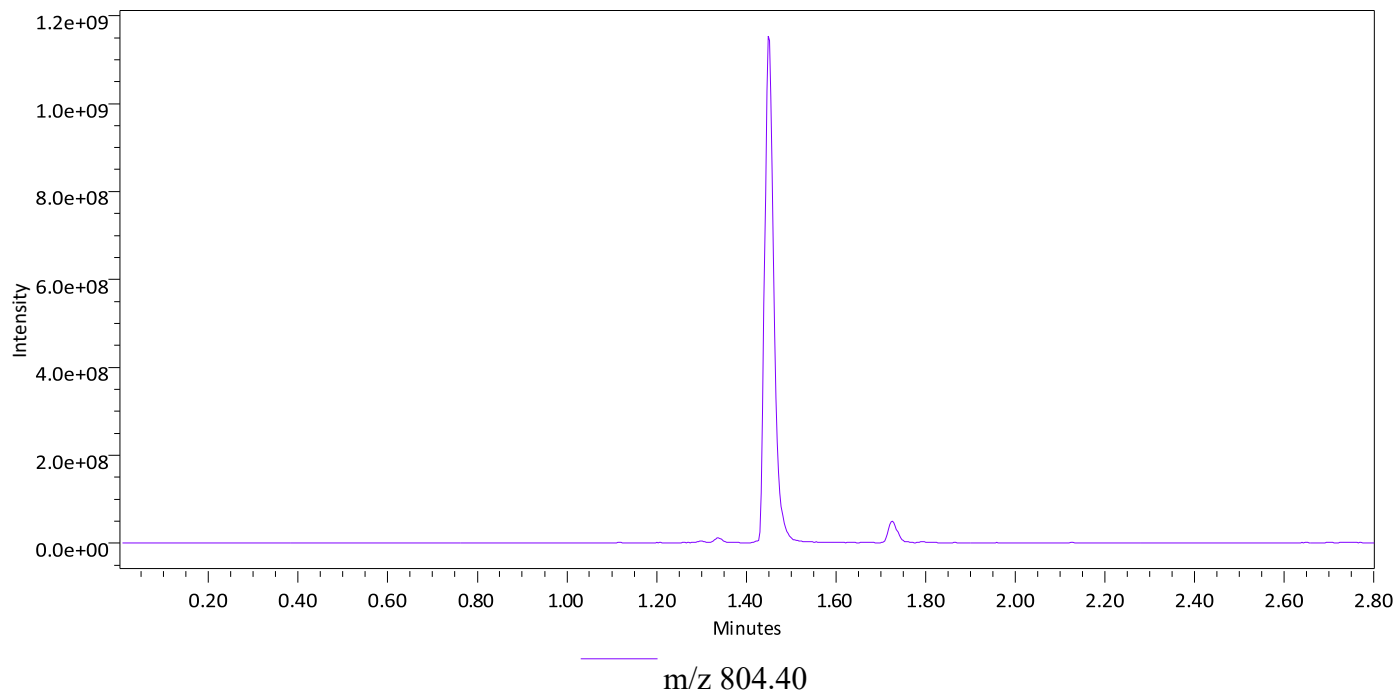
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B5
Vial: 2:B,5

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 8:00:52 PM PDT
4/26/2024 8:04:00 PM PDT

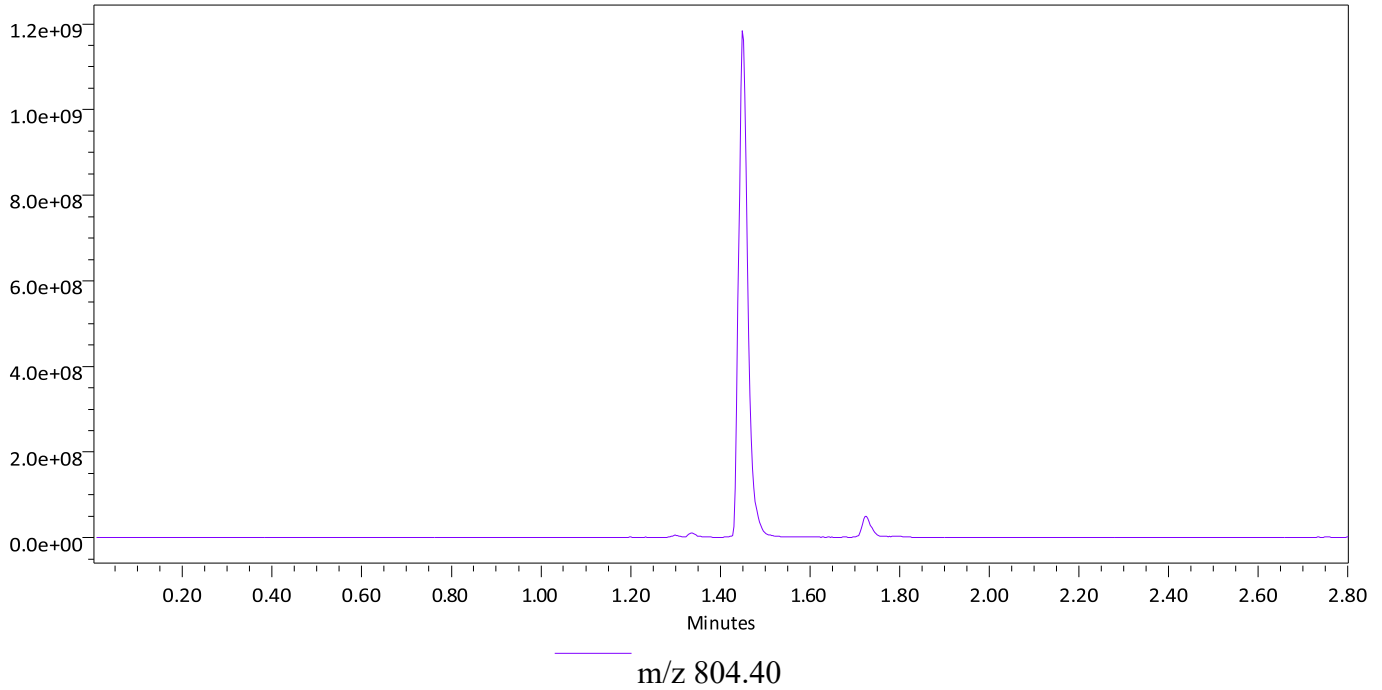
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B5
Vial: 2:B,5

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: B6
Vial: 2:B,6

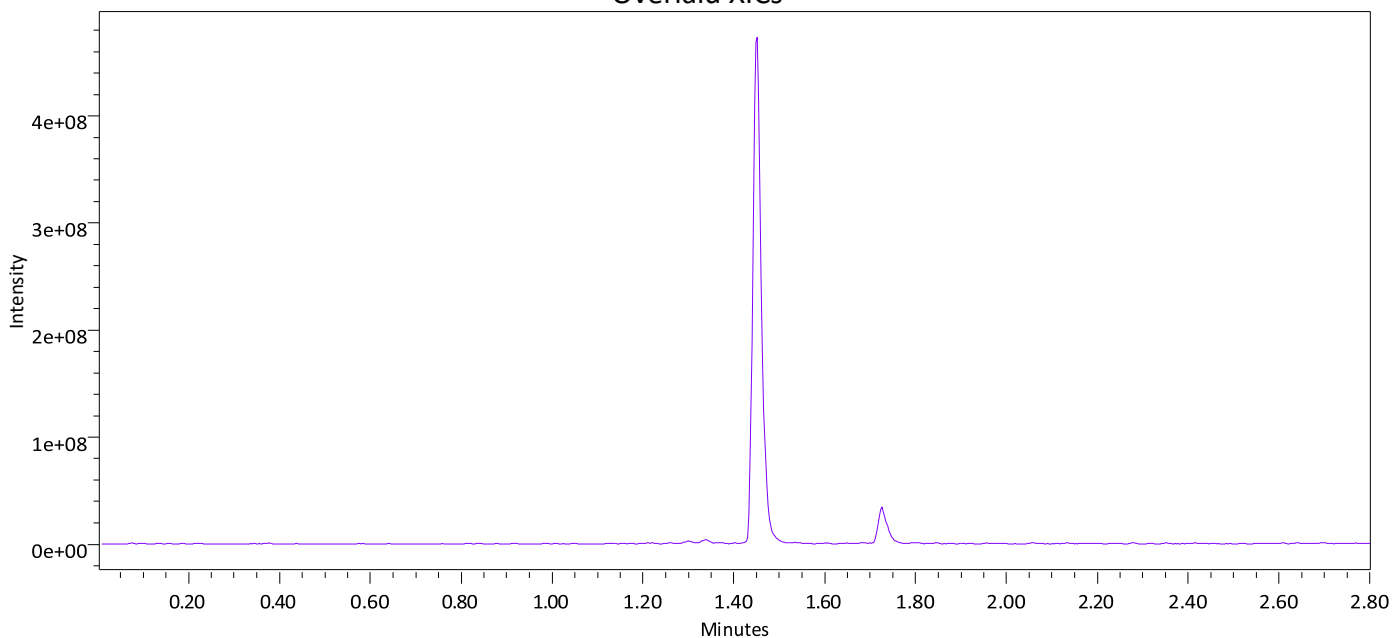
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 8:04:37 PM PDT

4/26/2024 8:07:46 PM PDT

Overlaid XICs



m/z 804.40

4/26/2024 8:08:22 PM PDT

4/26/2024 8:11:28 PM PDT

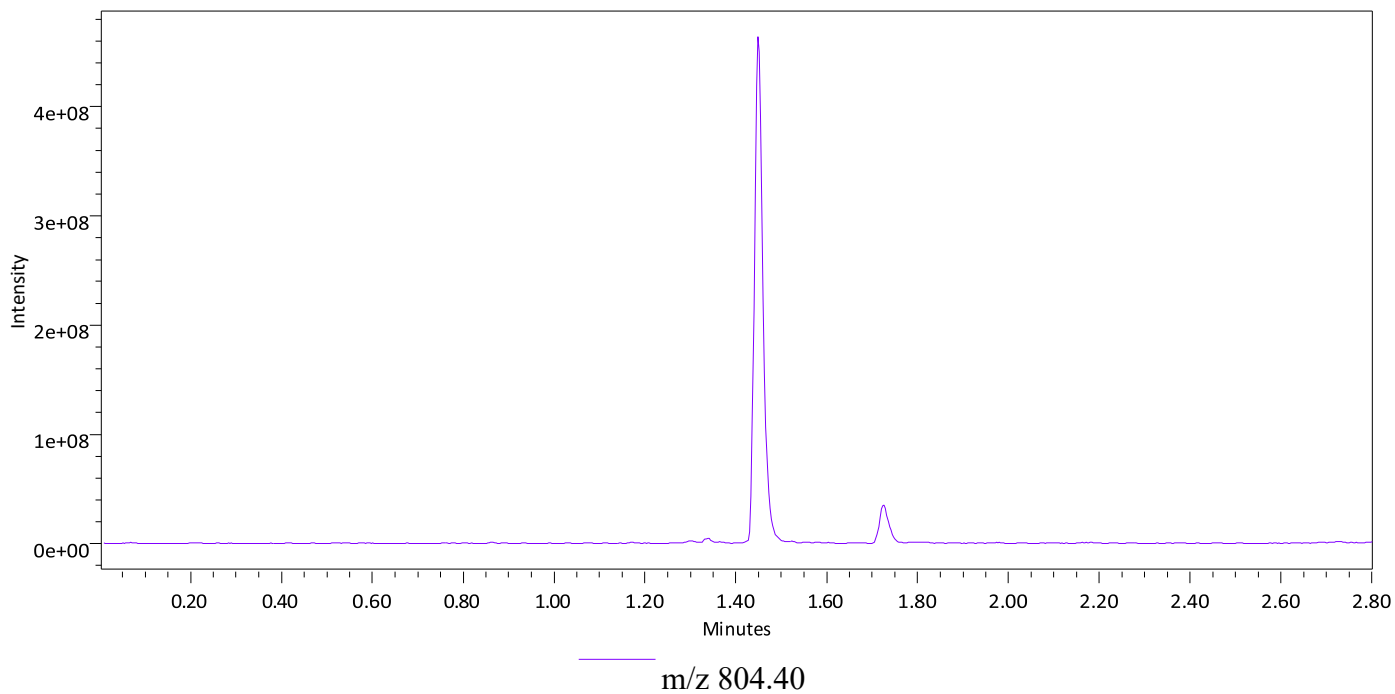
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B6
Vial: 2:B,6

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 8:12:04 PM PDT
4/26/2024 8:15:10 PM PDT

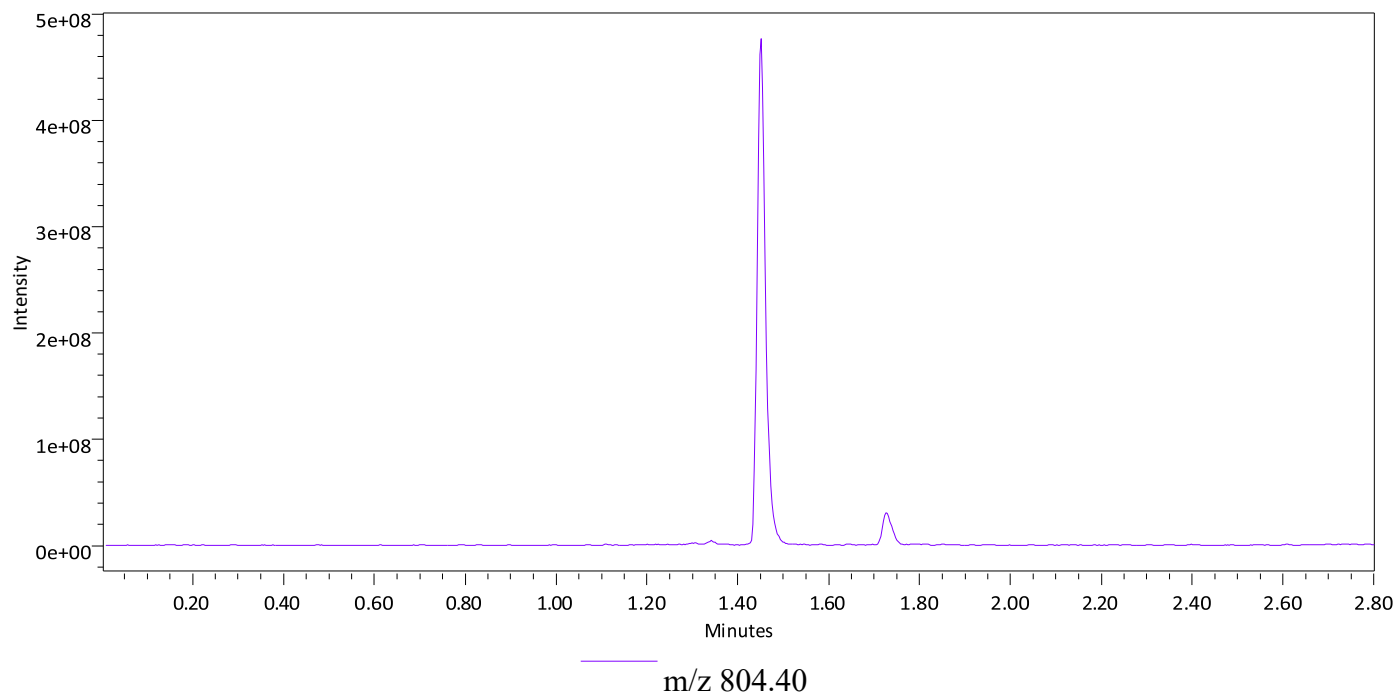
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B6
Vial: 2:B,6

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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TARGET MASS ANALYSIS

Sample Name: B7
Vial: 2:B,7

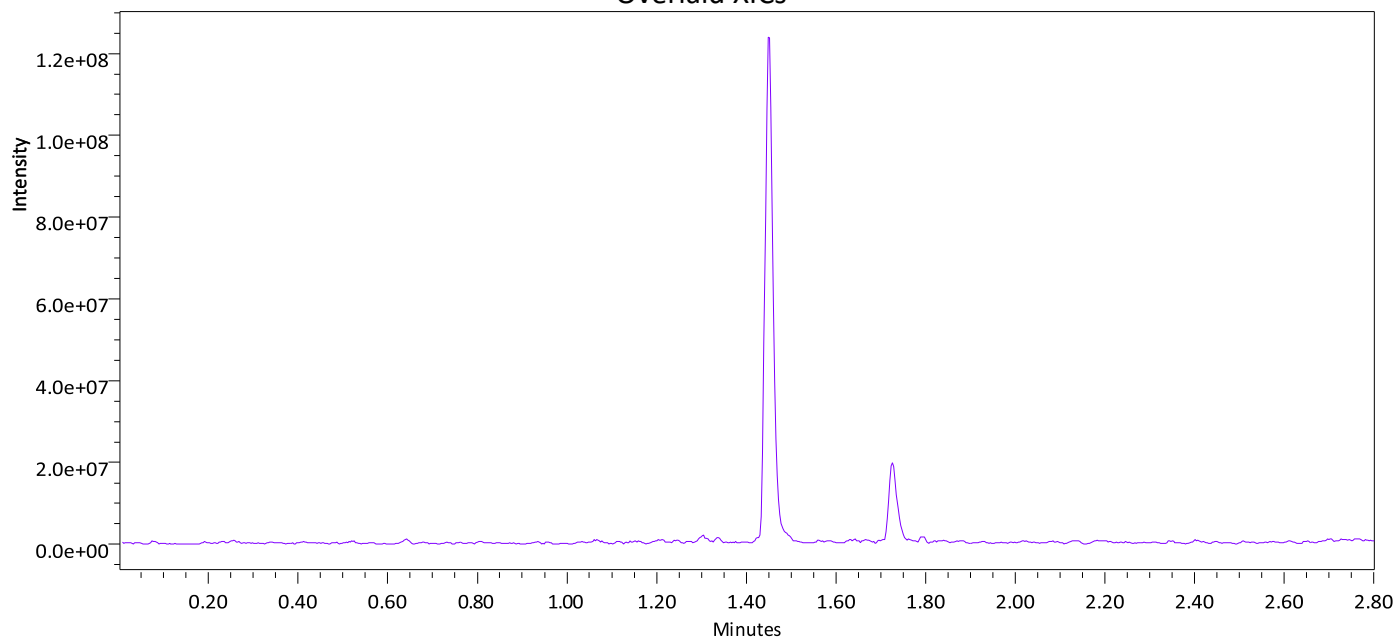
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 8:15:47 PM PDT

4/26/2024 8:18:55 PM PDT

Overlaid XICs



m/z 804.40

4/26/2024 8:19:31 PM PDT

4/26/2024 8:22:36 PM PDT

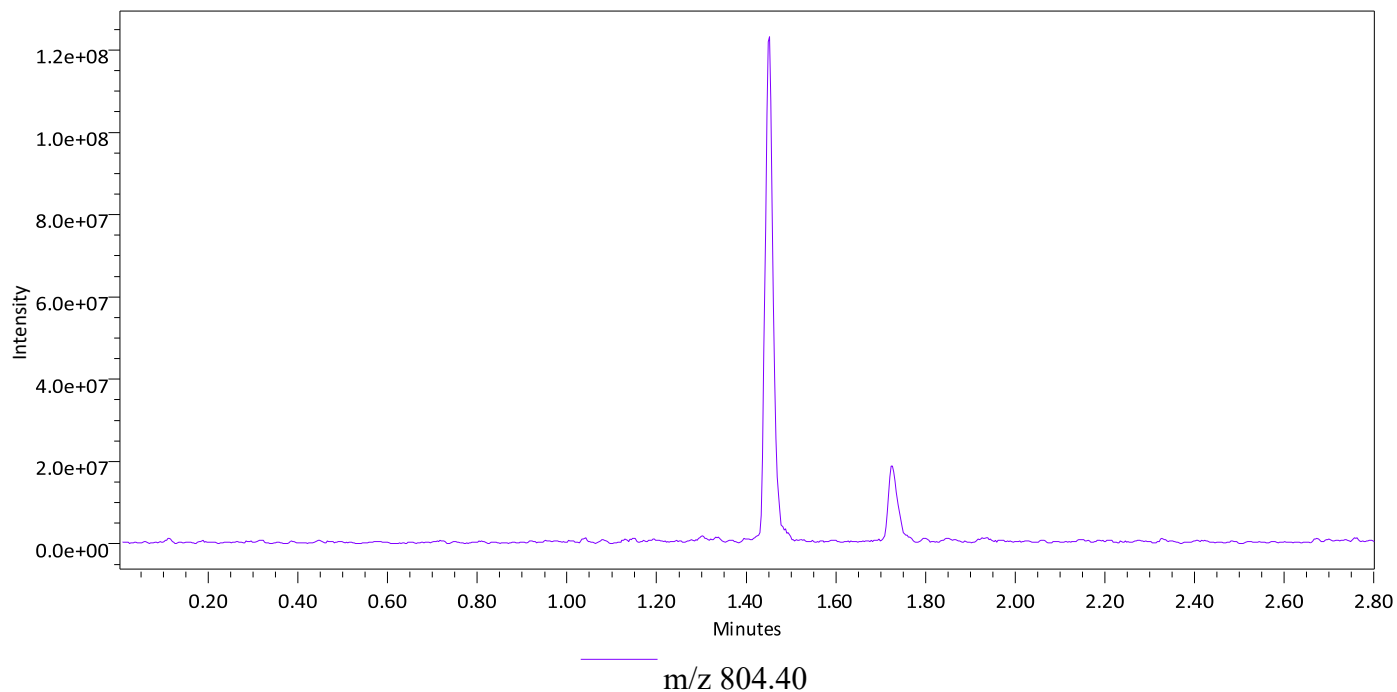
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B7
Vial: 2:B,7

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 8:23:11 PM PDT
4/26/2024 8:26:19 PM PDT

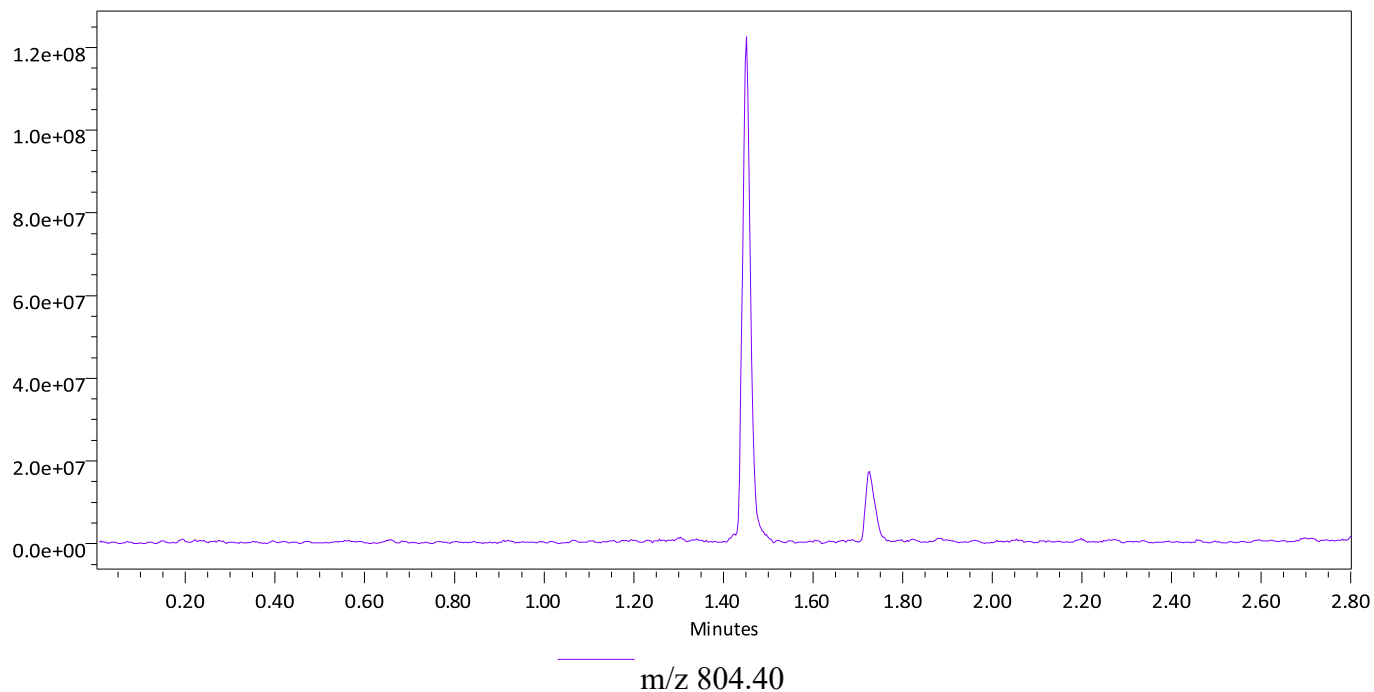
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B7
Vial: 2:B,7

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: B8
Vial: 2:B,8

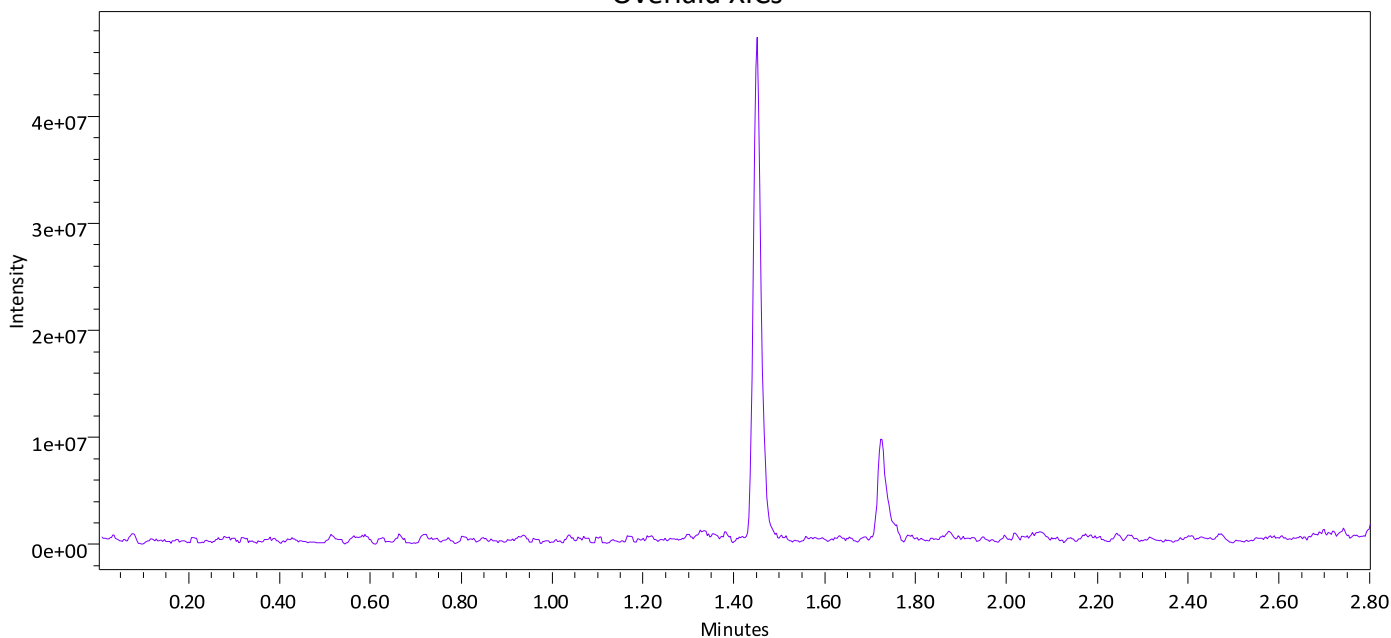
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 8:26:55 PM PDT

4/26/2024 8:30:02 PM PDT

Overlaid XICs



m/z 804.40

4/26/2024 8:30:39 PM PDT

4/26/2024 8:33:46 PM PDT

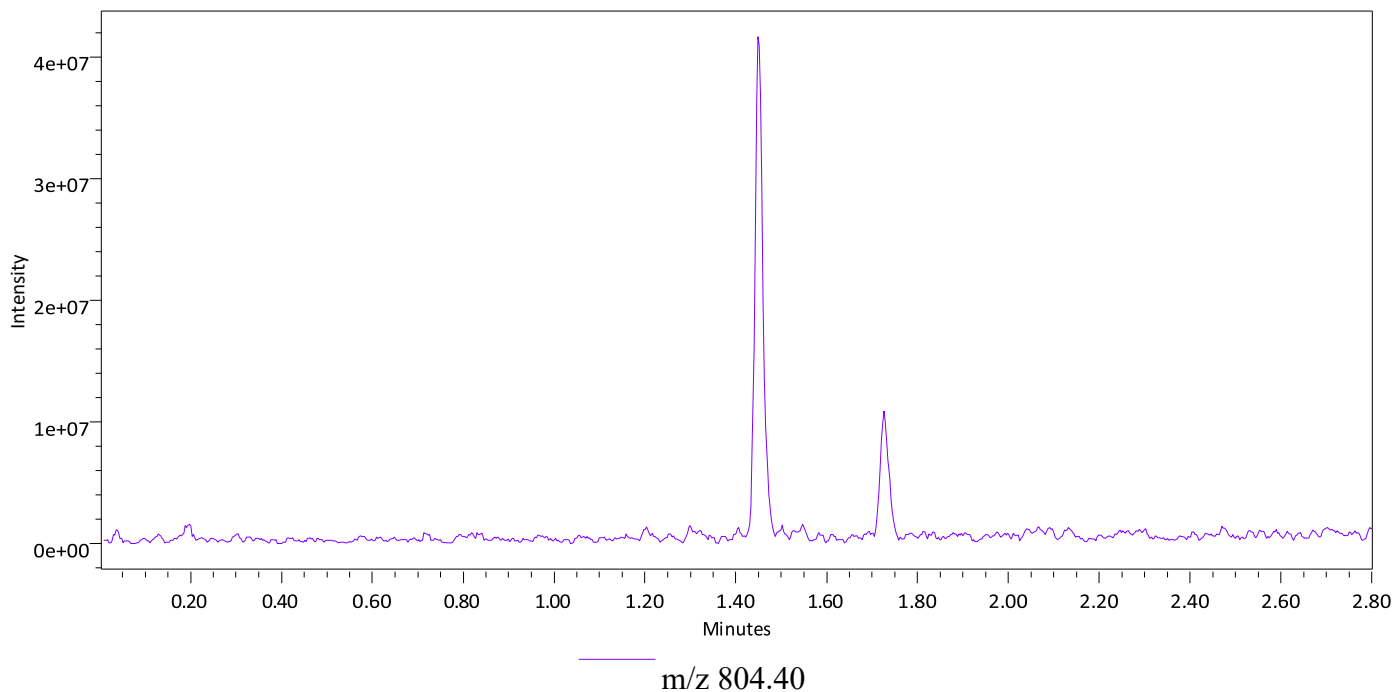
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B8
Vial: 2:B,8

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 8:34:23 PM PDT
4/26/2024 8:37:31 PM PDT

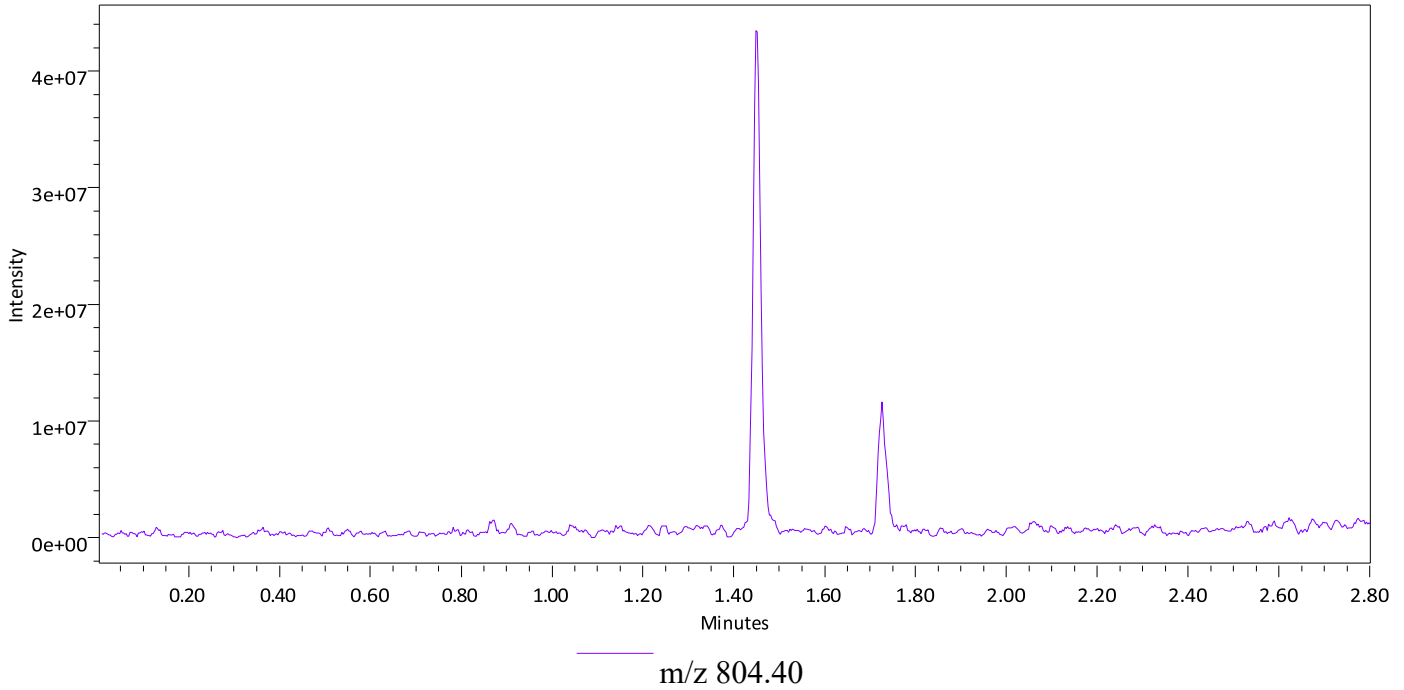
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B8
Vial: 2:B,8

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: B9
Vial: 2:B,9

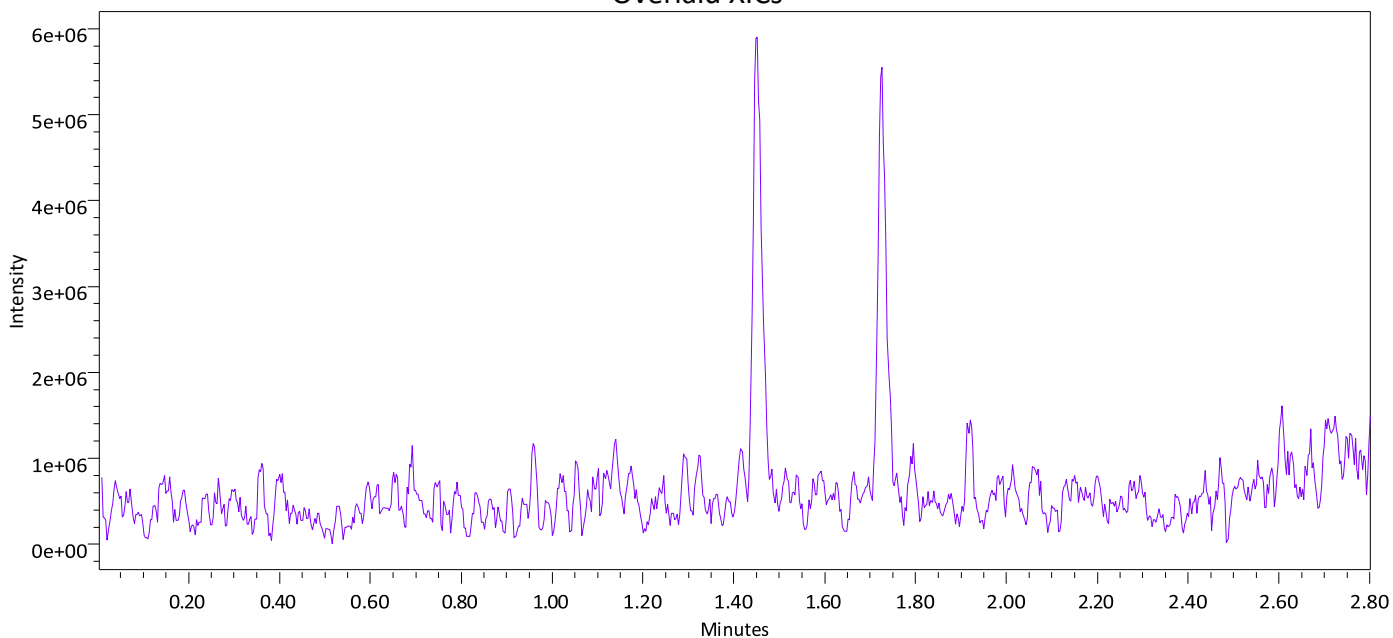
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 8:38:08 PM PDT

4/26/2024 8:41:16 PM PDT

Overlaid XICs



m/z 804.40

4/26/2024 8:41:50 PM PDT

4/26/2024 8:44:58 PM PDT

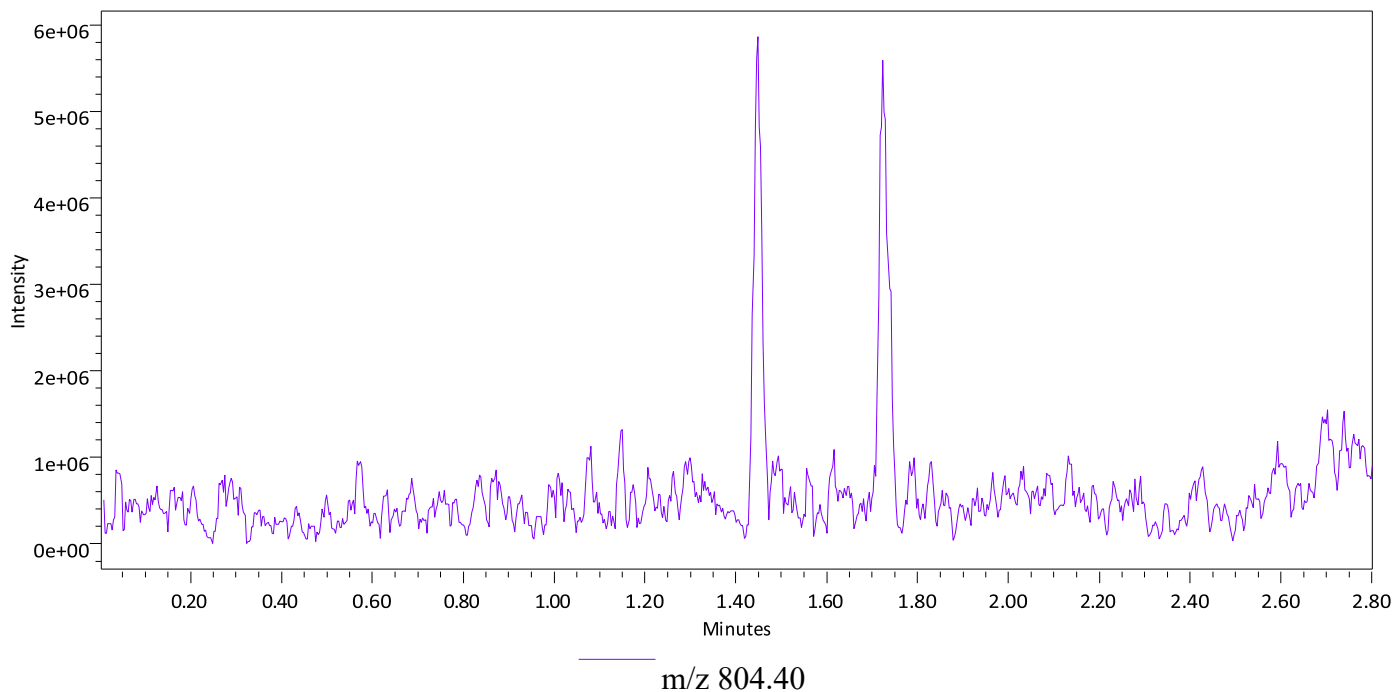
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B9
Vial: 2:B,9

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 8:45:36 PM PDT
4/26/2024 8:48:42 PM PDT

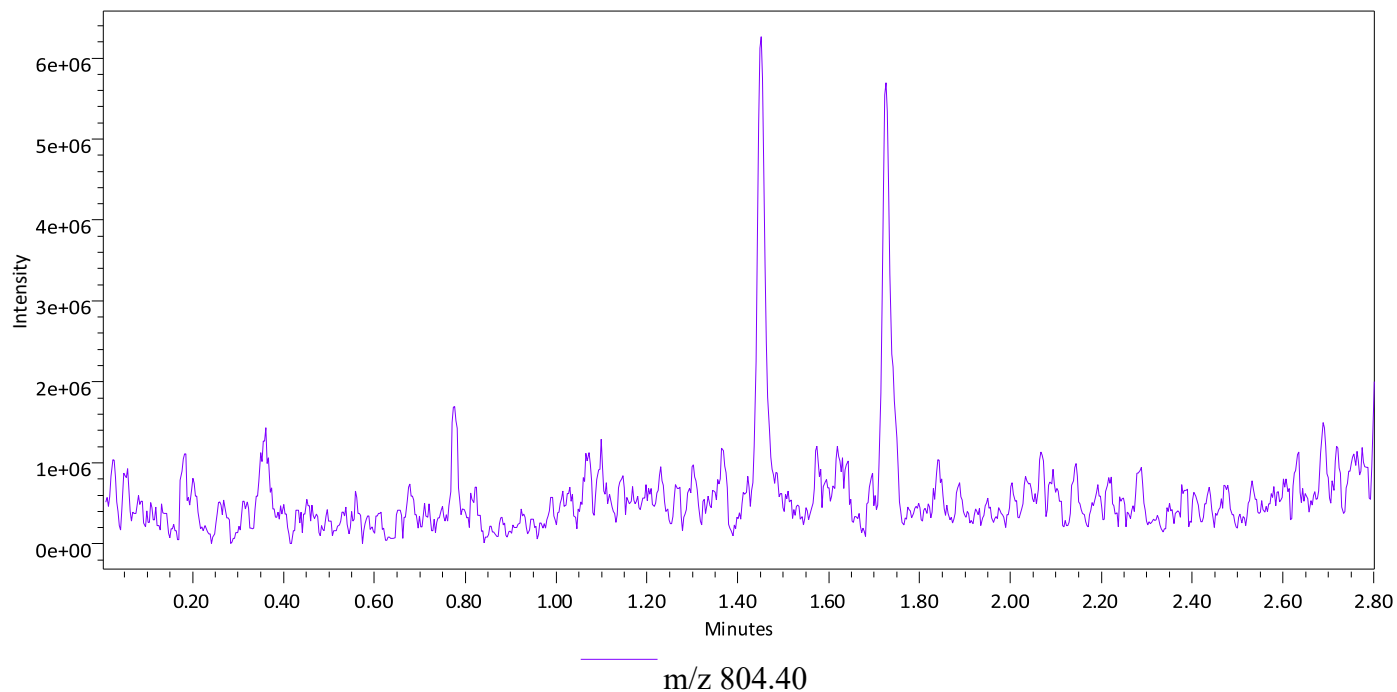
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: B9
Vial: 2:B,9

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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TARGET MASS ANALYSIS

Sample Name: C1
Vial: 2:C,1

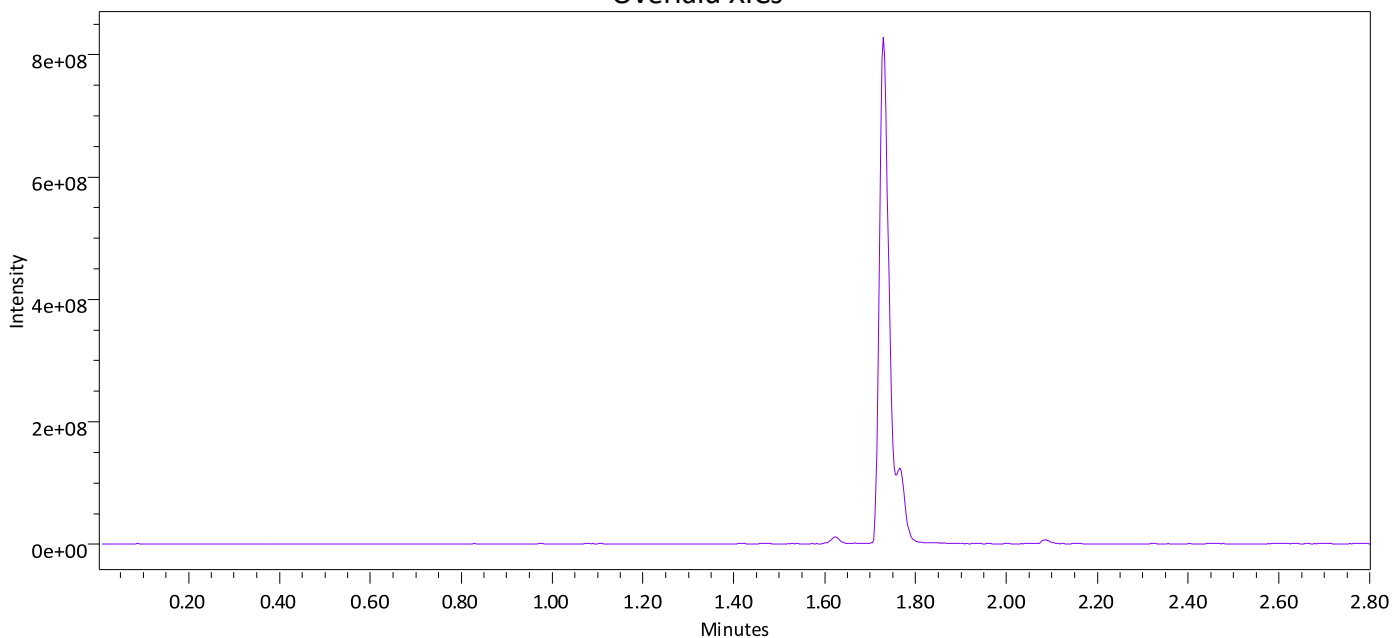
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 9:22:48 PM PDT

4/26/2024 9:25:54 PM PDT

Overlaid XICs



m/z 823.40

4/26/2024 9:26:29 PM PDT

4/26/2024 9:29:35 PM PDT

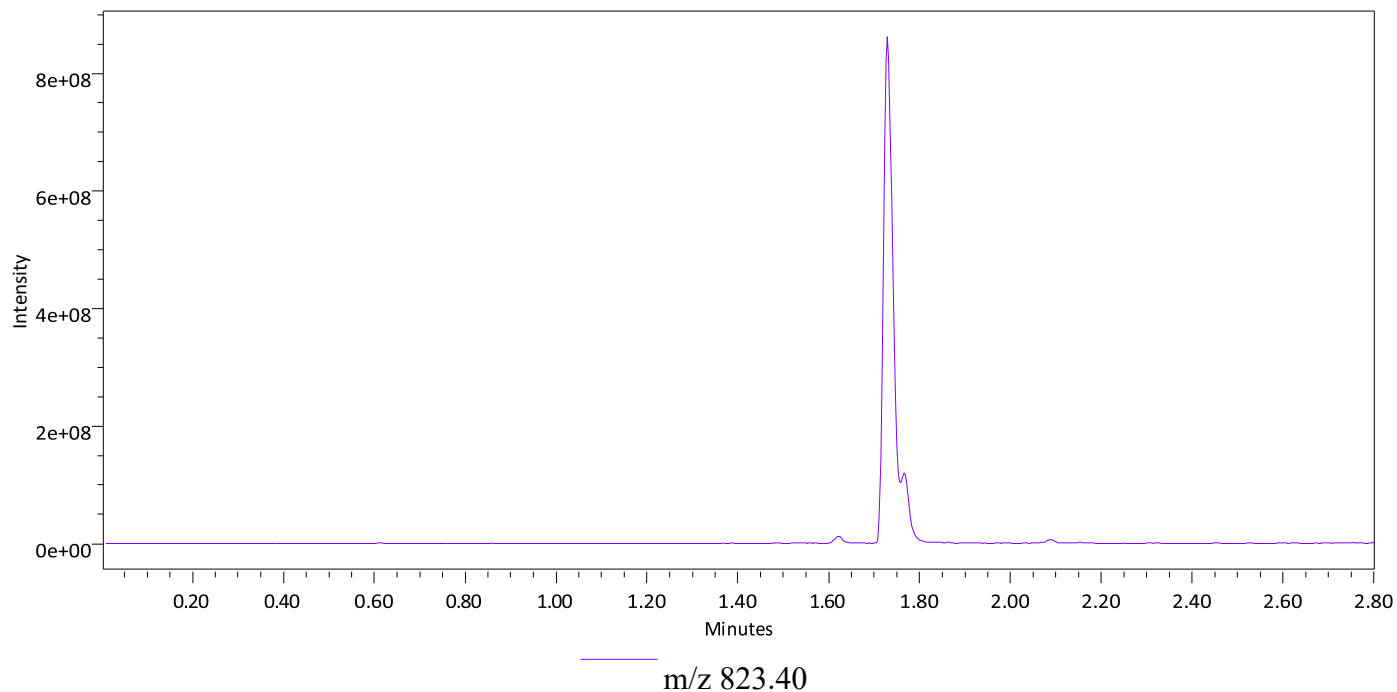
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C1
Vial: 2:C,1

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 9:30:10 PM PDT
4/26/2024 9:33:17 PM PDT

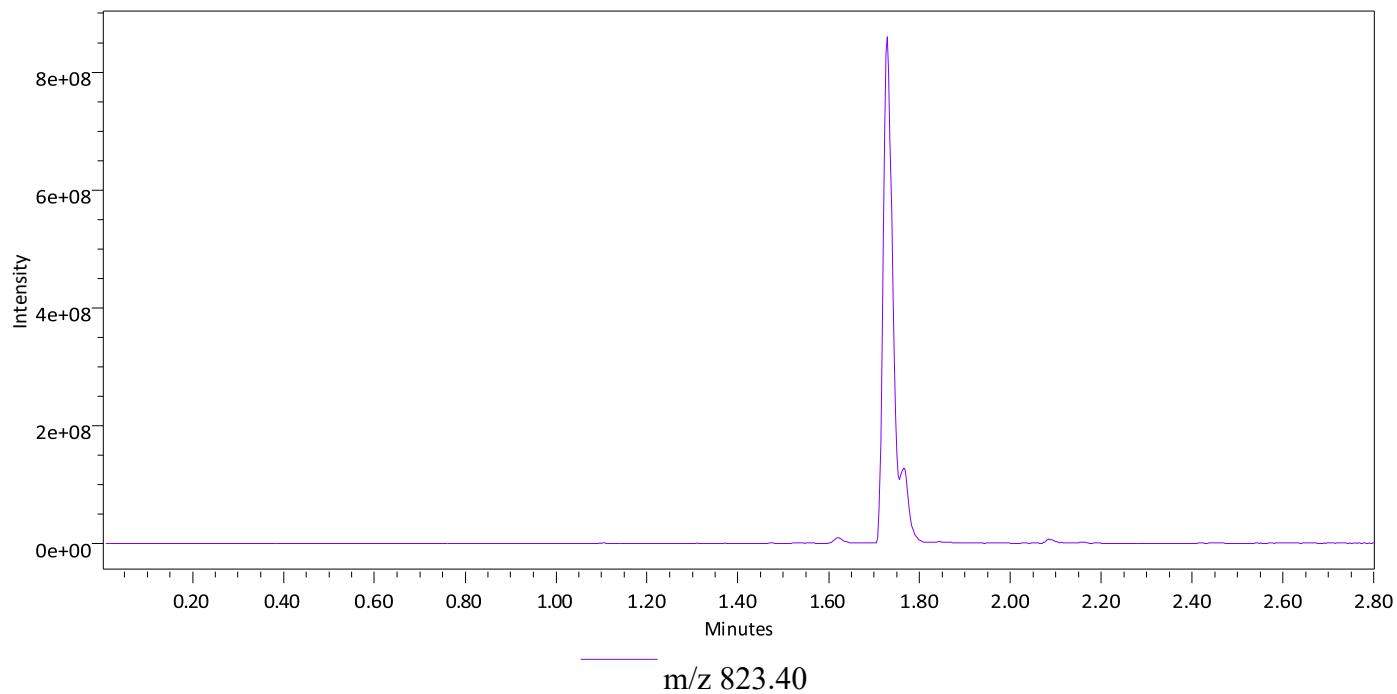
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C1
Vial: 2:C,1

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: C2
Vial: 2:C,2

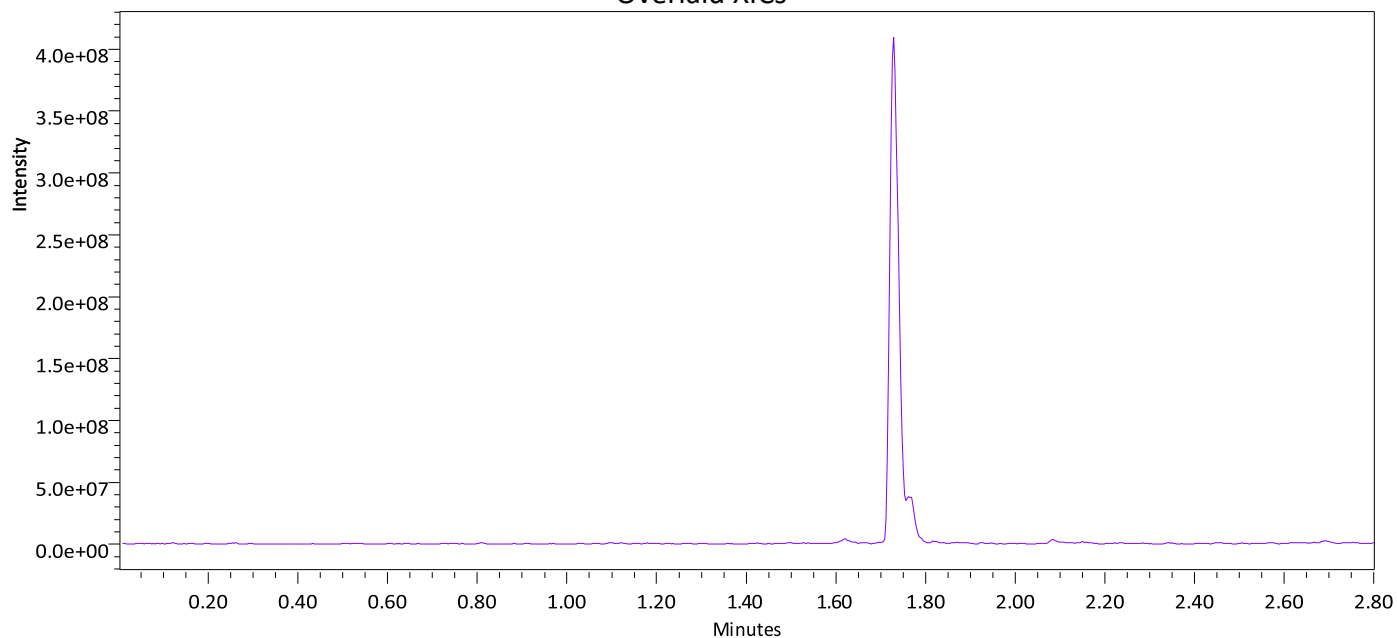
Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 9:33:53 PM PDT

4/26/2024 9:36:58 PM PDT

Overlaid XICs



m/z 823.40

4/26/2024 9:37:33 PM PDT

4/26/2024 9:40:41 PM PDT

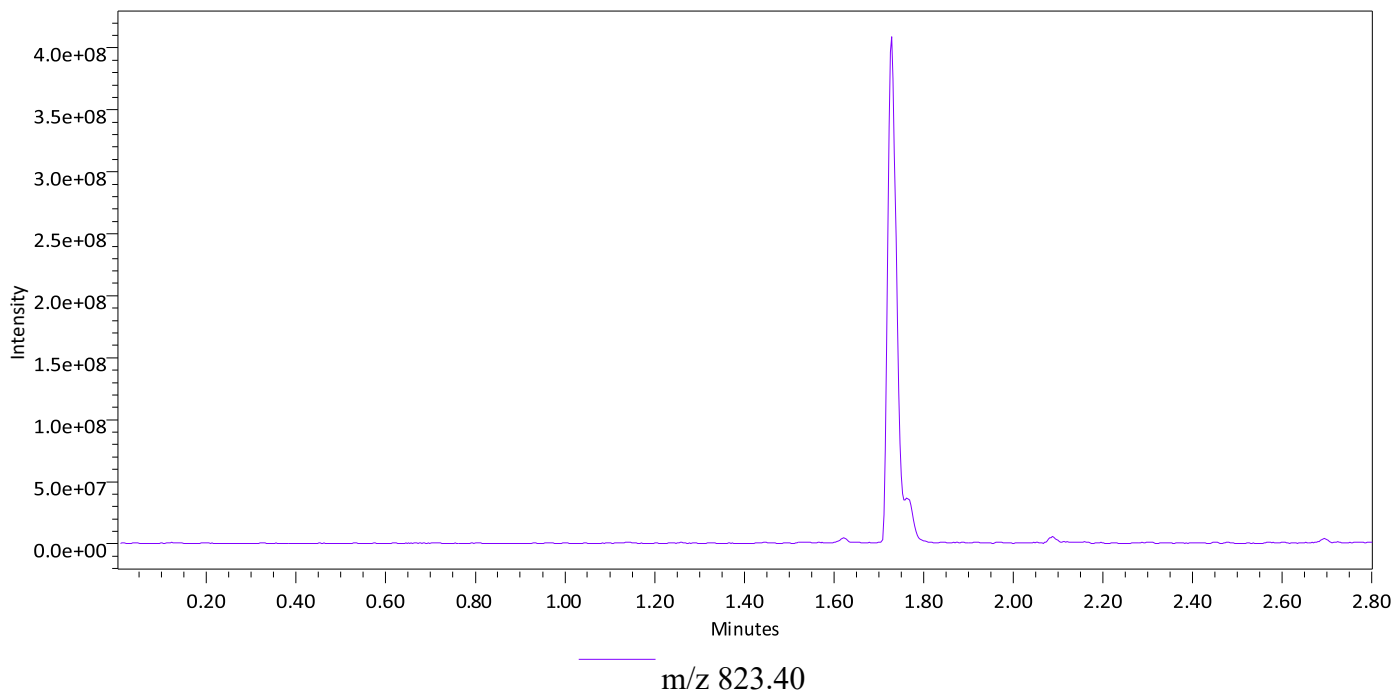
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C2
Vial: 2:C,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 9:41:17 PM PDT
4/26/2024 9:44:21 PM PDT

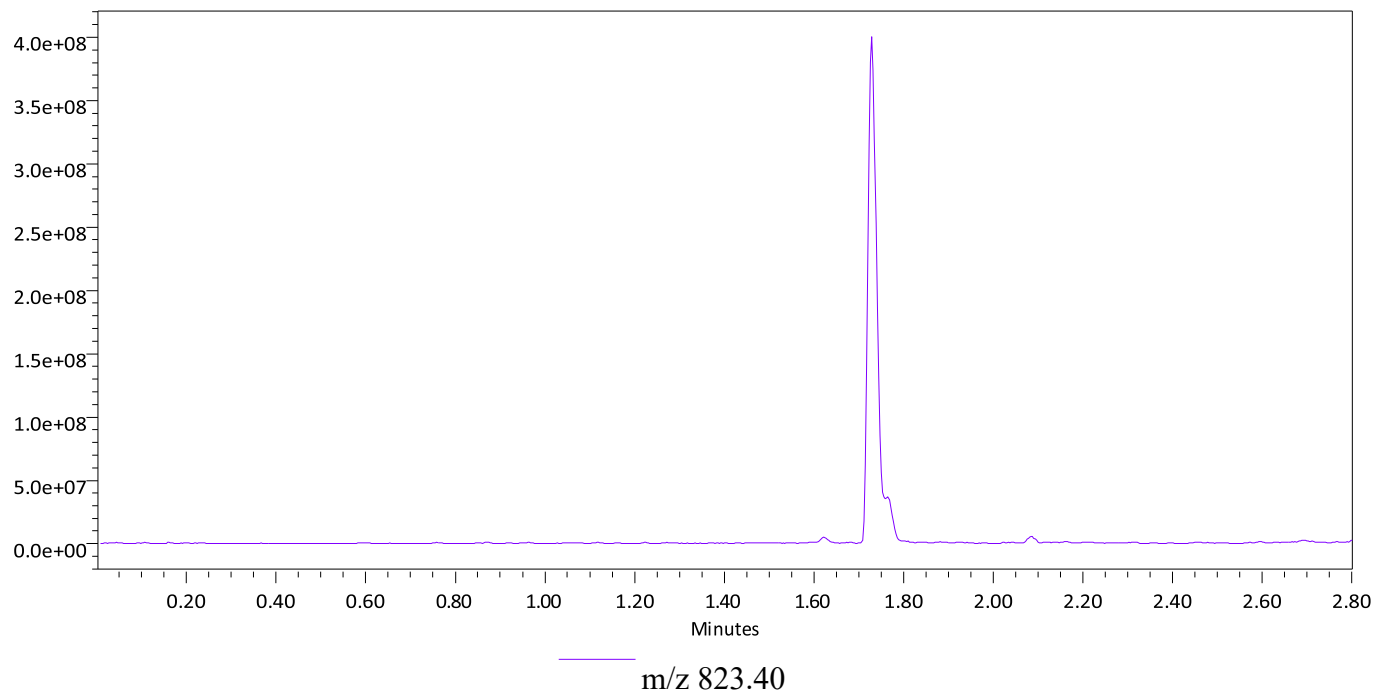
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C2
Vial: 2:C,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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TARGET MASS ANALYSIS

Sample Name: C3
Vial: 2:C,3

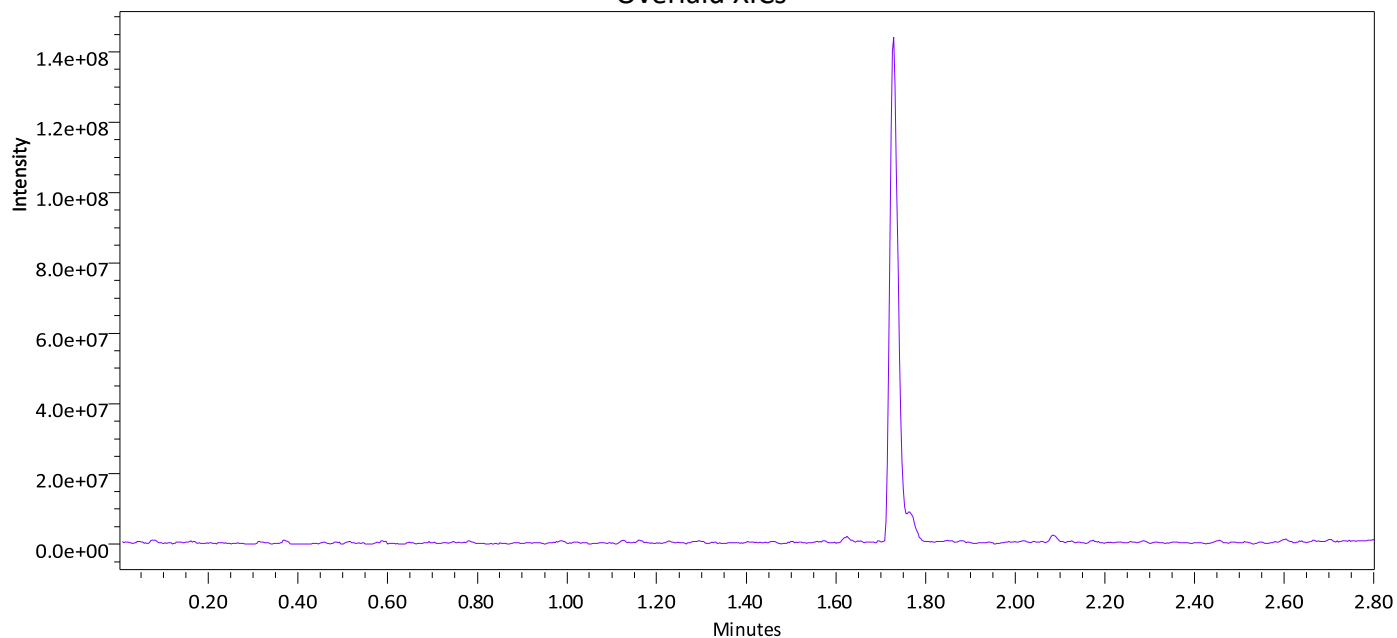
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 9:44:57 PM PDT

4/26/2024 9:48:03 PM PDT

Overlaid XICs



m/z 823.40

4/26/2024 9:48:37 PM PDT

4/26/2024 9:51:45 PM PDT

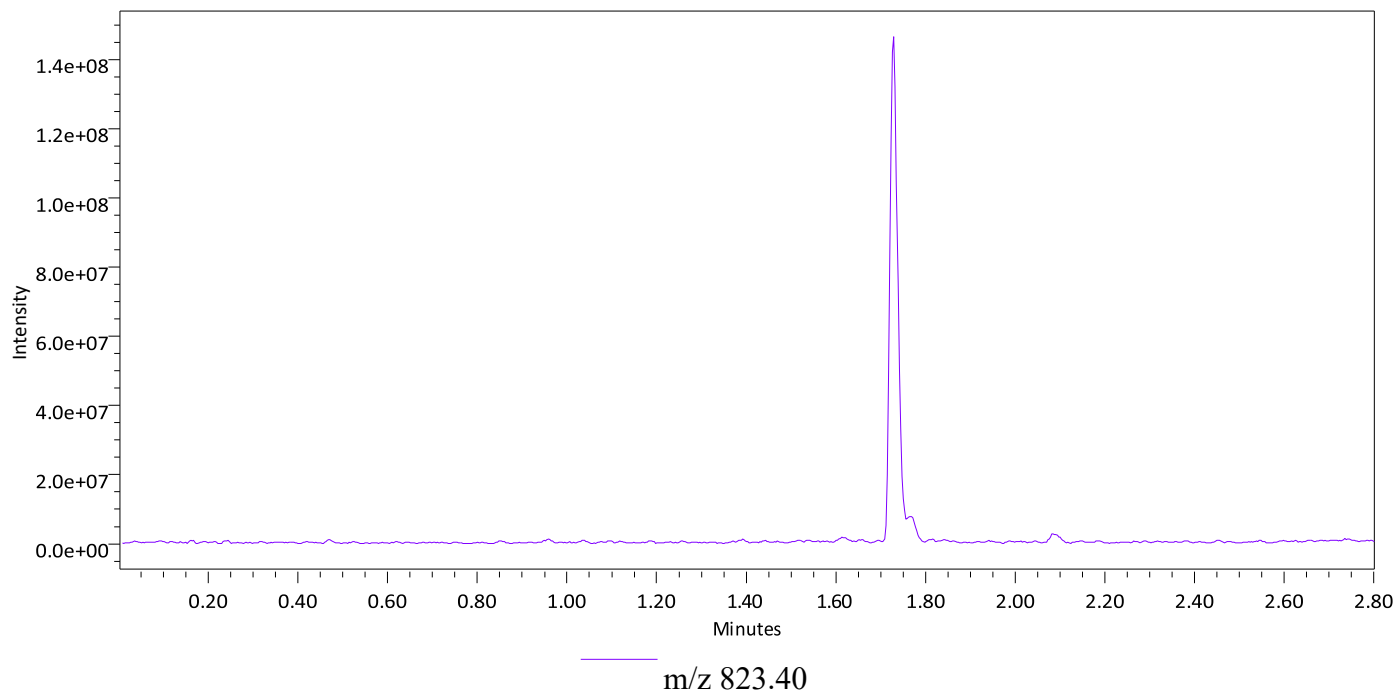
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C3
Vial: 2:C,3

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 9:52:20 PM PDT
4/26/2024 9:55:26 PM PDT

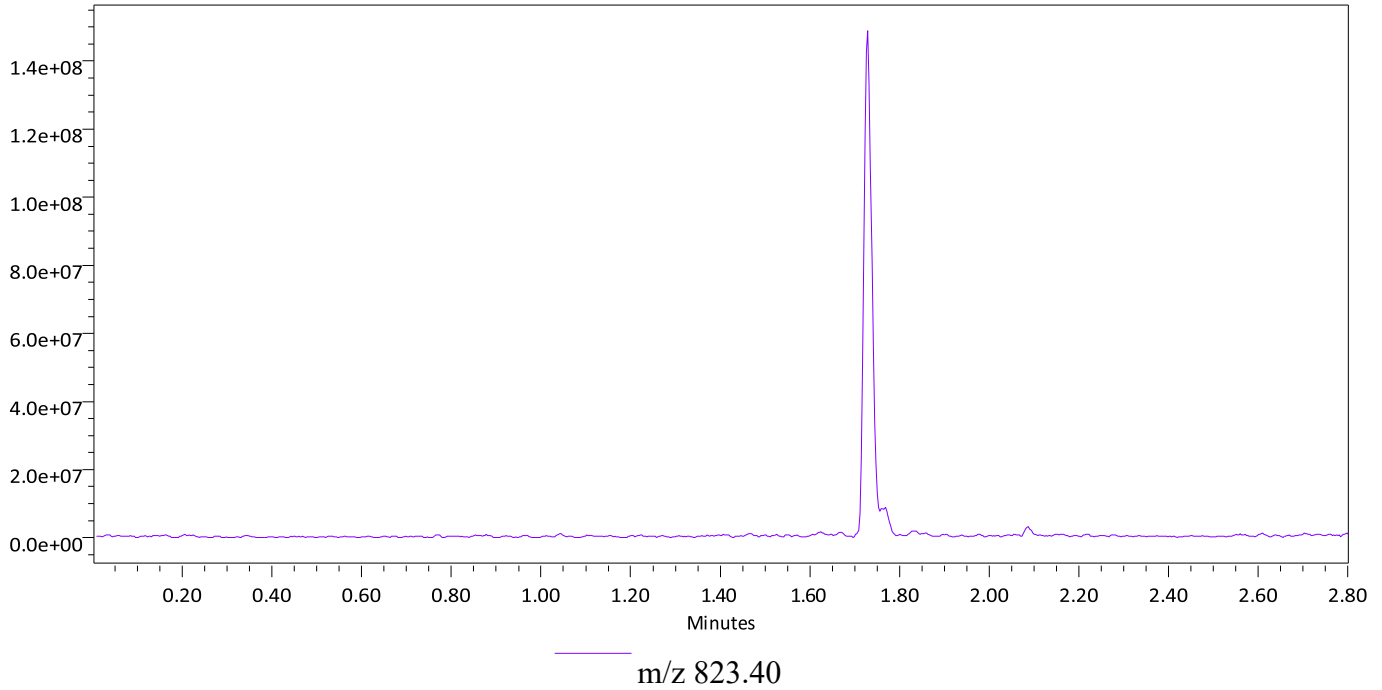
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C3
Vial: 2:C,3

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: C4
Vial: 2:C,4

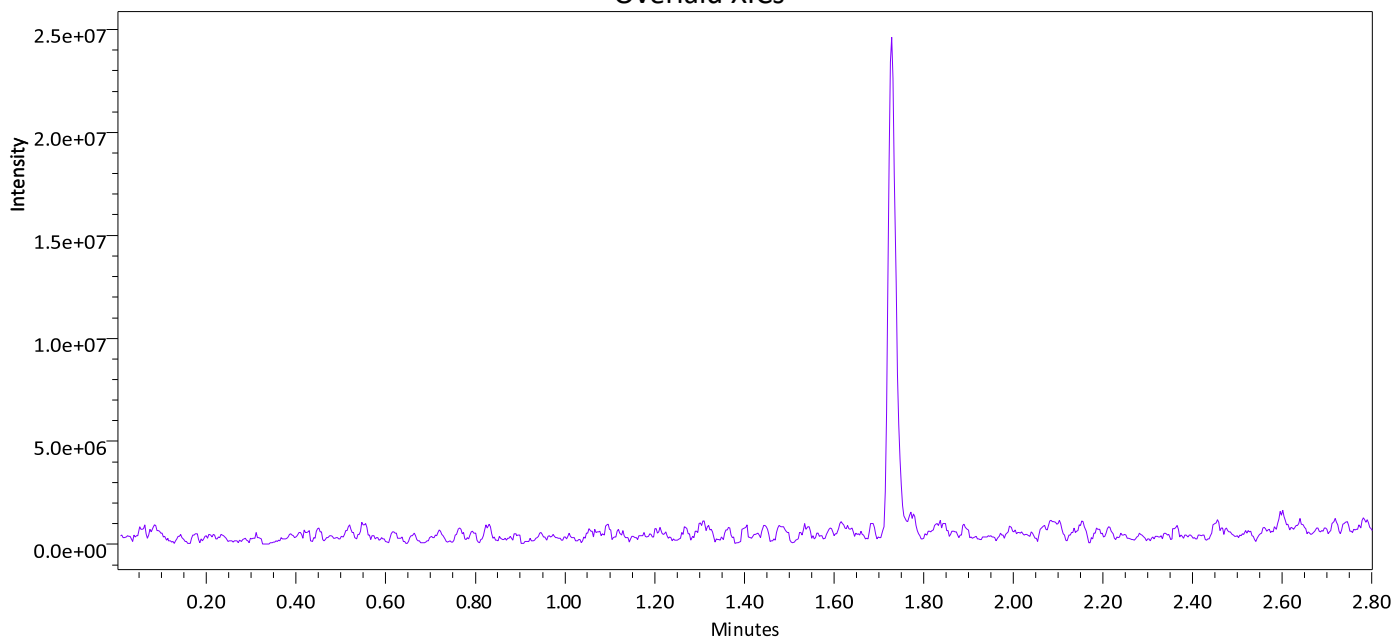
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 9:56:01 PM PDT

4/26/2024 9:59:07 PM PDT

Overlaid XICs



m/z 823.40

4/26/2024 9:59:43 PM PDT

4/26/2024 10:02:50 PM PDT

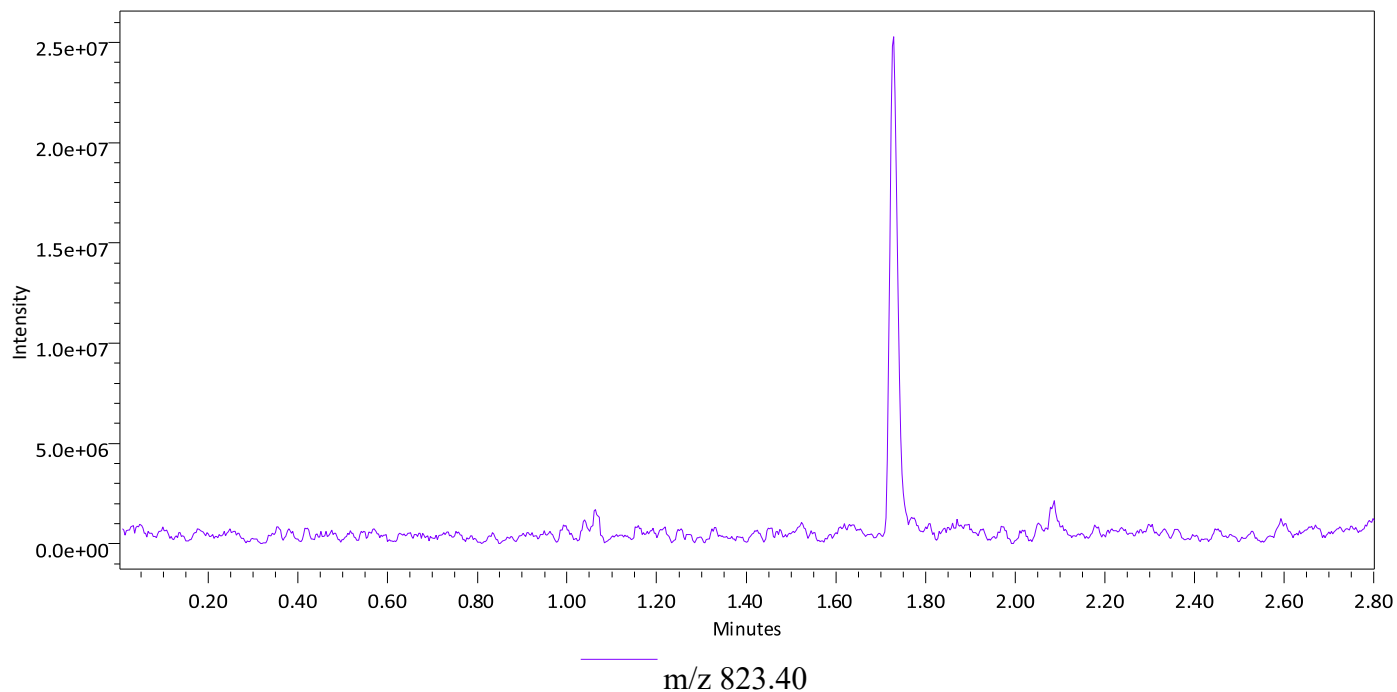
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C4
Vial: 2:C,4

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 10:03:24 PM PDT
4/26/2024 10:06:30 PM PDT

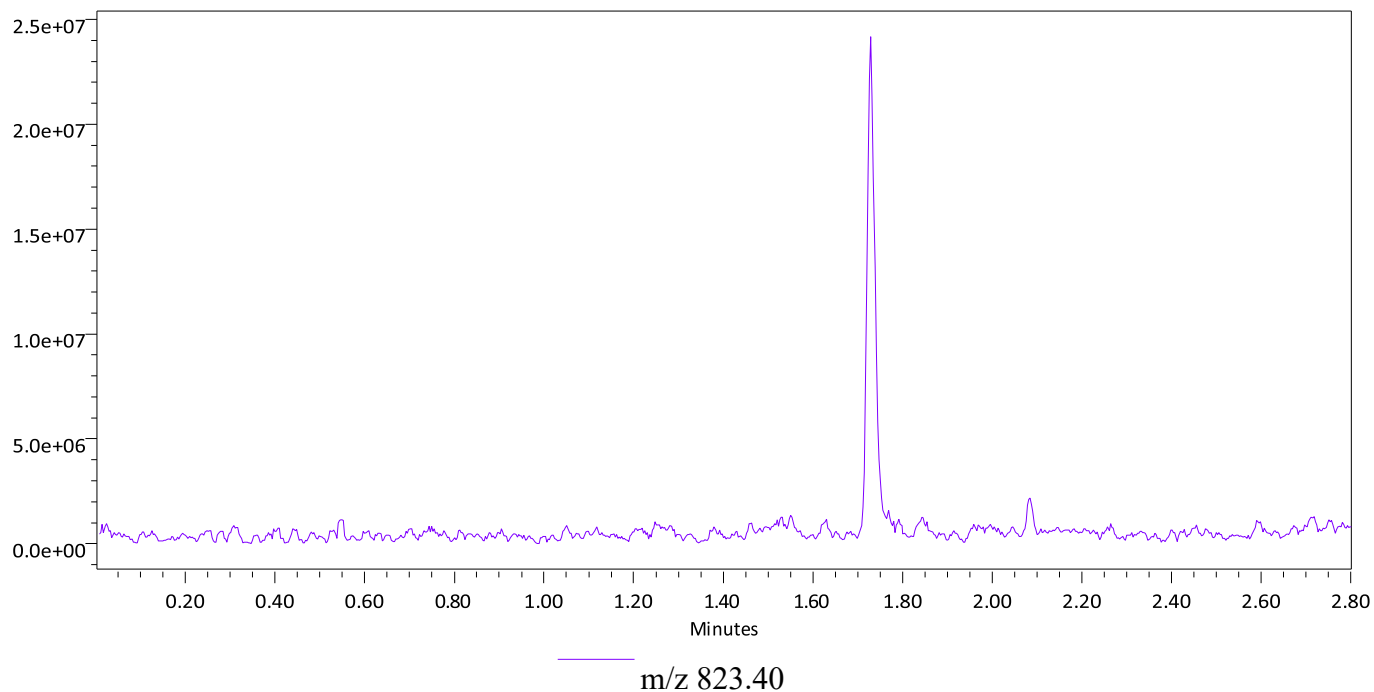
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C4
Vial: 2:C,4

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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TARGET MASS ANALYSIS

Sample Name: C5
Vial: 2:C,5

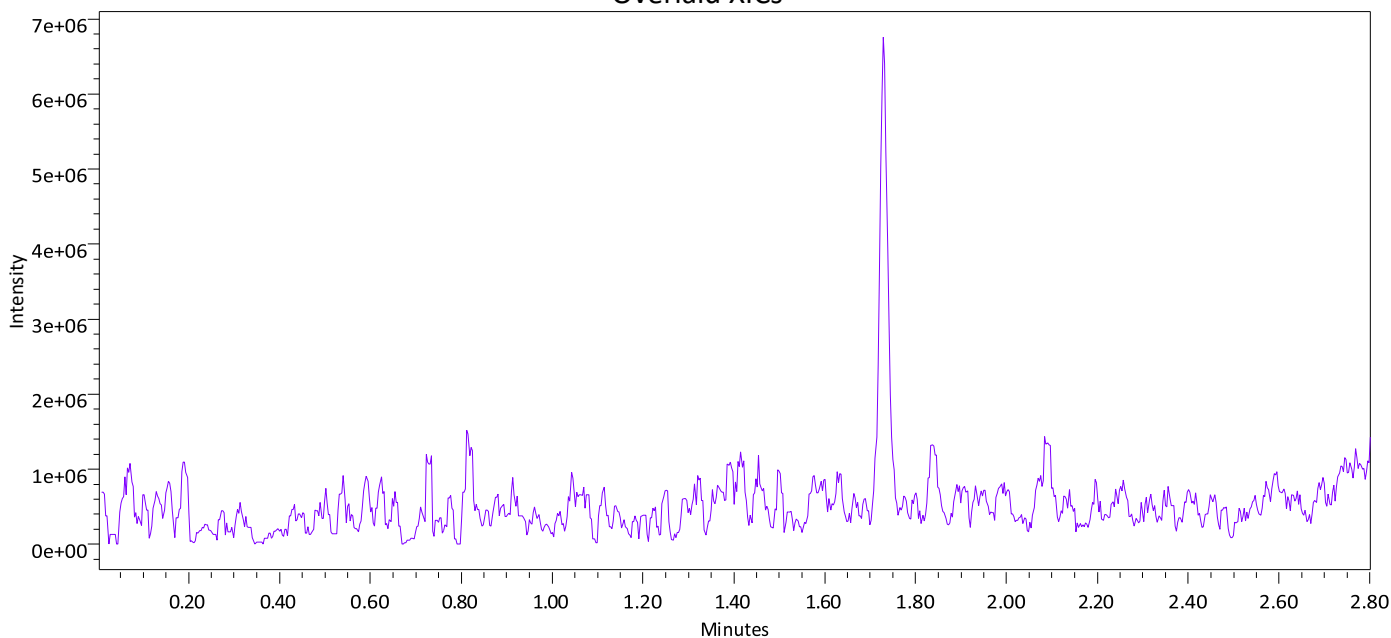
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 10:07:05 PM PDT

4/26/2024 10:10:11 PM PDT

Overlaid XICs



m/z 823.40

4/26/2024 10:10:48 PM PDT

4/26/2024 10:13:53 PM PDT

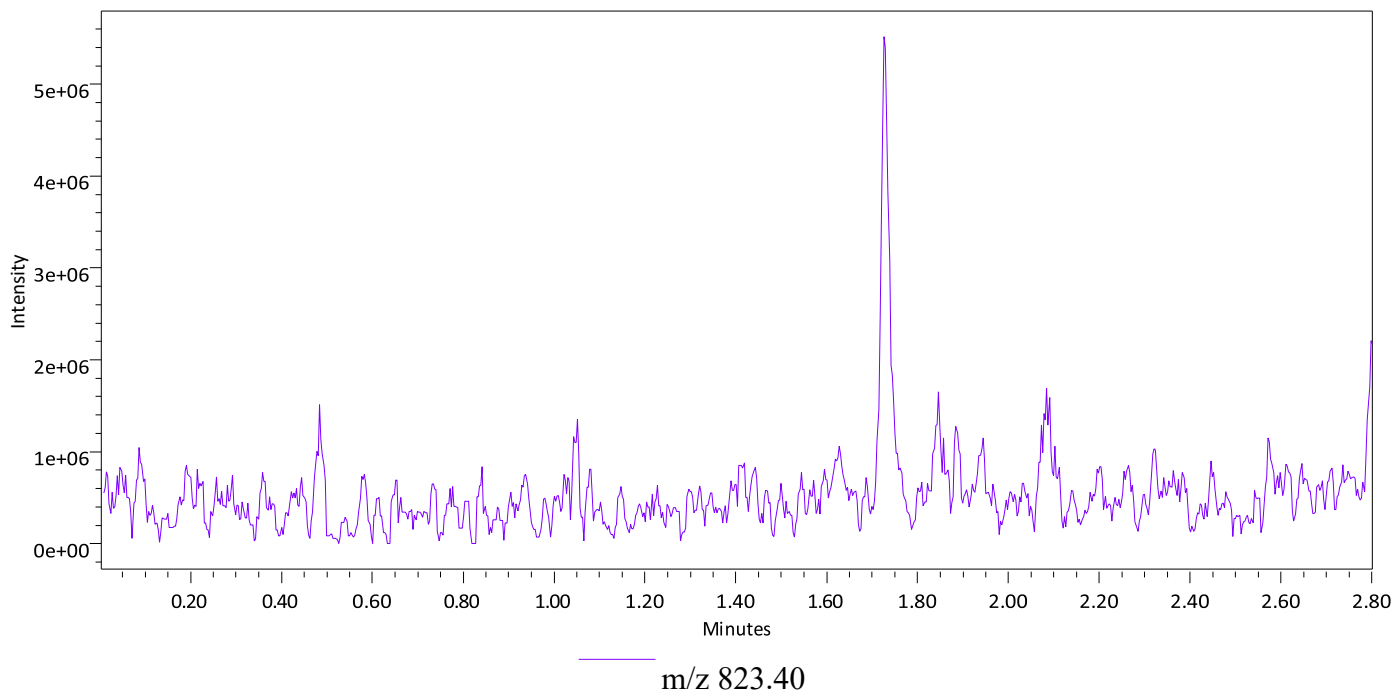
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C5
Vial: 2:C,5

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 10:14:29 PM PDT
4/26/2024 10:17:38 PM PDT

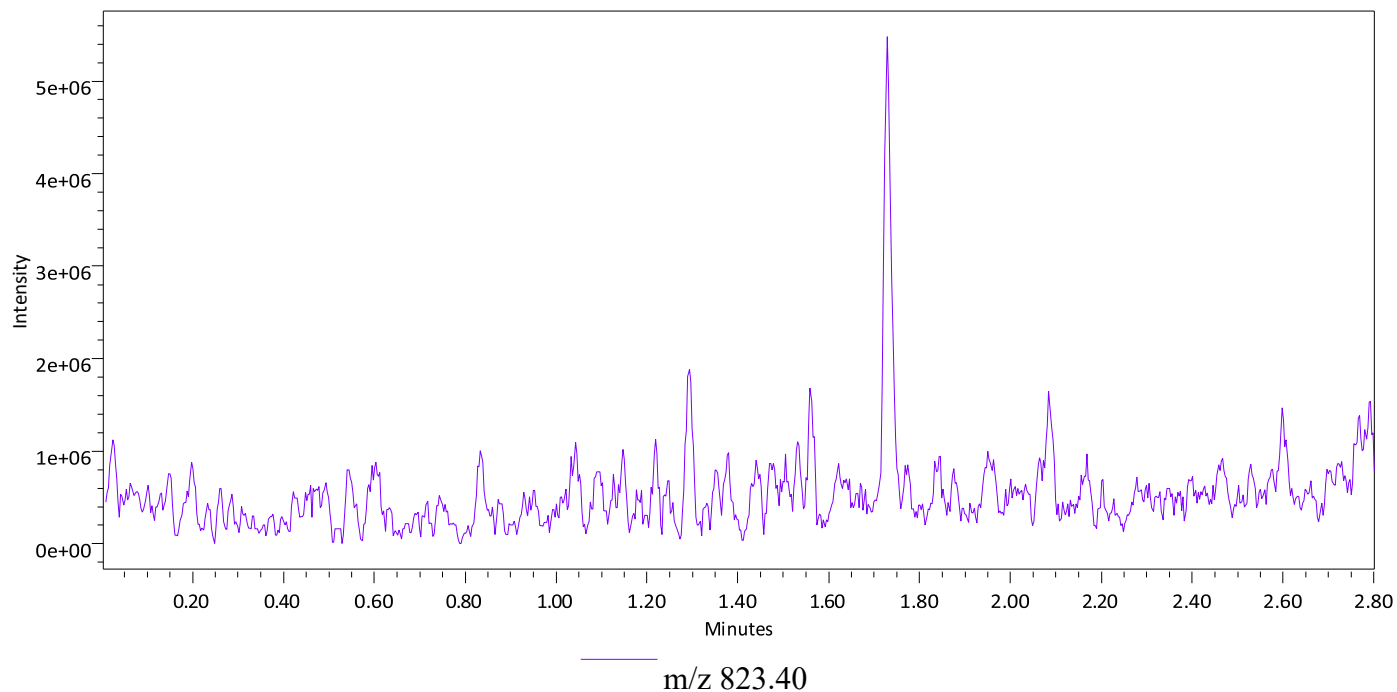
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C5
Vial: 2:C,5

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: C9
Vial: 2:C,9

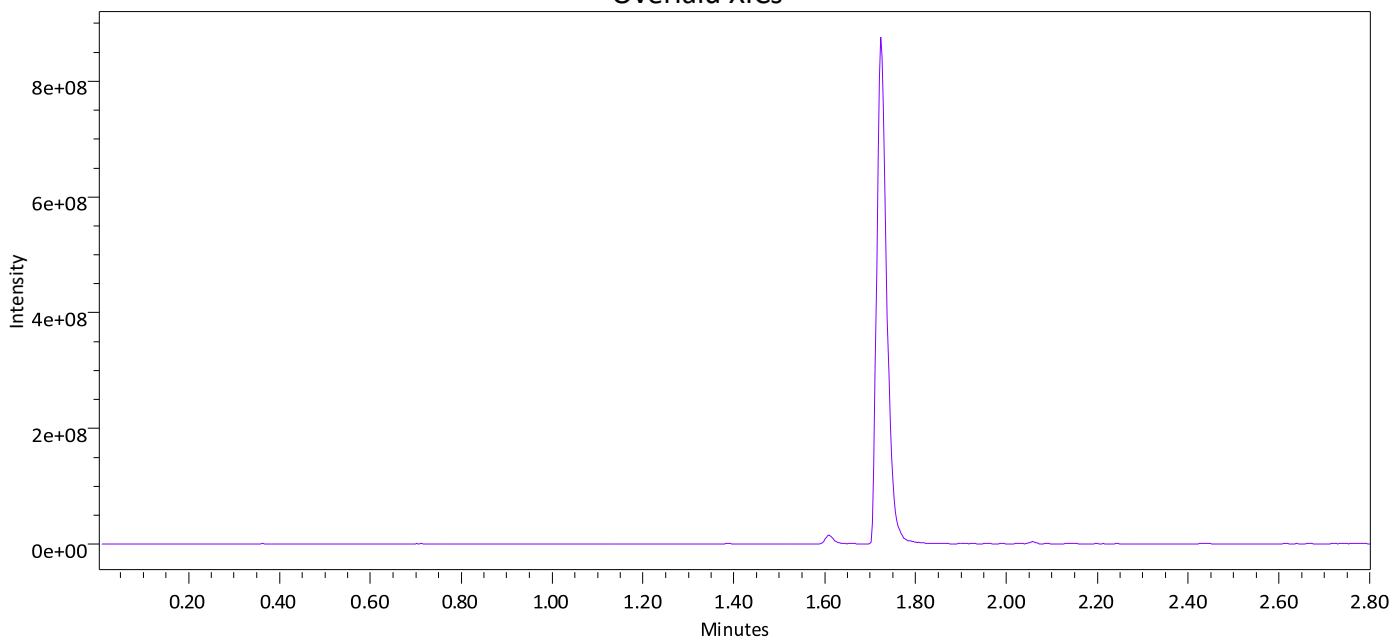
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 10:51:35 PM PDT

4/26/2024 10:54:41 PM PDT

Overlaid XICs



m/z 899.30

4/26/2024 10:55:17 PM PDT

4/26/2024 10:58:22 PM PDT

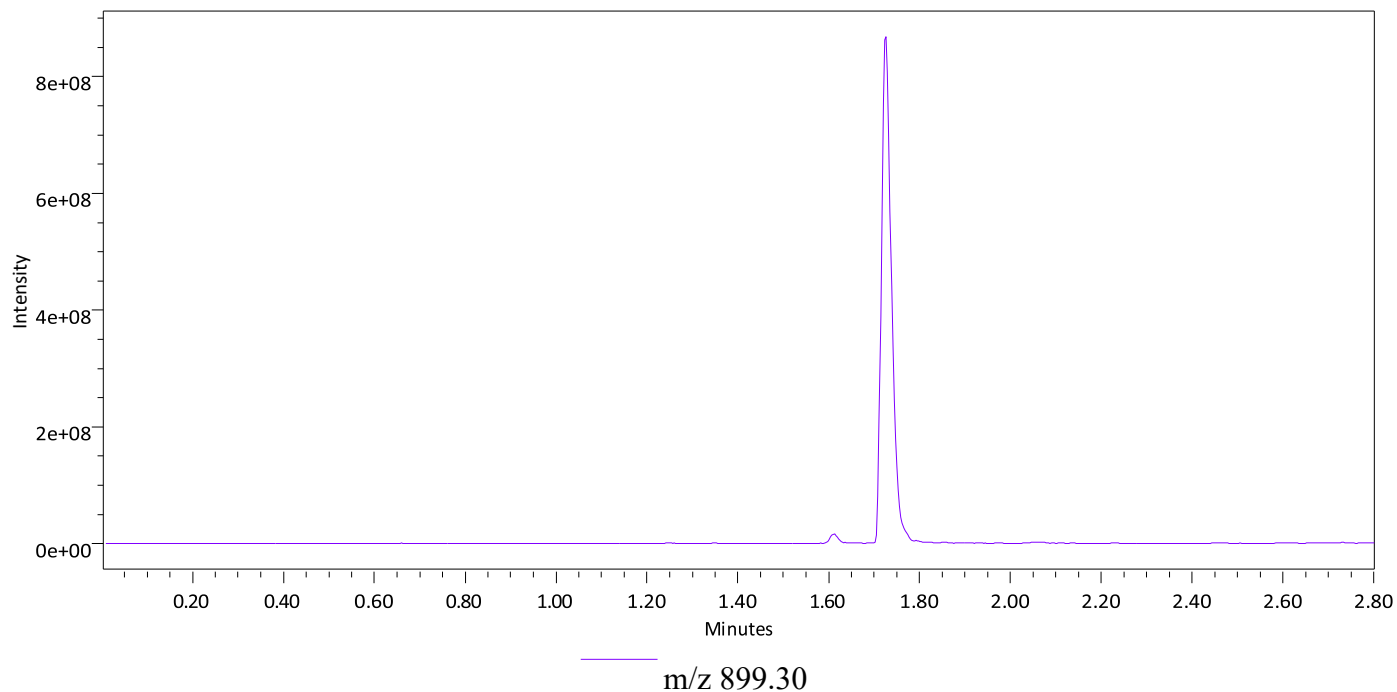
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C9
Vial: 2:C,9

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 10:58:56 PM PDT
4/26/2024 11:02:00 PM PDT

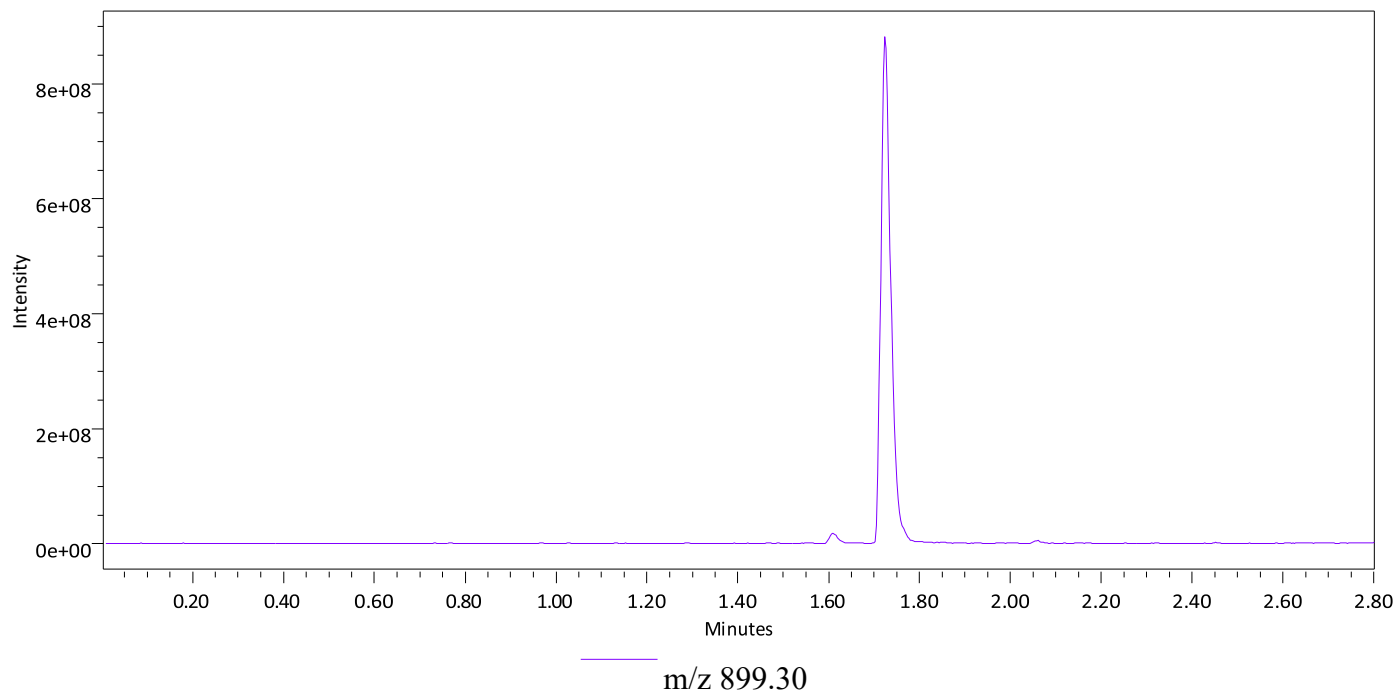
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C9
Vial: 2:C,9

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: C10
Vial: 2:C,10

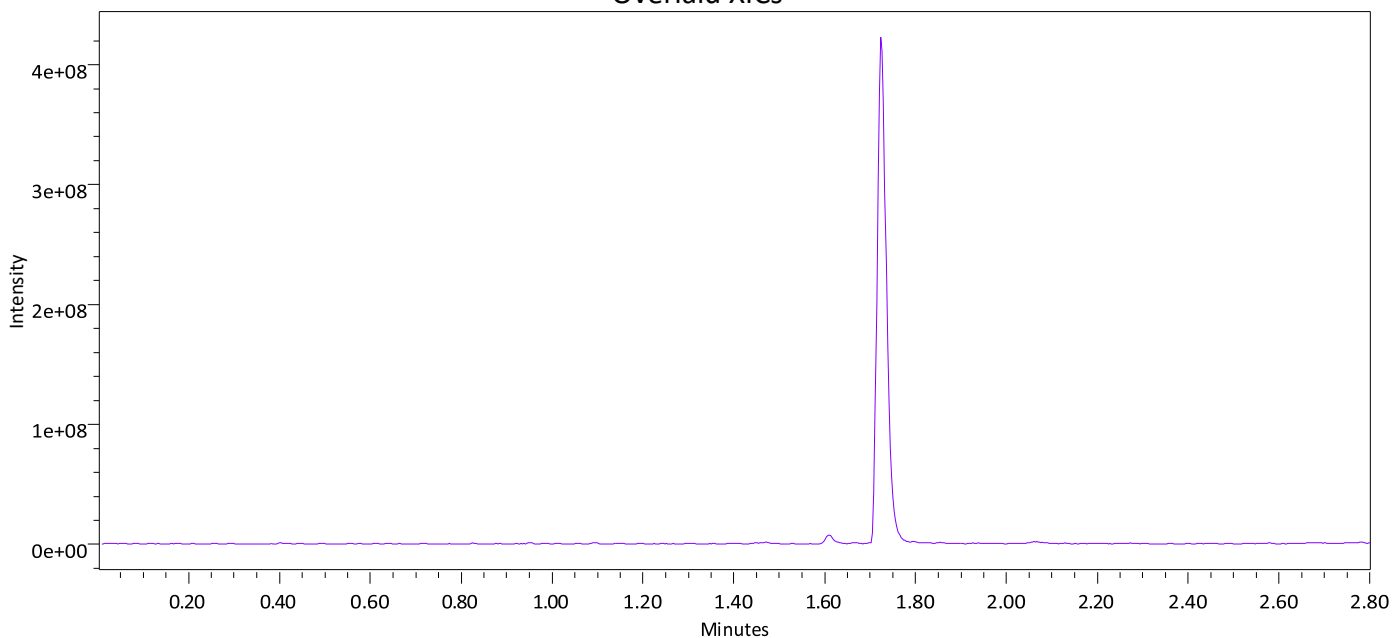
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 11:02:35 PM PDT

4/26/2024 11:05:43 PM PDT

Overlaid XICs



m/z 899.30

4/26/2024 11:06:19 PM PDT

4/26/2024 11:09:26 PM PDT

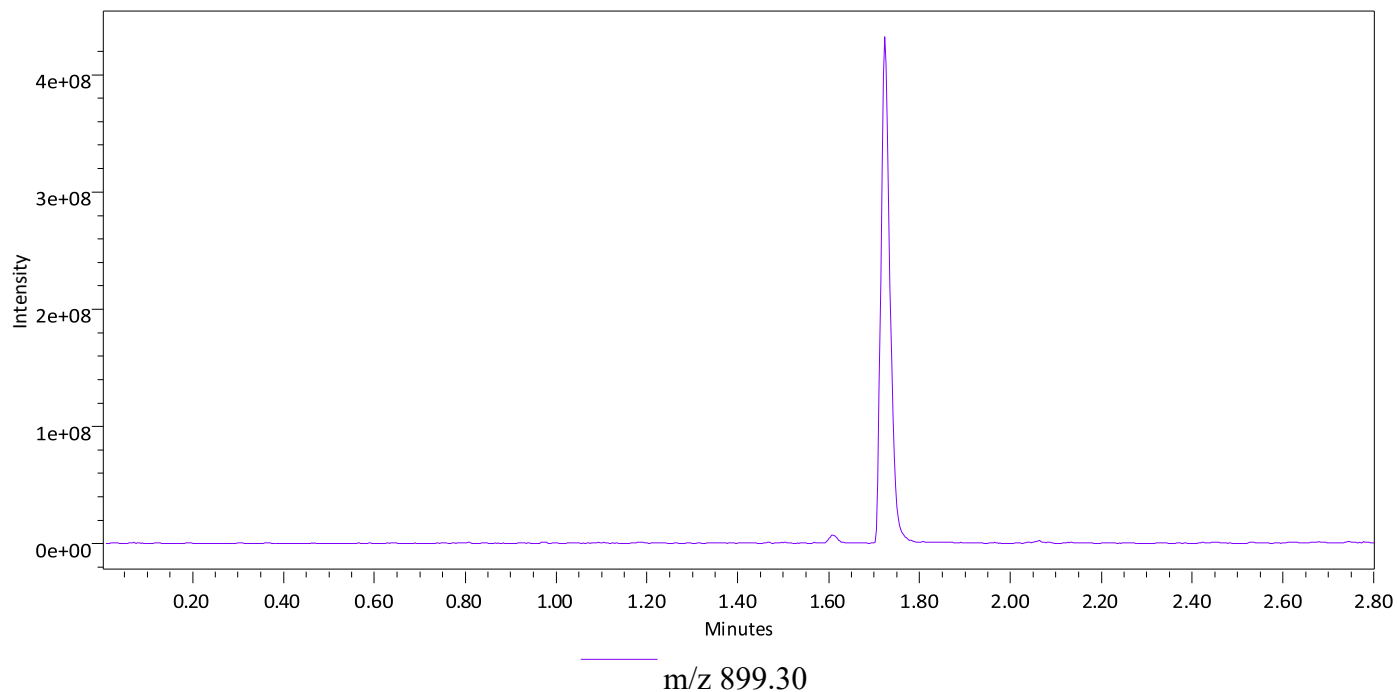
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C10
Vial: 2:C,10

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 11:10:01 PM PDT
4/26/2024 11:13:09 PM PDT

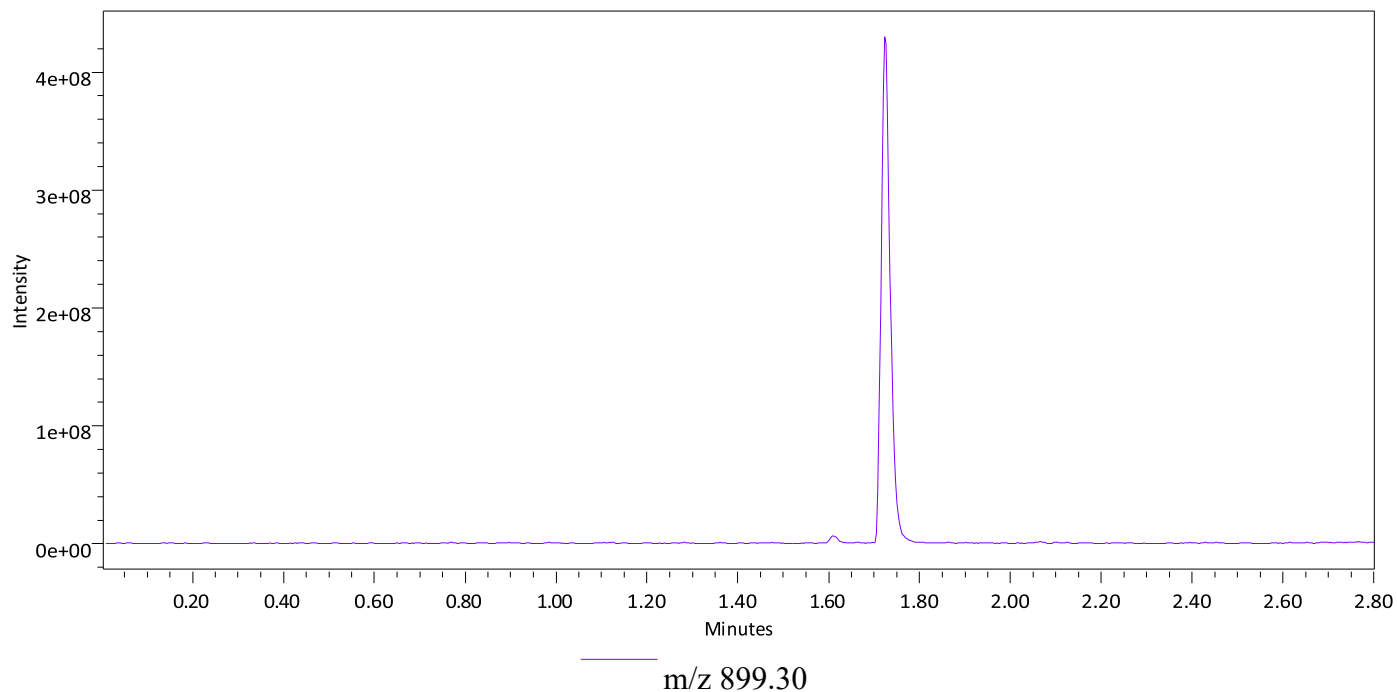
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C10
Vial: 2:C,10

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: C11
Vial: 2:C,11

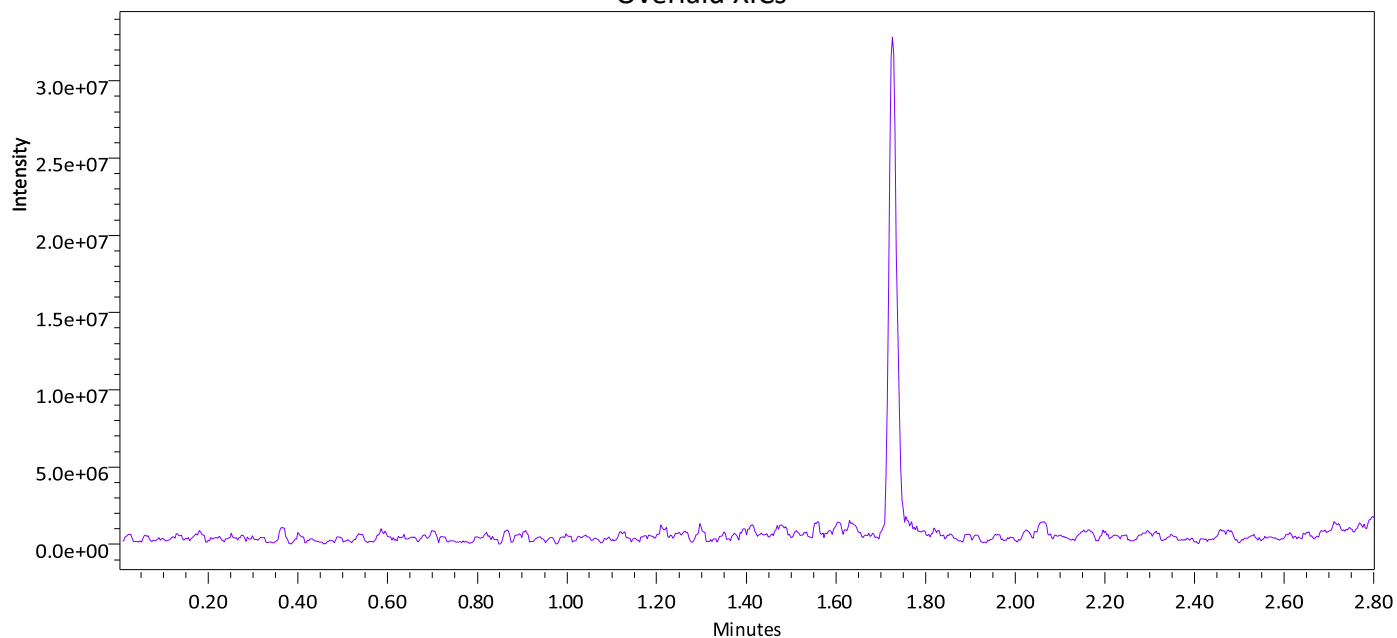
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 11:13:45 PM PDT

4/26/2024 11:16:52 PM PDT

Overlaid XICs



m/z 899.30

4/26/2024 11:17:29 PM PDT

4/26/2024 11:20:34 PM PDT

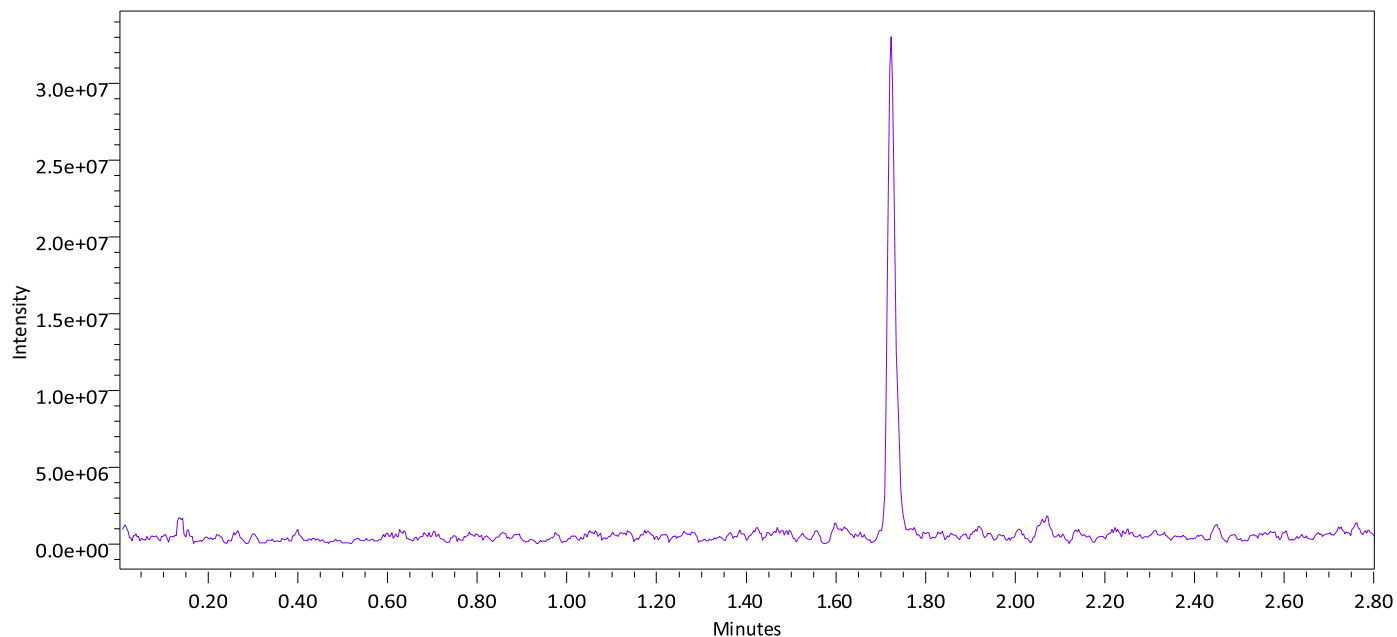
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C11
Vial: 2:C,11

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



m/z 899.30

4/26/2024 11:21:11 PM PDT

4/26/2024 11:24:18 PM PDT

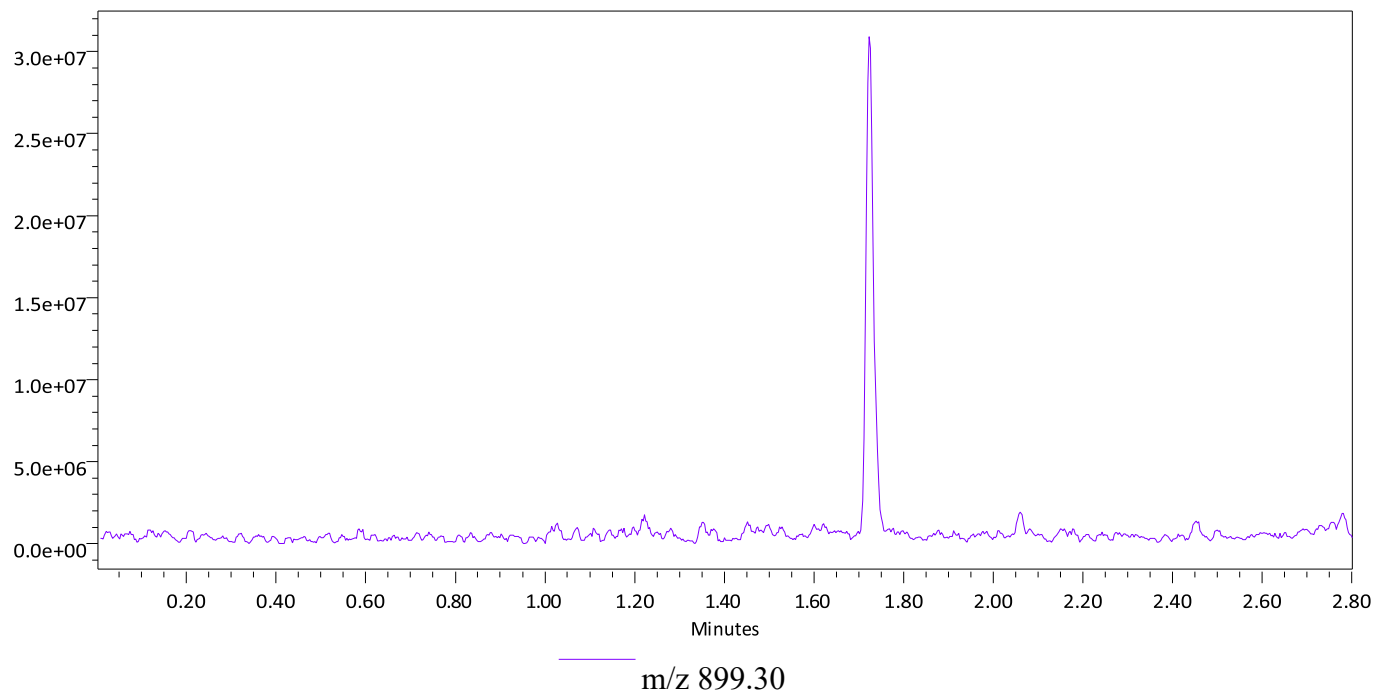
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C11
Vial: 2:C,11

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

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11:14:49 AM US/Pacific

TARGET MASS ANALYSIS

Sample Name: C12
Vial: 2:C,12

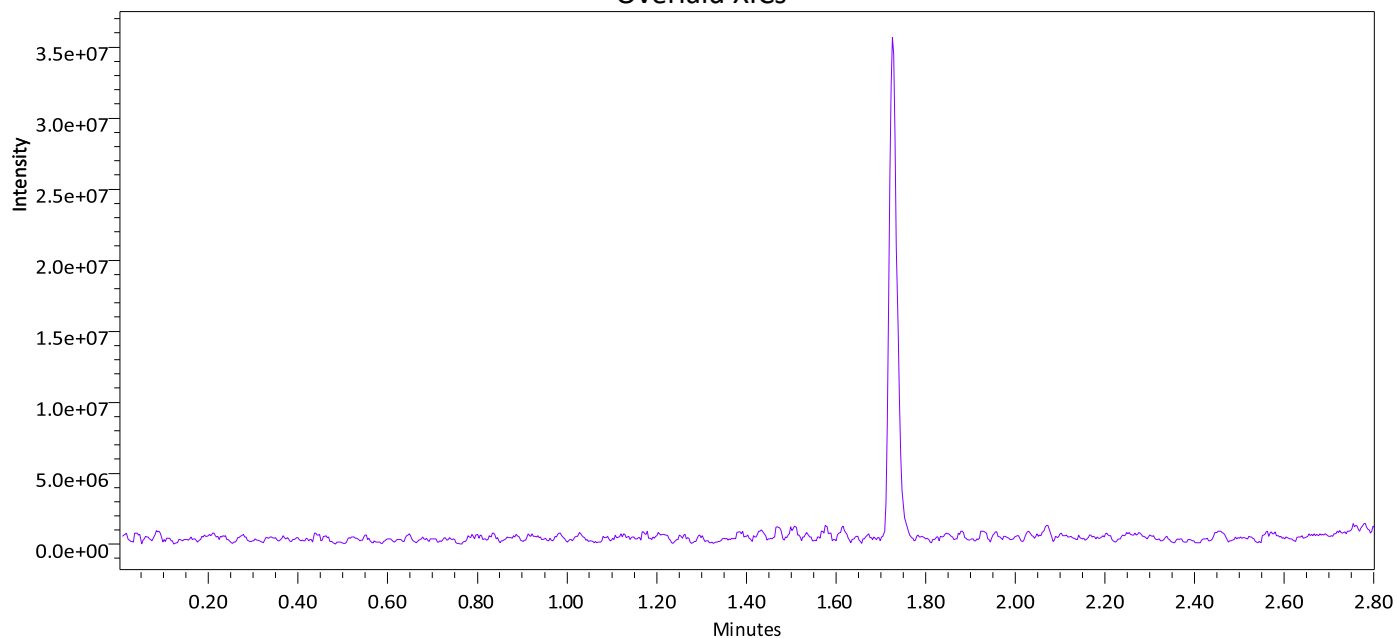
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 11:24:54 PM PDT

4/26/2024 11:28:04 PM PDT

Overlaid XICs



m/z 899.30

4/26/2024 11:28:41 PM PDT

4/26/2024 11:31:47 PM PDT

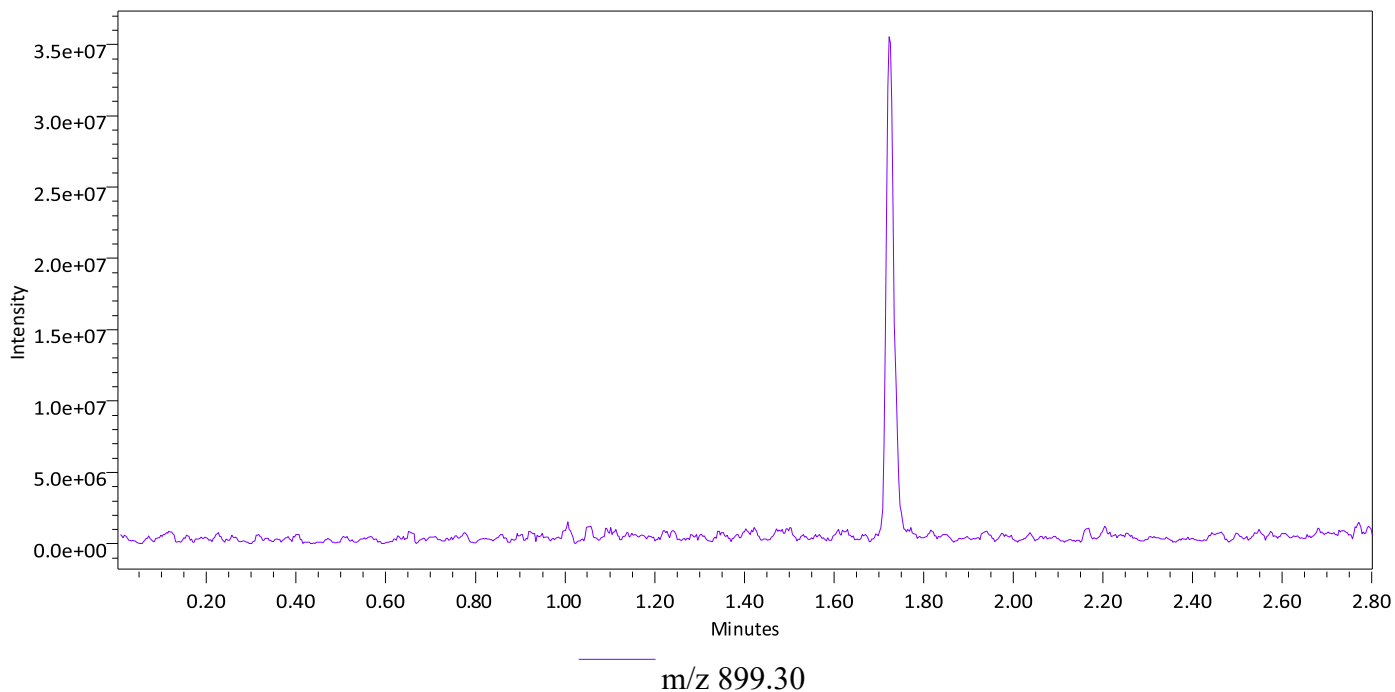
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C12
Vial: 2:C,12

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 11:32:23 PM PDT
4/26/2024 11:35:31 PM PDT

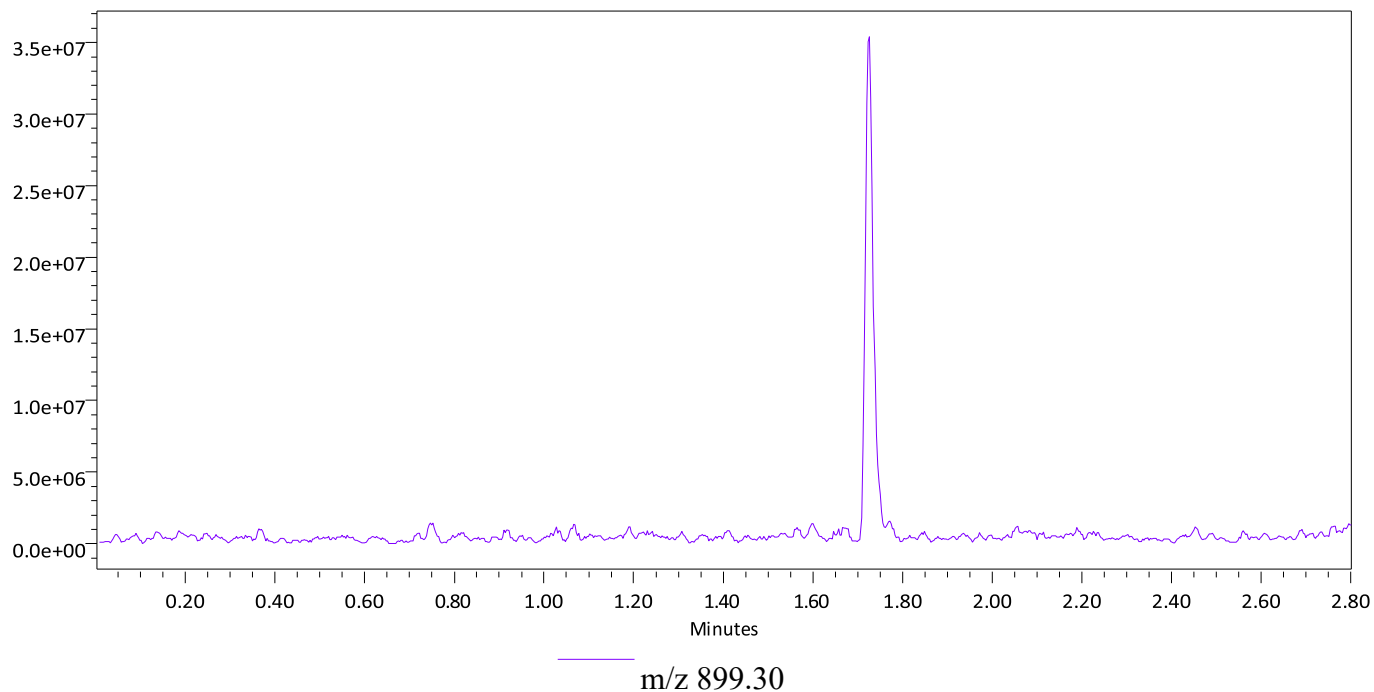
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: C12
Vial: 2:C,12

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

TARGET MASS ANALYSIS

Sample Name: D1
Vial: 2:D,1

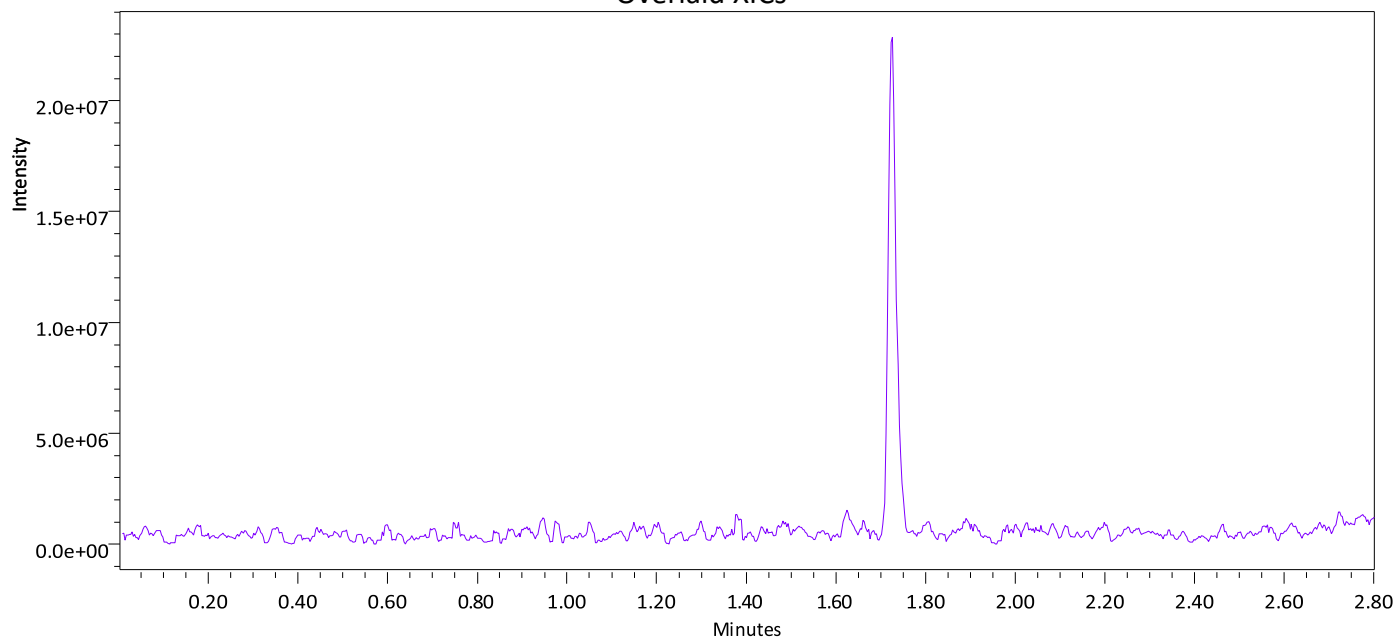
Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 11:36:07 PM PDT

4/26/2024 11:39:14 PM PDT

Overlaid XICs



m/z 899.30

4/26/2024 11:39:49 PM PDT

4/26/2024 11:42:54 PM PDT

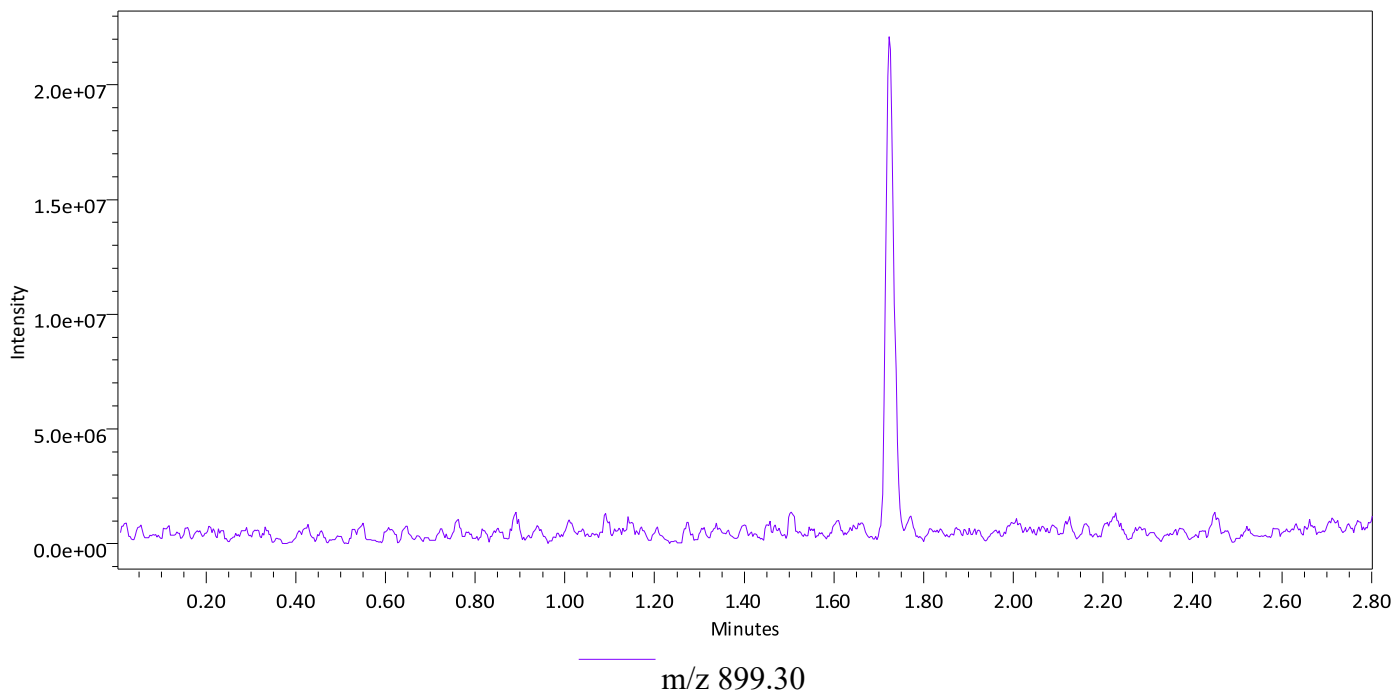
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D1
Vial: 2:D,1

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 11:43:29 PM PDT
4/26/2024 11:46:37 PM PDT

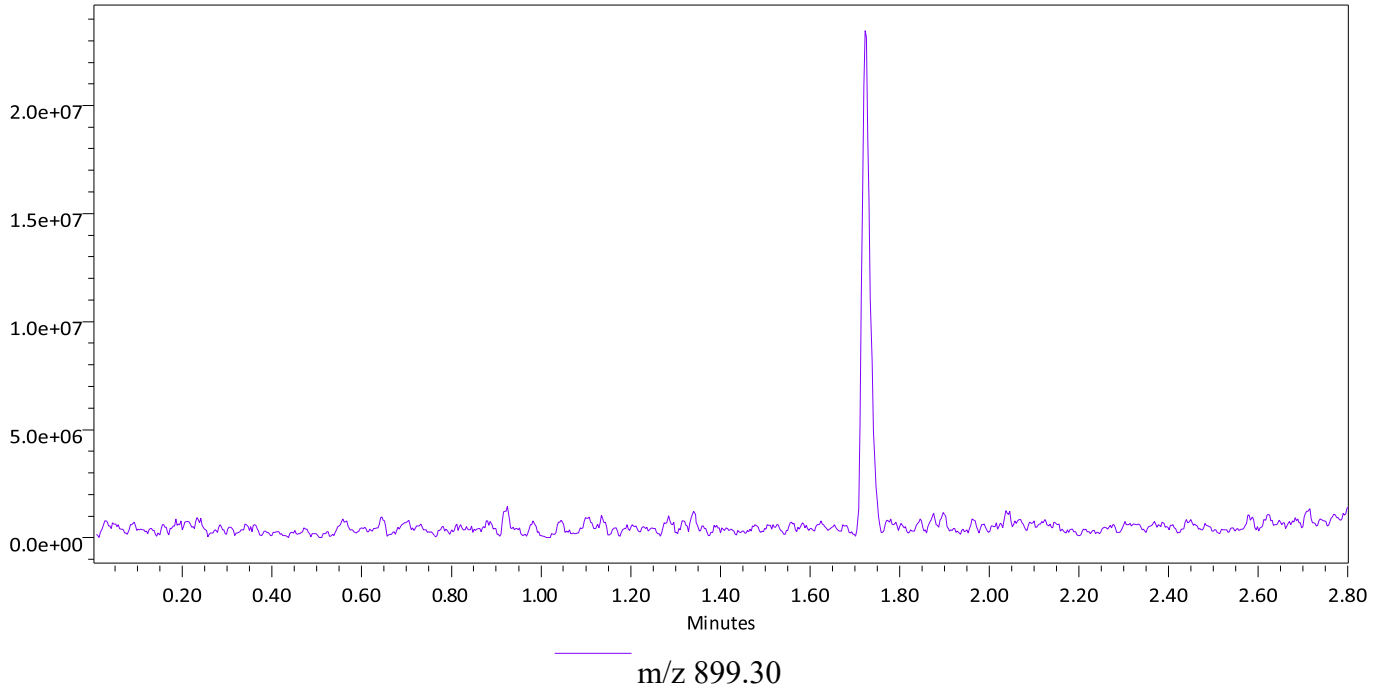
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D1
Vial: 2:D,1

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

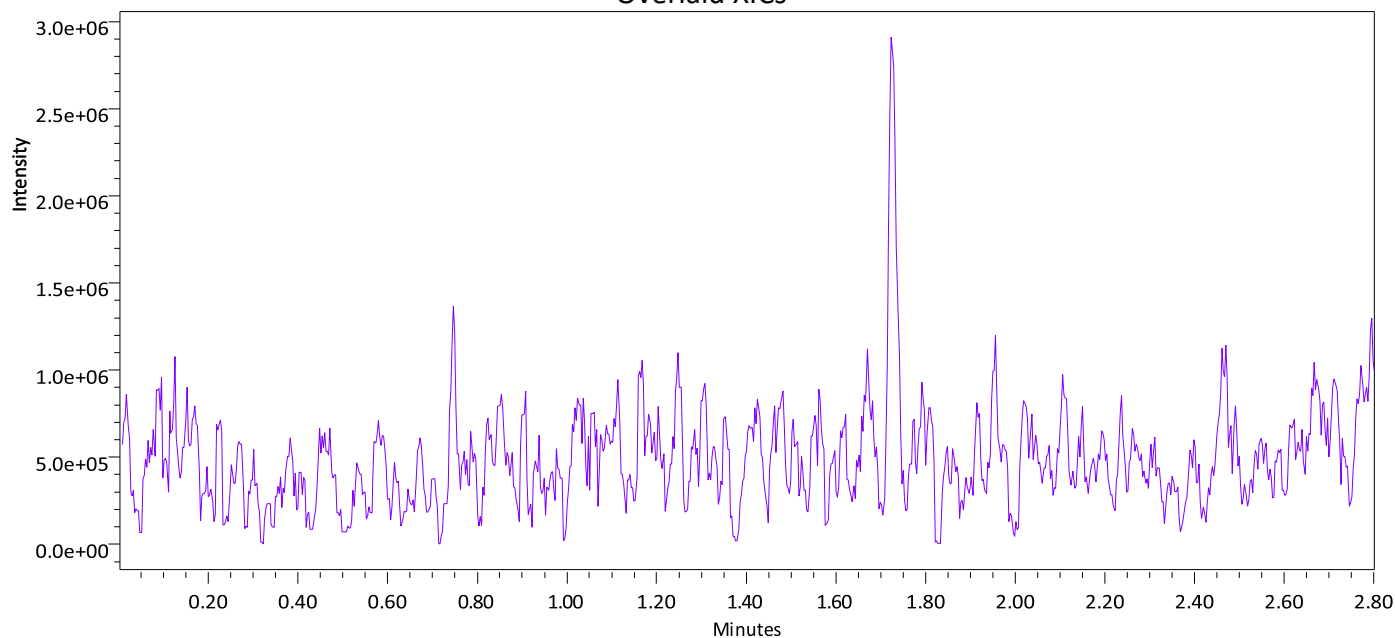
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

4/26/2024 11:47:12 PM PDT

4/26/2024 11:50:16 PM PDT

Overlaid XICs



m/z 899.30

4/26/2024 11:50:52 PM PDT

4/26/2024 11:53:59 PM PDT

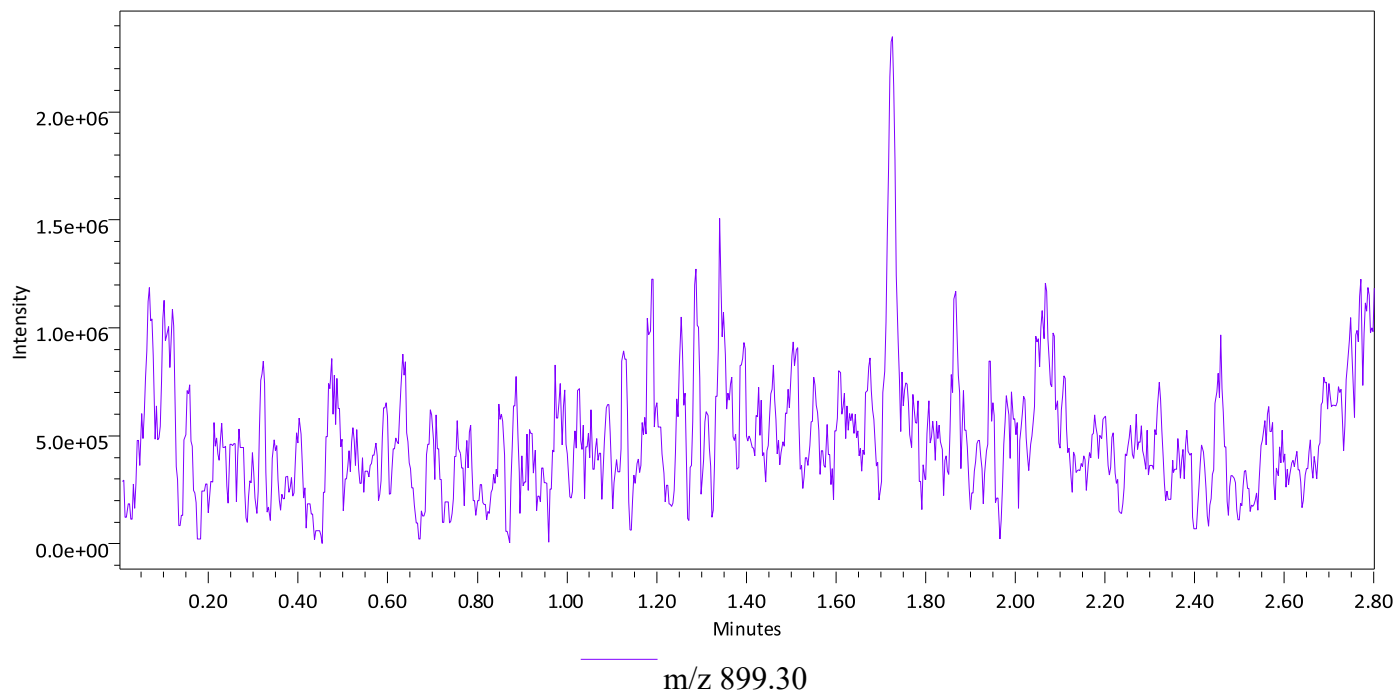
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



4/26/2024 11:54:35 PM PDT

4/26/2024 11:57:40 PM PDT

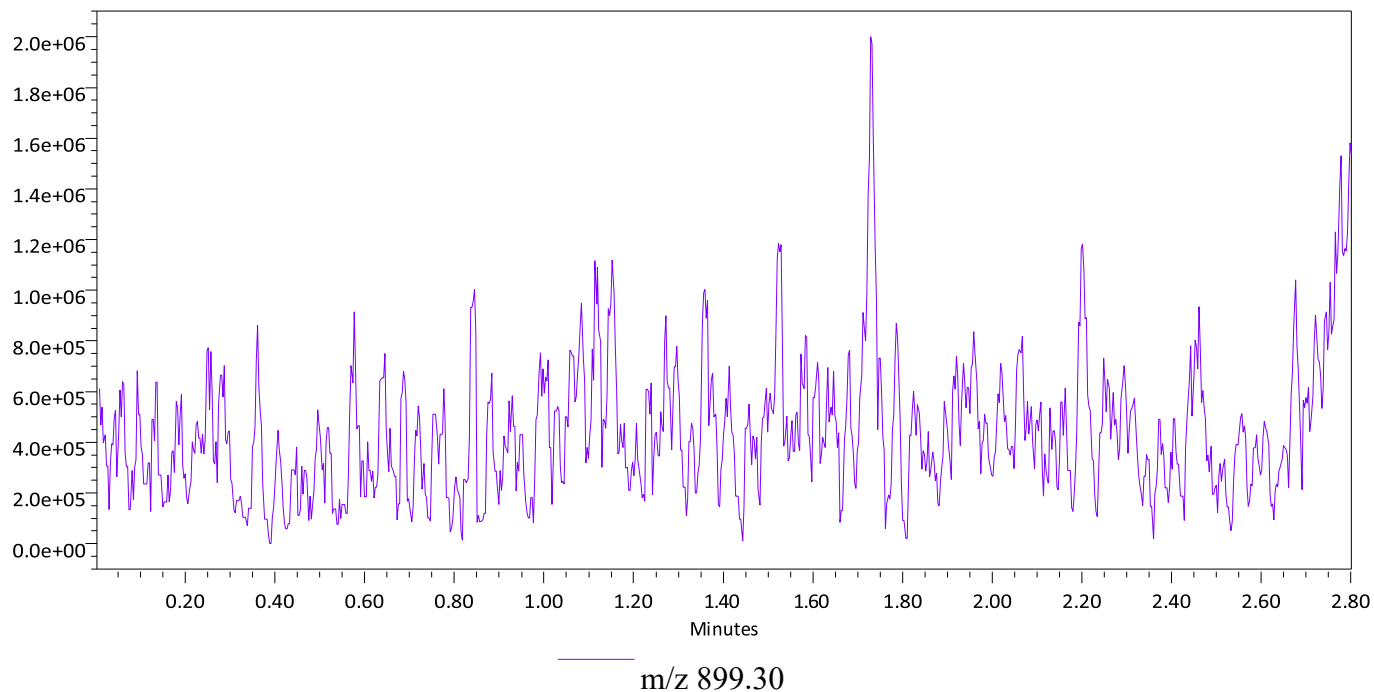
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

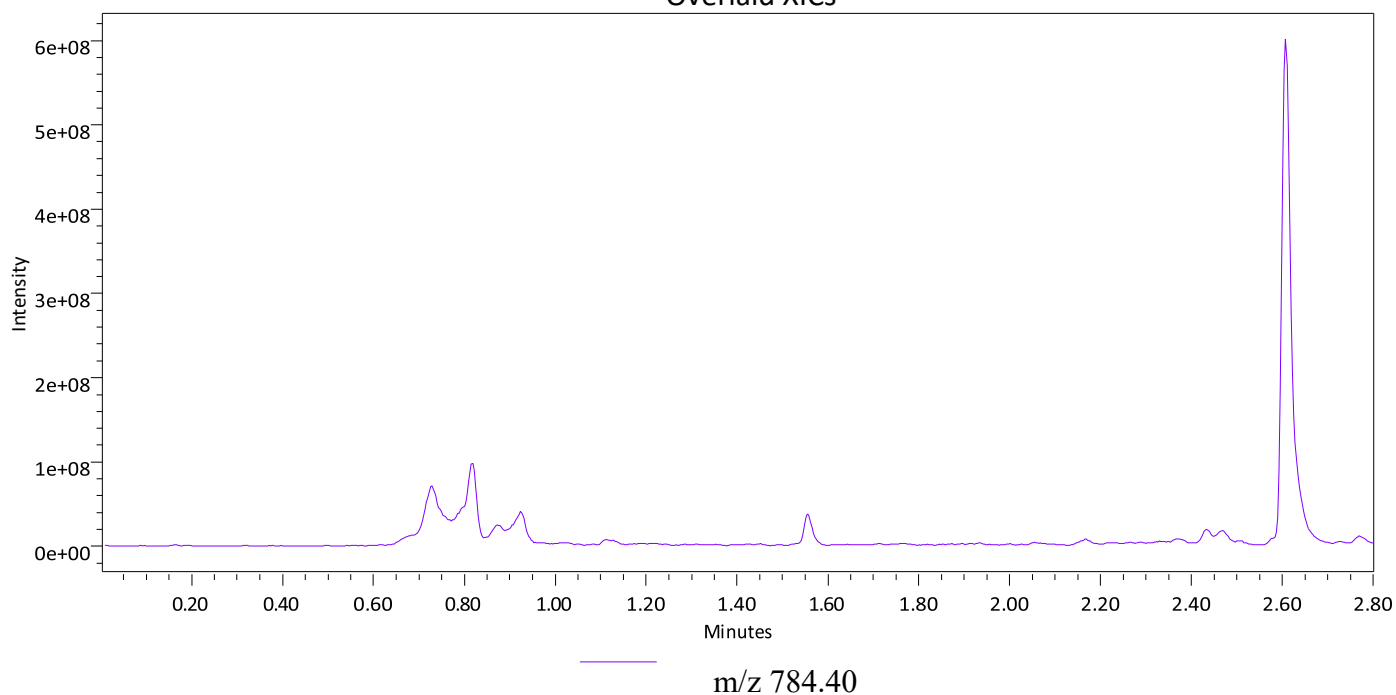
Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

D5
2:D,5

4/27/2024 12:20:23 AM PDT
4/27/2024 12:23:29 AM PDT

Overlaid XICs



D6
2:D,6

4/27/2024 12:24:03 AM PDT
4/27/2024 12:27:08 AM PDT

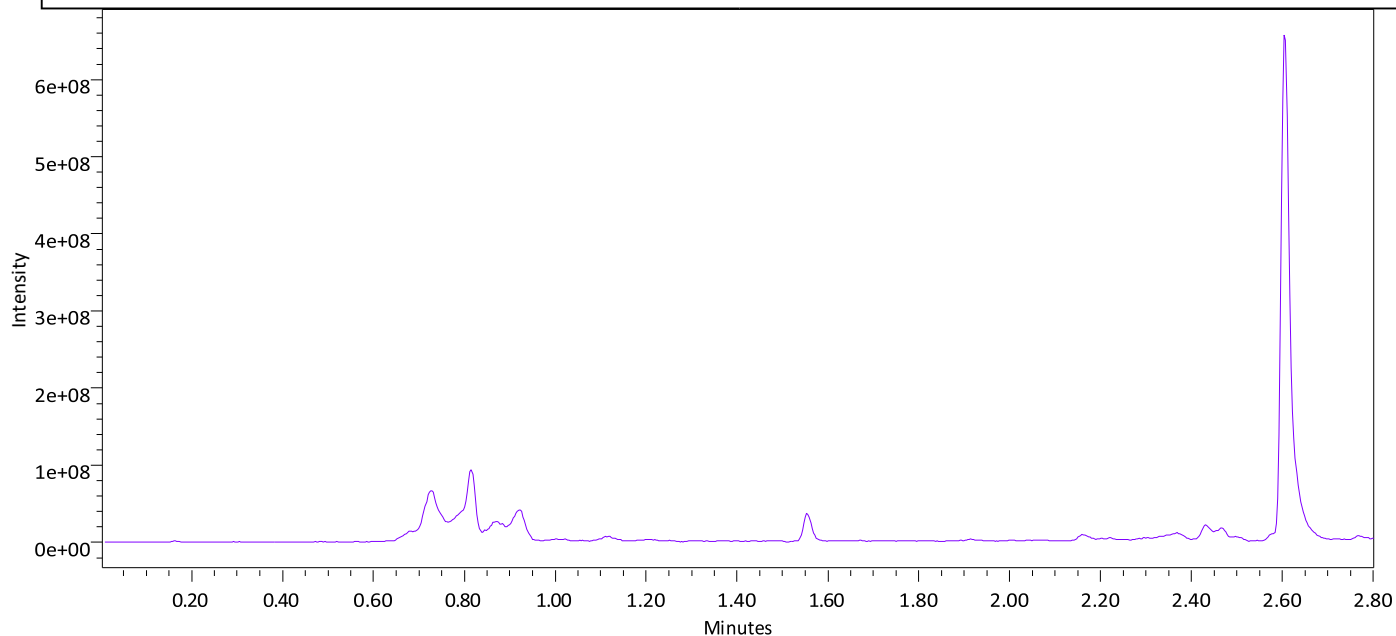
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 784.40

D7
2:D,7

4/27/2024 12:27:43 AM PDT

4/27/2024 12:30:52 AM PDT

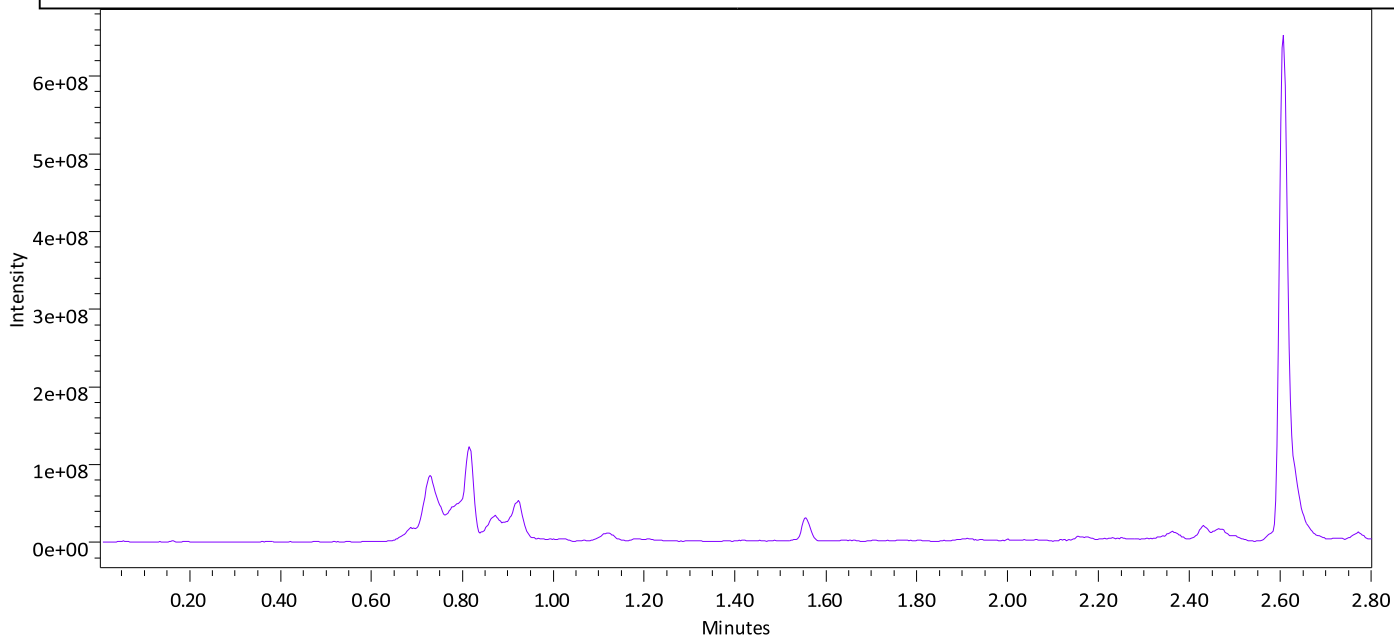
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 784.40

D8
2:D,8

4/27/2024 12:31:26 AM PDT

4/27/2024 12:34:34 AM PDT

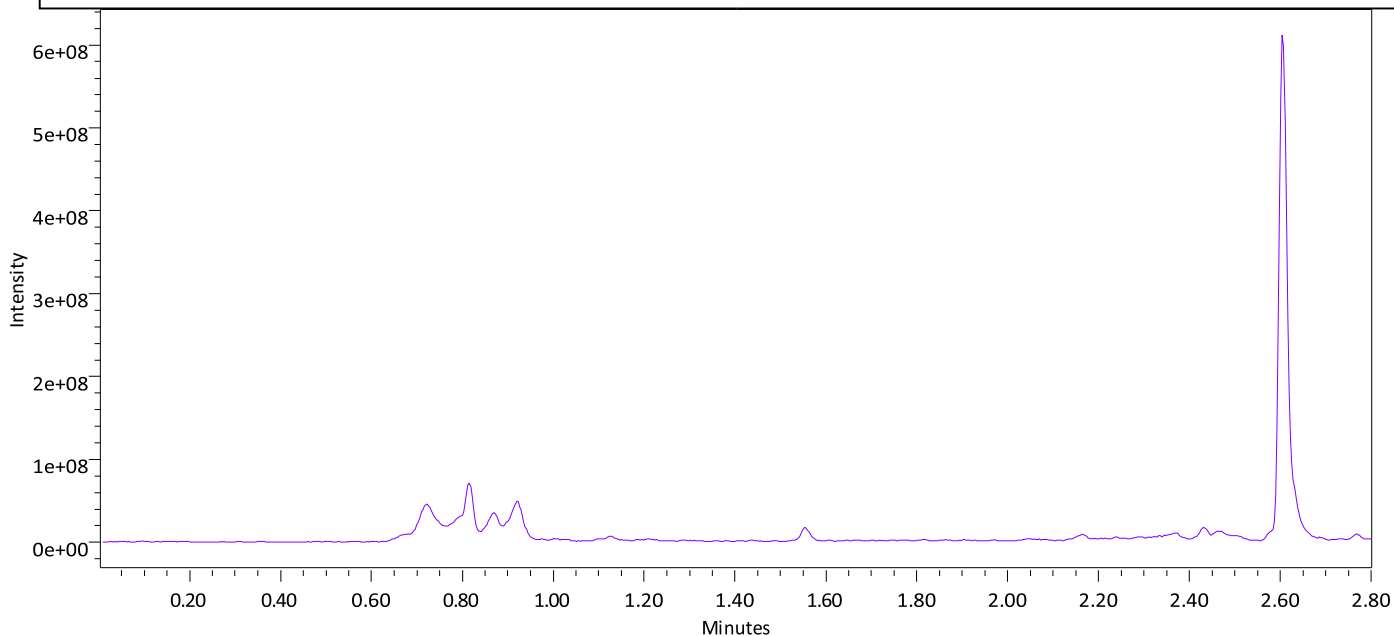
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



D9
2:D,9

4/27/2024 12:35:09 AM PDT

4/27/2024 12:38:17 AM PDT

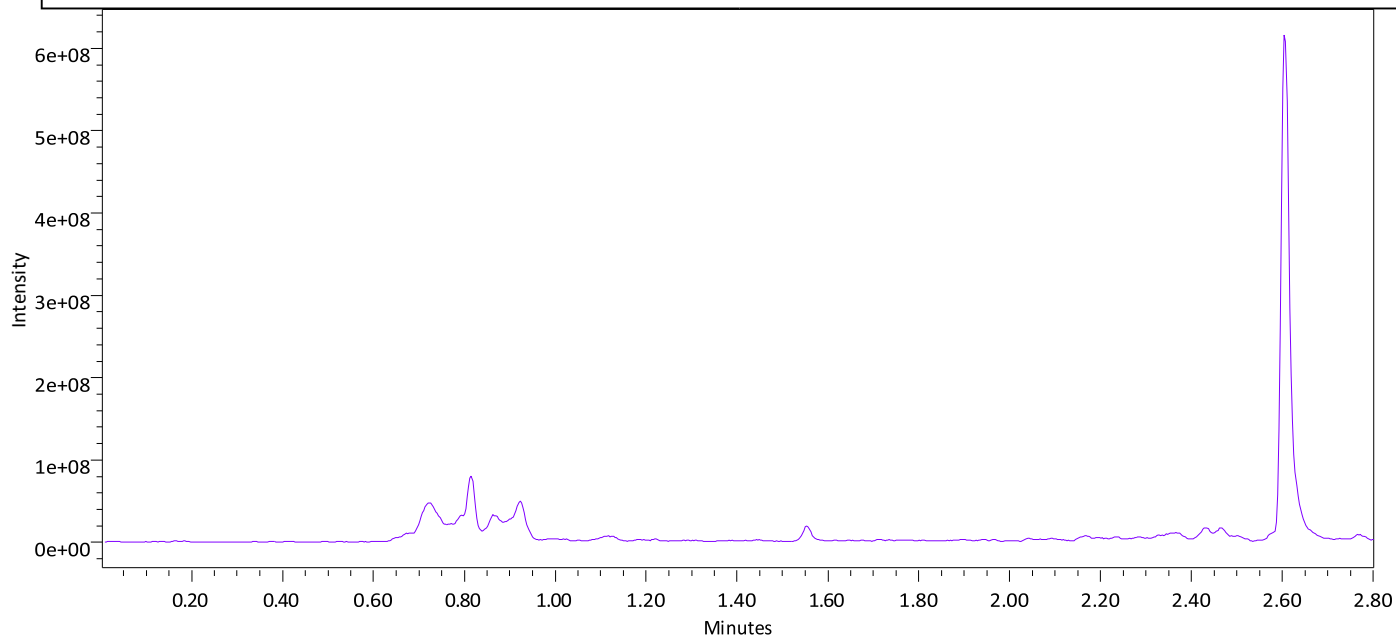
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



D10
2:D,10

4/27/2024 12:38:52 AM PDT
4/27/2024 12:42:00 AM PDT

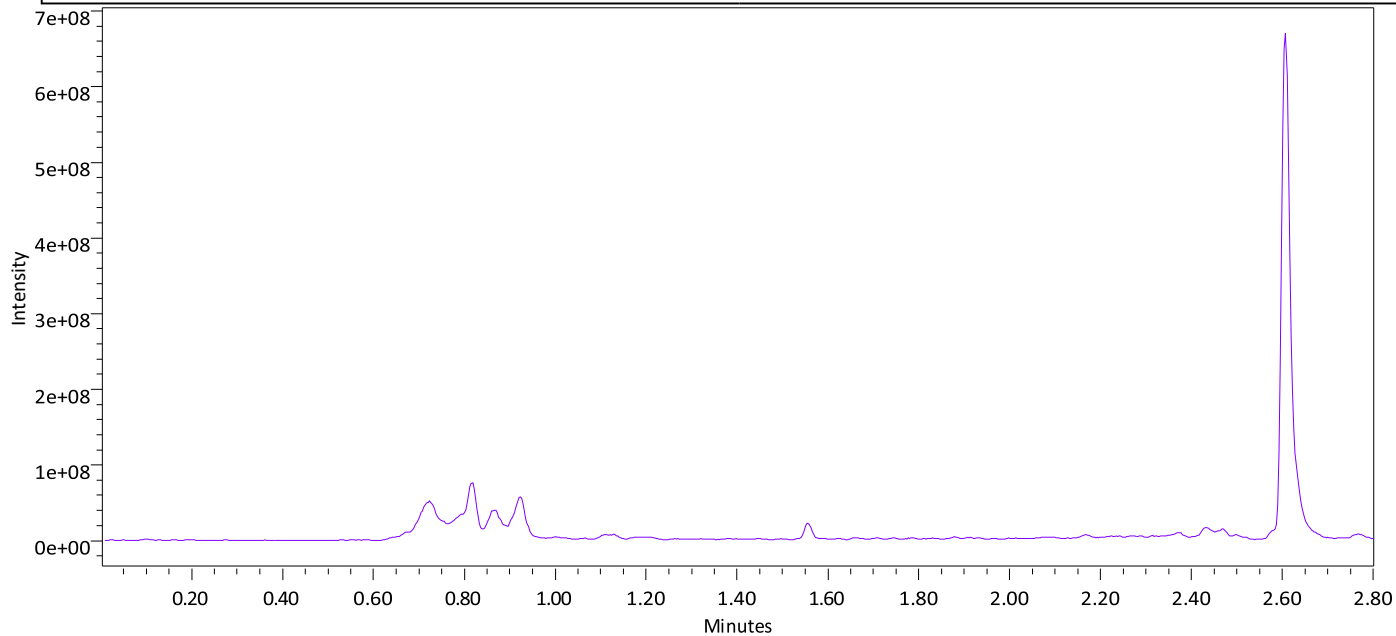
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 784.40

D11
2:D,11

4/27/2024 12:42:35 AM PDT

4/27/2024 12:45:41 AM PDT

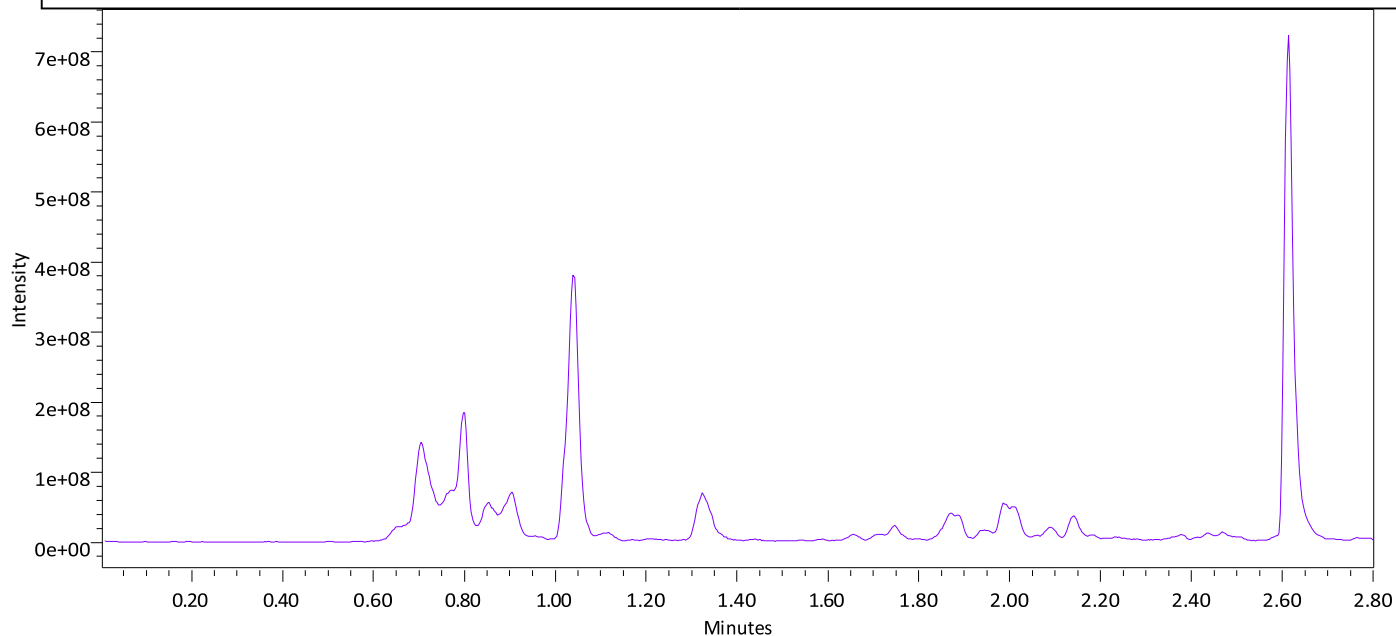
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 740.40

D12
2:D,12

4/27/2024 12:46:17 AM PDT

4/27/2024 12:49:24 AM PDT

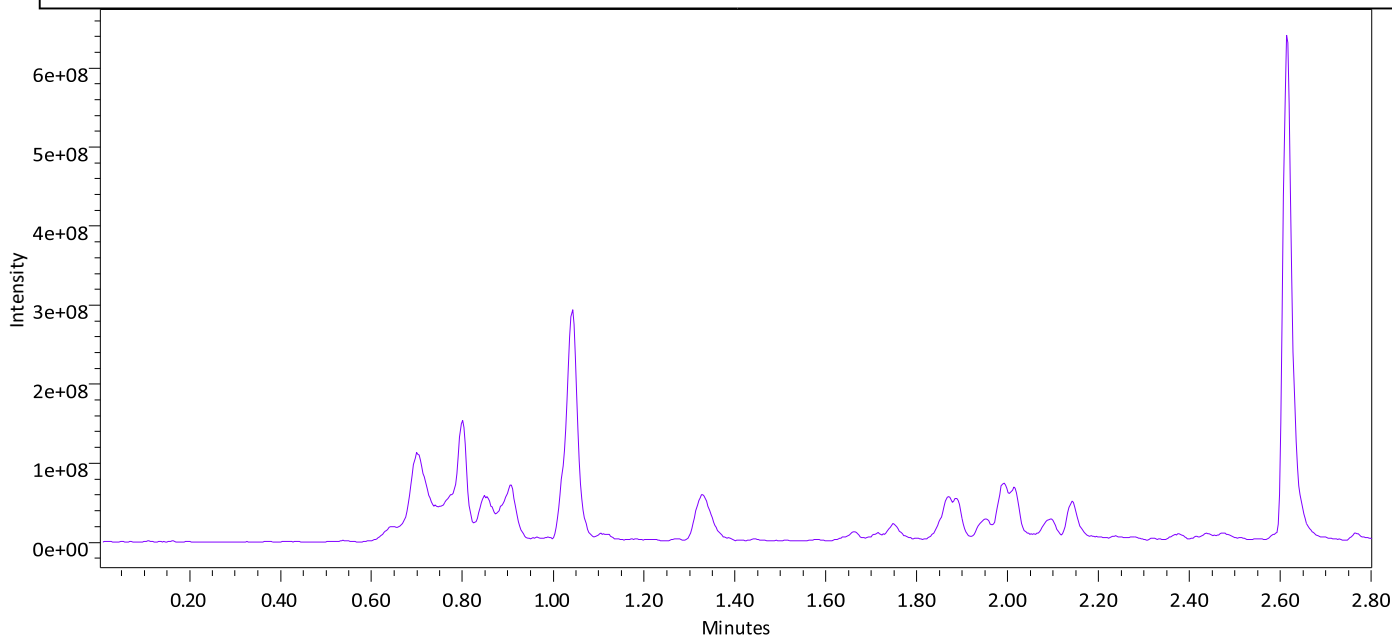
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



E1
2:E,1

4/27/2024 12:49:59 AM PDT

4/27/2024 12:53:07 AM PDT

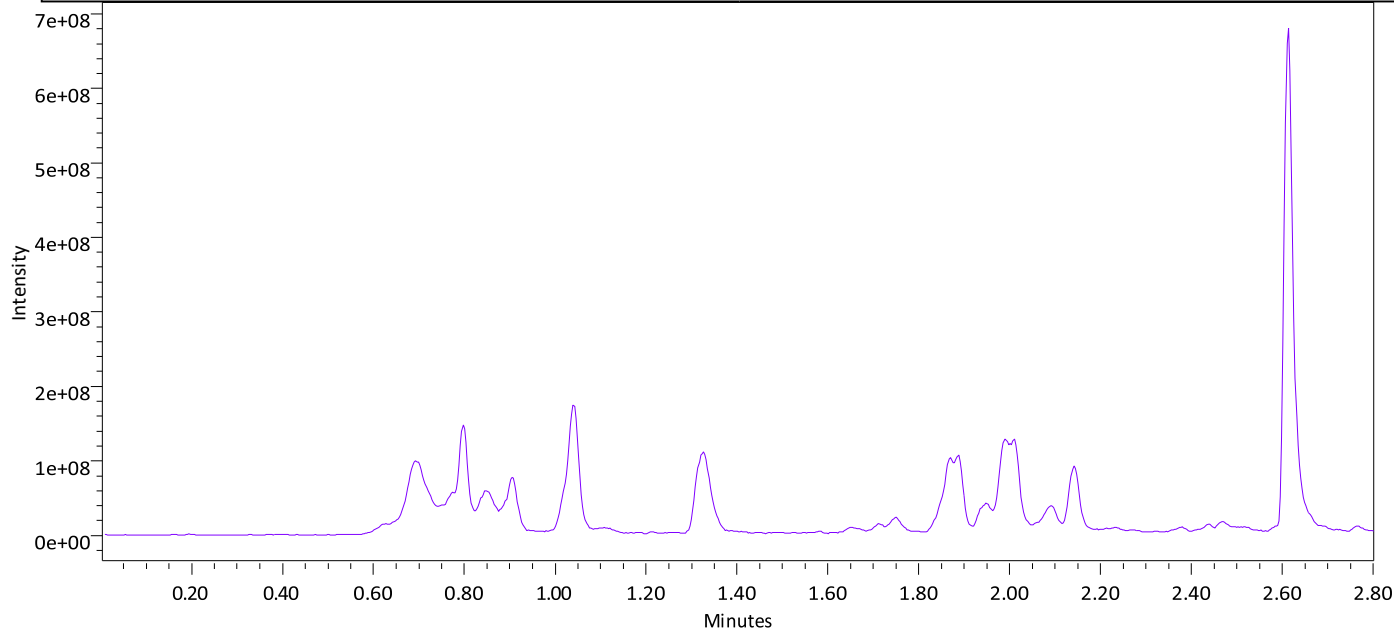
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



E2
2:E,2

4/27/2024 12:53:41 AM PDT
4/27/2024 12:56:47 AM PDT

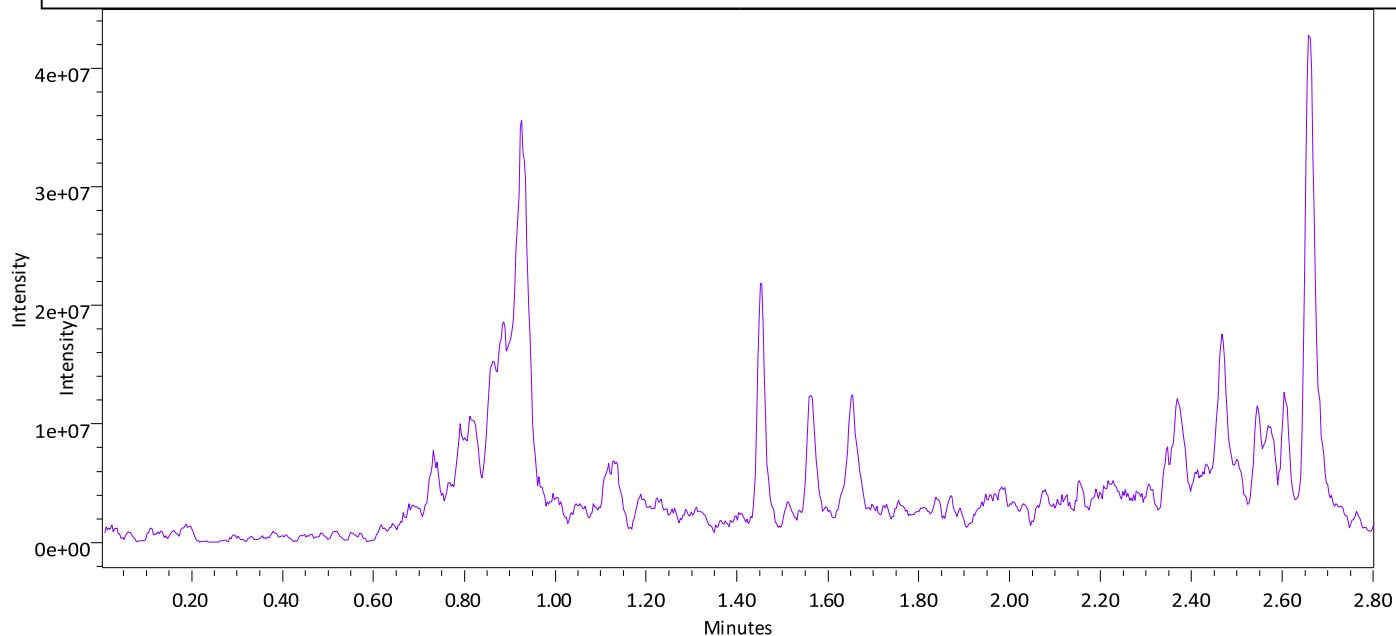
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 804.40

E3
2:E,3

4/27/2024 12:57:22 AM PDT

4/27/2024 1:00:29 AM PDT

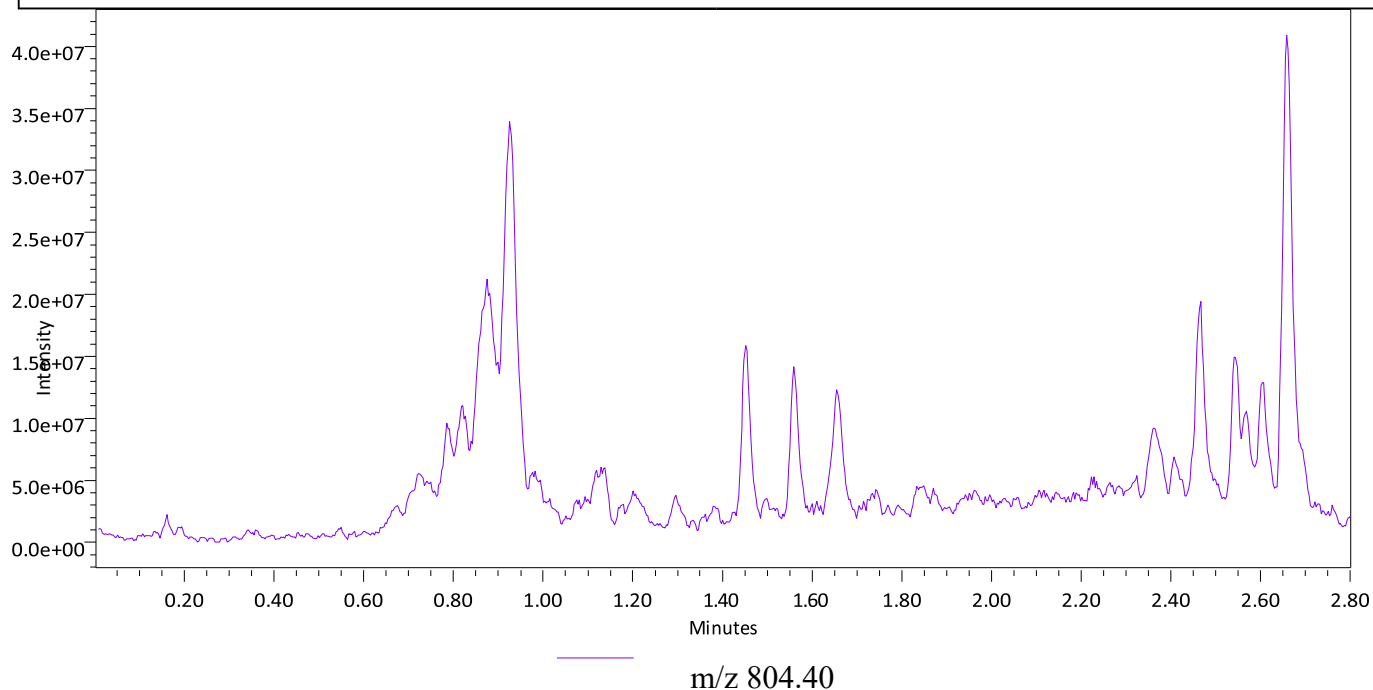
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



E4
2:E,4

4/27/2024 1:01:04 AM PDT
4/27/2024 1:04:10 AM PDT

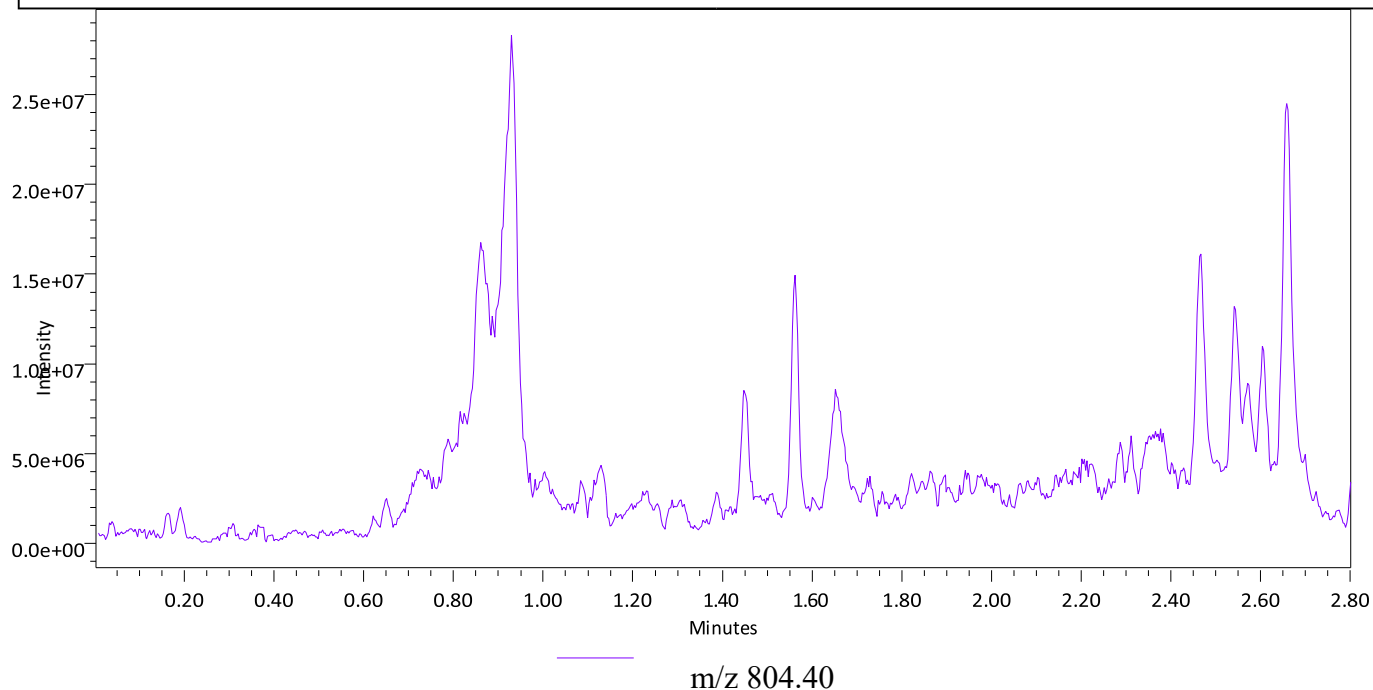
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



E5
2:E,5

4/27/2024 1:04:45 AM PDT
4/27/2024 1:07:50 AM PDT

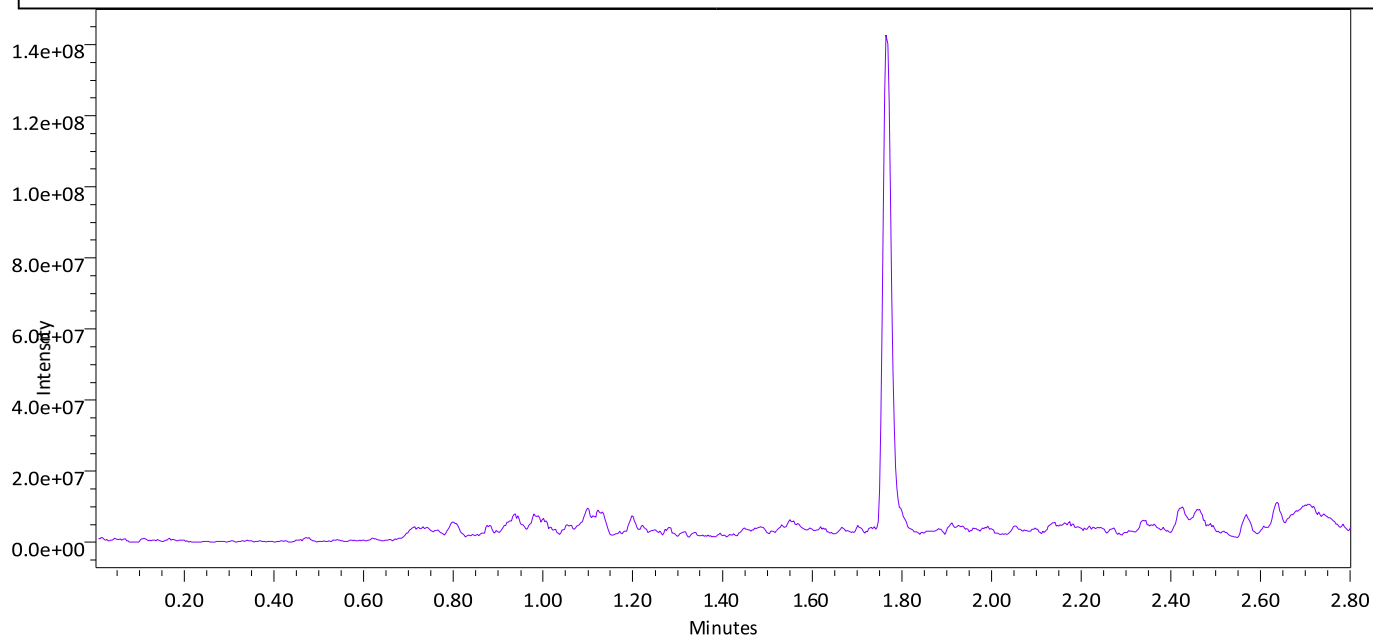
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 823.40

E6
2:E,6

4/27/2024 1:08:24 AM PDT

4/27/2024 1:11:33 AM PDT

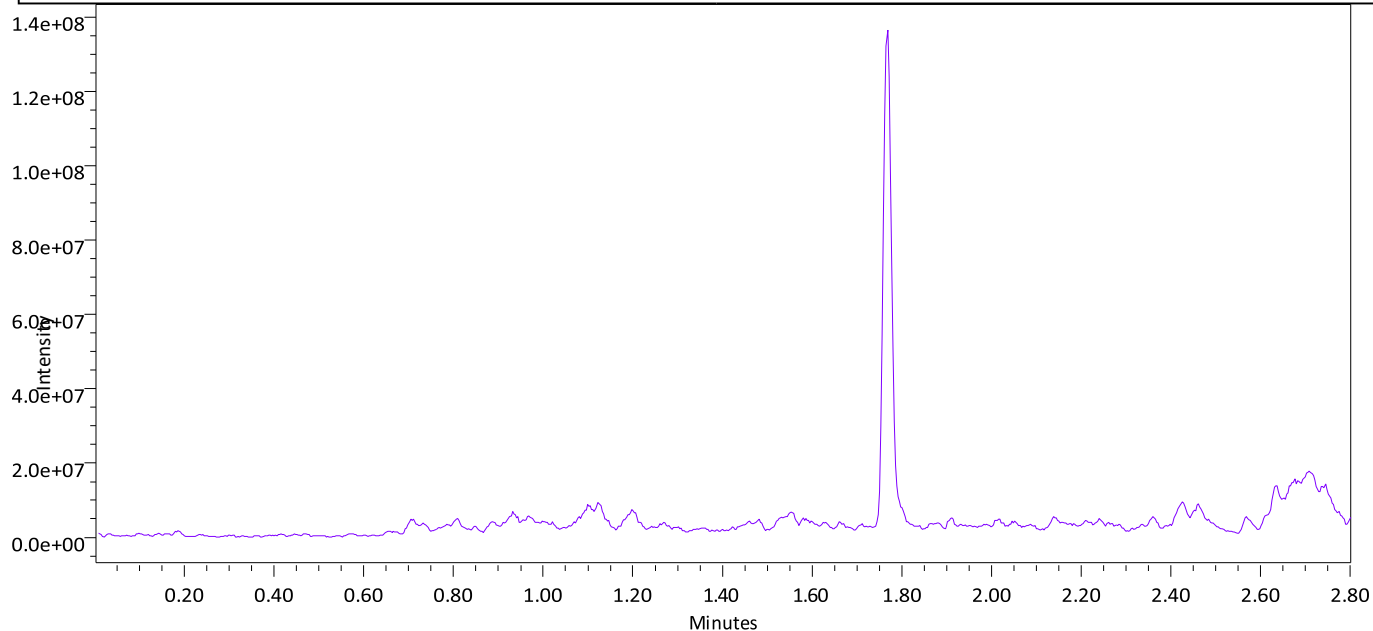
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 823.40

E7
2:E,7

4/27/2024 1:12:08 AM PDT

4/27/2024 1:15:16 AM PDT

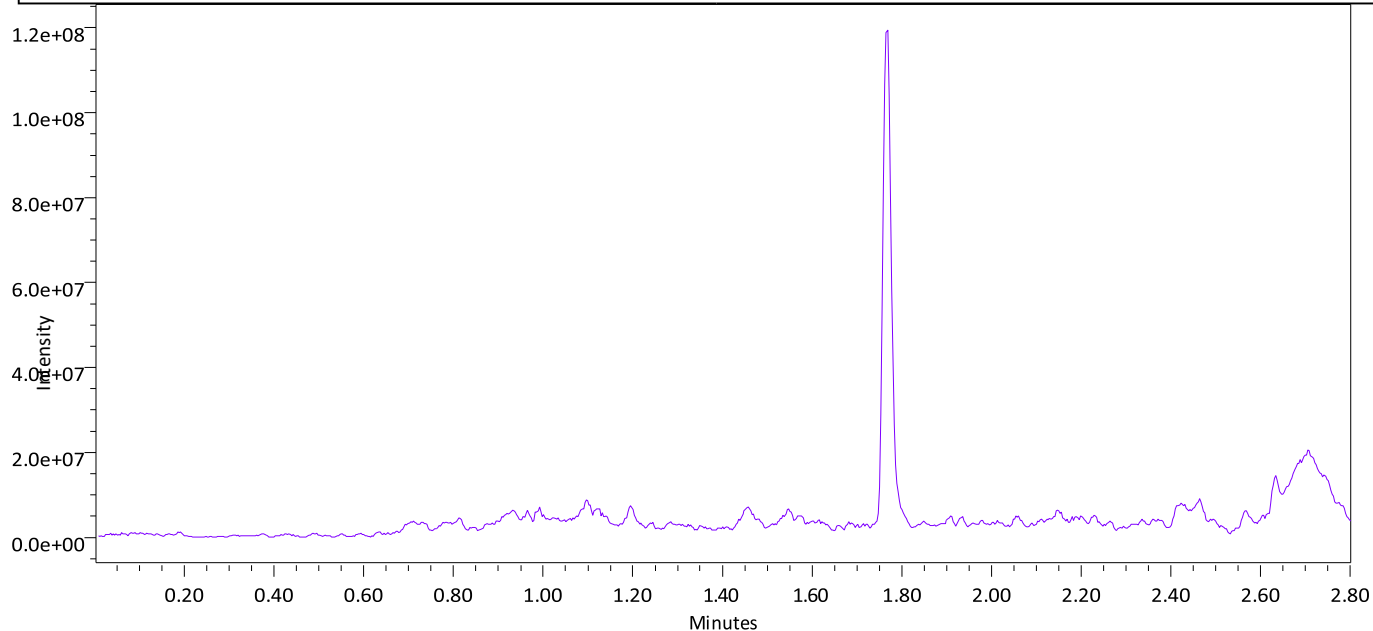
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 823.40

E8
2:E,8

4/27/2024 1:15:52 AM PDT

4/27/2024 1:19:01 AM PDT

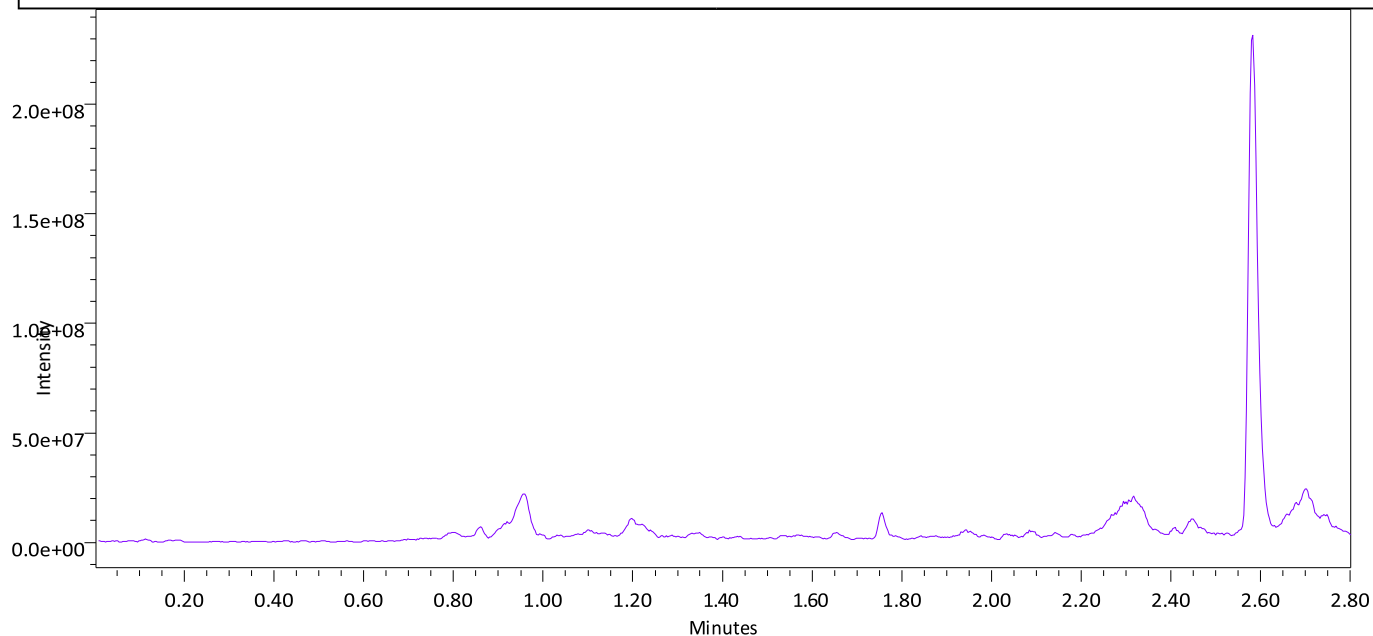
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 899.30

E9
2:E,9

4/27/2024 1:19:35 AM PDT

4/27/2024 1:22:43 AM PDT

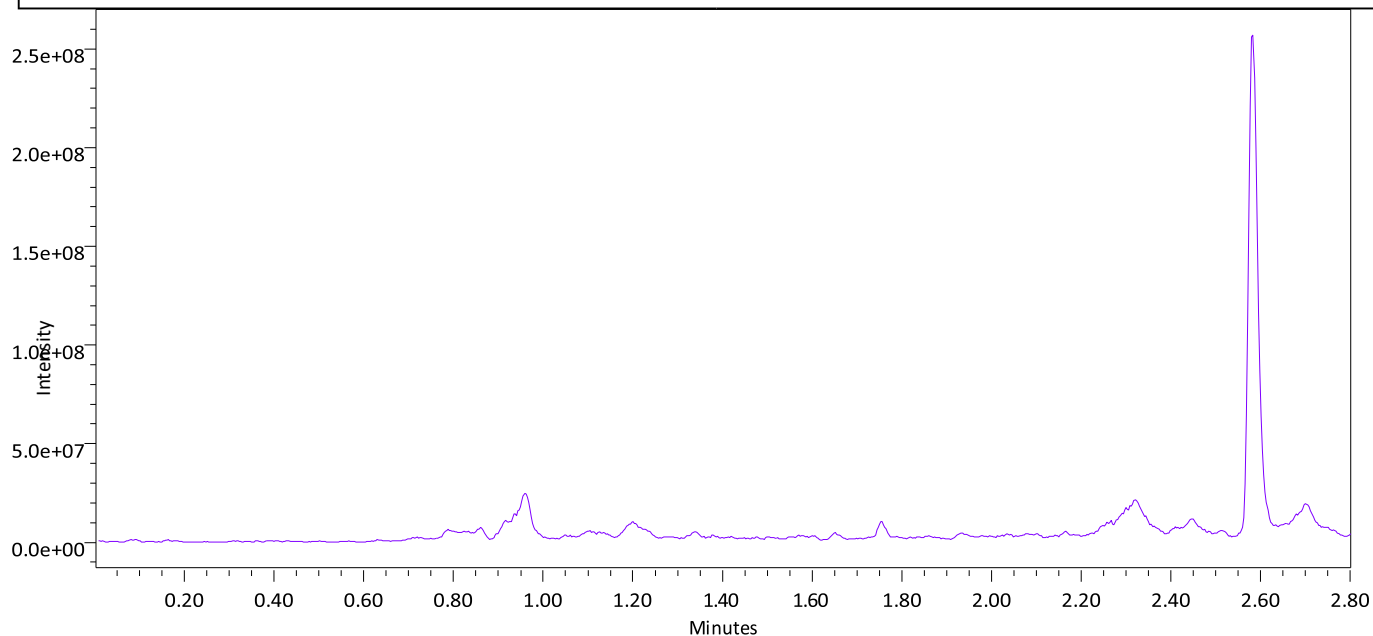
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



m/z 899.30

E10
2:E,10

4/27/2024 1:23:19 AM PDT

4/27/2024 1:26:24 AM PDT

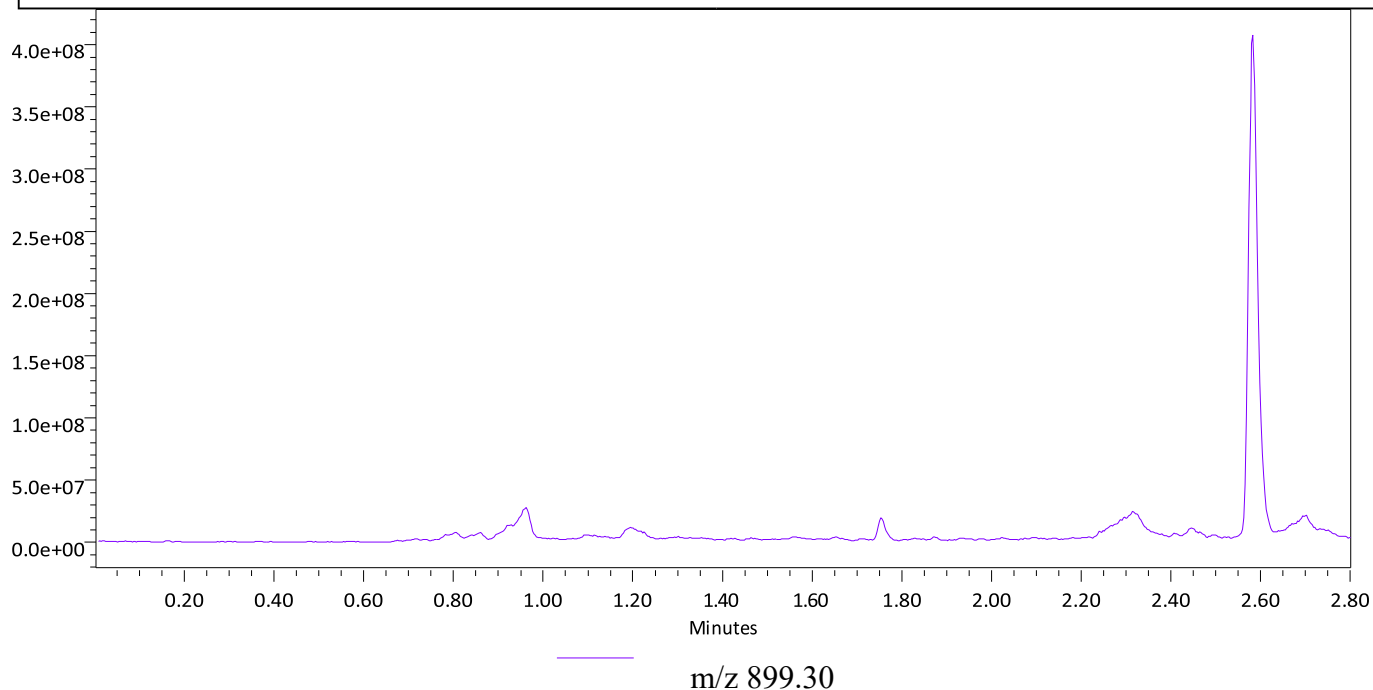
Overlaid XICs

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Supplementary Data 5 | Mass Spectrometry Data for Main Text Figure 6.

#	Macrocycle	ncAA	Mass	Cyclized Mass +OMe+Me+H
1	C(AGGA)(AGGA)FVY	3-methoxy-L-phenylalanine	866.37	867.37
2	C(AGGA)L(AGGA)VY	3-methoxy-L-phenylalanine	832.38	833.38

Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

3	C[AGGA]LF[AGGA]Y	3-methoxy-L-phenylalanine	880.38	881.38
4	CL[AGGA][AGGA]VY	3-methoxy-L-phenylalanine	832.38	833.38
5	CL(AGGA)F(AGGA)Y	3-methoxy-L-phenylalanine	880.38	881.38
6	CLL(AGGA)(AGGA)Y	3-methoxy-L-phenylalanine	846.4	847.4
7	C(AGGA)(AGGA)F(AGGA)Y	3-methoxy-L-phenylalanine	944.38	945.38

Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

8	C(AGGA)(AGGA)FV(AGGA)	3-methoxy-L-phenylalanine	894.4	895.4
9	C(AGGA)LF(CGGA)Y	3-methyl-L-histidine 6-methyl-L-tryptophan	877.39	878.39
10	CL(AGGA)F(CGGA)Y	3-methyl-L-histidine 6-methyl-L-tryptophan	877.39	878.39

Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

11	CLL(AGGA)(CGGA)Y	3-methyl-L-histidine 6-methyl-L-tryptophan	843.41	844.41
12	C(AGGA)(CGGA)FVY	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	889.38	890.411

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

13	C(AGGA)L(CGGA)VY	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	855.4	856.421
14	C(AGGA)LF(CGGA)Y	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	903.4	904.421

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

15	CL(AGGA)F(CGGA)Y	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	903.4	904.421
16	CL(AGGA)FV(CGGA)	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	839.4	840.431

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

17	CLL(AGGA)(CGGA)Y	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	869.41	870.441
18	CLL(AGGA)V(CGGA)	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	805.42	806.441

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

19	C(CGGA)(AGGA)FVY	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	889.38	890.411
20	C(CGGA)L(AGGA)VY	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	855.4	856.421

Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

21	CL(CGGA)(AGGA)VY	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	855.4	856.421
22	CL(CGGA)F(AGGA)Y	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	903.4	904.421

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

23	CL(CGGA)FV(AGGA)	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan	839.4	840.431
24	C(AGGA)(UAGA)FVY	3-methoxy-L-phenylalanine N6-alloc-L-lysine	901.4	902.481

Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

4/27/2024

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

25	C(AGGA)LF(UAGA)Y	3-methoxy-L-phenylalanine N6-alloc-L-lysine	915.42	916.501
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2
Date Acquired:
Date Processed:

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

26	CL(AGGA)(UAGA)VY	3-methoxy-L-phenylalanine N6-alloc-L-lysine	867.42	868.501
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

27	CL(AGGA)F(UAGA)Y	3-methoxy-L-phenylalanine N6-alloc-L-lysine	915.42	916.501
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T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

28	CL(AGGA)FV(UAGA)	3-methoxy-L-phenylalanine N6-alloc-L-lysine	851.43	852.501
29	CLL(AGGA)(UAGA)Y	3-methoxy-L-phenylalanine N6-alloc-L-lysine	881.41	882.511

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2
Date Acquired:
Date Processed:

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

30	CLLF(AGGA)(UAGA)	3-methoxy-L-phenylalanine N6-alloc-L-lysine	865.44	866.521
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

31	C(AGGA)L(CGGA)VY	3-methoxy-L-phenylalanine 3-(1-naphthyl)-L-alanine	852.39	853.411
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T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

32	CL(AGGA)(CGGA)VY	3-methoxy-L-phenylalanine 3-(1-naphthyl)-L-alanine	852.39	853.411
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2
Date Acquired:
Date Processed:

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

33	CL(AGGA)F(CGGA)Y	3-methoxy-L-phenylalanine 3-(1-naphthyl)-L-alanine	900.39	901.411
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T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

34	C(CGGA)L(AGGA)VY	3-methoxy-L-phenylalanine 3-(1-naphthyl)-L-alanine	852.39	853.411
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T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

35	CL(CGGA)(AGGA)VY	3-methoxy-L-phenylalanine 3-(1-naphthyl)-L-alanine	852.39	853.411
36	CLL(AGGA)(CGGA)Y	3-methoxy-L-phenylalanine 3-benzothienyl-L-alanine	872.36	873.391

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

37	CL(CGGA)(AGGA)VY	3-methoxy-L-phenylalanine 3-benzothienyl-L-alanine	858.43	859.371
38	CL(CGGA)F(AGGA)Y	3-methoxy-L-phenylalanine 3-benzothienyl-L-alanine	906.34	907.371

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2
Date Acquired:
Date Processed:

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

39	CL(CGGA)FV(AGGA)	3-methyl-L-histidine 3-(1-naphthyl)-L-alanine	810.39	811.39
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

40	CL(AGGA)F(CGGA)Y	3-methyl-L-histidine 3-benzothienyl-L-alanine	880.34	881.34
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T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

41	CLL(AGGA)(CGGA)Y	3-methyl-L-histidine 3-benzothienyl-L-alanine	846.36	847.36
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T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

42	C(AGGA)(UAGA)FVY	3-cyano-L-phenylalanine N6-alloc-L-lysine	896.39	897.45
43	C(AGGA)LF(UAGA)Y	3-cyano-L-phenylalanine N6-alloc-L-lysine	910.4	911.47

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

44	CL(AGGA)FV(UAGA)	3-cyano-L-phenylalanine N6-alloc-L-lysine	846.41	847.47
45	CLLF(AGGA)(UAGA)	3-cyano-L-phenylalanine N6-alloc-L-lysine	860.43	861.49

T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

46	C(AGGA)(CGGA)F(UAGA)Y	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan N6-alloc-L-lysine	1002.4	1003.501
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T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

47	C(AGGA)LF(CGGA)(UAGA)	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan N6-alloc-L-lysine	952.45	953.521
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

48	CL(AGGA)(CGGA)(UAGA)Y	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan N6-alloc-L-lysine	968.45	969.521
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T A R G E T M A S S A N A L Y S I S

Sample Name:	D2	Acq. Method Set:	G1 AAA0292
Vial:	2:D,2	Processing Method:	G1 AAA0292
Date Acquired:			
Date Processed:			

49	CL(AGGA)F(CGGA)(UAGA)	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan N6-alloc-L-lysine	952.45	953.521
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

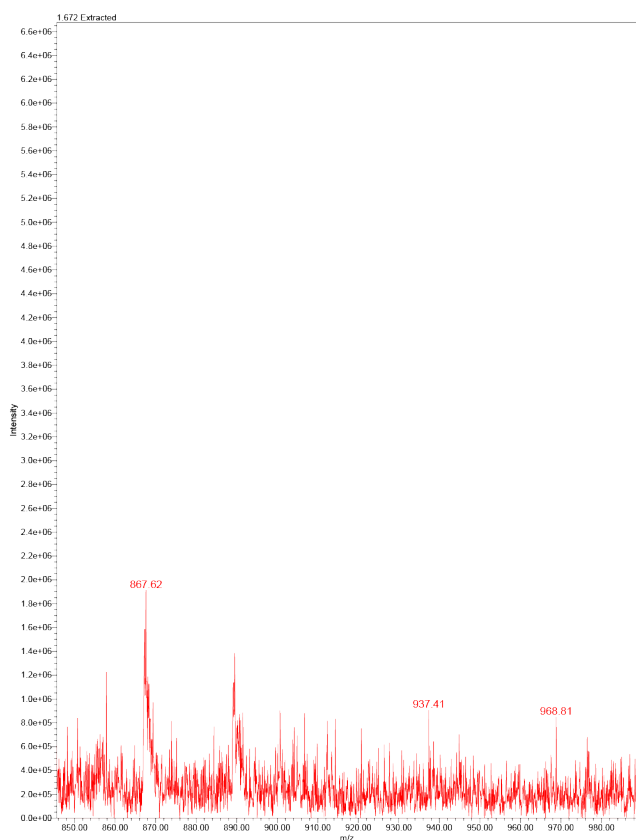
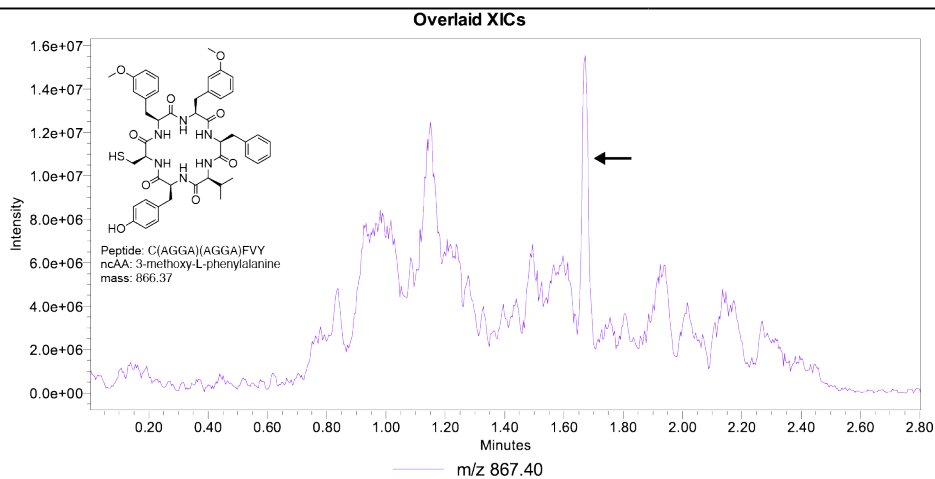
50	C(AGGA)(UAGA)F(CGGA)Y	3-methoxy-L-phenylalanine 6-methyl-L-tryptophan N6-alloc-L-lysine	1002.4	1003.501
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

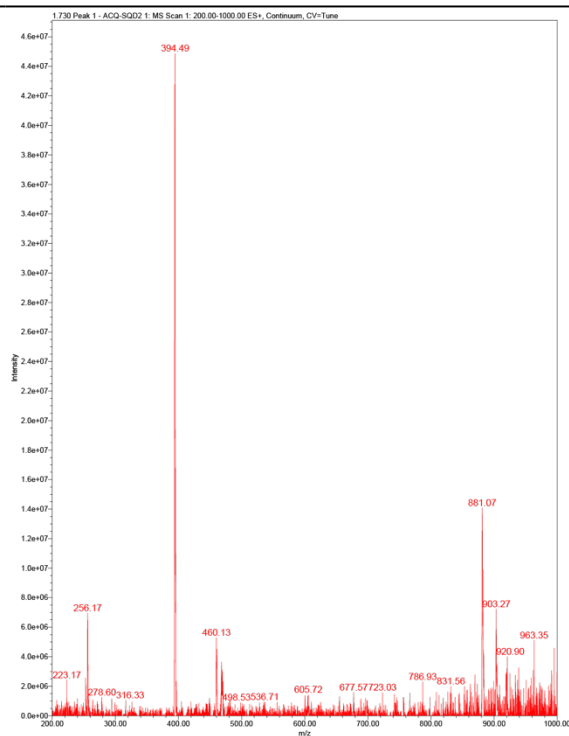
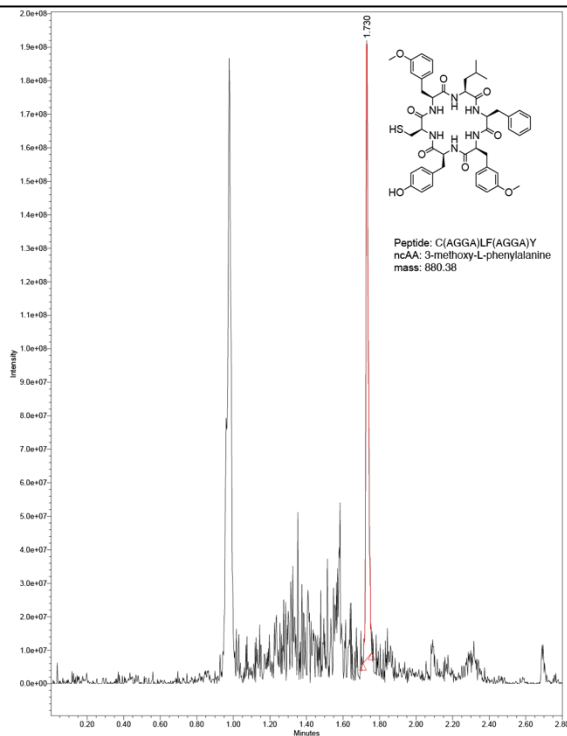


TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

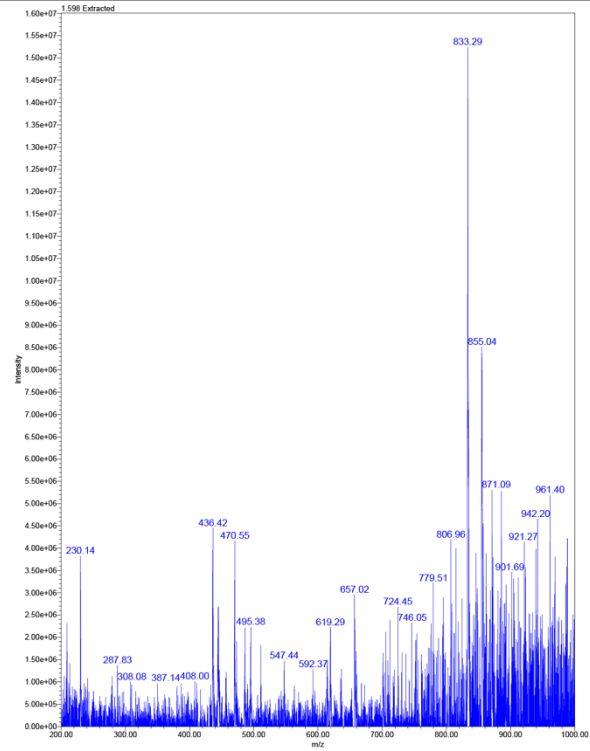
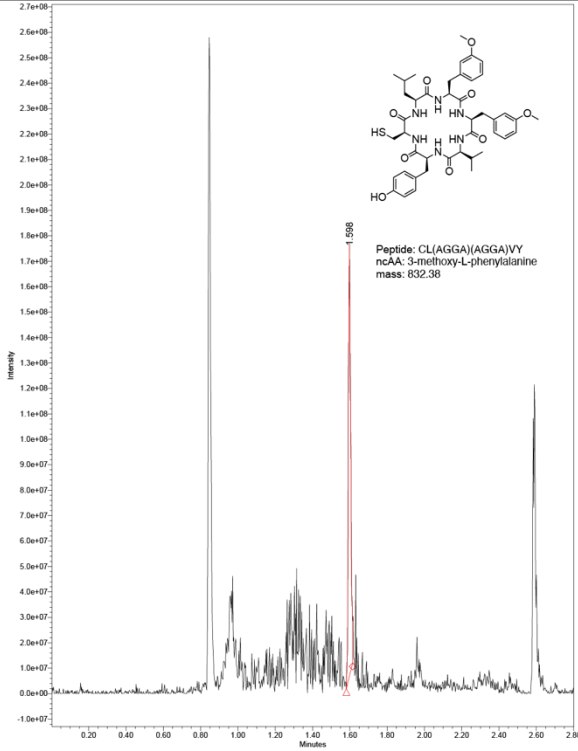


TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



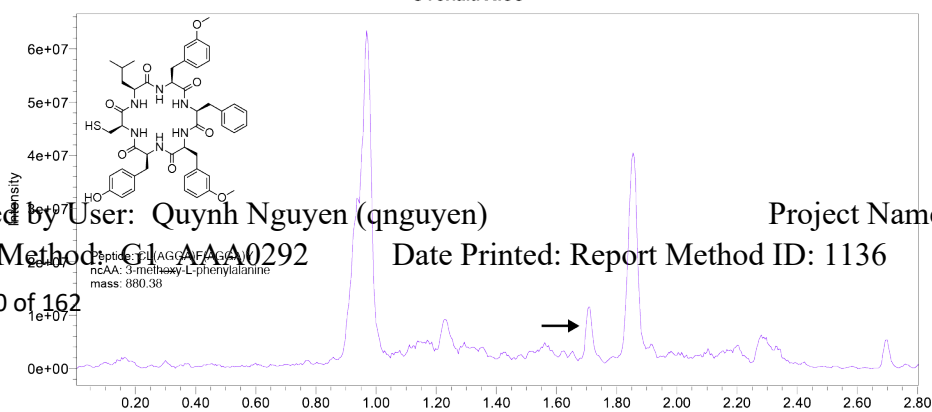
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Project Name: ASF\LCMS-1\2024_Q2

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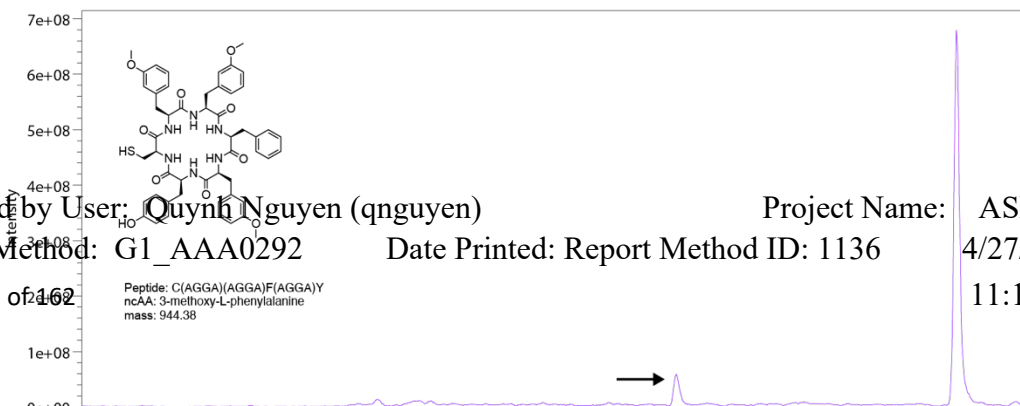
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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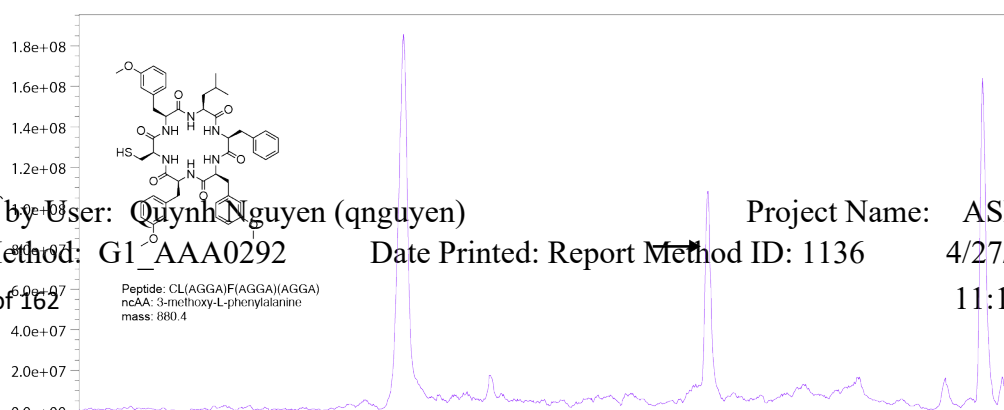
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Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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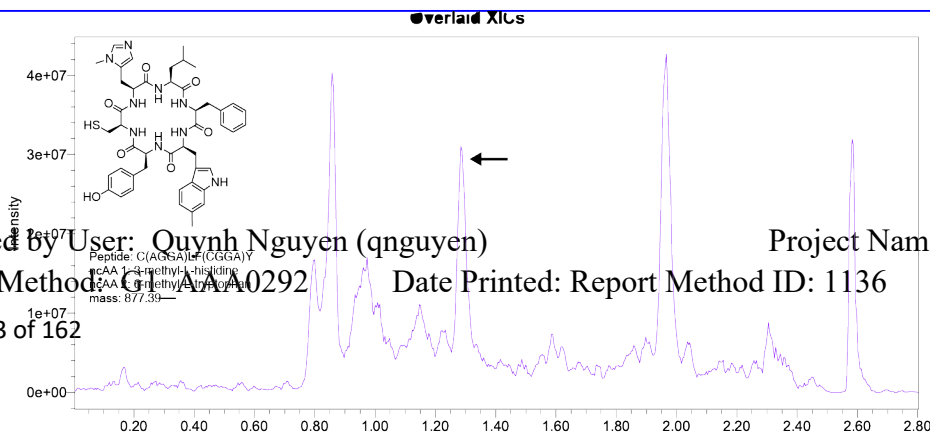
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Project Name: ASF\LCMS-1\2024_Q2

Date Printed: Report Method ID: 1136

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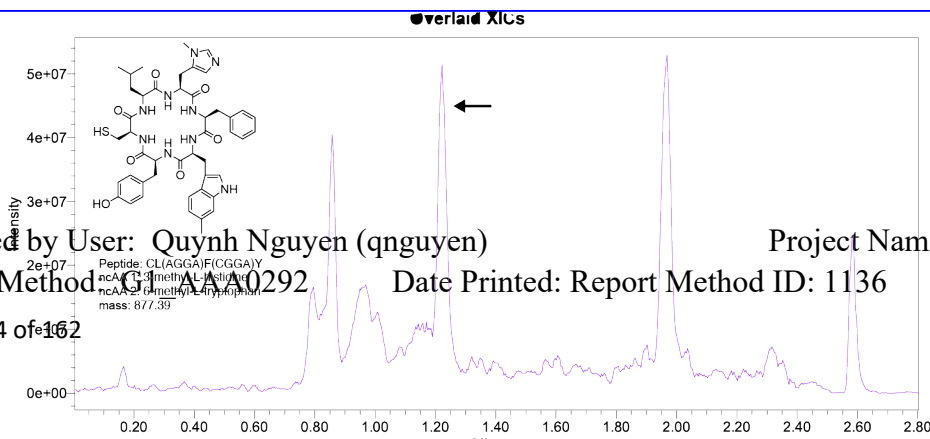
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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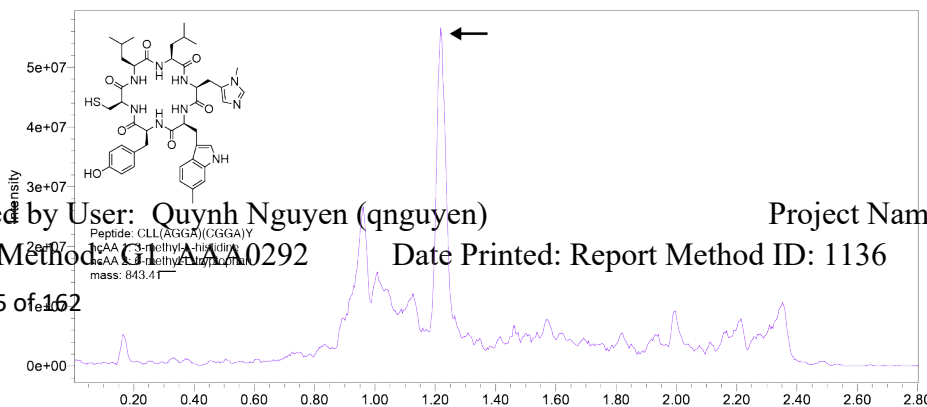
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Project Name: ASF\LCMS-1\2024_Q2

Date Printed: Report Method ID: 1136

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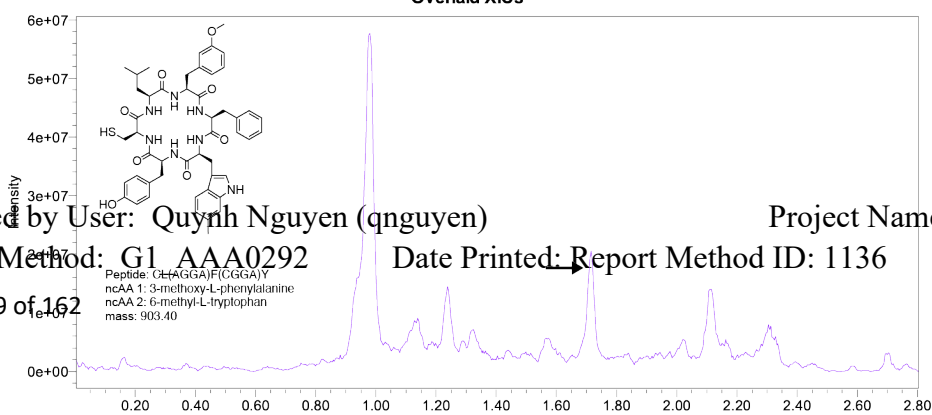
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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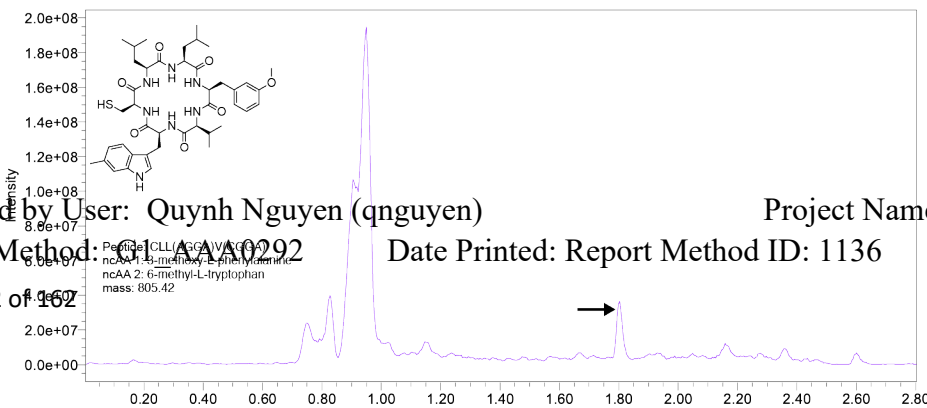
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: AAA0292

Date Printed: Report Method ID: 1136

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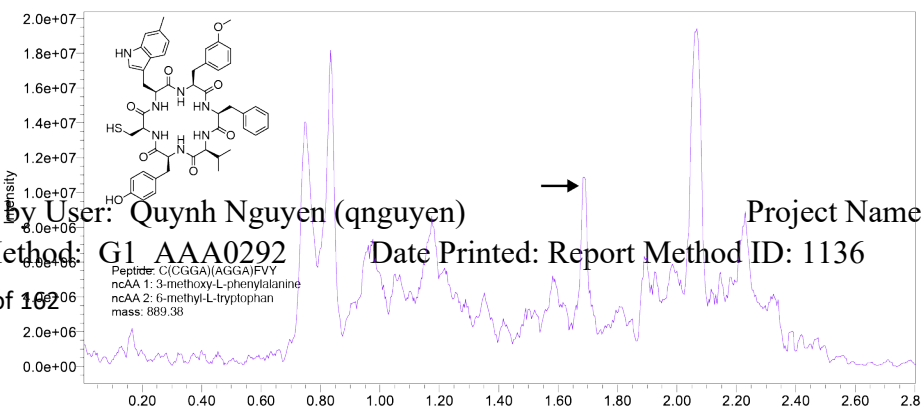
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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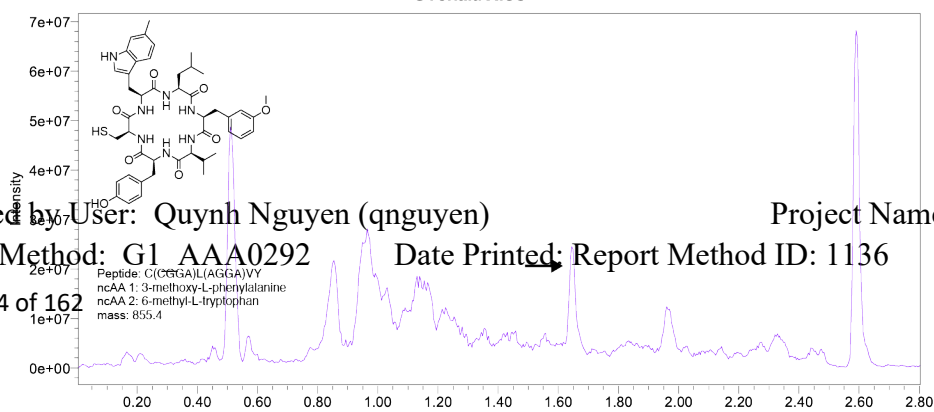
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2
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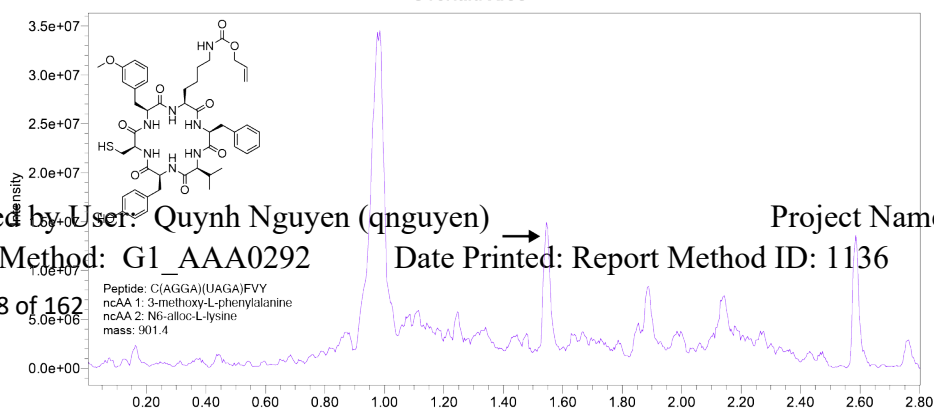
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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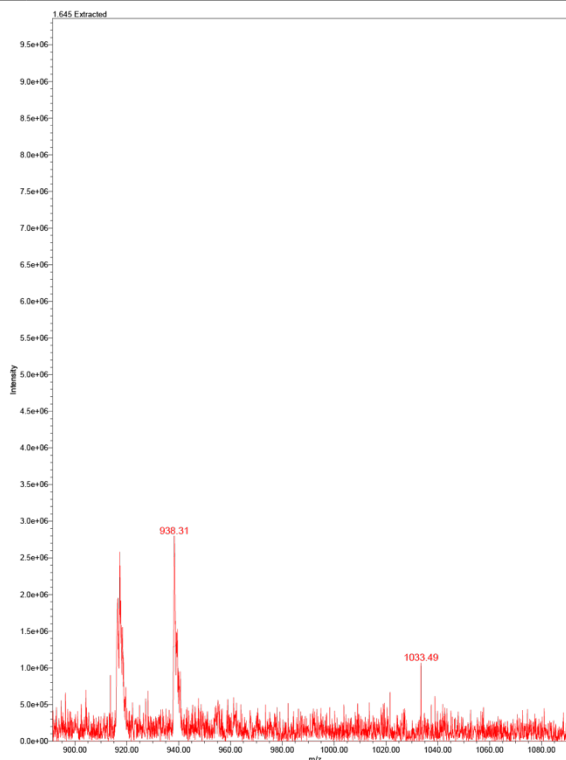
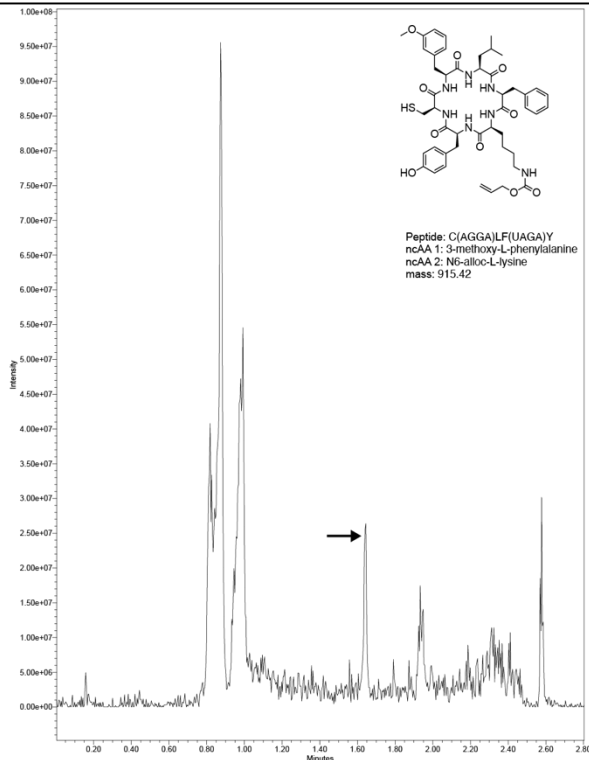
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:



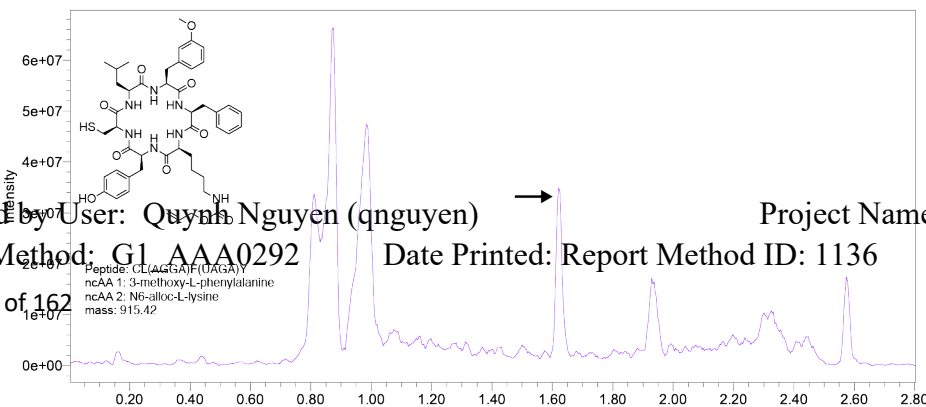
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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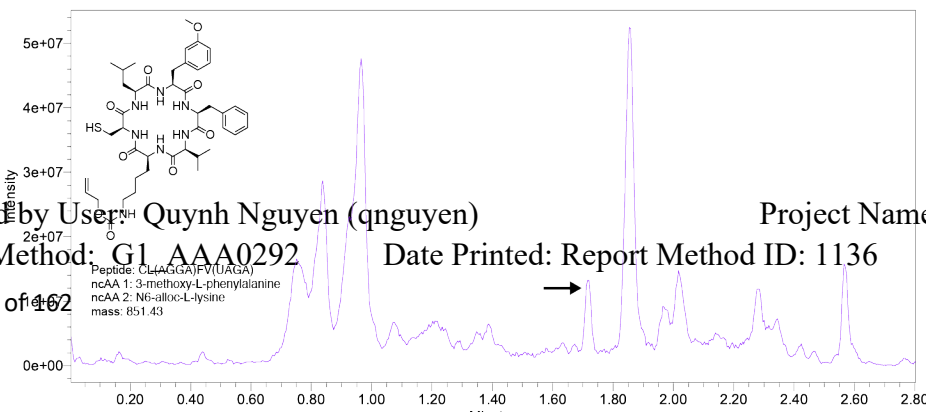
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

4/27/2024

11:14:49 AM US/Pacific

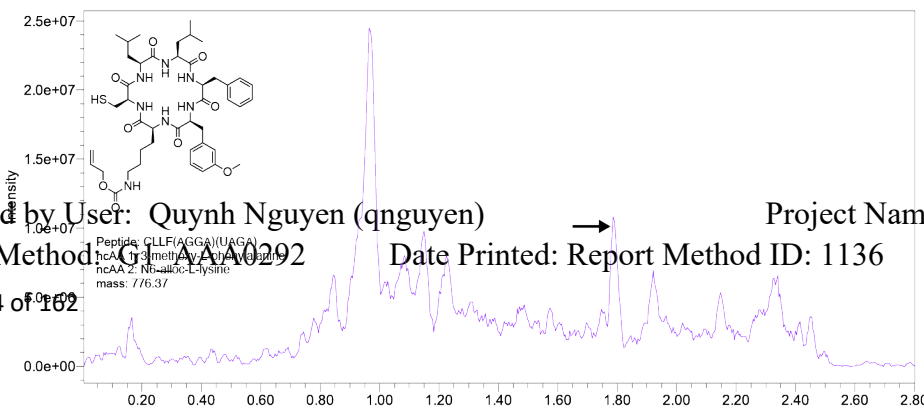
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

4/27/2024

11:14:49 AM US/Pacific

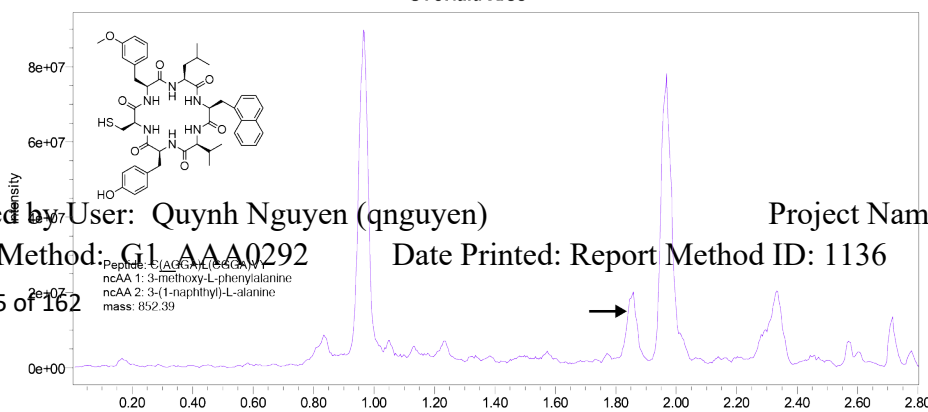
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

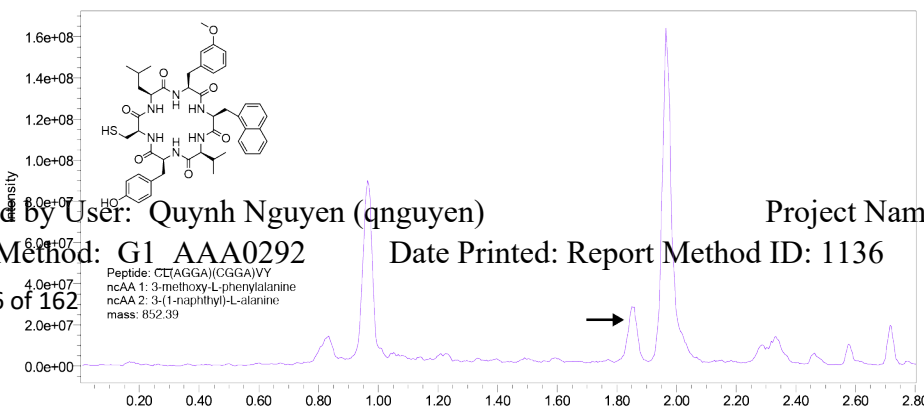
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

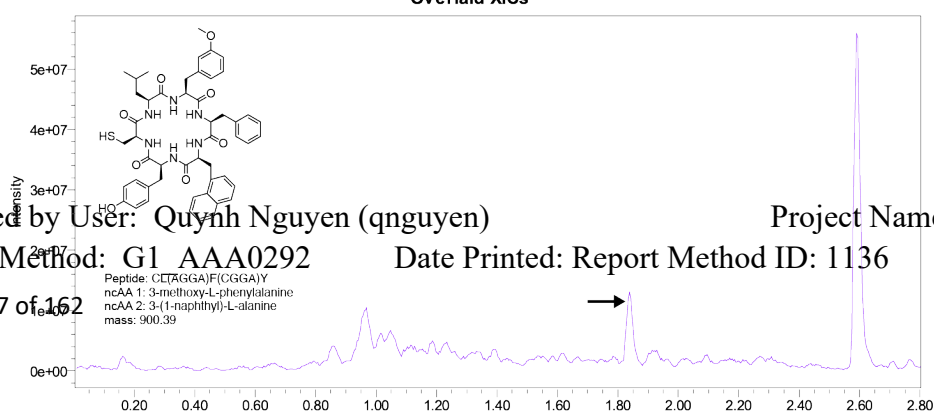
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

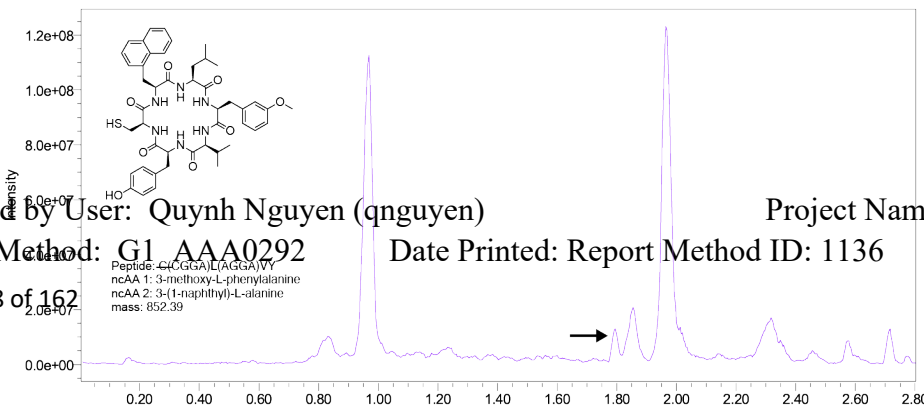
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

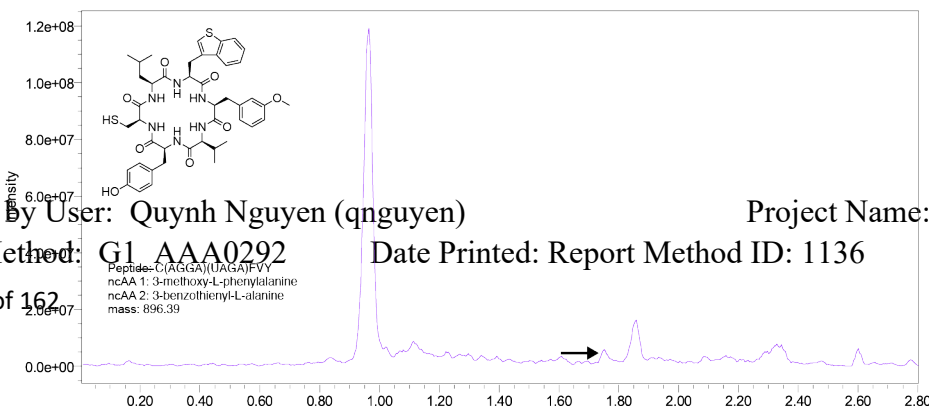
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported By User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

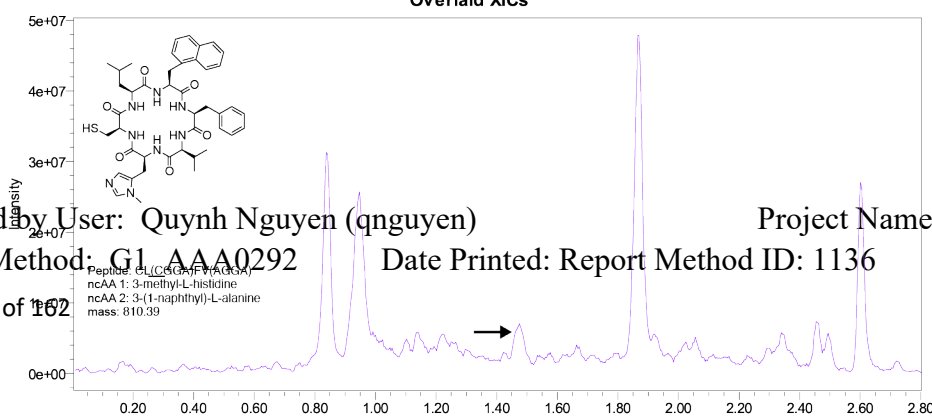
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Project Name: ASF\LCMS-1\2024_Q2

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

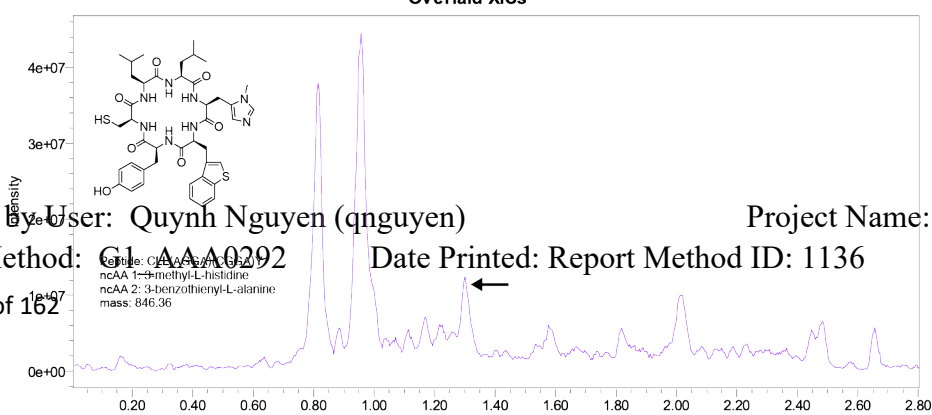
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Project Name: ASF\LCMS-1\2024_Q2

Date Printed: Report Method ID: 1136

4/27/2024

11:14:49 AM US/Pacific

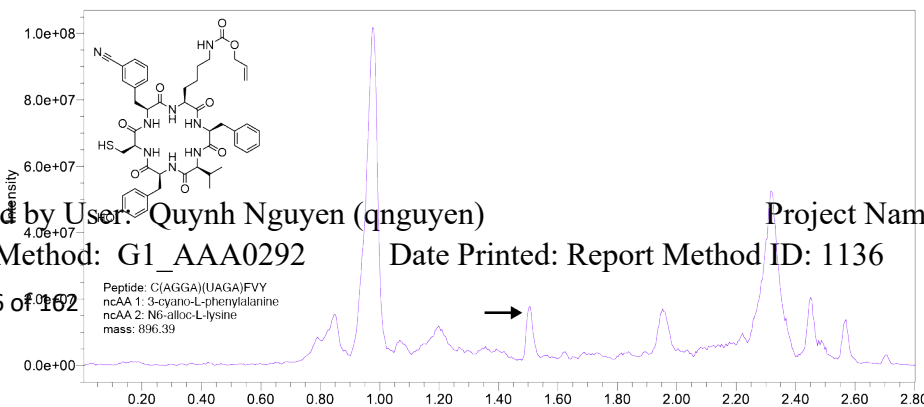
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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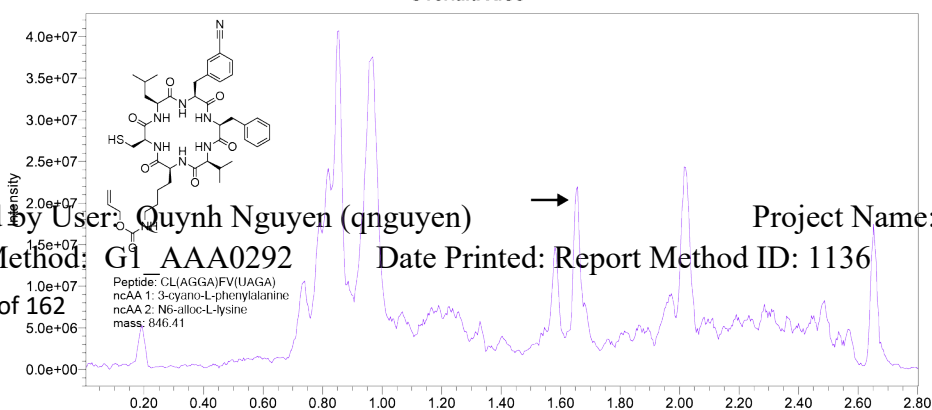
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2
4/27/2024

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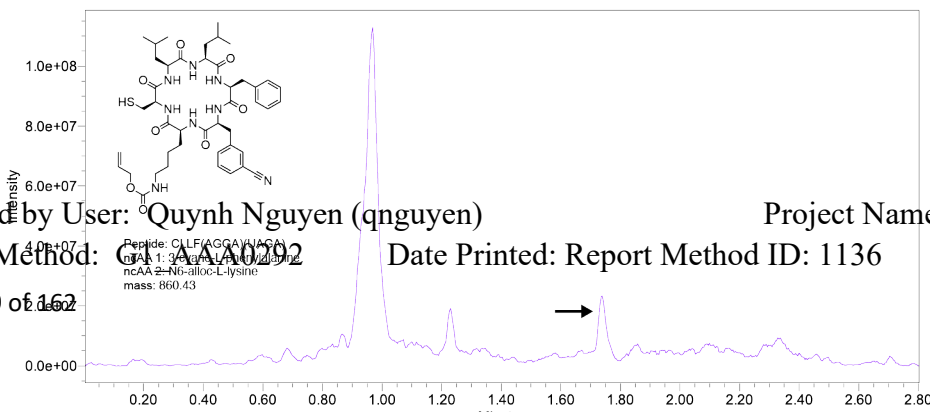
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

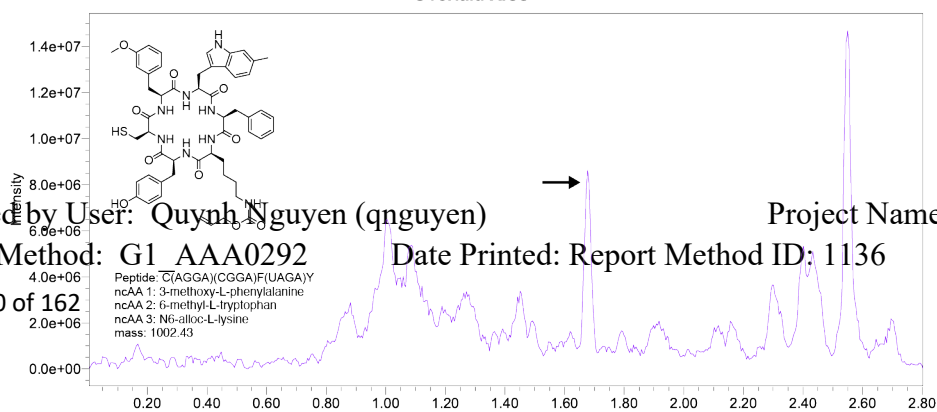
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

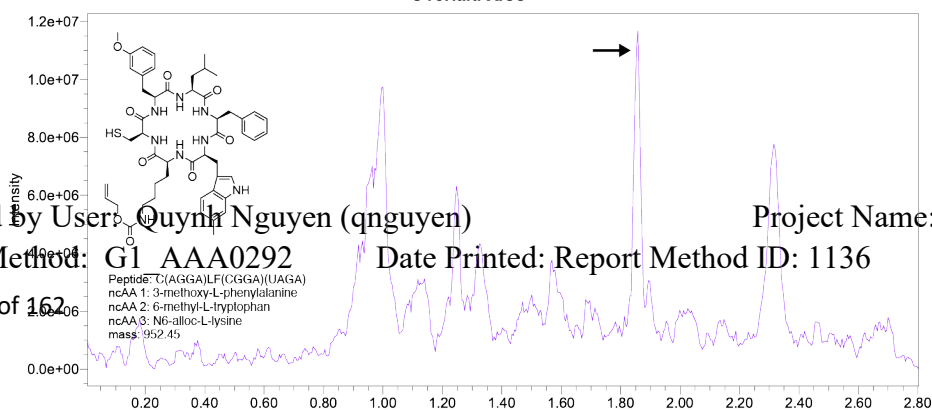
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

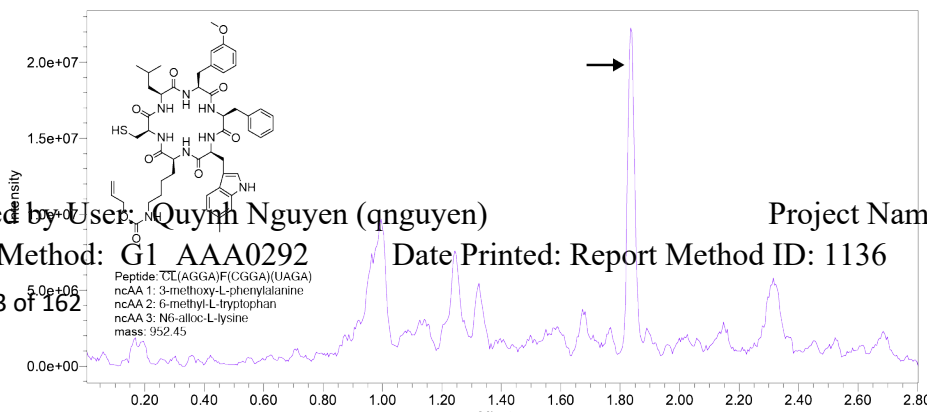
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

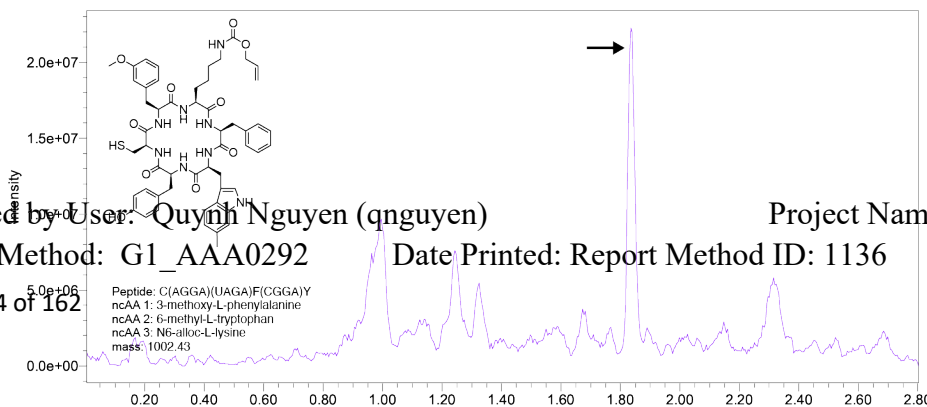
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

4/27/2024

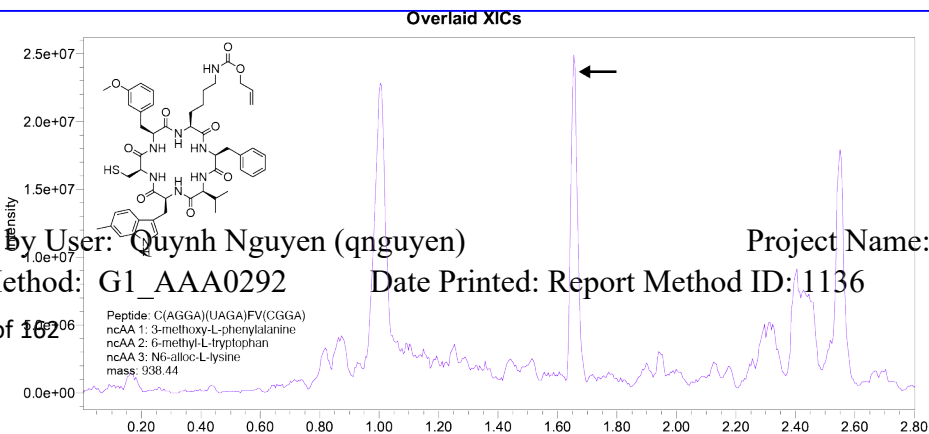
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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

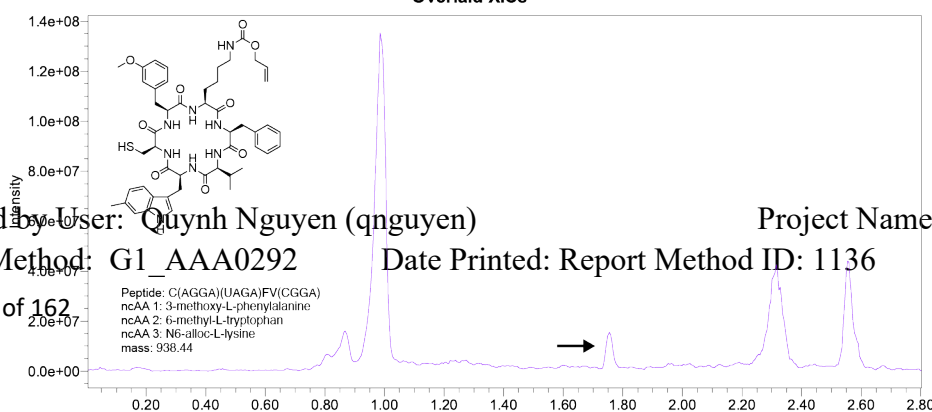
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

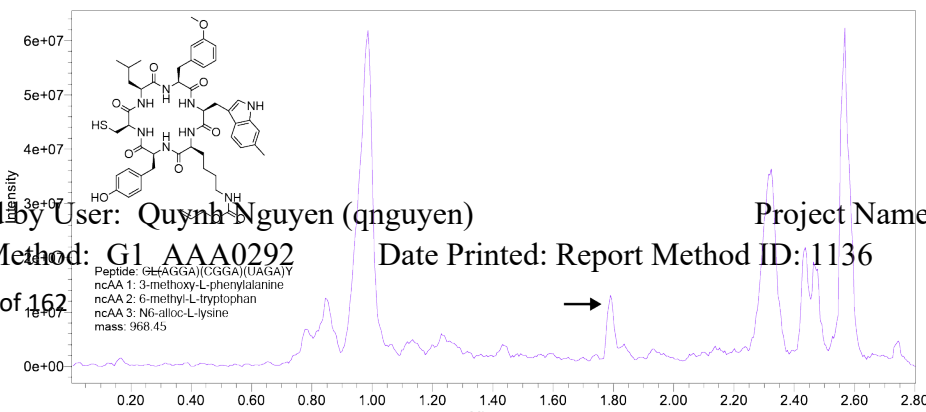
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

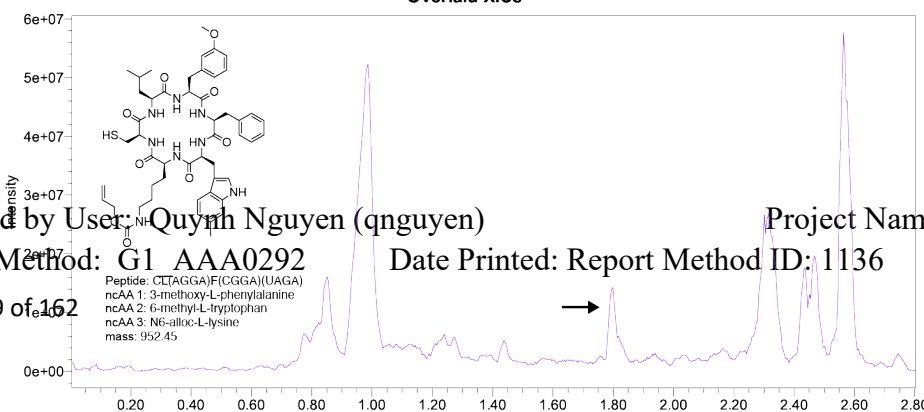
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

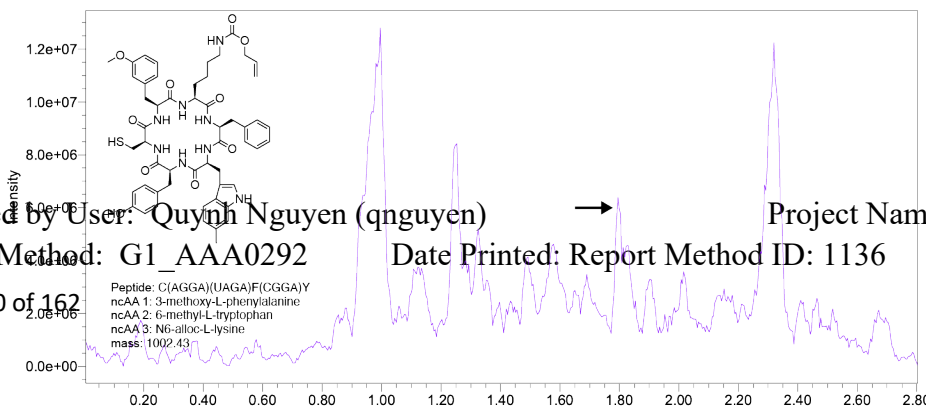
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

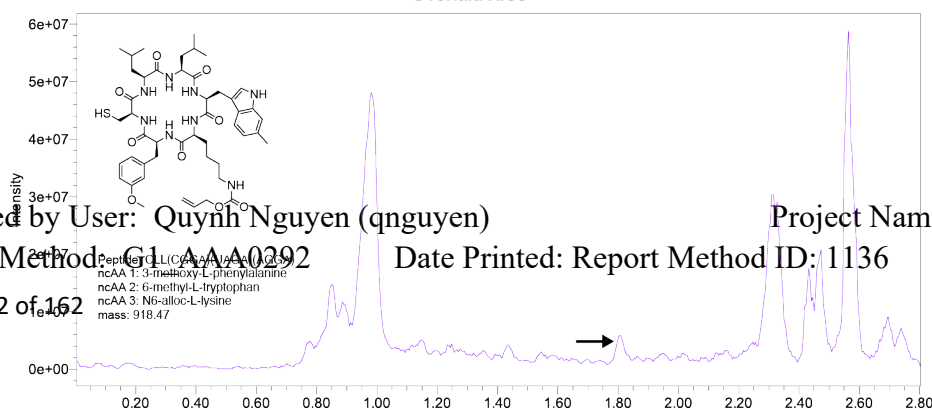
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

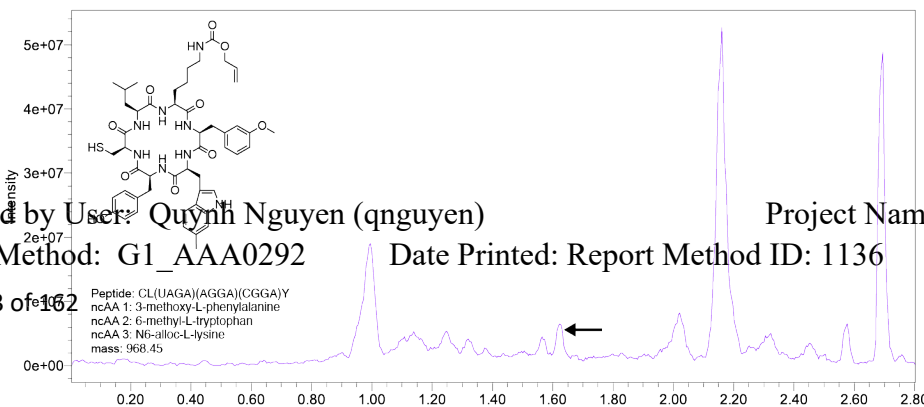
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

4/27/2024

11:14:49 AM US/Pacific

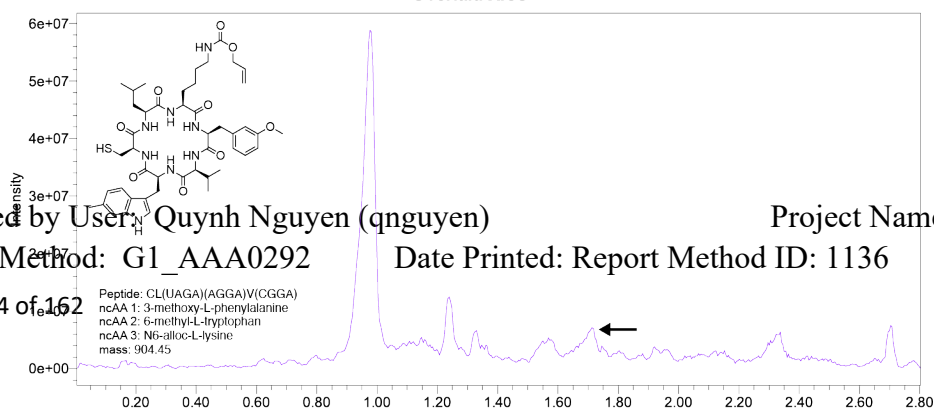
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

4/27/2024

11:14:49 AM US/Pacific

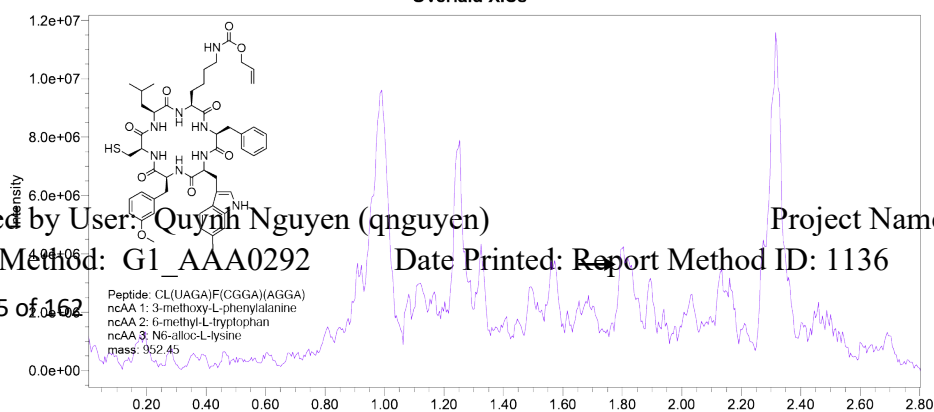
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: QuyDh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

4/27/2024

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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Samples from Main Text Figure 6 regenerated for HRMS analysis. Below is XIC and m/z for these samples:

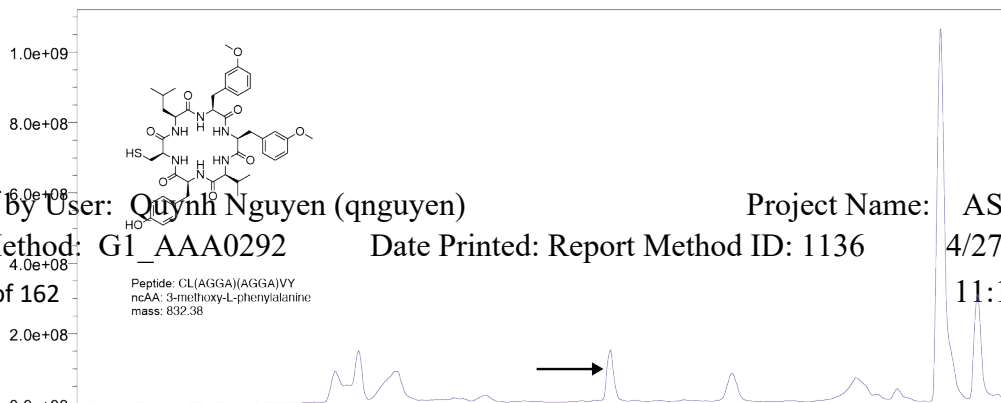
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

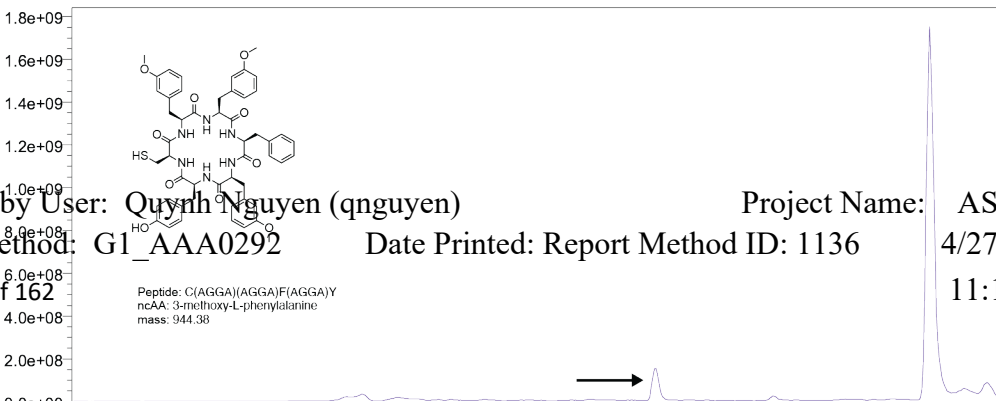
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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11:14:49 AM US/Pacific

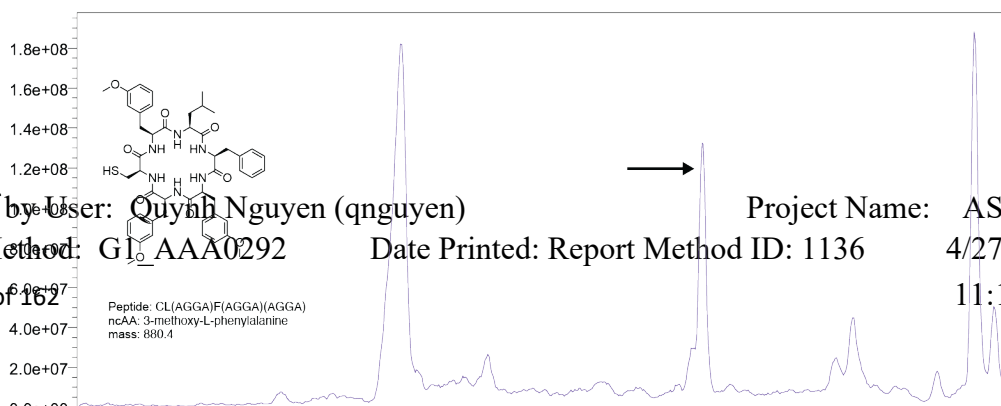
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

Page: 232 of 162

Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

4/27/2024

11:14:49 AM US/Pacific

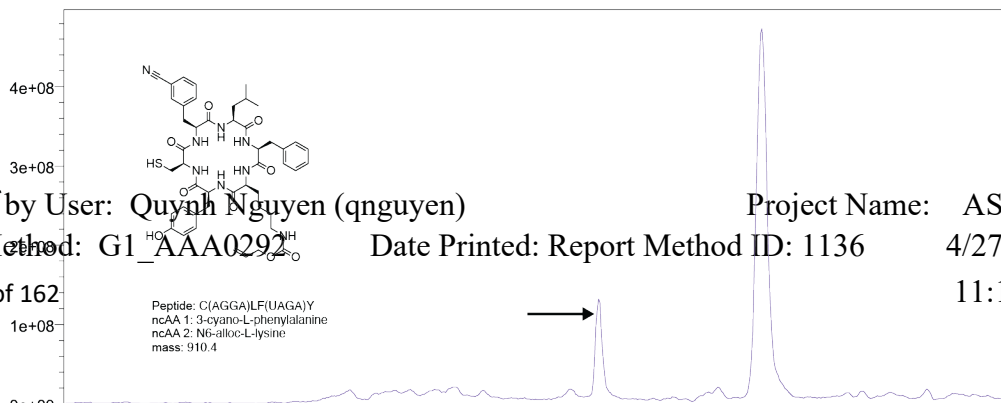
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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Peptide: G(AGGA)LF(UAGA)Y
nCAA 1: 3-cyano-L-phenylalanine
nCAA 2: N6-alloc-L-lysine
mass: 910.4

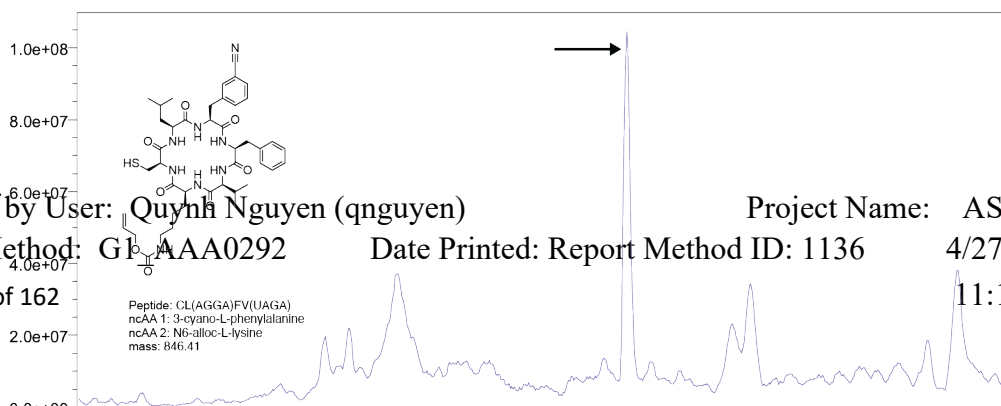
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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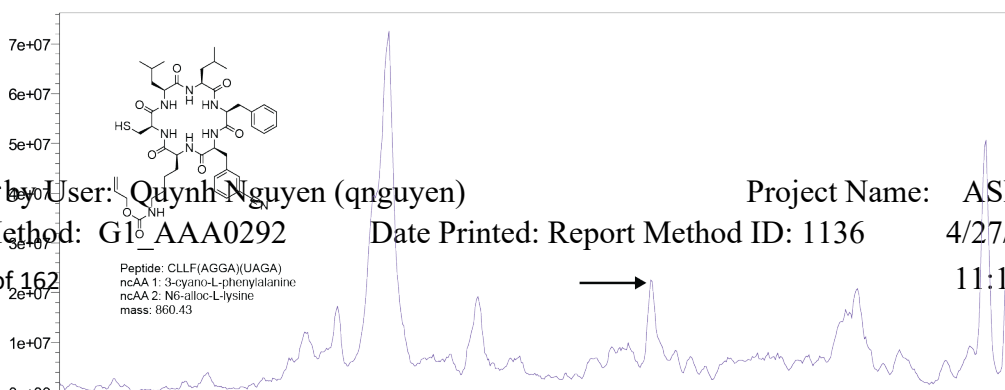
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs Y axis not normalized



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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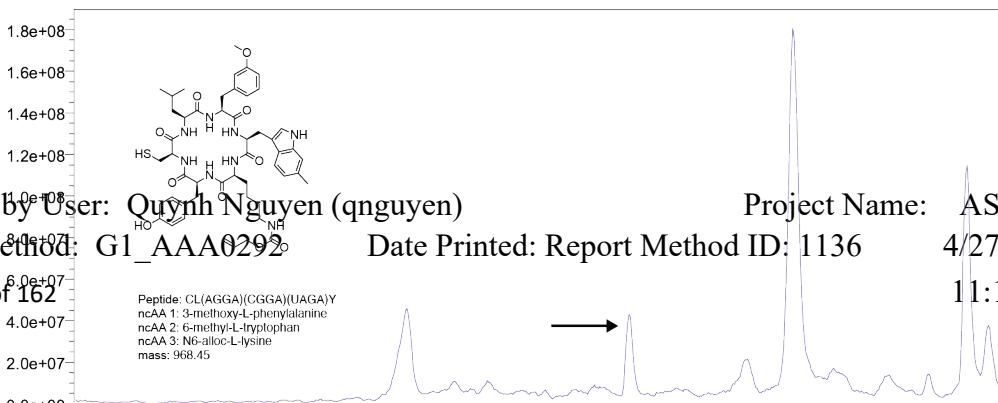
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

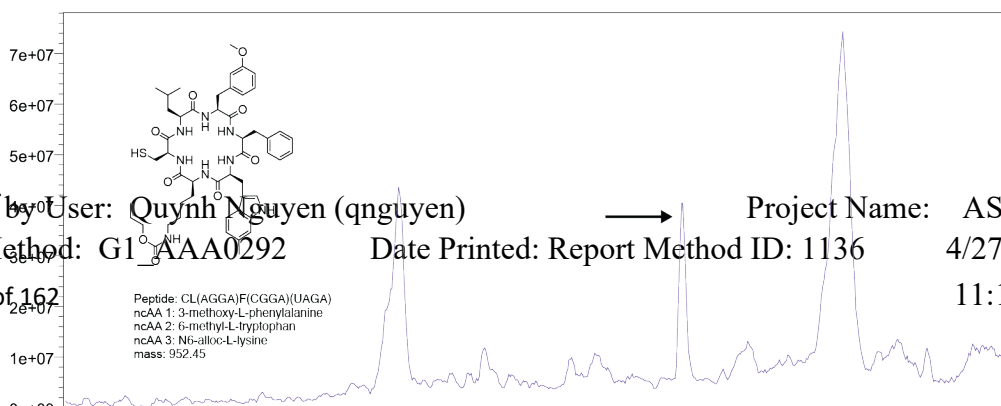
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1_AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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Peptide: CL(AGGA)F(CGGA)(UAGA)
ncAA 1: 3-methoxy-L-phenylalanine
ncAA 2: 6-methyl-L-tryptophan
ncAA 3: N6-allyl-L-lysine
mass: 952.45

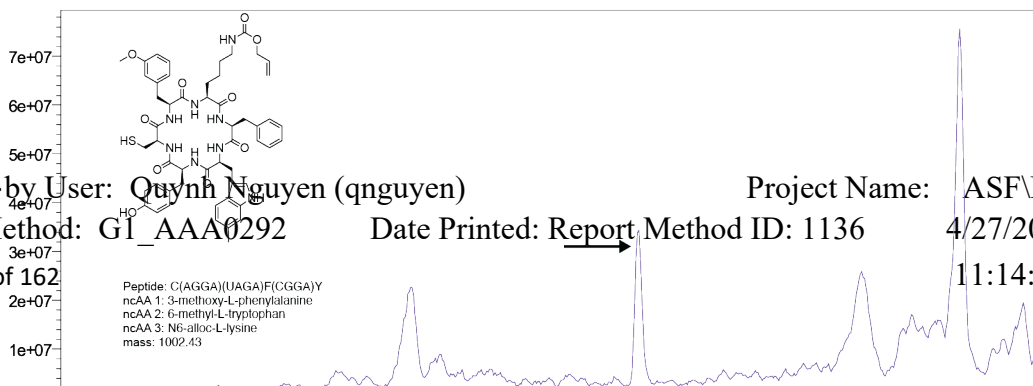
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs Y axis not normalized



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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11:14:49 AM US/Pacific

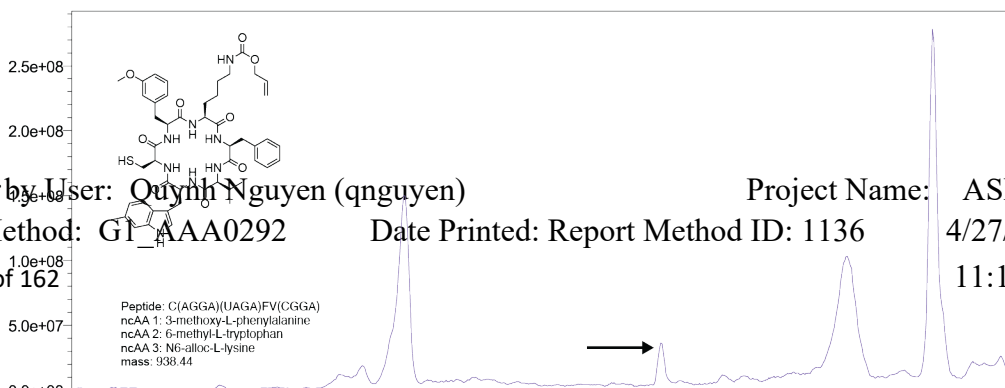
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs
Y axis not normalized



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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Peptide: C(AGGA)(UAGA)FV(CGGA)
ncAA 1: 3-methoxy-L-phenylalanine
ncAA 2: 6-methyl-L-tryptophan
ncAA 3: NG-alloc-L-lysine
mass: 938.44

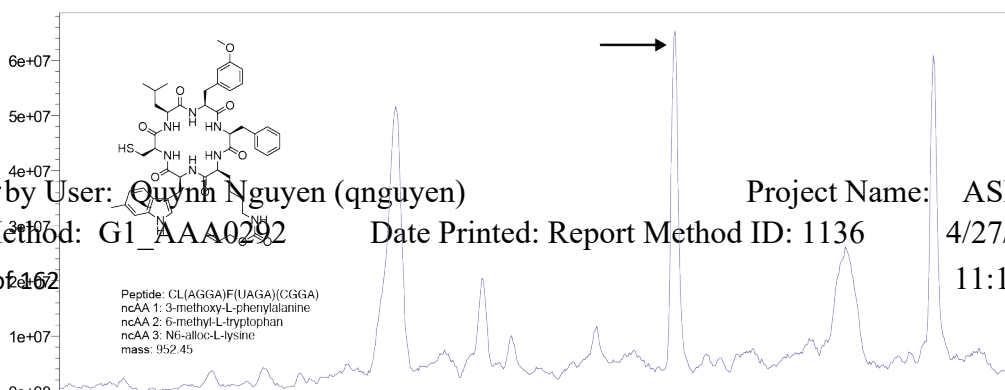
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs Y axis not normalized



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1_AAA0292

Date Printed: Report Method ID: 1136

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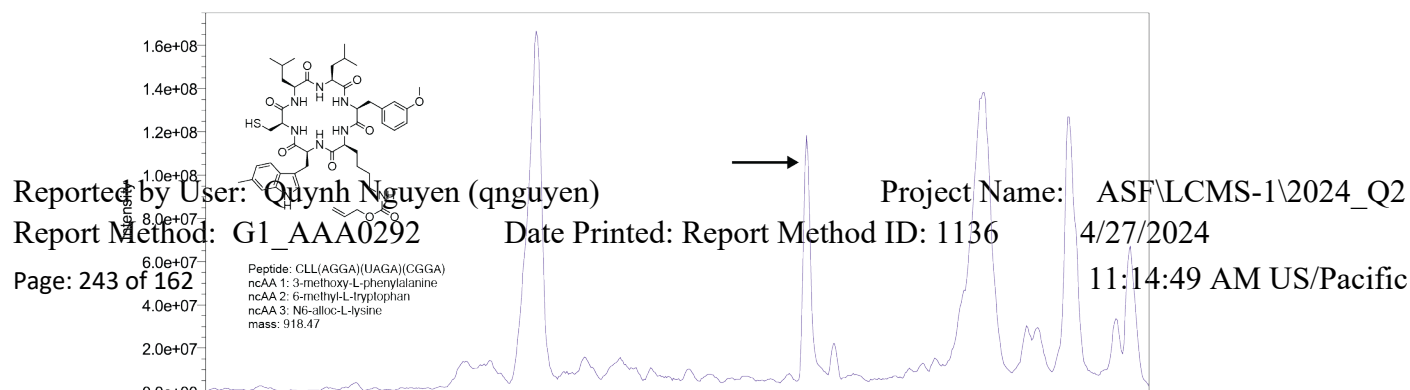
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs
Y axis not normalized



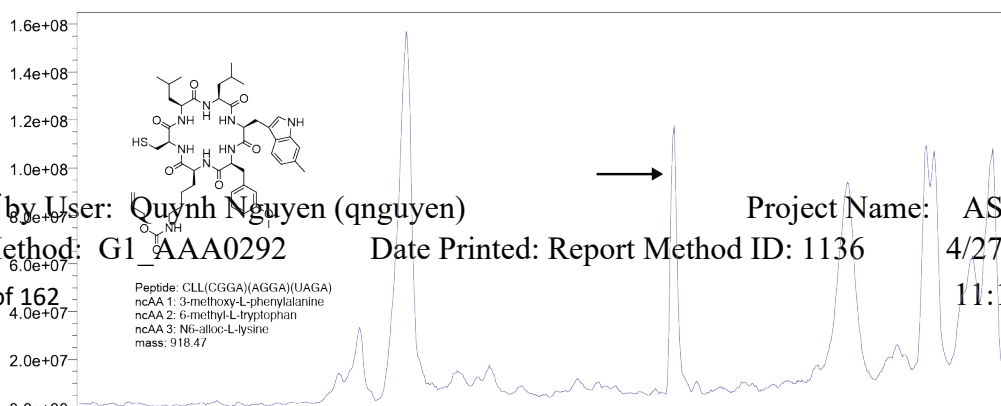
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Report Method: G1 AAA0292

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Date Printed: Report Method ID: 1136

Project Name: ASF\LCMS-1\2024_Q2

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Peptide: CLL(CGGA)(AGGA)(UAGA)
ncAA 1: 3-methoxy-L-phenylalanine
ncAA 2: 6-methyl-L-tryptophan
ncAA 3: N6-alloc-L-lysine
mass: 918.47

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

T A R G E T M A S S A N A L Y S I S

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Full XIC and m/z for samples from ED Figure 28:

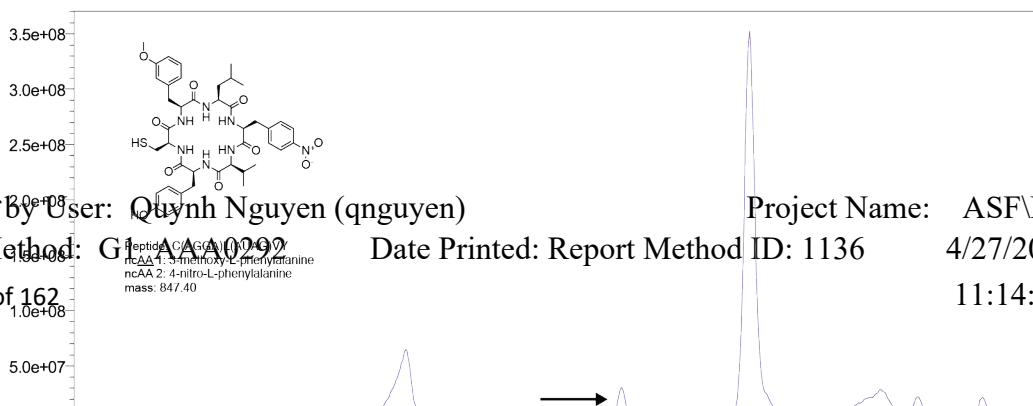
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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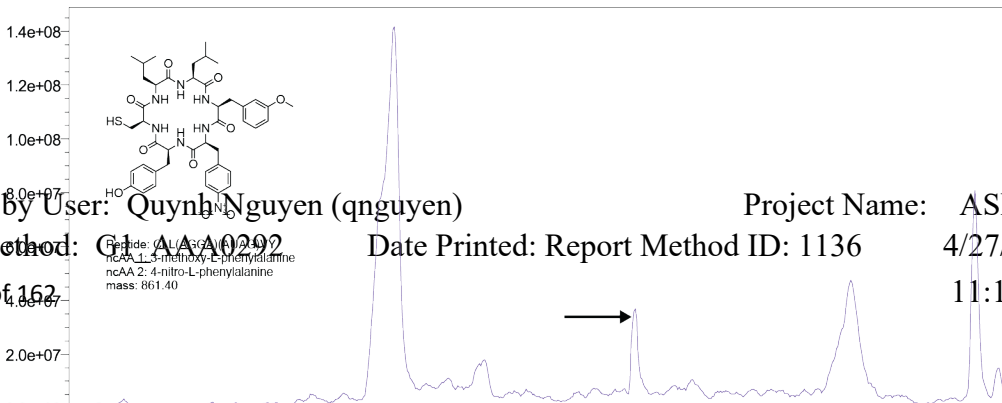
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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Grade: 99.99999999999999
ncAA 2: 4-nitro-L-phenylalanine
mass: 861.40

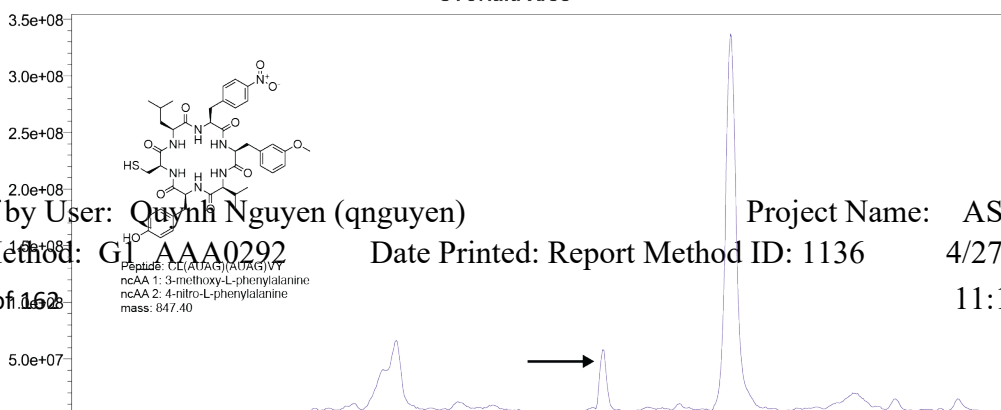
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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Peptide: G1(AAA0292)AAA0292
ncAA 1: 3-methoxy-L-phenylalanine
ncAA 2: 4-nitro-L-phenylalanine
mass: 847.40

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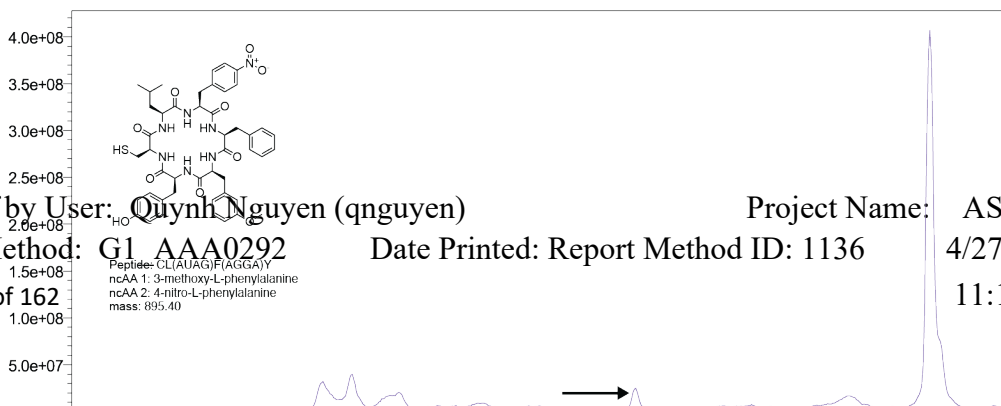
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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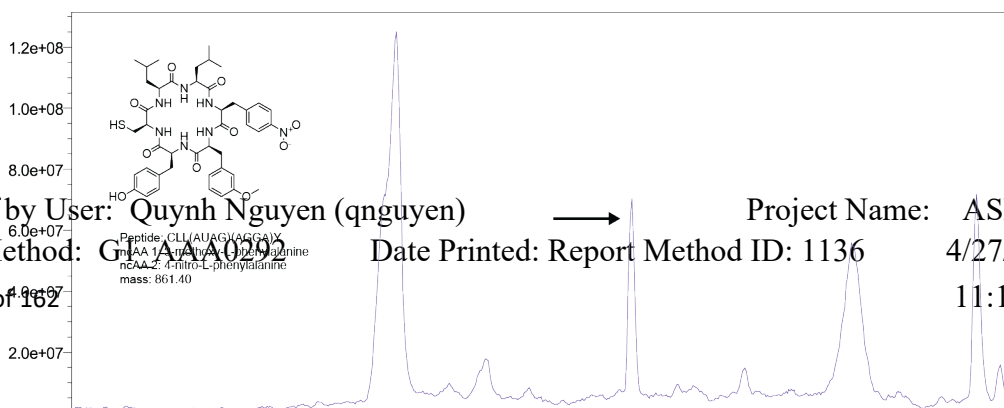
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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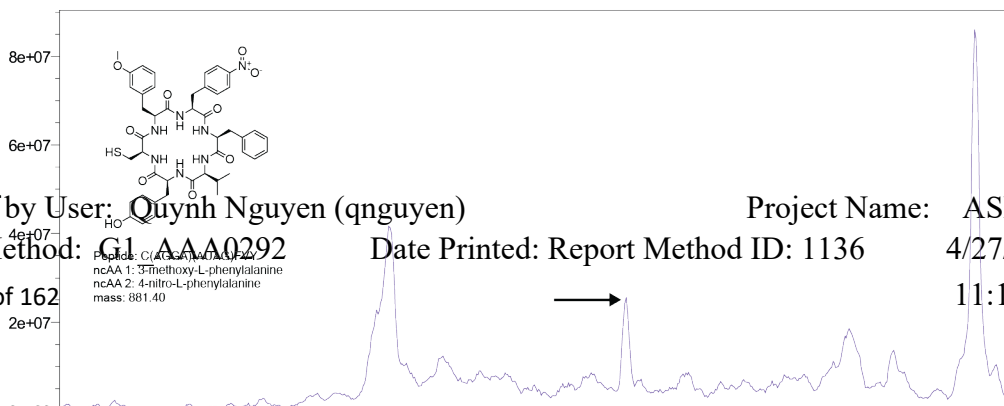
TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

Overlaid XICs



Reported by User: Quynh Nguyen (qnguyen)

Project Name: ASF\LCMS-1\2024_Q2

Report Method: G1 AAA0292

Date Printed: Report Method ID: 1136

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TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method:G1 AAA0292

Date Acquired:
Date Processed:

TARGET MASS ANALYSIS

Sample Name: D2
Vial: 2:D,2

Acq. Method Set: G1 AAA0292
Processing Method: G1 AAA0292

Date Acquired:
Date Processed:

References:

- 1 Thompson, M. G. *et al.* Isolation and characterization of novel mutations in the pSC101 origin that increase copy number. *Scientific Reports* **8** (2018). <https://doi.org:10.1038/s41598-018-20016-w>
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