Page contents

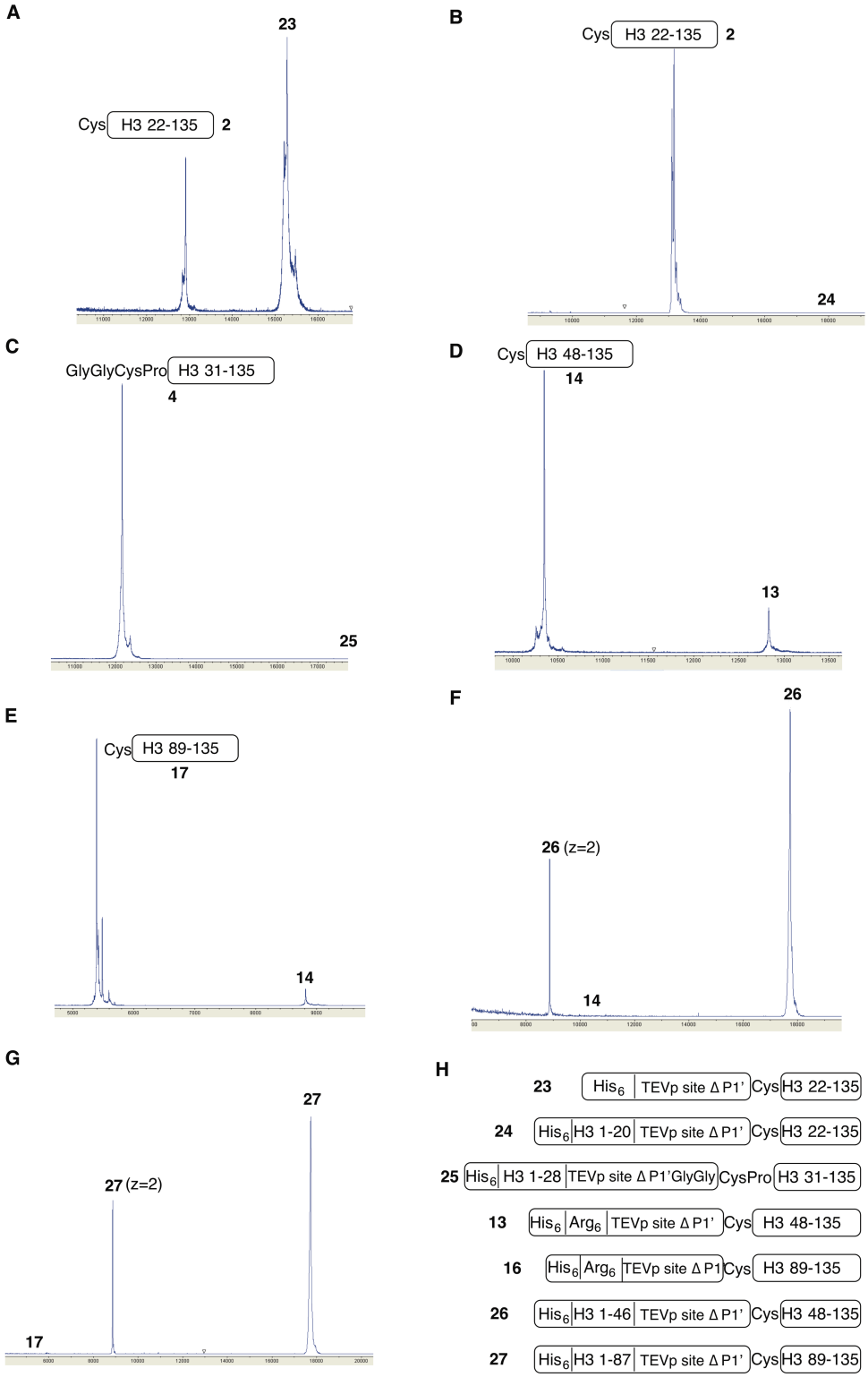


Figure S1. Monitoring the TEV protease digestion of His_6-tagged truncated histone constructs by MALDI-TOF. The spectra are taken 12 hours after setup of the digestion; precise conditions are described in the 'Generation of truncated histones for protein ligation' section. A, B, C, D, E, F, G show respectively the digestion for constructs 23, 24, 25, 13, 16, 26, 27 diagrammed in H. The full-length constructs 26 and 27 do not show any digestion even when TEV protease is present in 1:1 mass ratio.

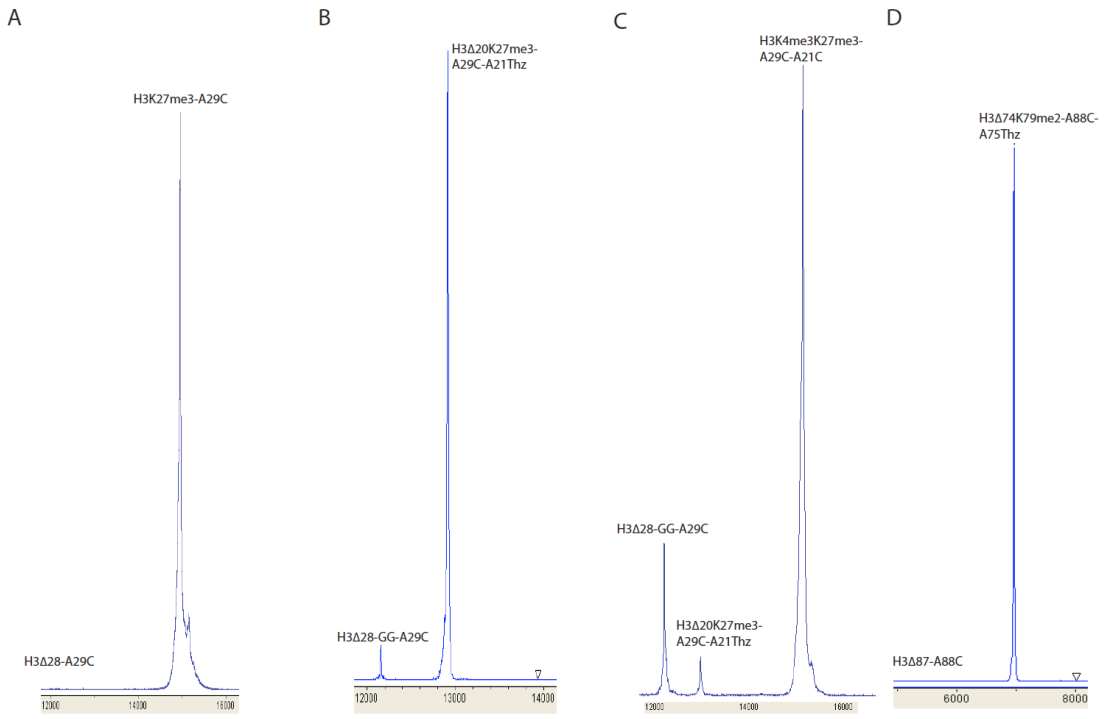


Figure S2. MALDI-TOF monitoring of ligations for (A) H3K27me3 (**7**, goes to completion), (B) first ligation for H3K4me3K27me3 (**8**, goes to completion), (C) second ligation for H3K4me3K27me3 (**8**, goes to near completion), (D) first ligation for H3K79me2 (**22a**, goes to completion).

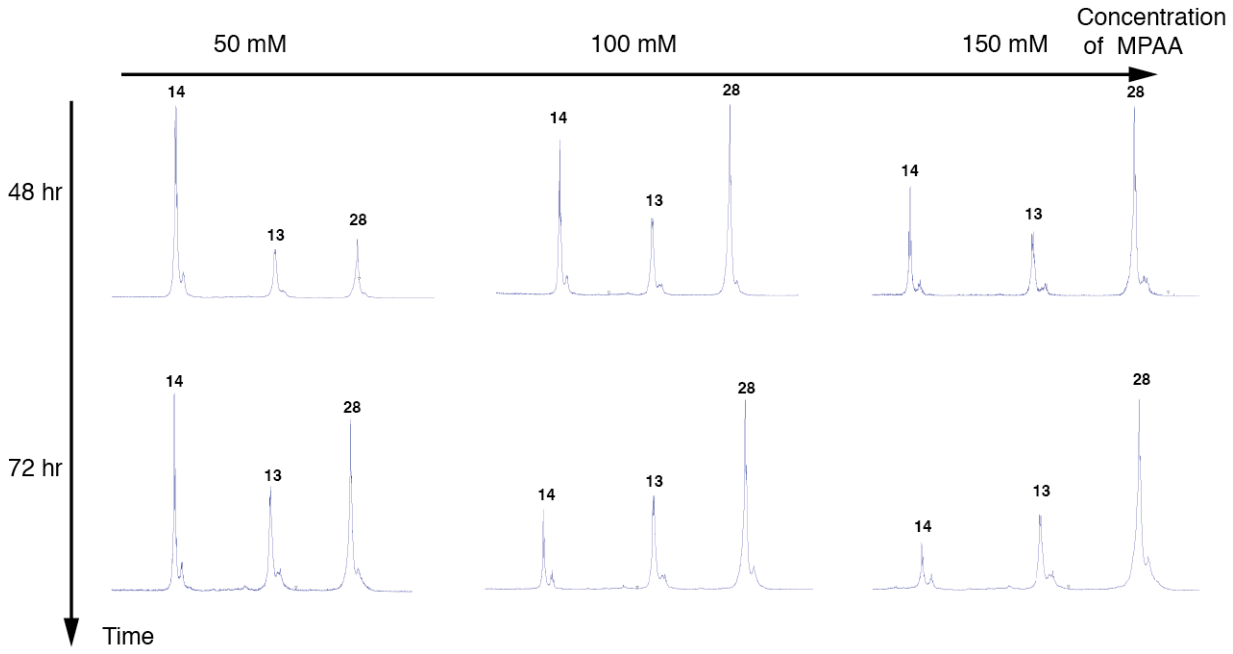


Figure S3. Up to 150 mM of MPAA and 72 hours of reaction time are required for the second ligation for H3K36me3 (**15**) at the β-branched valine. MALDI-TOF spectra are taken at the time and condition indicated. H3Δ46-A47C (**14**), His_6-tagged H3Δ46-A47C (**13**), H3K36me3-A29C-A47C (**28**).

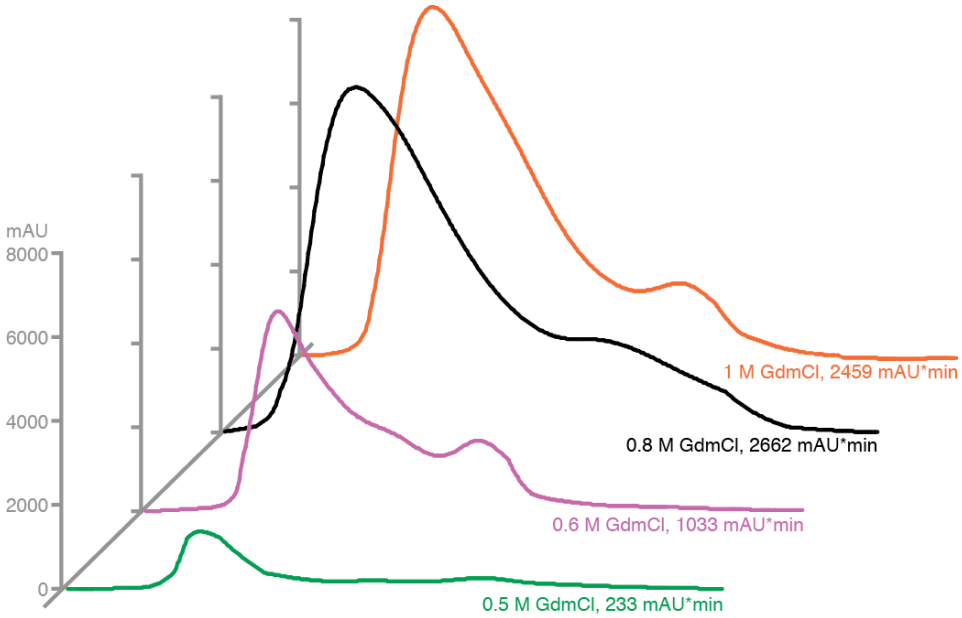


Figure S4. 0.8-1 M of GdmCl is optimal for the refolding and MesNa cleavage of H3(1-74)-Intein-CBD (20). 2.5 ml of H3(1-74)-Intein-CBD inclusion body (IB) extract is dialyzed to refolding buffers containing 0.5 M, 0.6 M, 0.8 M, and 1.0 M GdmCl, cleaved with MesNa, and subjected to HPLC. The peaks and integrated peak area corresponding to H3(1-74)-MES for each condition are shown, and offset in retention time and UV baseline by 20% in each dimension. See 'generation of H3(1-74)-MES by the intein cleavage method' section for details.

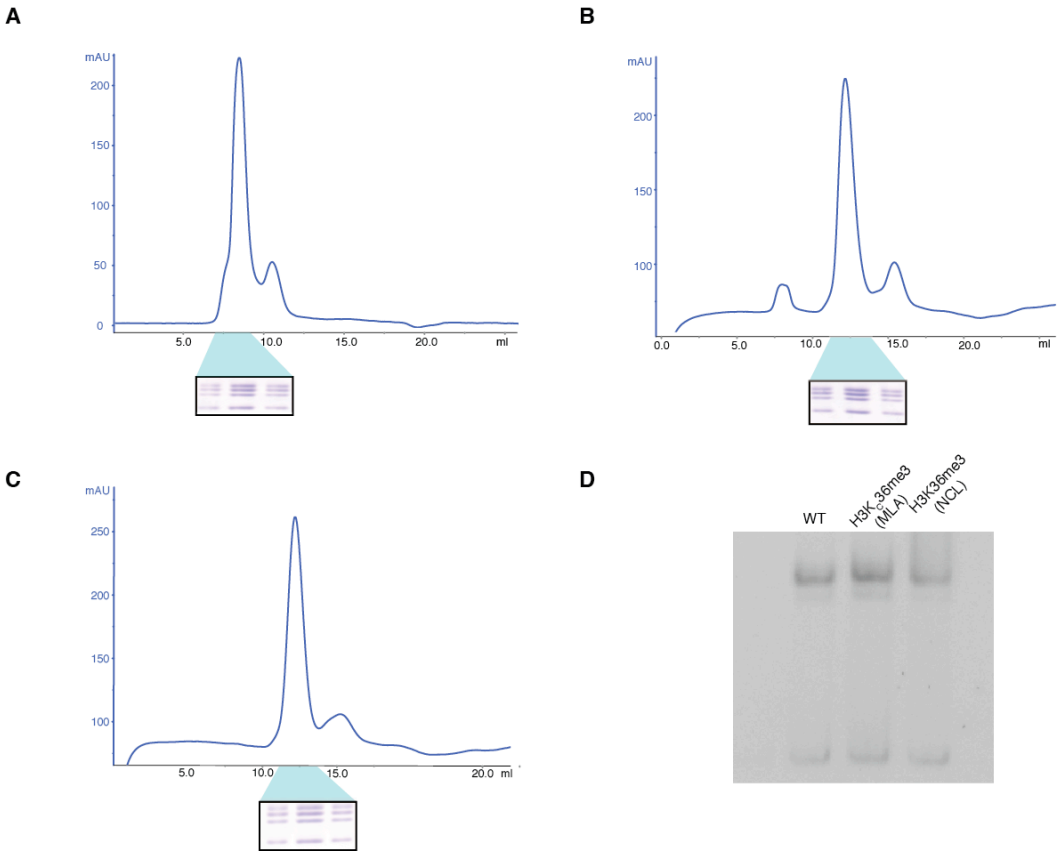


Figure S5. Purification of octamers bearing (A) wild type H3, (B) MLA of H3K36me3, (C) native H3K36me3 prepared in this study by NCL, and (D) reconstitution of nucleosomes with these three octamers. Refer to later section 'Characterization of intermediates and products' for details.

General materials and equipments

Boc-Lys(me)2-OH is purchased from Bachem (Torrance, CA); the remaining of Boc-amino acids and coupling reagents are purchased from EMD Millipore (Billerica, MA). Sodium 2-mercaptoethanesulfonate (MesNa), N,N-diisopropylethylamine (DIPEA), 4-mercaptophenyl acetic acid (MPAA), Triisopropylsilane, Guanidinium chloride (GdmCl), and Trifluoroacetic acid (TFA) are purchased from Sigma-Aldrich (St. Louis, MO). Isopropyl β-D-1-thiogalactopyranoside (IPTG), Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) are purchased from Goldbio technology (St. Louis, MO). 2,2'-Azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) is purchased from Wako (Richmond, VA). N,N-dimethylformamide (DMF) are purchased from VWR international (Radnor, PA). Ni-NTA resin is purchased from Qiagen (Gaithersburg, MD). The QuikChange site-directed mutagenesis kit is purchased from Agilent (La Jolla, CA). All primers are ordered from IDT (Coralville, IA). In-house prepared electrocompetent DH10B cells are used as cloning hosts. ESI-MS spectra in positive-ion-mode are taken on Thermo scientific LTQ-FT at Mass Spectrometry, Metabolomics & Proteomics Facility (MMPF) of University of Illinois at Chicago (UIC) with an analytical HPLC run up-front. 20 ul of filtered solution of histone fragments in milliQ water (~0.1 mg/ml) is loaded on the analytical HPLC column (Agilent, poroshell 120 SB-C18 2.7 um) each time. The method runs at 0.5 ml/min with the gradient ramping from 5% B to 100 % B in 10 min (buffer A, 0.1% formic acid in water; buffer B, 0.1% formic acid, 90% ACN in water). MALDI-TOF spectra in positive-ion-mode are taken either on Bruker Ultraflextreme MALDI-TOF-TOF or AB SCIEX Voyager DE Pro MALDI-TOF. Masses of peptides are acquired in reflector mode if below 2500 Da, otherwise in linear mode.

Peptides/histones are purified on semipreparative HPLC on a Varian 940 LC system. All runs use 0.1% TFA in water as solvent A and 90% acetonitrile in water with 0.1% TFA as solvent B. Samples are centrifugation-clarified or filtered through 0.22 μm filters before loading. All methods run at 4 mL/min from beginning to end, and fractions of 2 ml are collected. YMC Pack Pro C18 semipreparative column (250x10 mmI.D., S-5 μm, 12 nm, AS12S05-2510WT), YMC Triart C18 semipreparative column (250x10 mm I.D., S-10 μm, 12 nm, TAS12S11-2510WT), and YMC semipreparative C8 column (250x10 mmI.D., S-5 μm, 30 nm, OC30S05-2510WT) are used.

Preparation of Boc-Lys(me)3-OH monomer

Boc-Lys-OH (12.2 g), 500 mL of MeOH, 50 g of $KHCO_3$ and 32 mL of MeI are added to a 2 L round bottom flask. The mixture is stirred at room temperature. Under these conditions the $KHCO_3$ only partially dissolves. An empty balloon bound to a needle is inserted into the septum of the flask as a reservoir for the CO_2 generated. The

reaction is monitored every 12 hour by HPLC (aliquots of reaction mixture are acidified with 4M HCl and filtered) until the ratio between Boc-Lys(me)3-OH and Boc-Lys-OH exceeds 25:1 (~72 hours). Boc-Lys(me3)-Ome is also generated as side product.

The reaction mixture is vacuum-filtered through a Büchner funnel and evaporated to dryness on rotary evaporator (Büchi). The residue is taken up in 50 mL of water, acidified to pH 1.0 with 4M HCl under constant stirring. The crude mixture is left stirring for half an hour, degassed by sonication under vacuum for 5 minutes, and purified by preparative HPLC in batches. HPLC purification is necessary to remove all the inorganic salts that will inhibit coupling during SPPS. For our preparative column (YMC C18 pro pack, 150 mm*20 mm I.D., S-5 μm, 12 nm), 3 ml of the crude mixture is loaded each time. The method, running at 13 mL/min, entails a 13 minutes wash with 98% solvent A and 2% solvent B followed by the ramp up of gradient from 2% B to 100% B in 15 minutes. The long wash-step is necessary to remove the inorganic salts. The fractions with the Boc-Lys(me)3-OH are pooled, lyophilized, and used directly in peptide synthesis. ^{1}H NMR (500 MHz, D_2O): δ 3.44-3.48 (t, 2H), 3.24(s, 9H), 3.05-3.08 (t, 1H), 1.75-1.94 (m, 6H), 1.54 (s, 9H); ^{13}C NMR (400 MHz, D_2O) δ 179.9, 157.7, 81.1, 66.6, 55.9, 53.3, 39.7, 31.5, 28.1, 27.2; MS (ESI) Calculated for $[C_{14}H_{29}N_2O_4]^+$: 289.2; Found: 289.4.

Synthesis of peptidyl thioesters

Apart from H3(1-74)-MES (**21**), all peptidyl thioesters are synthesized on S-trityl-β-mercatopronionyl-p-methyl-benzhydrylamine resin from peptides international (Louisville, KY).[1] The resin is swelled in dry DMF for 1 hour in a 50 ml peptide synthesis vessel (Chemglass), undergoes three 4-minute treatments with S-trityl deprotection cocktail of 95% TFA, 2.5% Triisopropylsilane, and 2.5% H_2O, followed by extensive DMF flow washing. For coupling of an amino-acid residue, 4 equivalents of the amino acid, 3.9 equivalents of HBTU (0.5 M in DMF) are first mixed and vortexed, followed by addition of 6 equivalents of DIPEA and brief vortexing to fully solubilize and activate the amino acid. The resin is incubated with the activated monomer solution for a minimum of 10 minutes (30 minutes for the coupling of the first residue) with occasional stirring. After the ninhydrin test[2] (works only for primary amine) indicates the completion of coupling, the coupling solution is drained and the resin is flow-washed three times with DMF. The Boc group of newly coupled amino acid is removed by three washes of TFA (~10 ml each wash with the first wash being flow-wash). This coupling-deprotecting cycle is iterated to assemble the whole peptide. A Teflon water aspirator coupled to the water faucet is utilized to generate

the vacuum required for draining the reaction vessel during peptide synthesis. Before TFA is applied to de-Boc the α-amine of glutamine, the resin undergoes three extra washes with CH_2Cl_2 after being washed with DMF. After removal of the Boc group on the last amino acid coupled, the resin is washed sequentially with copious DMF, CH_2Cl_2, MeOH, and is left on vacuum for half an hour. HF treatment to cleave the peptide from the resin and afford global deprotection is performed at Midwest Bio-Tech (Fishers, IN).

All peptidyl thioesters are purified on YMC Pack Pro C18 semipreparative column as follows: for H3(1-20)K9me3-SR (**1**) and H3(1-20)K4me3-SR, the gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 12% B from 5 to 15 minutes, then ramps up linearly to 100% B from 15 to 28.9 minutes, stays at 100% B for 3 minutes and equilibrates at B% for 5 minutes. For H3(1-28)K27me3-SR, the gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 8% B from 5 to 5.5 minutes, then ramps up linearly to 14% B from 5.5 to 15.5 minutes and to 100% B from 15.5 to 29.1 minutes, then stays at 100% B for 3 minutes and reequilibrates at 5% B for 5 minutes. For H3(29-46)K36me3-A29C-SR (**11**), the gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 12% B from 5 to 6.1 minutes, then ramps up linearly to 18% B from 6.1 to 16.1 minutes, then to 100% B from 15.8 to 29.1 minutes, stays at 100% B for 3 minutes and reequilibrates at 5% B for 5 minutes. For H3(75-87)K79me2/3-A75Thz-SR (**18a and 18b**), the gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 22% B from 5 to 7.7 minutes, then ramps up linearly to 88% B from 7.7 to 17.7 minutes, then to 100% B from 17.7 to 29.1 minutes, stays at 100% B for 3 minutes and reequilibrates at 5% B for 5 minutes.

Expression and purification of His_6-tagged truncated histones

The four requisite plasmids (kanamycin resistant, based on pET vectors) are made by the Quikchange method from the previously published pRuth5H3.2 C110A vector[3] and transformed into a chemically competent BL2(DE3)pRARE2 *E.Coli* strain (pRARE2 is a plasmid that encodes t-RNAs for the rare condons and confers chloroamphenicol resistance, isolated from the Rosetta2 (DE3) strain, EMD). A single colony is inoculated into 20 ml LB media and grown overnight at 37°C at 240 rpm with antibiotic selection. 10 ml of this starter culture is added to 1 L of LB media with the appropriate antibiotics, grown at 37°C at 240 rpm to OD_{600} of 0.6-0.7, and induced by IPTG added to a final concentration of 1 mM. After 3-4 hours of induction, the cells are harvested by centrifugation, resuspended in lysis buffer (50 mM Tris•HCl pH 8.0, 100 mM NaCl, 5 mM ß-mercaptoethanol, 1 mM PMSF) and lysed by Emulsiflex C5 (Avestin). Inclusion bodies (IBs) are isolated from the lysate by centrifugation at 18000 g for 15 minutes in 40 mL Oak Ridge tubes in an SS-34 Rotor (Sorvall). Glass beads

(5mm, Fisher) are added to aid resuspension by vortexing of IBs and retained in following steps. The IBs are first washed by homogenization of the pellet in lysis buffer plus 1% (v/v) Triton-X100, centrifuged (13,000 g for 10 minutes), and then washed twice with lysis buffer without detergent with an interposed centrifugation as above. The IBs are swelled and homogenized in 1 ml of DMSO then solubilized in 20-30 ml of D1000 buffer (6M GdmCl, 1M NaCl, 50 mM Tris pH 8.0, 5 mM ß-mercaptoethanol) on a Nutator for one hour. This IB extract is centrifuged at 30000 g for 20 min to remove any insoluble material, then the clarified supernatant is applied to 1 ml bed volume of Ni-NTA resin (Qiagen) pre-washed with D1000, then nutated for 1 hour in a 50 mL glass column (Bio-Rad). Histone-depleted supernatant is allowed to flow through the resin and the resin is washed twice with 30 mL D1000, followed by 3 x 2 ml elutions (elution buffer, D1000 + 250 mM imidazole, pH 8.0). The purity of the histones is assessed by MALDI-TOF and SDS-PAGE; the concentration measured by Nanodrop spectrometer (Thermo Scientific). For SDS-PAGE, since Guanidinium precipitates with SDS, the sample is first diluted 15 fold with water then mixed with loading dye, boiled, and loaded hot into the gel. To buffer-exchange into non-denaturing conditions, 0.25 volume of 2 M triethylammonium acetate (TEAA) buffer, pH 4.0 (prepared by adding acetic acid slowly to a stirred triethylamine solution in an ice bath), is added to the pooled histone fractions. 2.5 ml of this solution is loaded on one PD-10 desalting column pre-equilibrated with 25 ml of 150 mM TEAA buffer, pH 4.0. The column is then eluted with 3.5 ml of 150 mM TEAA, pH 4.0. The elution is flash-frozen in liquid N_2 and lyophilized to dryness (Labconco).

Generation of truncated histones for protein ligation

For the full-length, His_6-tagged construct of H3Δ20-A21C (**24**) and H3Δ28-GG-A29C (**25**), lyophilized histones are resuspended to 6-10 mg/ml in 50 mM Tris buffer, pH 8.0. 1/100 mass equivalent of TEV protease and 4 mM DTT are added. The digestion usually finishes overnight at room temperature. If not, check the pH to make sure it is between 7.6-8.0, and add another batch of DTT/TEV protease. The TEV protease for removing the His_6-tag was prepared in-house following Waugh's protocol, often with an additional Superdex 75 purification step.[4] To the TEV digestion mixture for H3Δ20-A21C (**2**), 6M GdmCl, 400 mM $MeONH_2$•HCl, 20 mM TCEP are added, followed by overnight incubation at room temperature to resolve the pyruvate adduct[5] of N-terminal cysteine and HPLC purification. To the digestion mixture for H3Δ28-GG-A29C, 6M GdmCl is first added, followed by HPLC purification to yield H3Δ28-GG-A29C (**4**). The GlyGly dipeptide is removed using Qiagen's TAGzyme kit. Briefly, 50 μl of provided DAPase is added to 0.45 ml of storage buffer (50 mM MES, pH 6.70,

150 mM NaCl), and 100 µl provided 20 mM Cystamine to 0.9 ml of storage buffer. The two solutions are then mixed, incubated at room temperature for 5 minutes, and added to 6-10 mg of lyophilized H3Δ28-GG-A29C (**4**). The resulting mixture is incubated at room temperature for about 2 hours. Once MALDI-TOF indicates complete removal of the GlyGly dipeptide, 6M GdmCl, 400 mM $MeONH_2$•HCl, and 20 mM TCEP are added to the mixture to deblock any newly formed pyruvate adduct, followed by HPLC purification. The three HPLC purifications in this sequence are performed on the YMC Pack Pro C18 column as follows: the gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 100% B from 5 to 20 minutes, stays at 100% B for 3 minutes and reequilibrates at 5% B for 5 minutes.

For the His_6Arg_6H3Δ46-A47C (**13**) and His_6Arg_6H3Δ87-A88C (**16**) fragments, the lyophilized histones are resuspended to 6-10 mg/ml in 500 mM MES, pH 6.50. 1.0 (for **13**) and 0.5 (for **16**) mass equivalents of TEV protease (without final size-exclusion purification step) are added respectively with 4 mM of DTT, followed by overnight incubation at room temperature. The pyruvate adduct formed *in-situ* is resolved as described above. The HPLC purifications are done on YMC Triart C18 semipreparative column as follows: the gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 100% B from 5 to 20 minutes, stays at 100% B for 3 minutes and reequilibrates at 5% B for 5 minutes. The small amount of residual undigested histone in these two purifications is inert in ligation chemistry and removed in subsequent purification steps.

Generation of H3(1-74)-MES (23) by the intein cleavage method

The plasmid encoding H3(1-74)-intein-CBD (**20**) is generated using NEB's impact kit. The plasmid (ampicillin resistant) is transformed to BL21(DE3)pRARE. The protein expresses well in inclusion bodies (IBs). The IB extract is prepared the same as for His_6-tagged truncated histones. The IB extract is diluted with one volume of the refolding buffer (0.8 M GdmCl, 500 mM NaCl, 100 mM HEPES, pH 7.30, 1 mM TCEP). This solution is then dialyzed against 25 volume equivalents of refolding buffer overnight at room temperature. After the dialysis, 0.5 M MesNa is added to the partially soluble (milky) dialysate. After 3-4 hours, the mixture is centrifuged at 20000 g for 20 minutes, with the supernatant (cleaved histone and folded intein-CBD fragments) collected and the precipitate (unfolded and uncleaved protein) resolubilized in IB extraction buffer 0.8 fold of the original volume. This refolding and cleavage process may be repeated up to 4 times and still yield significant amounts of histone thioester. The combined supernatant is acidified to pH 1.0 with 4 M HCl and let stand for 0.5 hour, which efficiently precipitates out the cleaved intein-CBD fragment. After centrifugal clarification (20000 g for 20

minutes), 12 ml of the supernatant is loaded directly on to the YMC semipreparative C8 column. The purification is as follows: the gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 40% B from 5 to 10.5 minutes, then ramps up linearly to 50% B from 10.5 to 25.5 minutes and to 100% B from 25.5 to 33.4 minutes, stays at 100% B for 3 minutes and reequilibrates at 5% B for 5 minutes. In total, one liter of culture yields around 15 mg of H3(1-74)-MES. MALDI-TOF and ESI-MS demonstrate that the N-terminal formyl-methionine is processed off by endogenous exopeptidase activity and hydrolysis of the thioester occurs if the cleavage goes too long.

Native Chemical ligation (NCL) and kinetically controlled ligation (KCL)

Ligations are monitored by MALDI-TOF. To make H3K9me3 (**3**), and H3K27me3 (**7**), equimolar peptidyl 3-mercapto-propionamide thioester (-SR) and truncated H3s are added to an Eppendorf tube. NCL buffer (6 M GdmCl, 200 mM phosphate, pH 7.0) is added to bring the concentration of the reactants to 2 mM. 30 mM MPAA and 20 mM TCEP (both as 0.5 M stock solution in NCL buffer, pH 7.0) are added and the final pH is adjusted between 7.0-7.3. The vial is sealed with Parafilm and incubated overnight.

To make H3K4me3K27me3 (**8**), the first ligation is set up the same as above with the concentration of peptide and C-terminal protein fragment at 5 mM. After MALDI-TOF indicates the completion of the ligation, 200 mM $MeONH_2$•HCl is added to the ligation mixture, followed by overnight incubation at room temperature. 1.0 equivalent of H3(1-20)K4me3-SR and 20 mM TCEP are added to the reaction mixture, and the pH is brought back to 7.0-7.3 with 5 M NaOH. After overnight at room temperature, the second ligation goes to near completion.

For the preparation of H3K36me3, 0.2 mL of the 0.5 M TCEP stock and 0.6 ml of the aforementioned 0.5 M MPAA stock are added to 10-20 mg of crude H3(1-28)-SR (**9**), followed by overnight incubation at room temperature and HPLC purification on YMC Pack Pro C18 semipreparative column. The gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 10% B from 5 to 5.8 minutes, then ramps up linearly to 16% B from 5.8 to 15.8 minutes and then to 100% B from 15.8 to 29.1 minutes, stays at 100% B for 3 minutes and reequilibrates at 5% B for 5 minutes. 1.0 equivalent of the lyophilized product, H3(1-28)-SR'(**10**, the MPAA thioester) is added to H3(29-46)K36me3-A29C-SR (**11**). NCL buffer is added to make the final concentration of the peptides around 5 mM, then 20 mM of TCEP. The reaction is monitored by MALDI-TOF and finishes within 2-3 hours. There is a side reaction where the internal cysteine of the product appears to cyclize with the C-terminal thioester to form thiolactone, which is resolved in the next step by MPAA-mediated transthioesterification. H3Δ46-A47C

(1.0 equivalent), 150 mM of MPAA and 100 mM TCEP (as a stock solution of 300 mM MPAA + 200 mM TCEP, pH 7.0 in NCL buffer) are added, and pH is adjusted between 7.0-7.3 with 2M NaOH (this ligation occurs at a thioester carbonyl carbon vicinal to a β-branched valine side chain). The second ligation takes 72 hours to reach ~80% completion. During this reaction 20 mM TCEP is added after every 24 hours to ensure reduced cysteine nucleophiles.

To make H3K79me2/3 (**22a and 22b**), the first ligation is set up the same as for H3K4me3 with concentrations of peptides around 5 mM. The ligation goes to completion after overnight at room temperature. After addition of 200 mM $MeONH_2$•HCl and incubation at room temperature overnight, 1.5 molar equivalents of H3(1-74)-SR" (**21**, the MES thioester), 150 mM of MPAA and 100 mM of TCEP (as a stock solution of 300 mM MPAA+200 mM TCEP, pH 7.0 in NCL buffer) are added to the reaction mixture and pH is brought back to 7.0-7.3 (this ligation is at β-branched isoleucine) with 5M NaOH. The ligation is incubated for 72 hours and 20 mM TCEP is added after every 24 hours.

After the ligations finish, the ligation mixture is diluted 5 fold with NCL buffer, quenched with 100 mM ß-me, incubated at RT for 0.5 hour, acidified to pH 1.0 with 4M HCl, centrifugation-clarified (MPAA may precipitate out at low pH), and purified on the YMC semipreparative C8 column as follows: the gradient stays at 5% B from 0 to 5 minutes, ramps up linearly to 52% B from 5 to 12.4 minutes, then ramps up linearly to 62% B from 12.4 to 27.4 minutes, then to 100% B from 27.4 to 33.4 minutes, stays at 100% B for 3 minutes and reequilibrates at 5% B for 5 minutes.

Desulfurization

To 2-10 mg of fully ligated H3 are added 300 μl of NCL buffer, 300 μl of 0.5 M stock of TCEP, 20 μl of 10% ß-me (v/v in NCL buffer), and 20 μl of 200 mM VA-044 dissolved in MilliQ grade water. The resulting solution is agitated at 45°C in a Thermoshaker (Eppendorf) for 40 minutes and purified the same way as the final ligation product (*vide supra*). Temperature above 50°C leads to side reactions. Excessive ß-me and temperature below 42°C prevent complete reaction. The desulfurization is monitored by LTQ-FT.

Pulldown of LEDGF and western blot

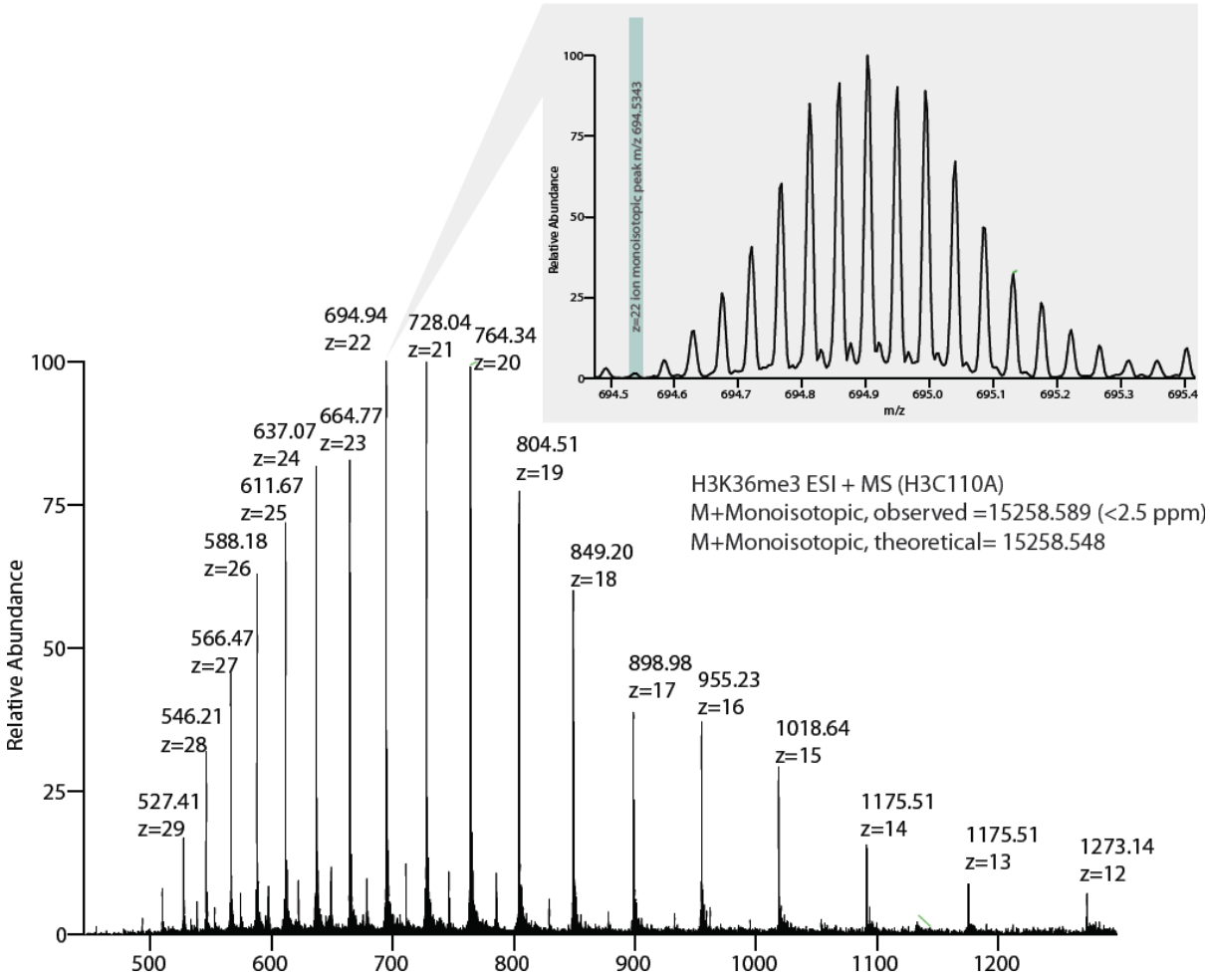
Nuclear extract of HeLa S3 cells is prepared and used.[6] For each pulldown experiment, HeLa S3 cell pellet corresponding to 50 ml of culture (0.6-1.0 million cells/ml) from a spinner flask culture is used. Nucleosomes bearing wild type H3, $H3K_C36me3$ (MLA), and native-like H3K36me3 (NCL) are reconstituted on the same

biotinylated DNA sequence with human H2A, H2B and H4 as described previously.[3] The MLA of H3K36me3 is prepared as described[7] except that the H3 with lysine 36 mutated to cysteine is His_6-tagged at N-terminus, and this tag is TEV cleavable to reveal a native alanine in the first position upon cleavage. This histone is expressed and purified as described before. The aminoethylation is implemented before TEV protease digestion to side-step the most common side reaction in the original protocol: the alkylation of α-amino group at the N-terminus. The H3 peptide used for pulldown is H3(27-46) K36me3 with a TEG-biotin conjugated to ε-amino group of lysine 27 at the N-terminus. The nucleosomal pulldown for LEDGF is carried out as described except that 40 µl of Dynabeads® MyOne™ Streptavidin T1bead (Invitrogen) is used for each pulldown; Tris is used as the buffering reagent; a total of 4 washes with concentration of NaCl at 160 mM and a tube transfer after the second wash are carried out.[8] Nuclear extract was first fractionated on 5ml Heperin Hitrap column (GE healthcare). The fraction eluted with 300 mM NaCl was used for pull down assays where the total protein is imaged by silver stain. Primary Anti-LEDGF antibody (Bethyl A300-848A, 1:2000 dilution) and secondary goat-anti-rabbit HRP-conjugated antibodies (ThermoFisher 31462, 1:15000 dilution) are diluted in 2% ECL Advance Blocking agent (GE Healthcare). The blot is developed in ECL Ultra (TMA-100, Lumigen) and imaged with an ImageQuant LAS-4000 (GE healthcare).

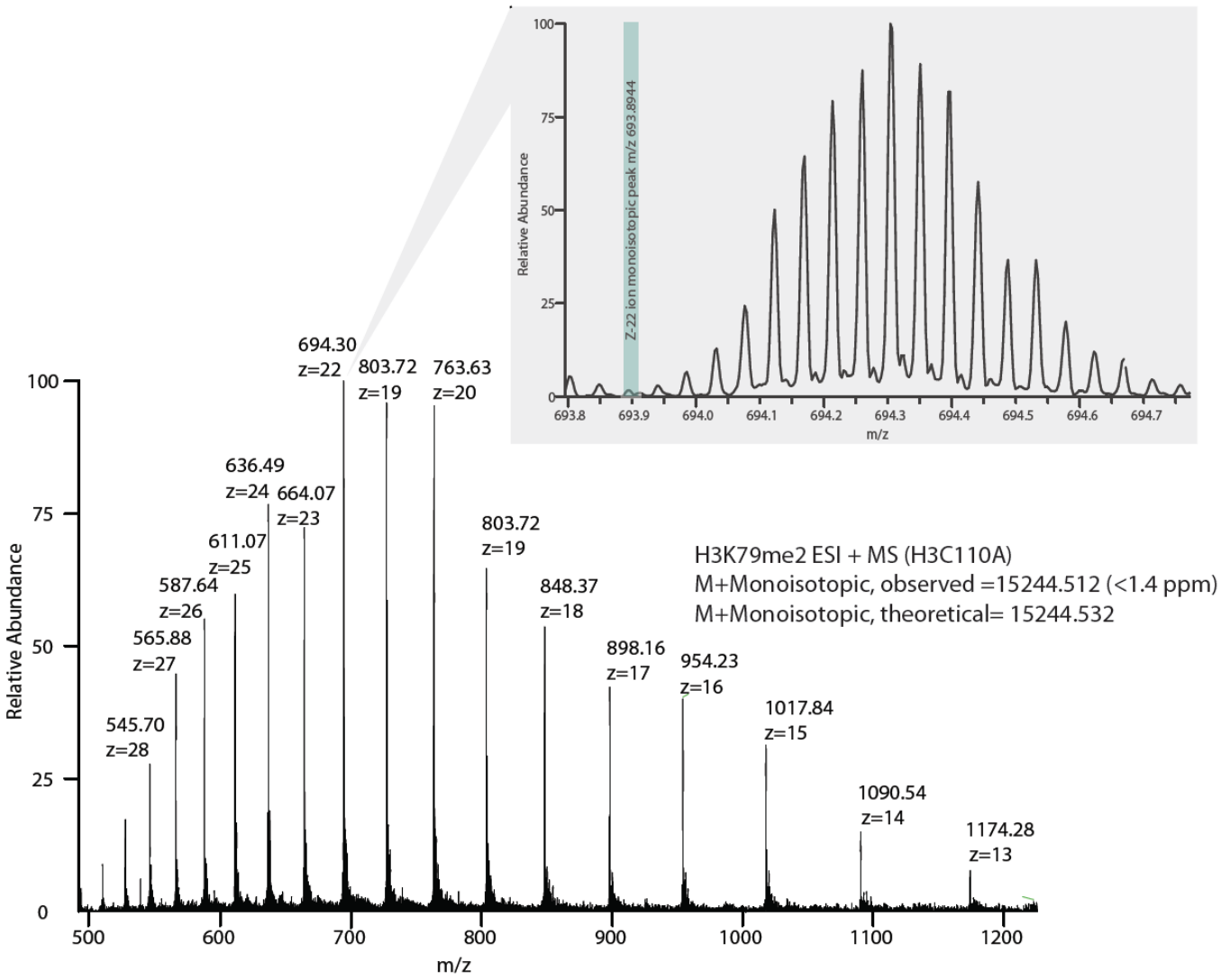
Characterization of intermediates and products

We carried out monoisotopic analysis of the final desulfurized H3K36me3, and H3K79me2, and these three species matches to the calculated masses to ppm accuracy (± 0.05 Da). Masses are calculated with UCSF prospector program (http://prospector.ucsf.edu/). For all other species, Masses (either by ESI-MS or MALDI-TOF that is appropriate) and analytical HPLC traces of the purified materials are provided below.

Monoisotopic analysis of H3K36me3 (15)

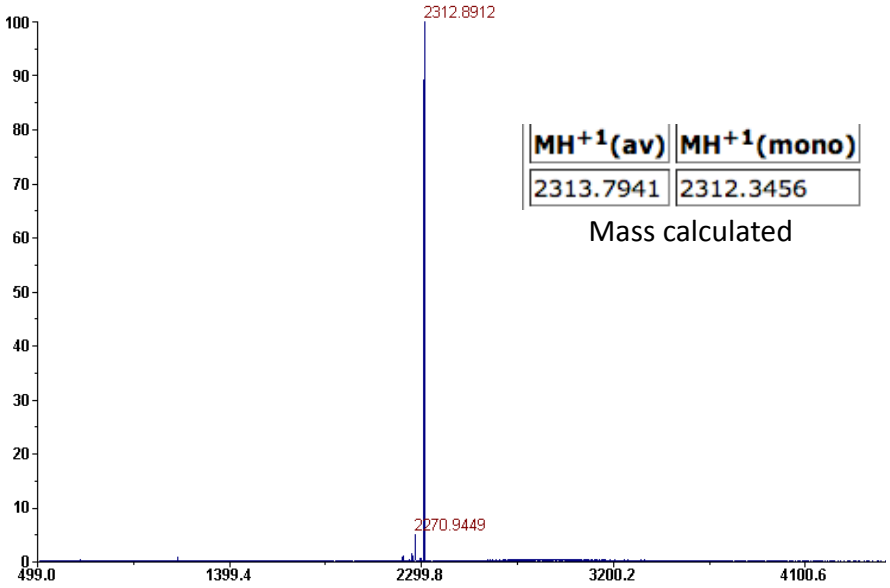


Monoisotopic analysis of H3K79me2 (22a)



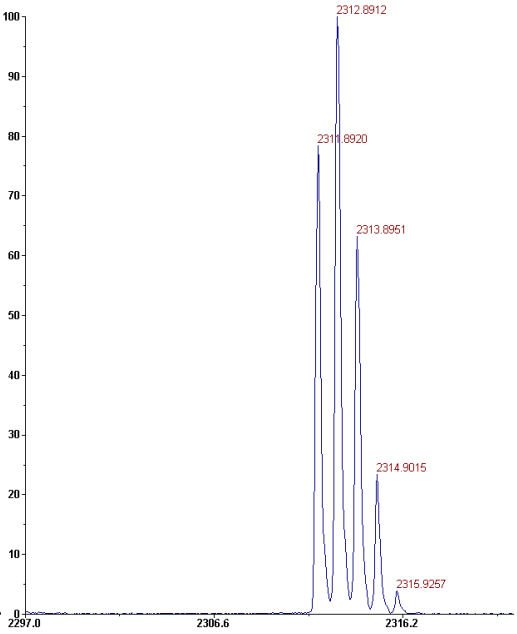
H3(1-20)K4me3-SR

Mass observed by MALDI-TOF in reflector mode



MH^{+1}(av)	MH^{+1}(mono)
2313.7941	2312.3456

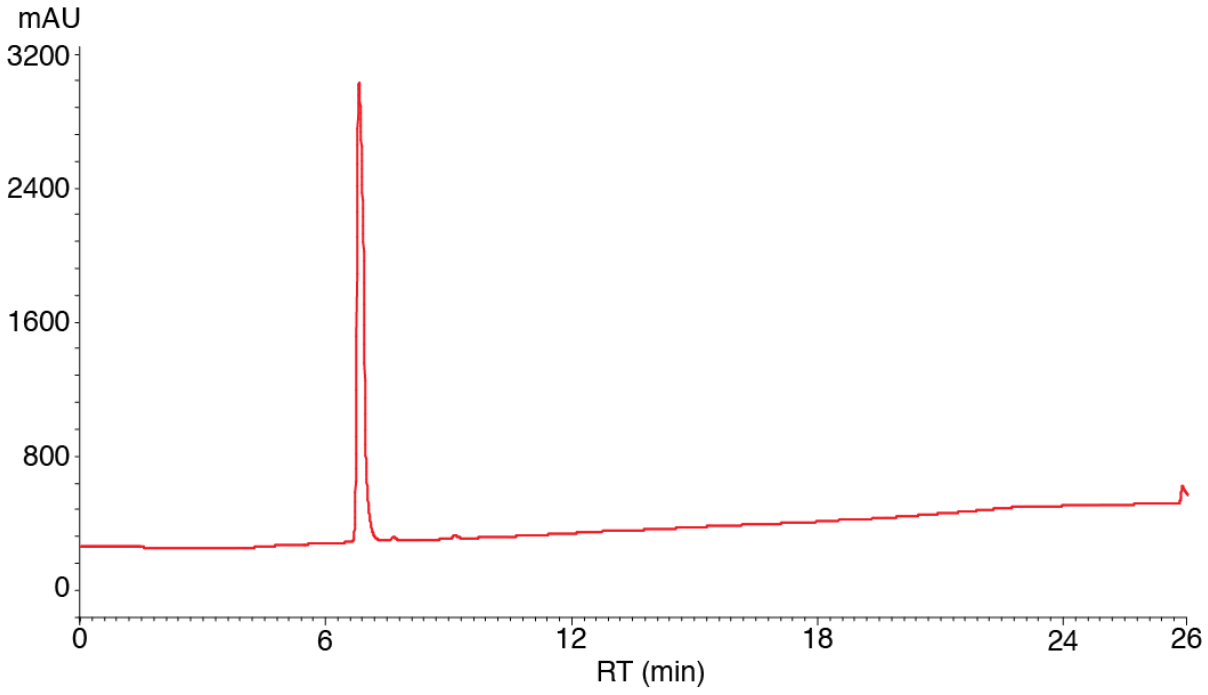
Mass calculated



Zoom-out view

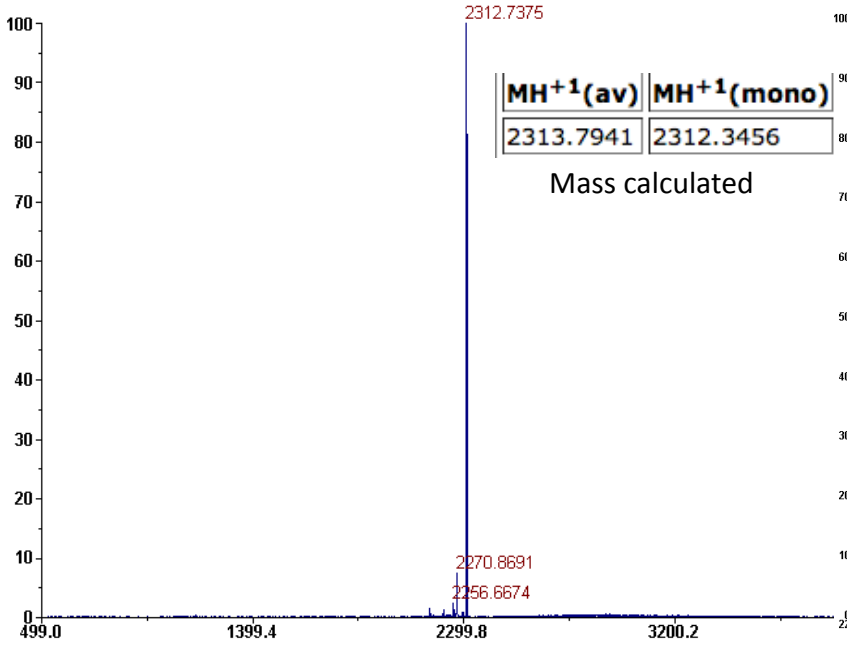
Zoom-in view

Analytical HPLC trace



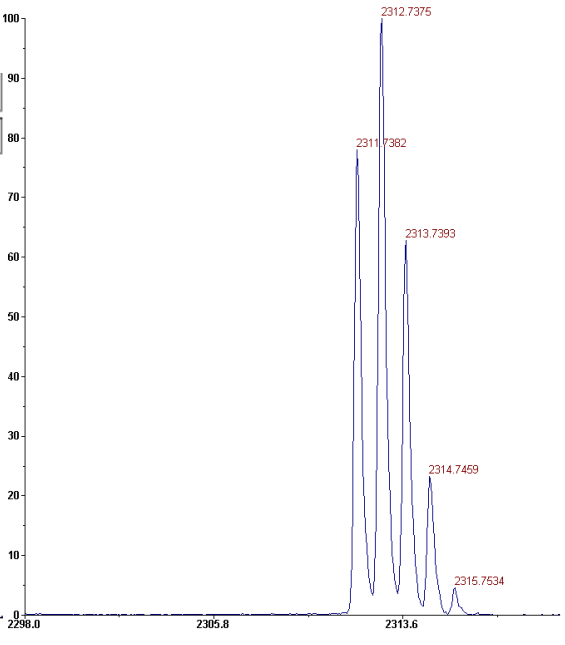
H3(1-20)K9me3-SR (1)

Mass observed by MALDI-TOF in reflector mode



MH^{+1}(av)	MH^{+1}(mono)
2313.7941	2312.3456

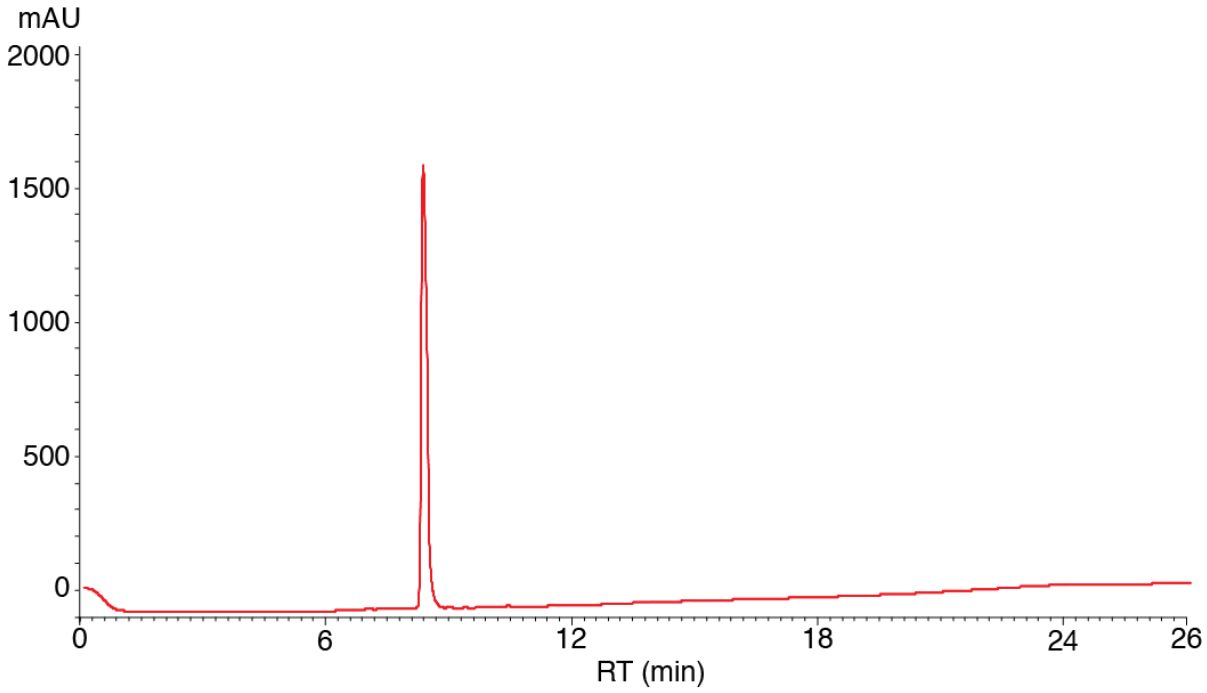
Mass calculated



Zoom-out view

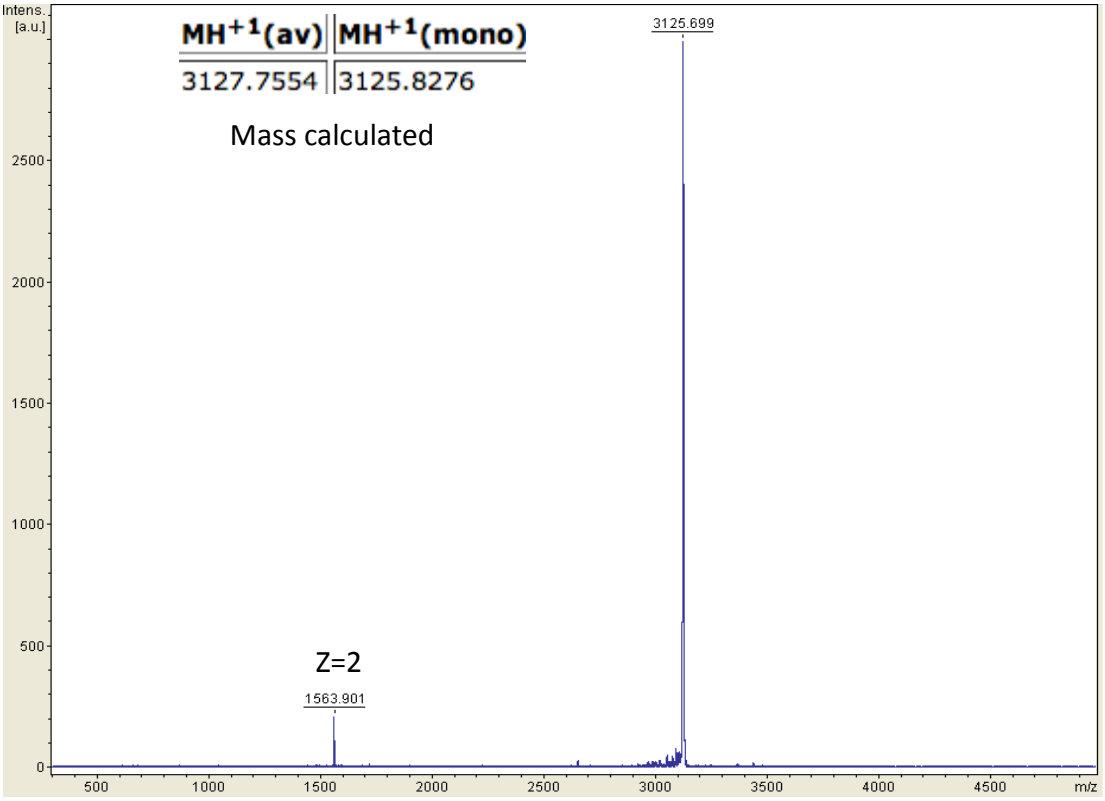
Zoom-in view

Analytical HPLC trace

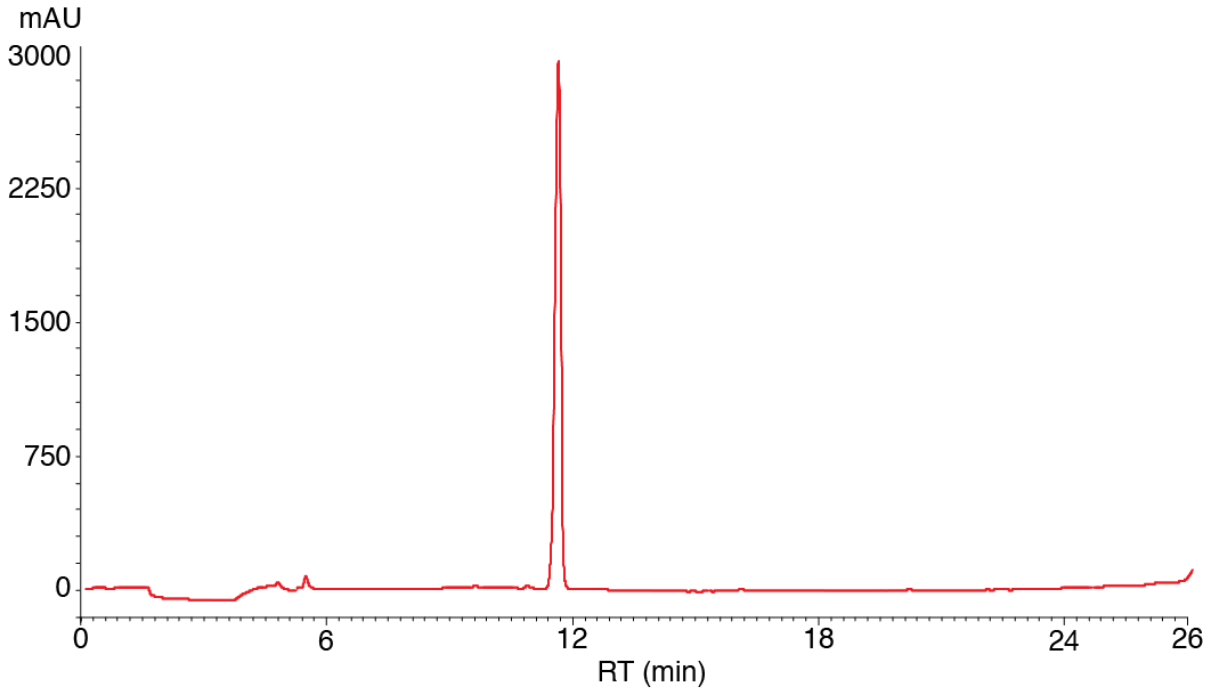


H3(1-28)K27me3-SR

Mass observed by MALDI-TOF in linear mode, zoom out view

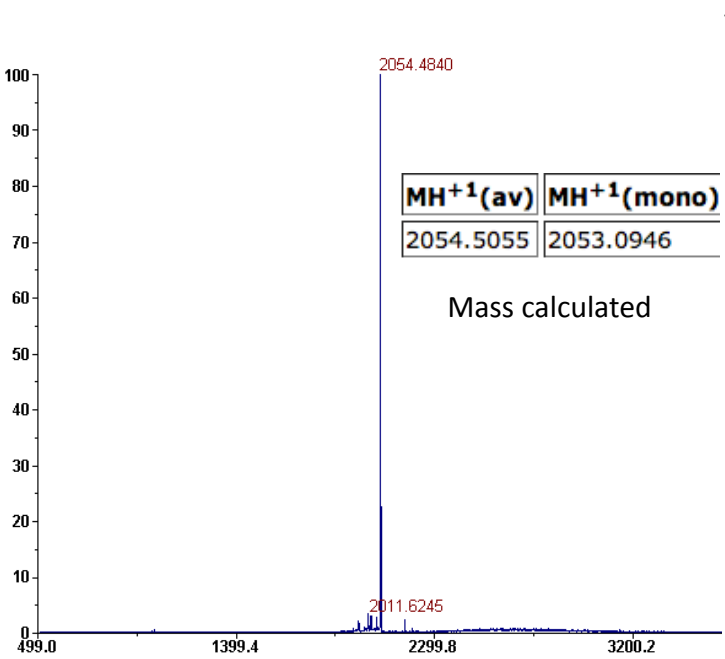


Analytical HPLC trace



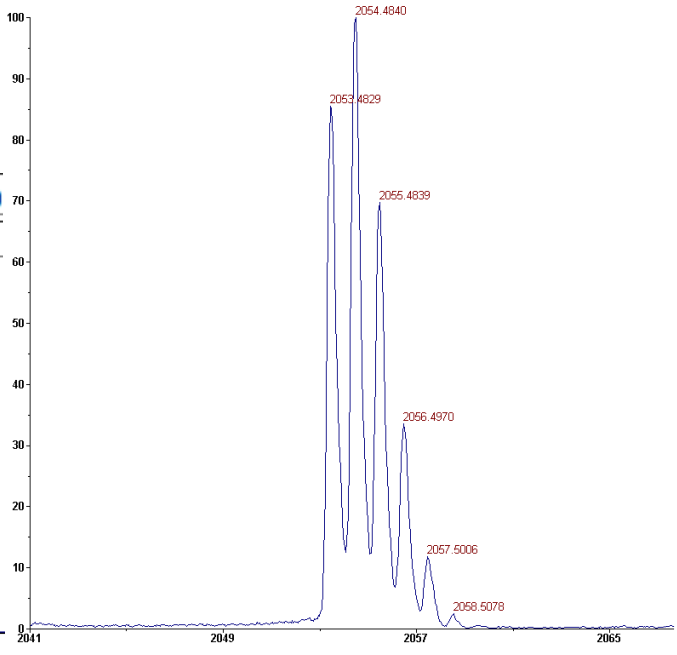
H3(29-46)K36me3-A29C-SR (11)

Mass observed by MALDI-TOF in reflector mode



MH^{+1}(av)	MH^{+1}(mono)
2054.5055	2053.0946

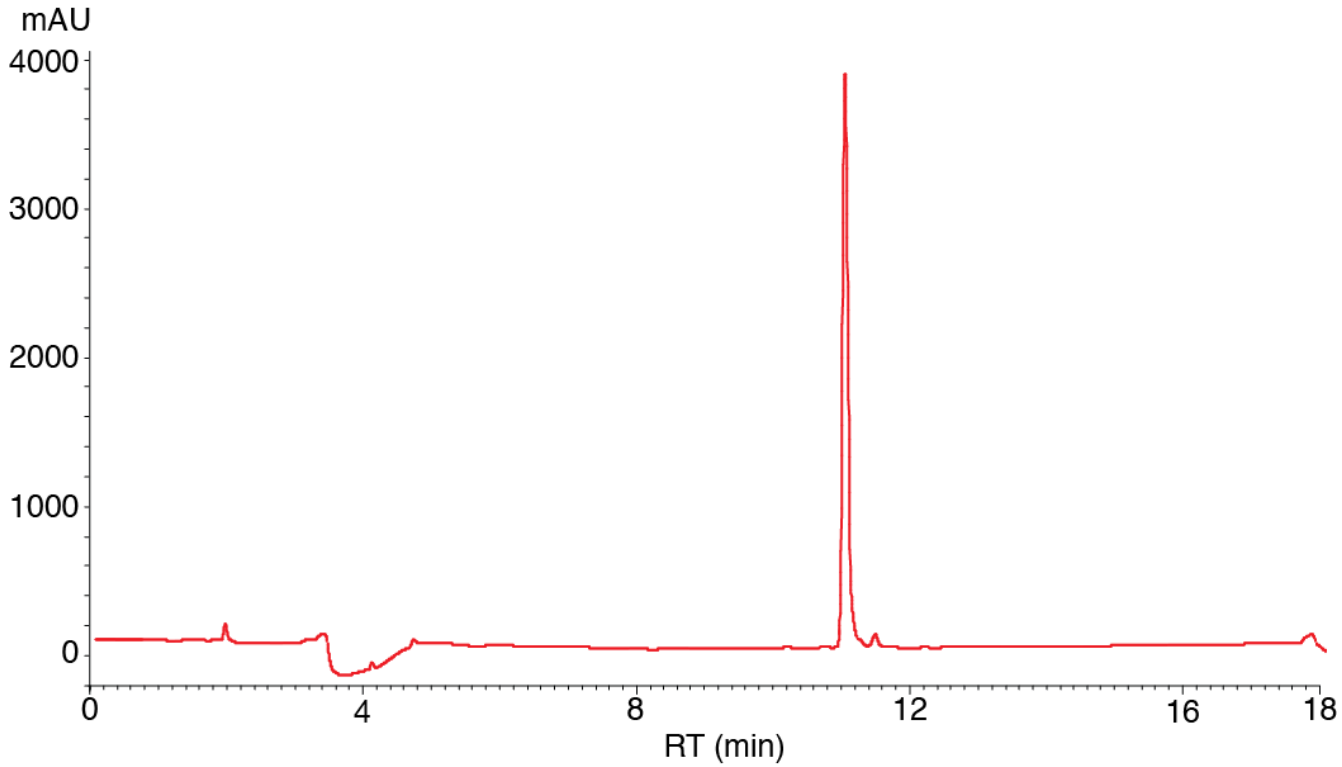
Mass calculated



Zoom-out view

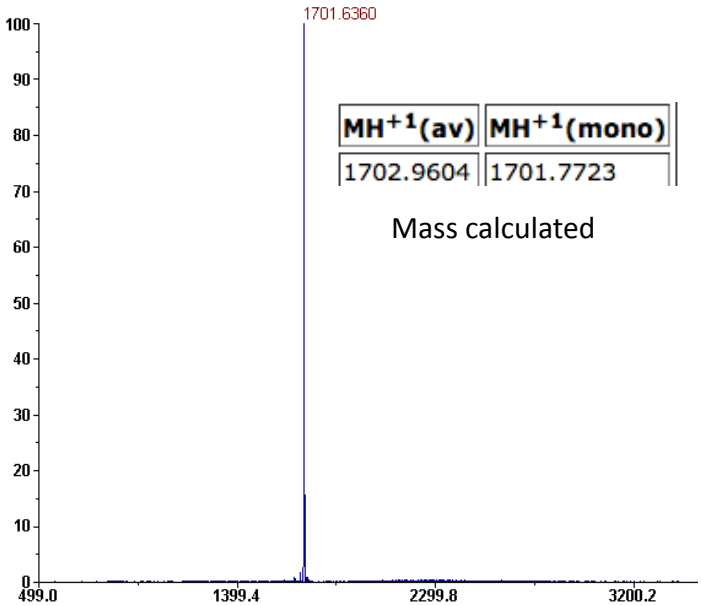
Zoom-in view

Analytical HPLC trace



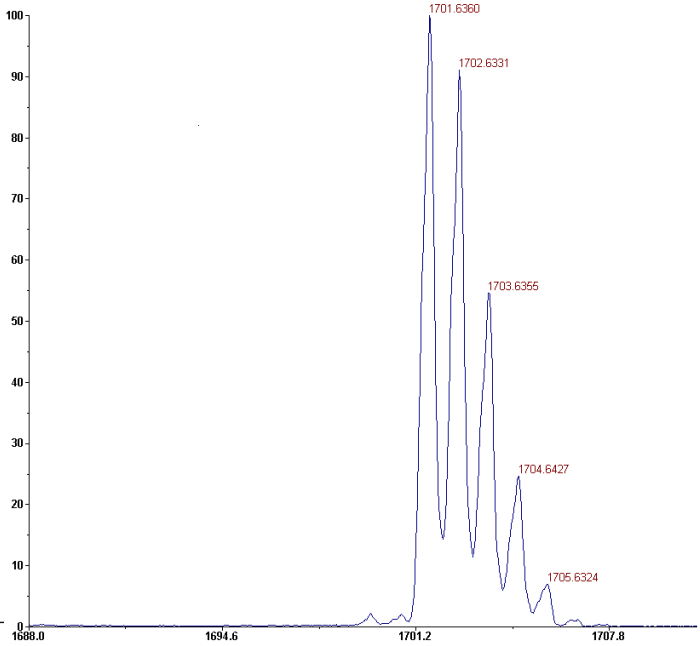
H3(75-87)K79me2-A75Thz-SR (18a)

Mass observed by MALDI-TOF in reflector mode



MH^{+1}(av)	MH^{+1}(mono)
1702.9604	1701.7723

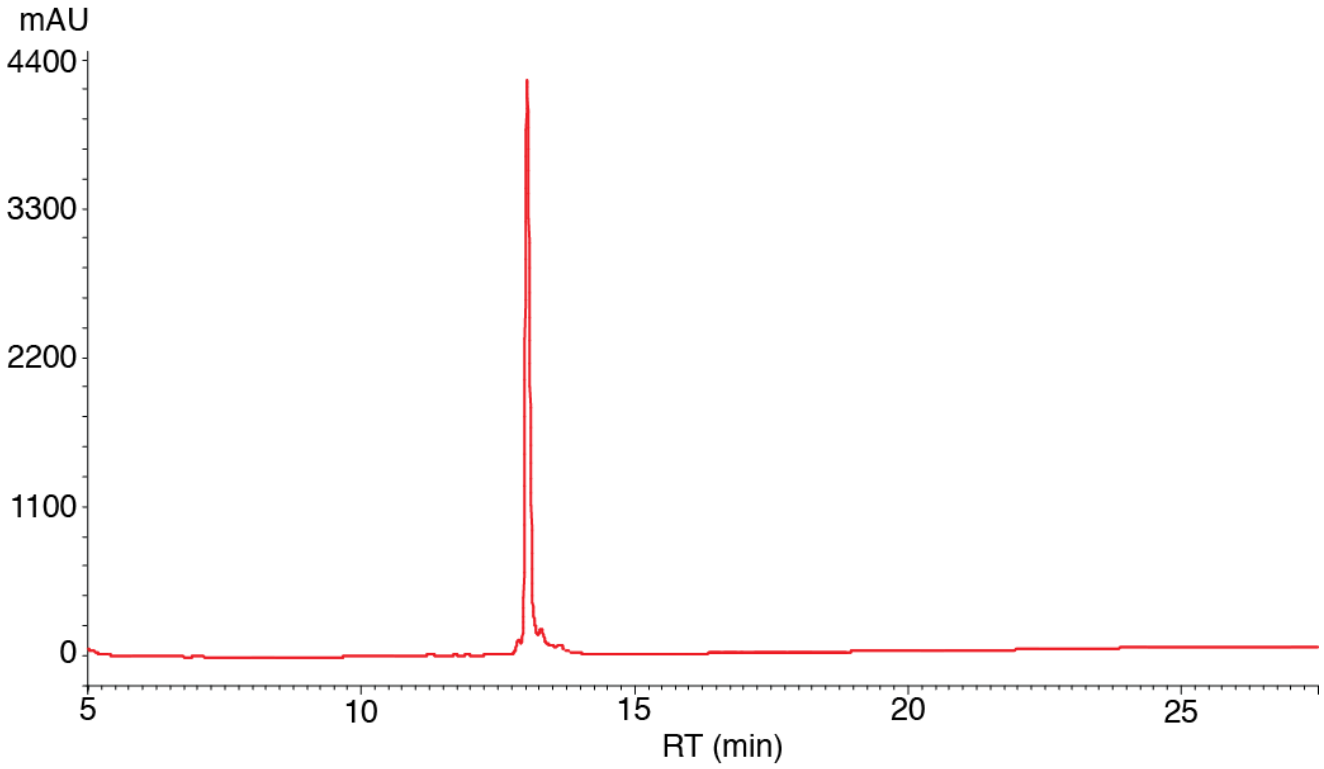
Mass calculated



Zoom-out view

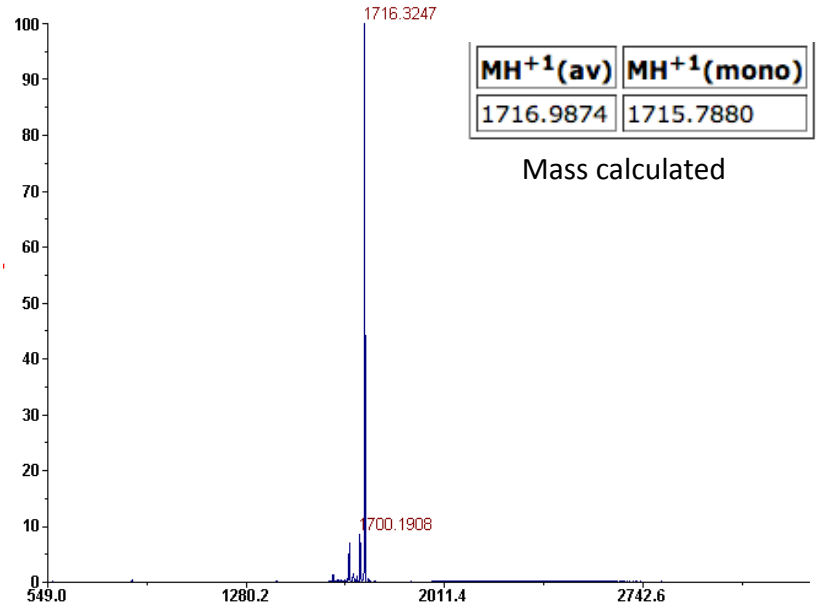
Zoom-in view

Analytical HPLC trace



H3(75-87)K79me3-A75Thz-SR (18b)

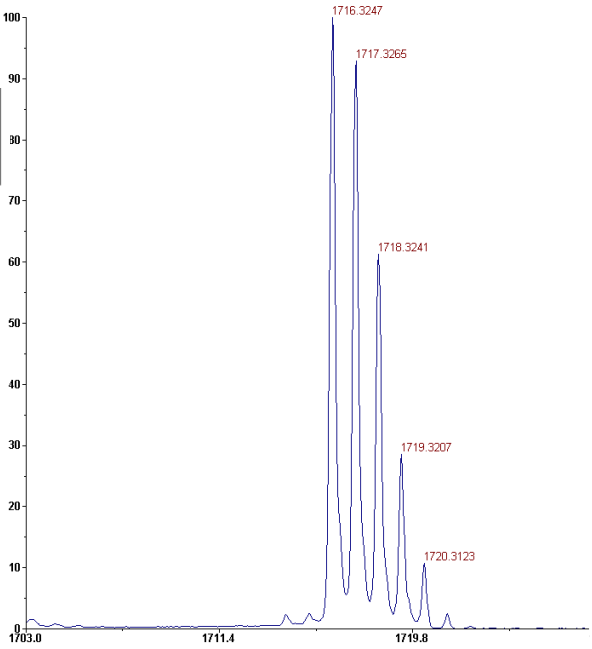
Mass observed by MALDI-TOF in reflector mode



MH^{+1}(av)	MH^{+1}(mono)
1716.9874	1715.7880

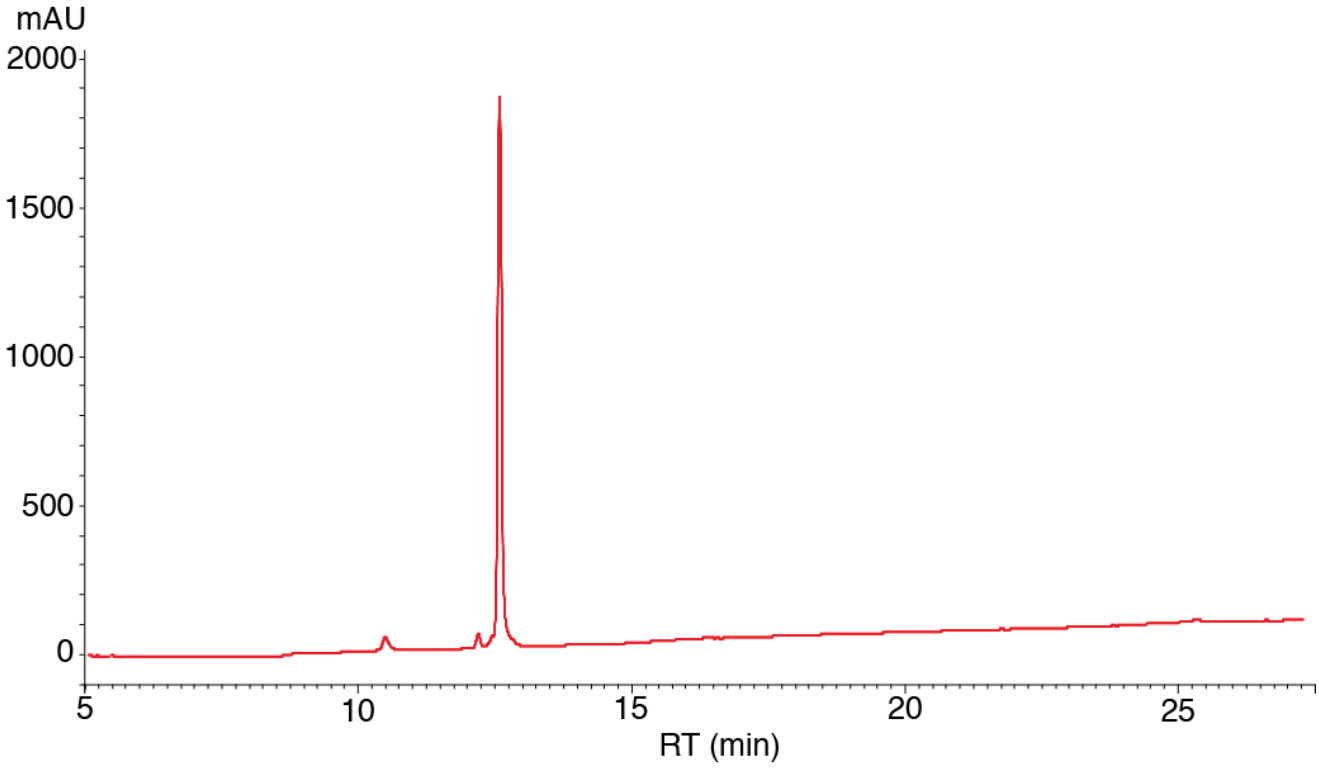
Mass calculated

Zoom-out view



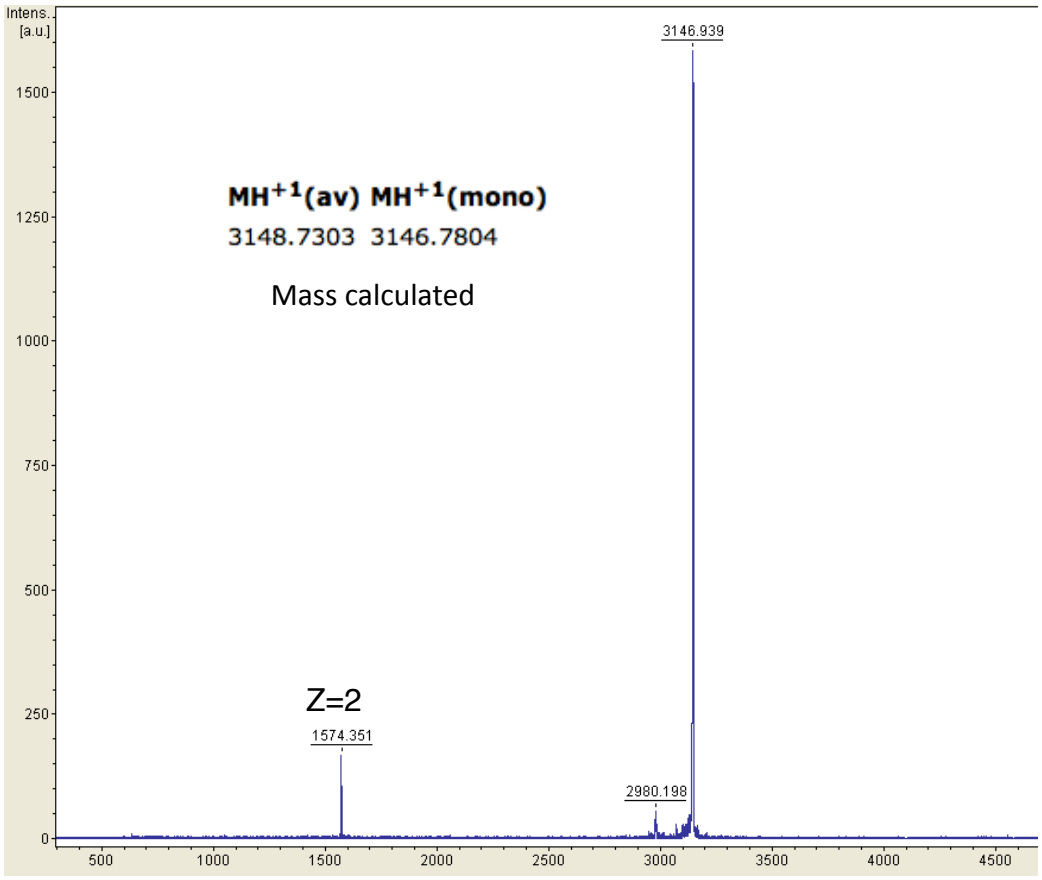
Zoom-in view

Analytical HPLC trace

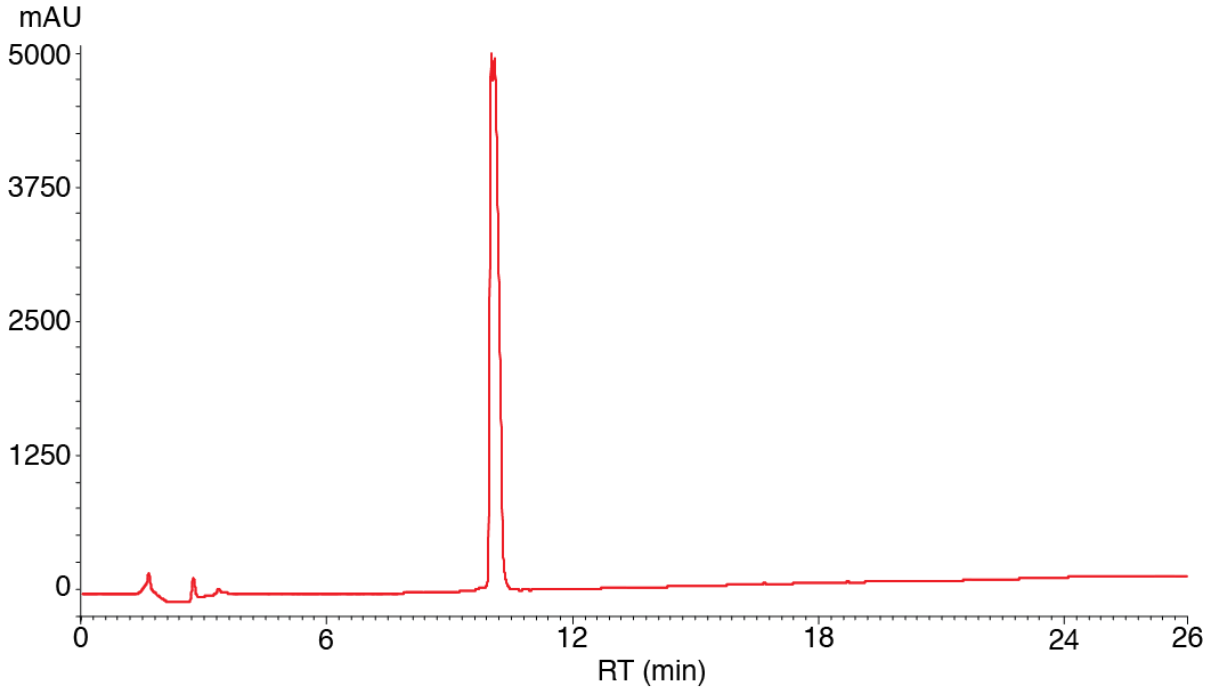


H3(1-28)-SR’ (10)

Mass observed by MALDI-TOF in linear mode

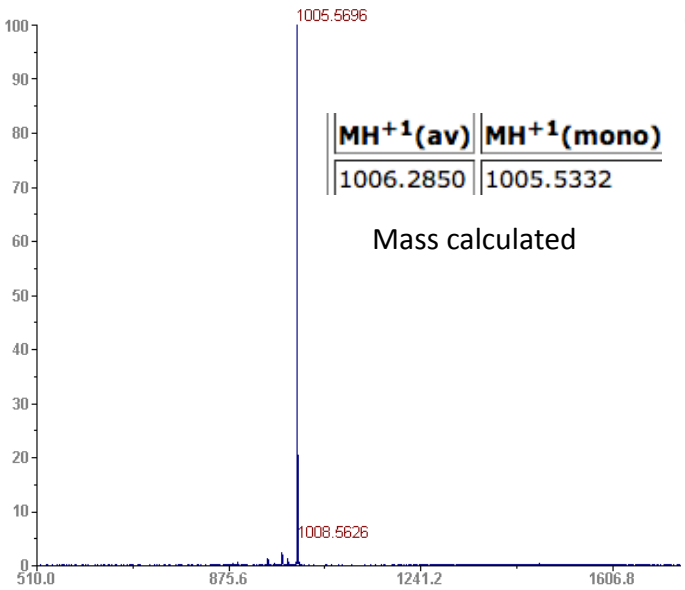


Analytical HPLC trace



H3(21-28)K27me3-A21Thz-SR

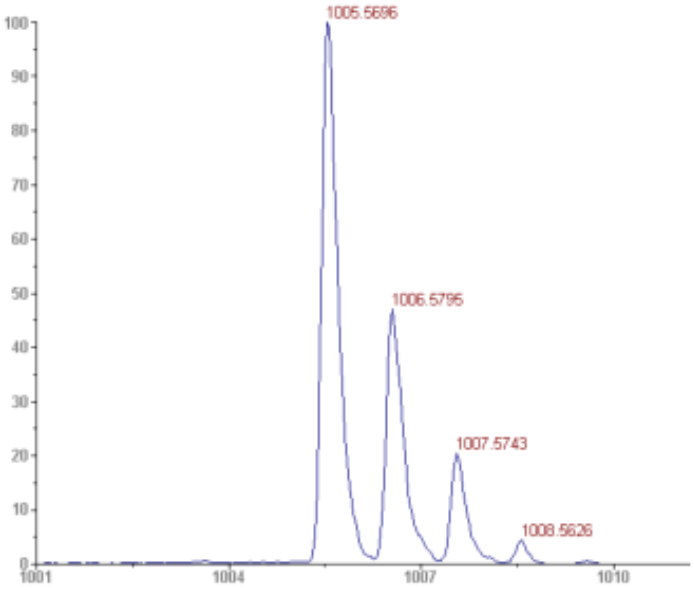
Mass observed by MALDI-TOF in reflector mode



MH^{+1}(av)	MH^{+1}(mono)
1006.2850	1005.5332

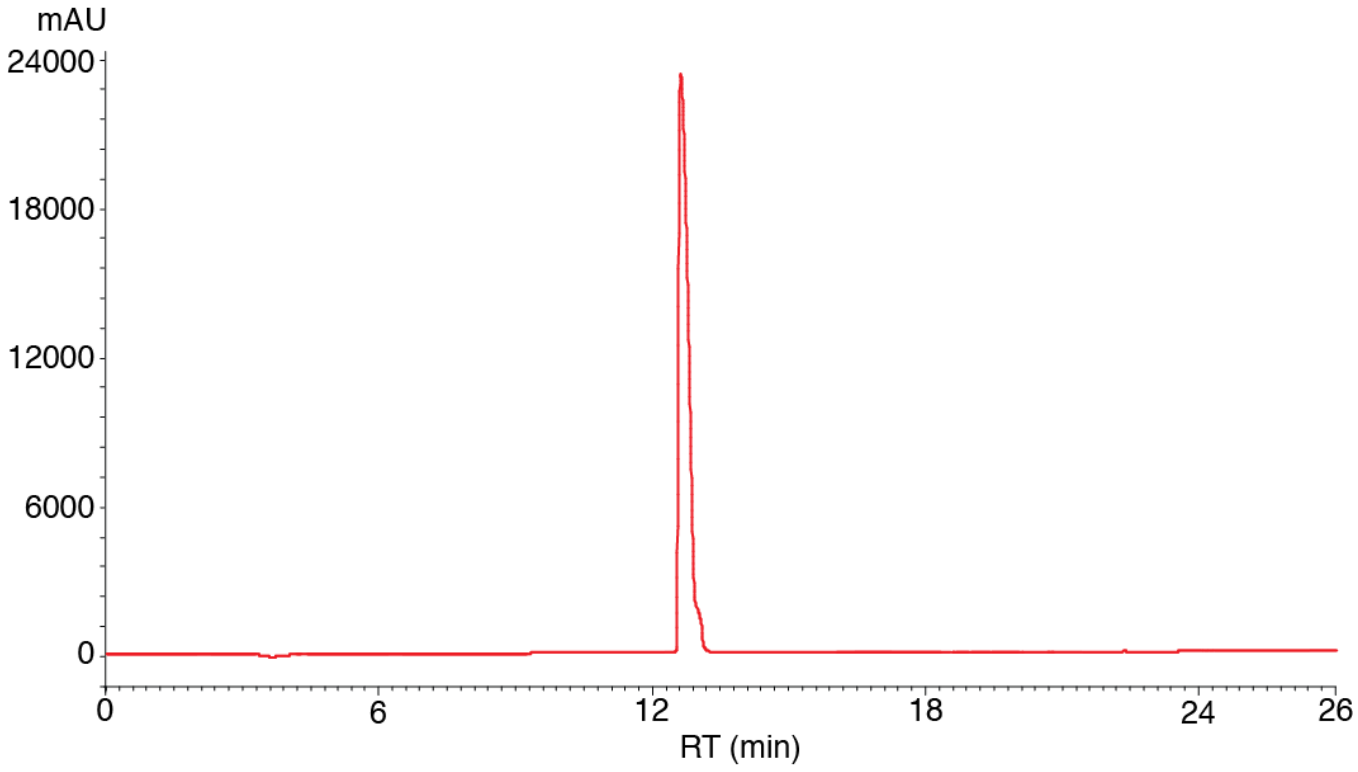
Mass calculated

Zoom-out view



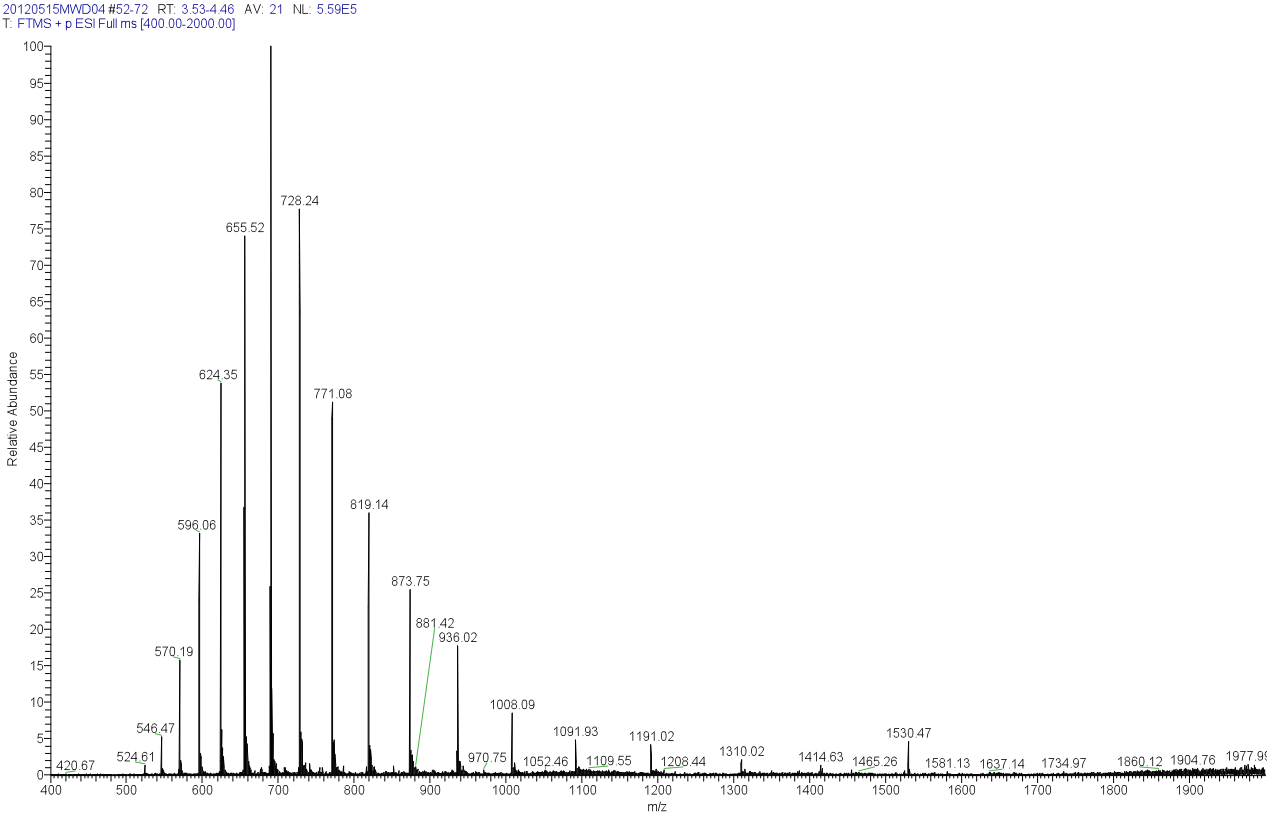
Zoom-in view

Analytical HPLC trace

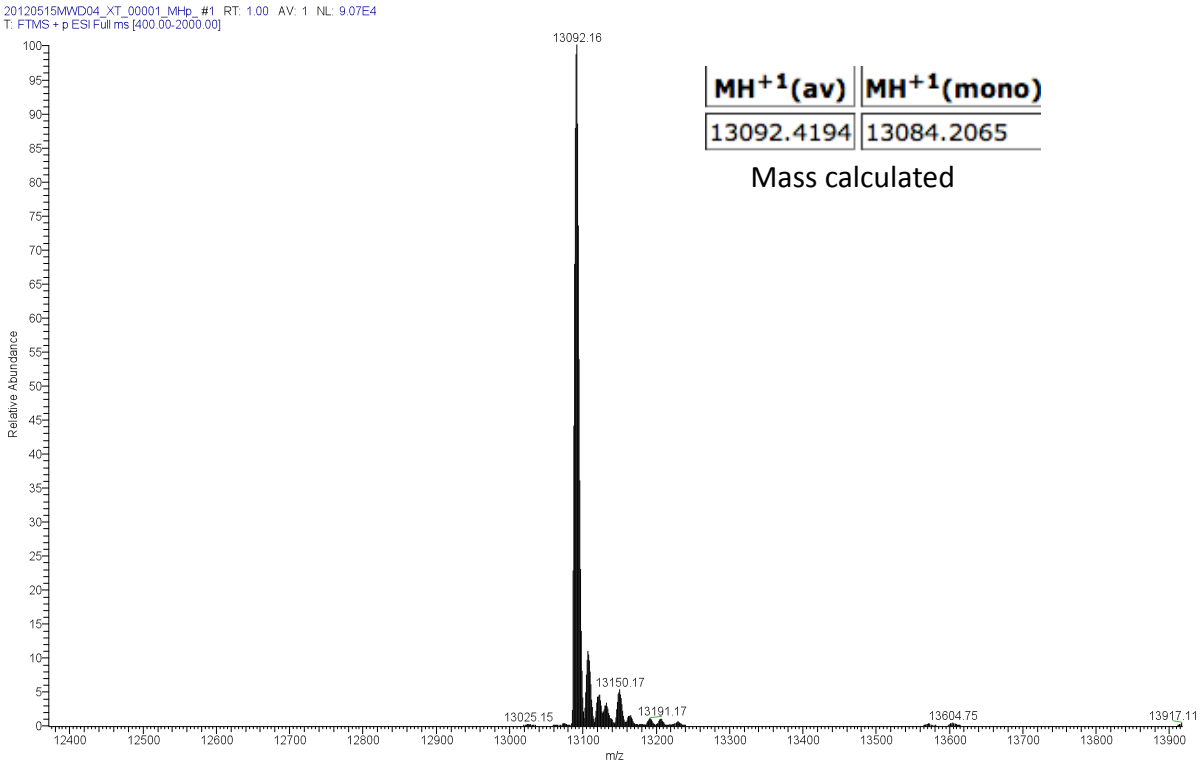


H3Δ20-A21C (2)

ESI-MS before deconvolution



ESI-MS after deconvolution

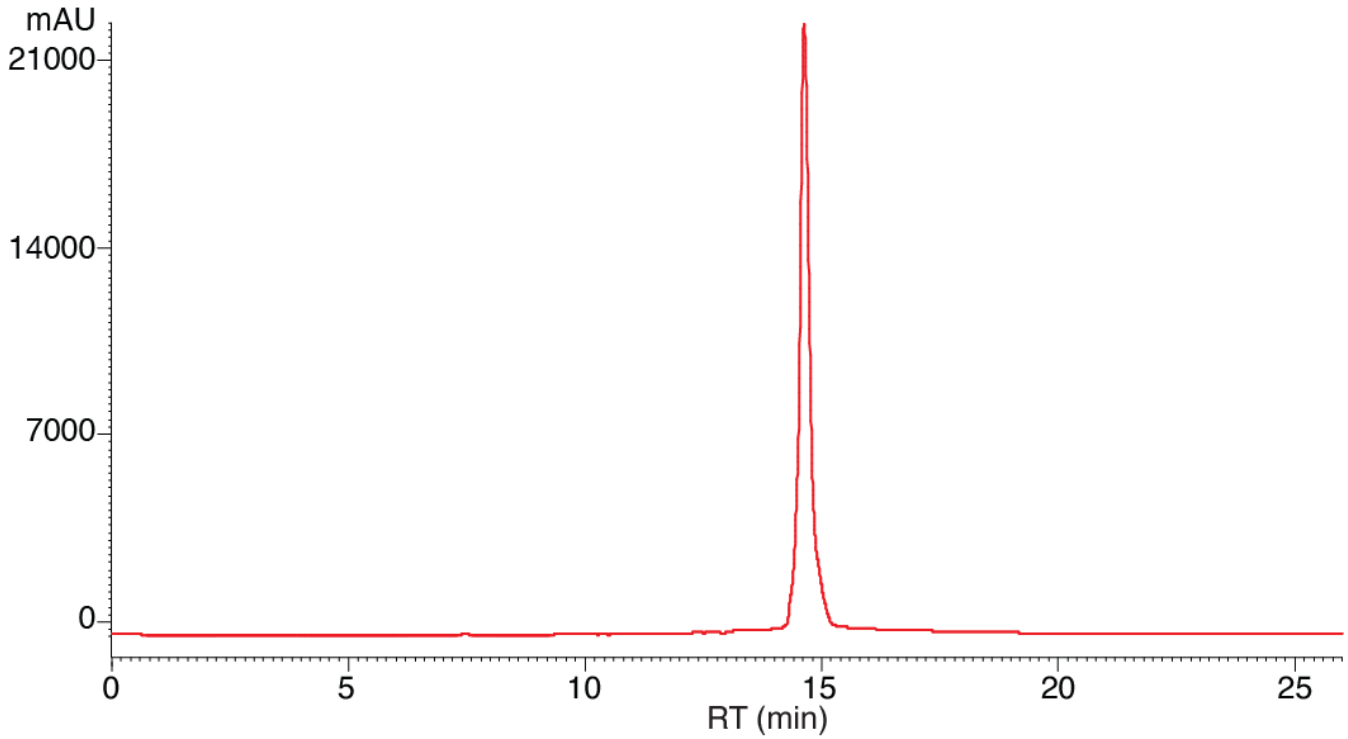


MH^{+1}(av)	MH^{+1}(mono)
13092.4194	13084.2065

Mass calculated

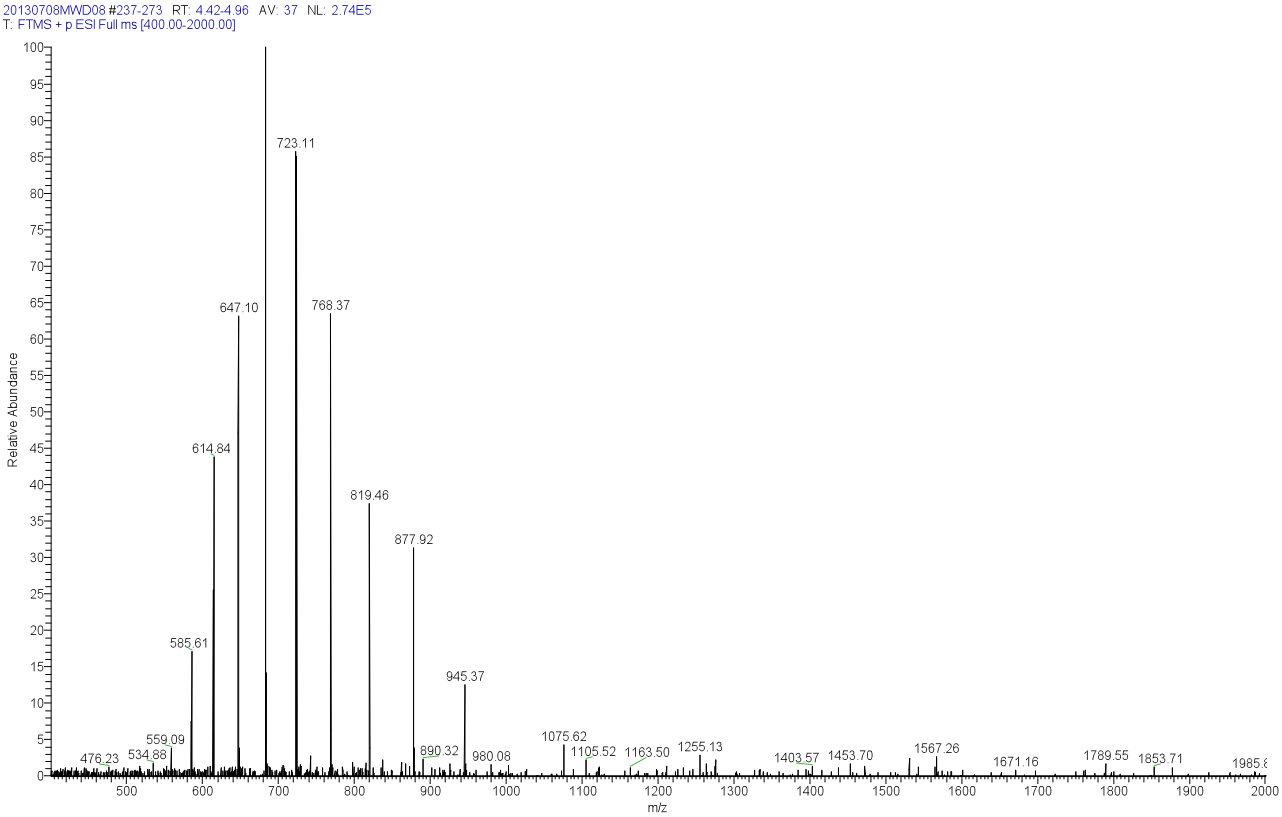
H3Δ20-A21C (2)

Analytical HPLC trace

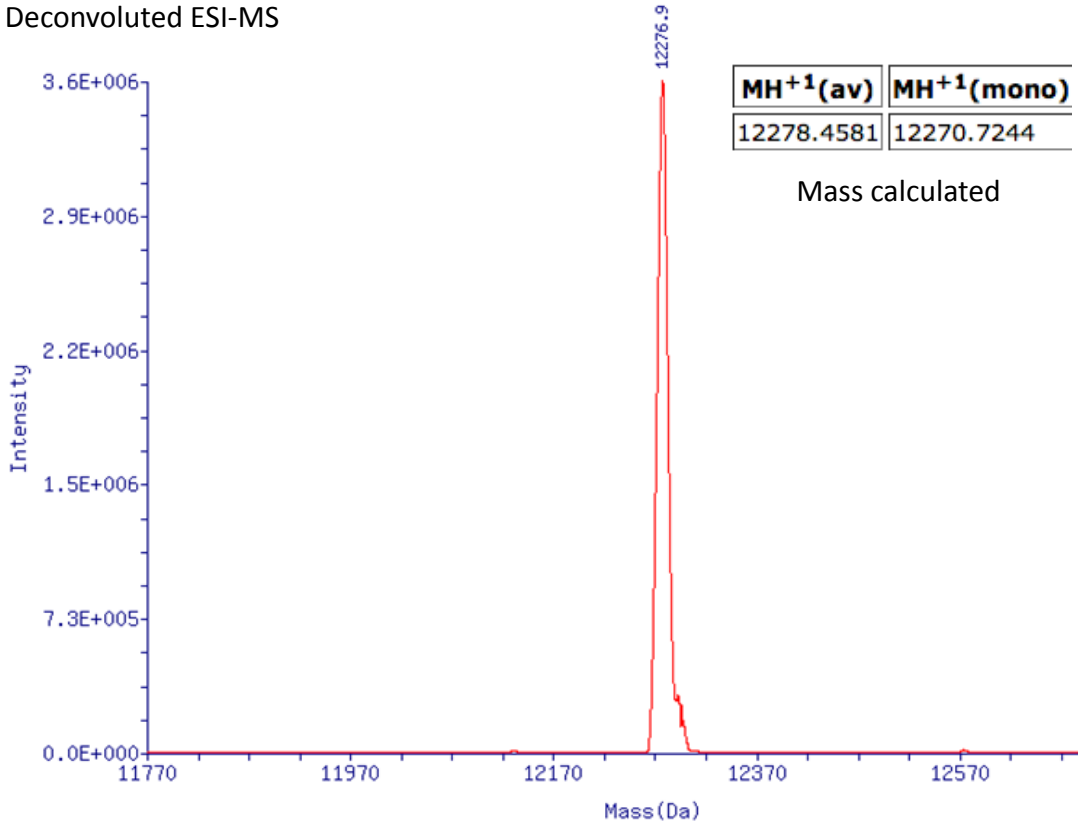


H3Δ28-A29C

ESI-MS before deconvolution



Deconvoluted ESI-MS

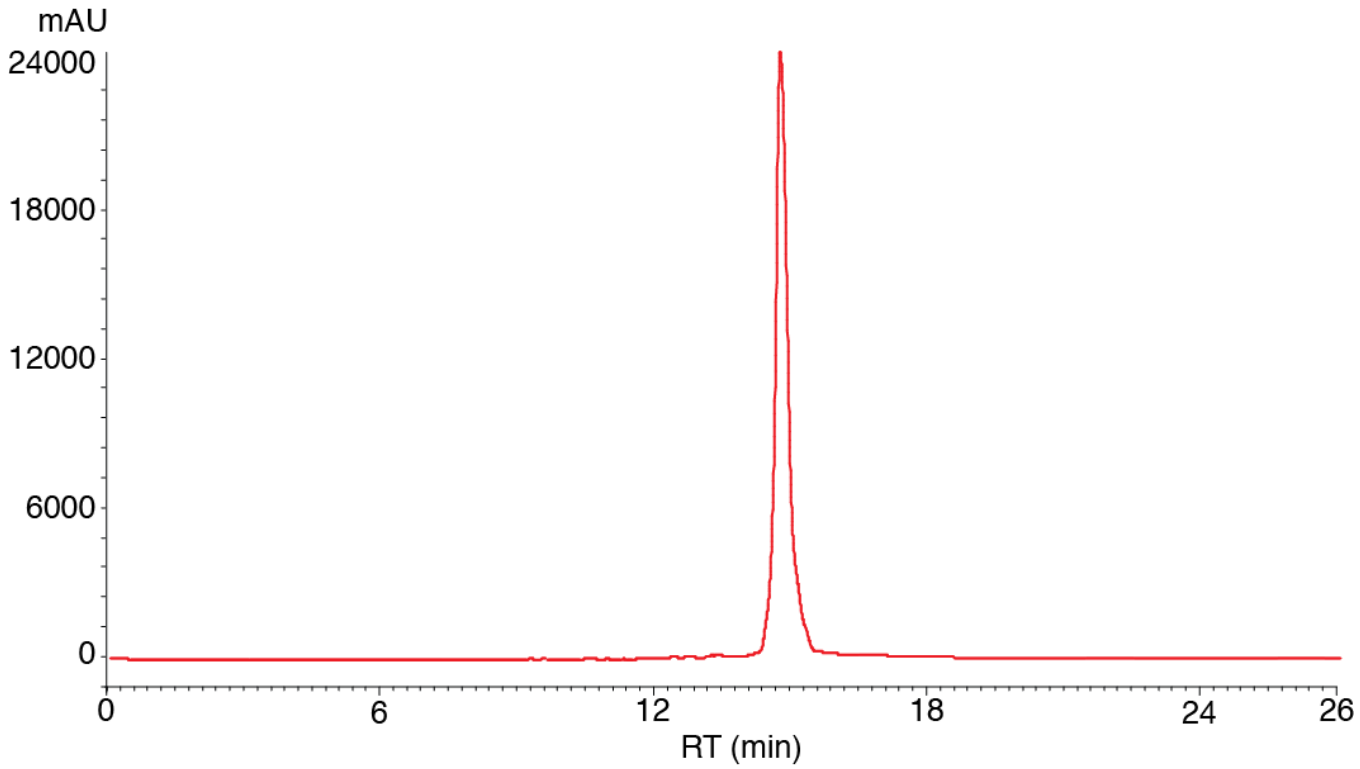


MH^{+1}(av)	MH^{+1}(mono)
12278.4581	12270.7244

Mass calculated

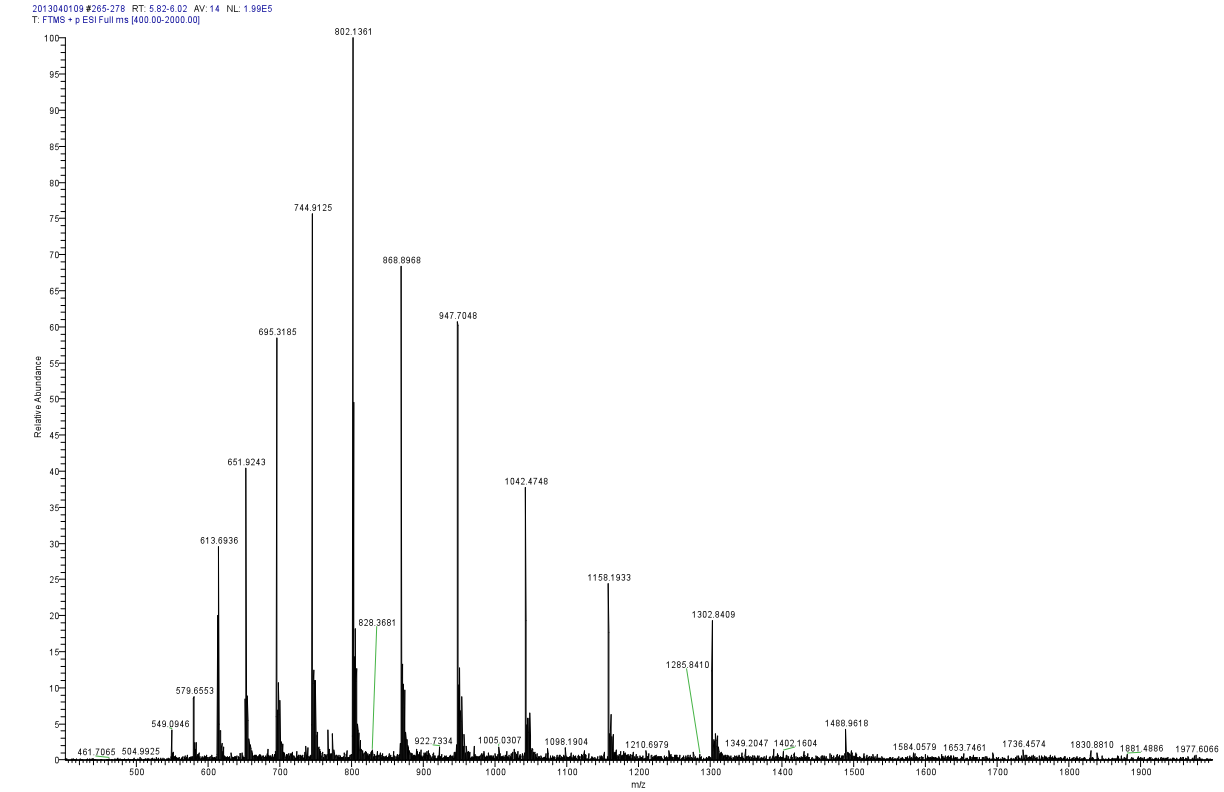
H3Δ28-A29C

Analytical HPLC trace

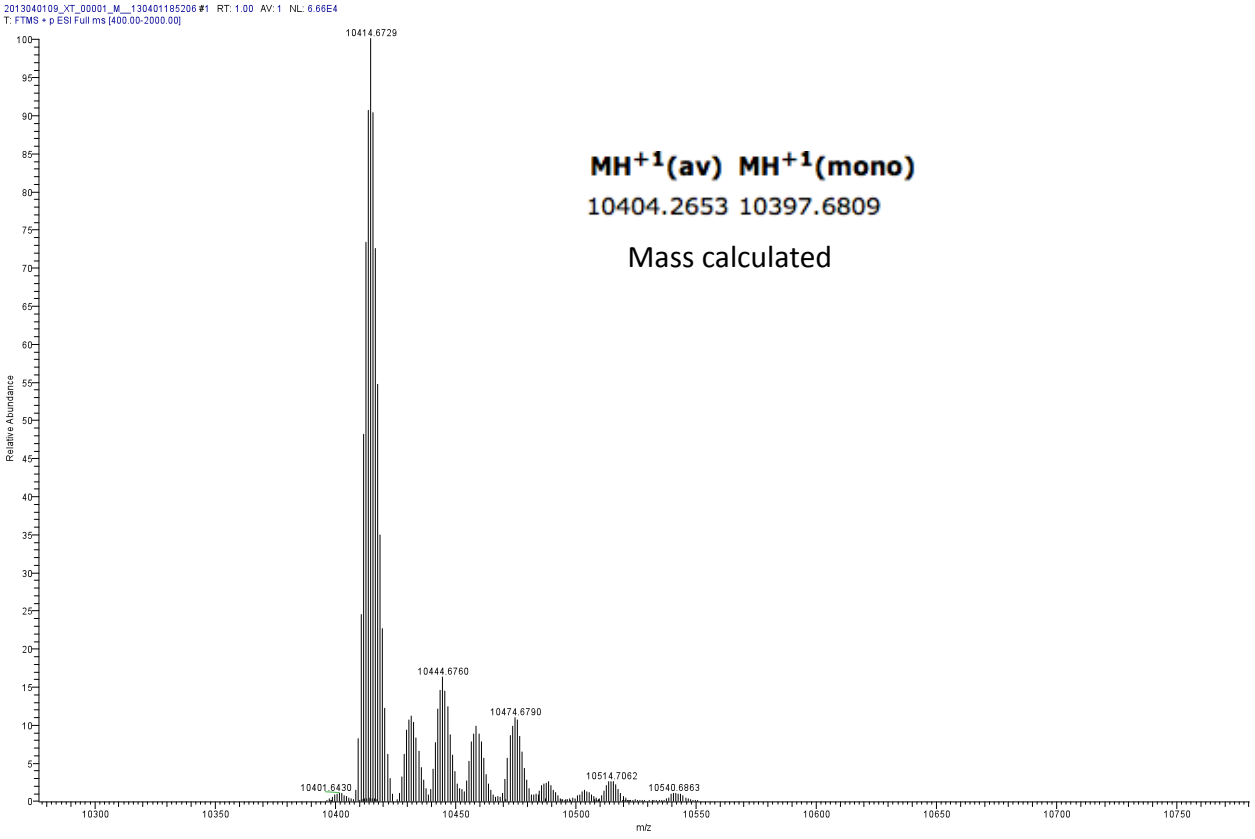


H3Δ46-A47C (14)

ESI-MS before deconvolution



Deconvoluted ESI-MS

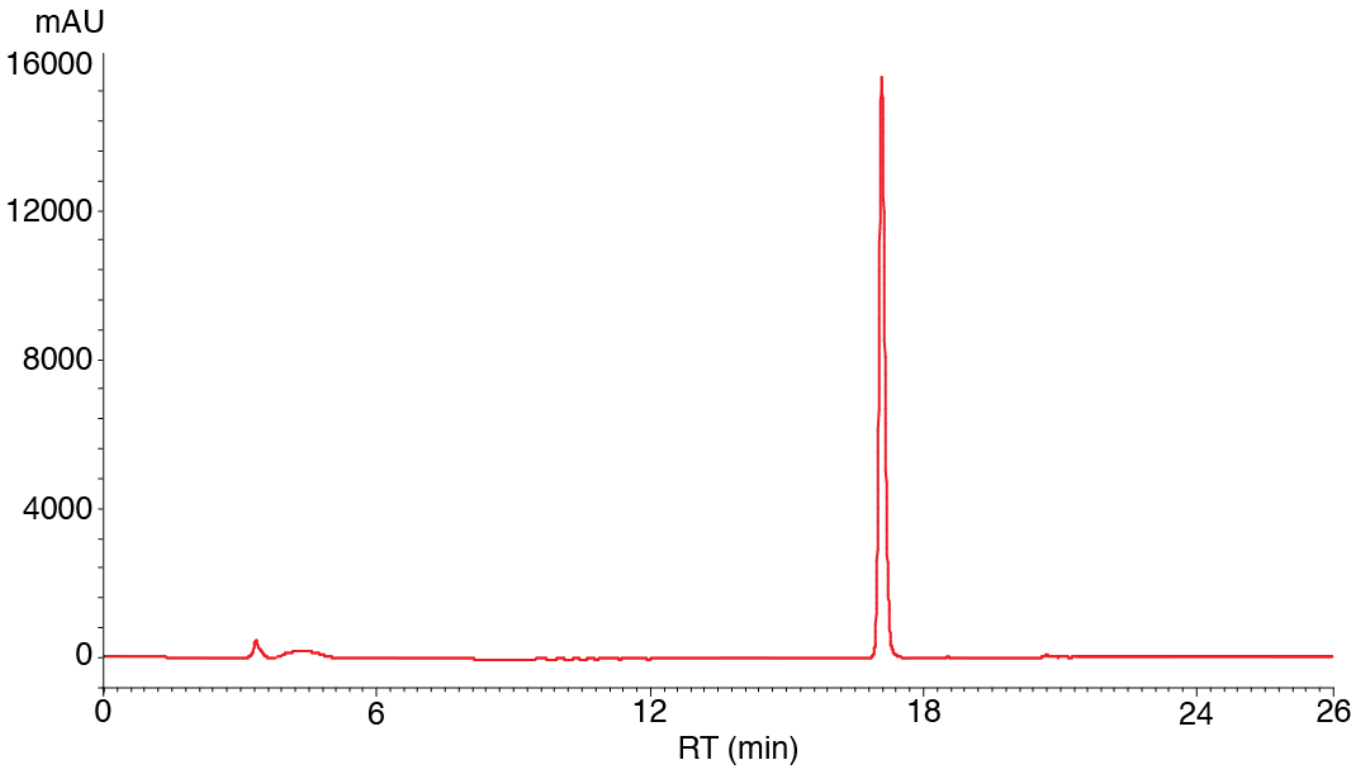


MH^{+1}(av)	MH^{+1}(mono)
10404.2653	10397.6809

Mass calculated

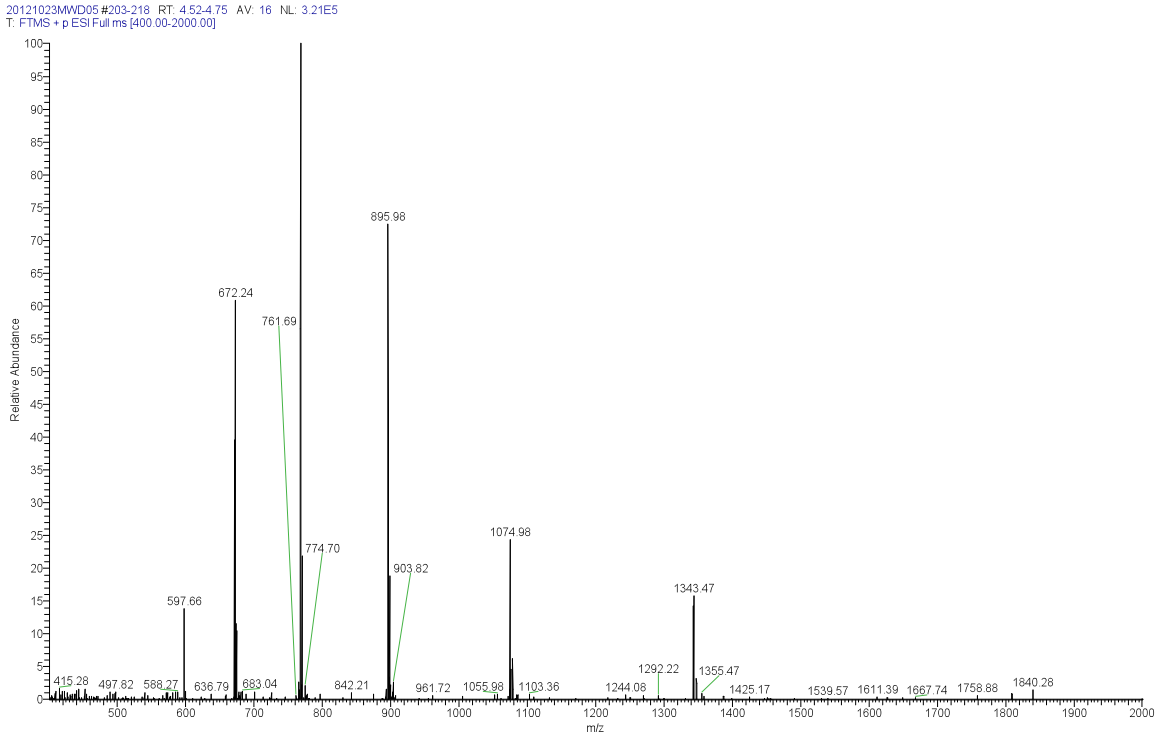
H3Δ46-A47C (14)

Analytical HPLC trace

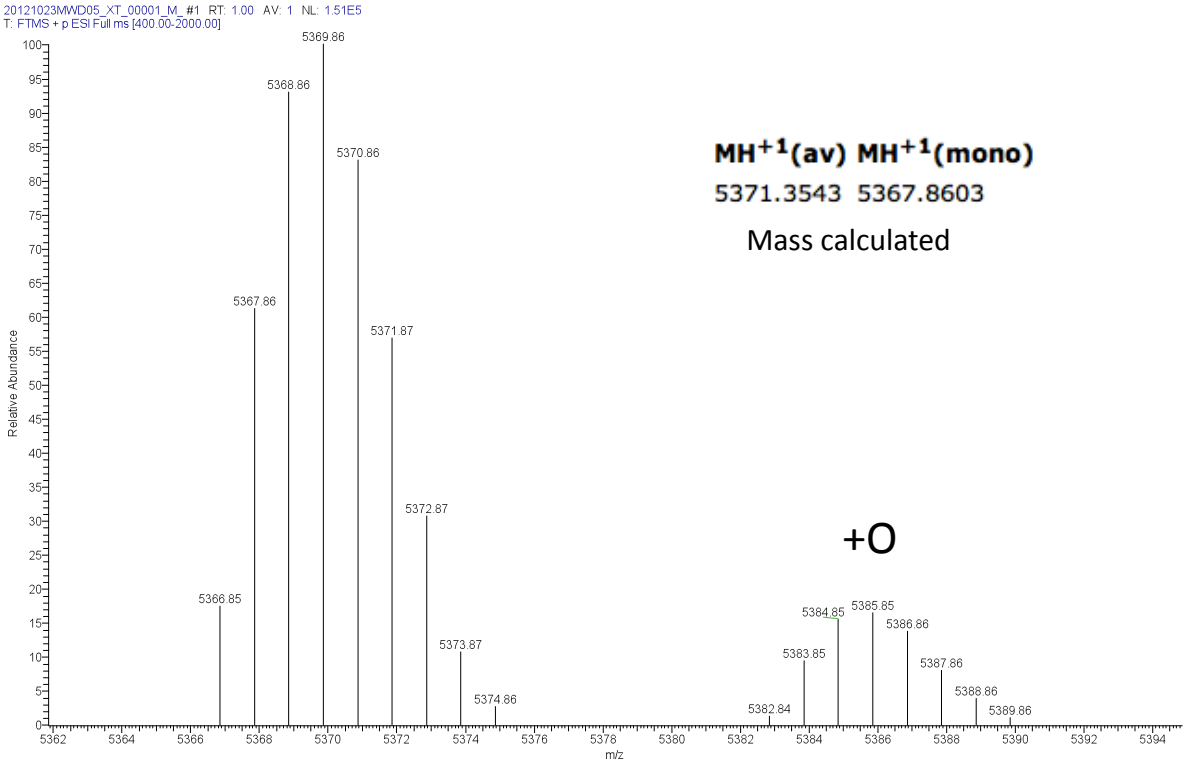


H3Δ87-A88C (17)

ESI-MS before deconvolution



Deconvoluted ESI-MS



MH^{+1}(av) MH^{+1}(mono)

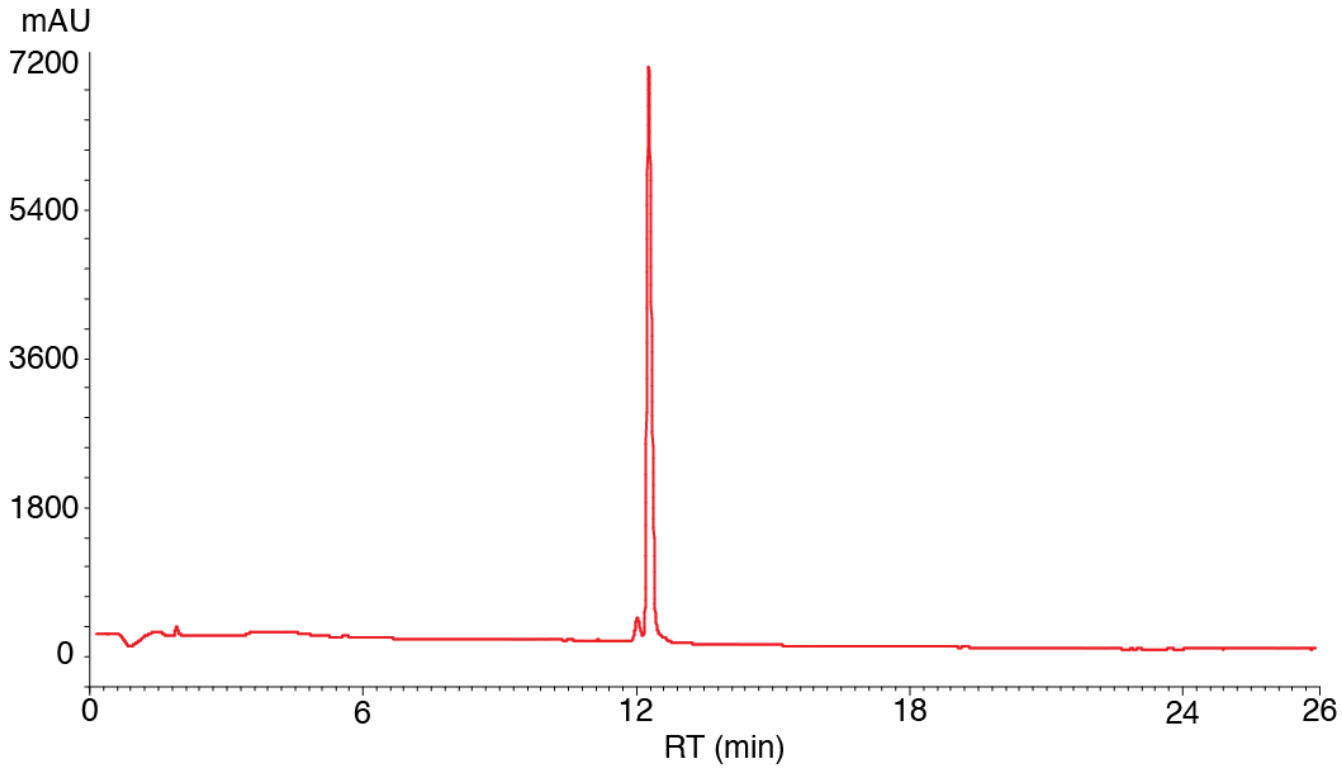
5371.3543 5367.8603

Mass calculated

+O

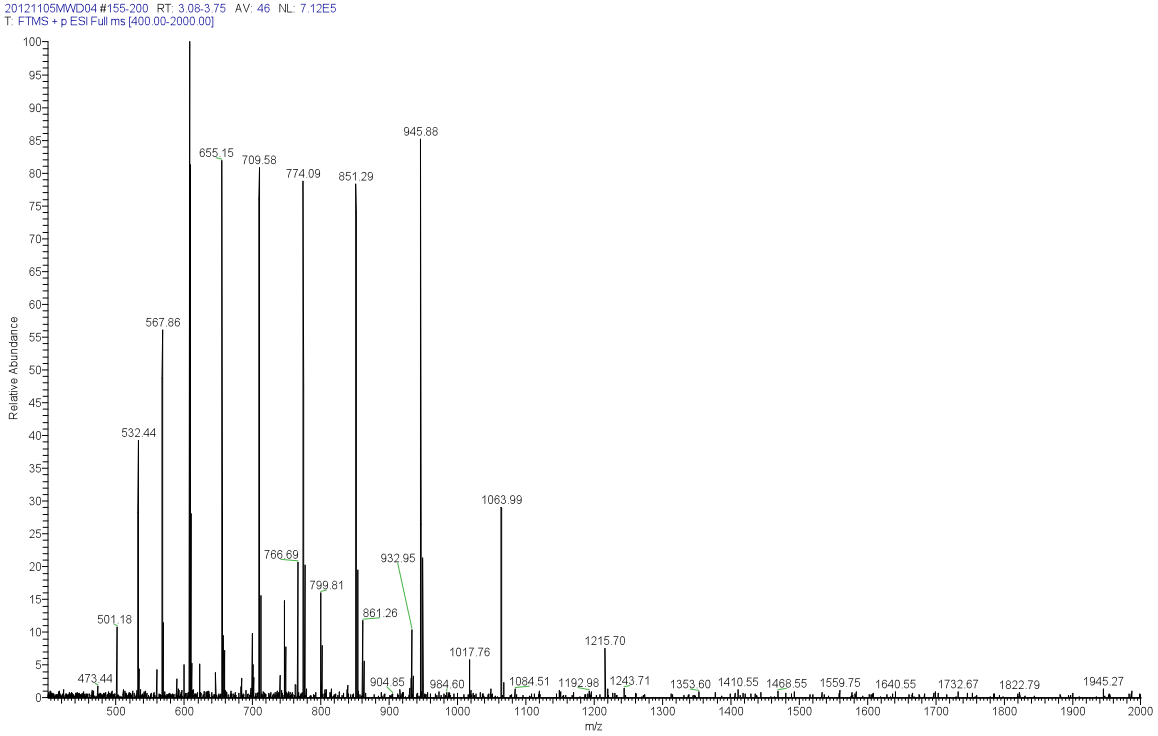
H3Δ87-A88C (17)

Analytical HPLC trace

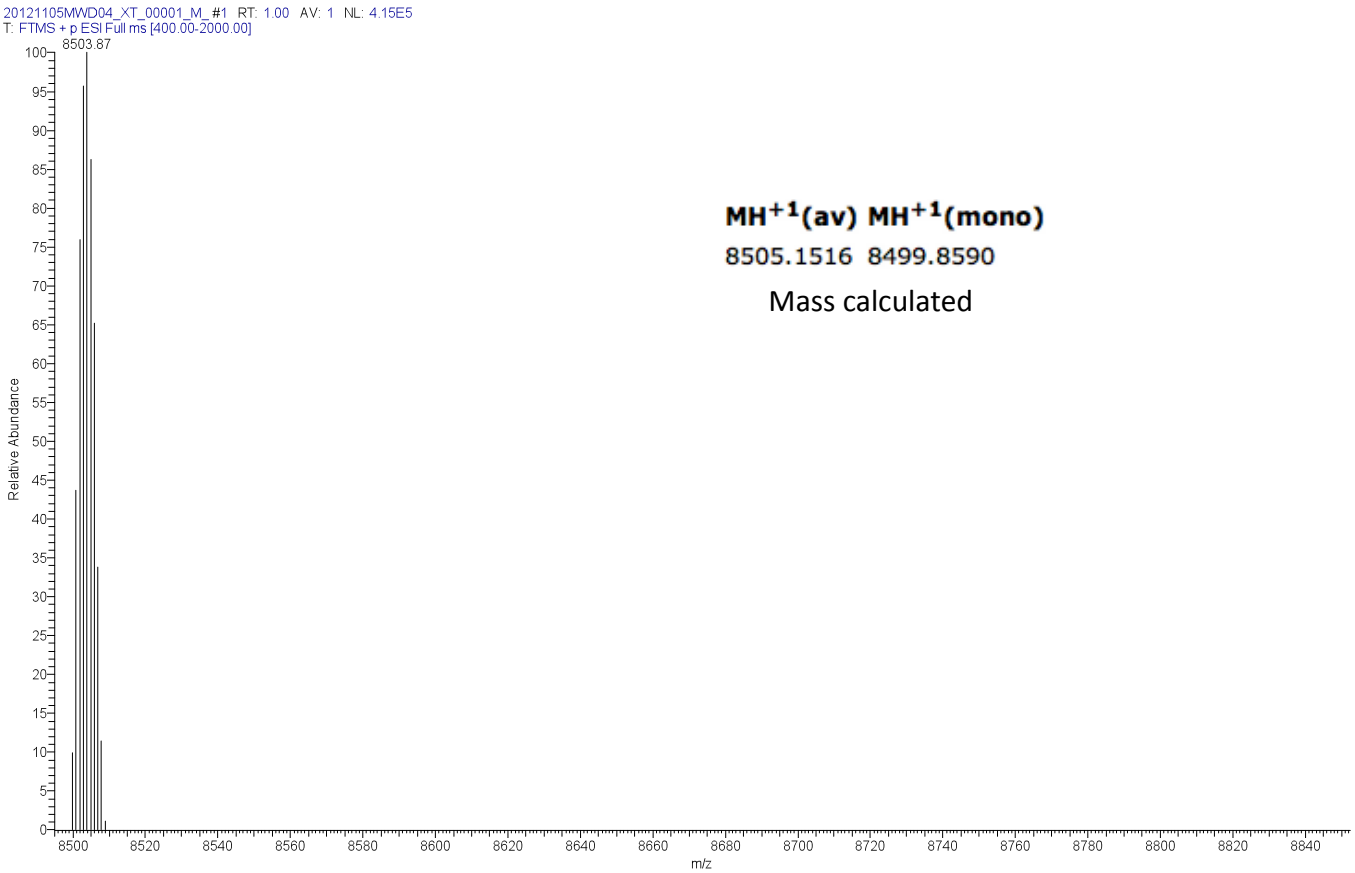


H3(1-74)-MES (21)

ESI-MS before deconvolution



Deconvoluted ESI-MS

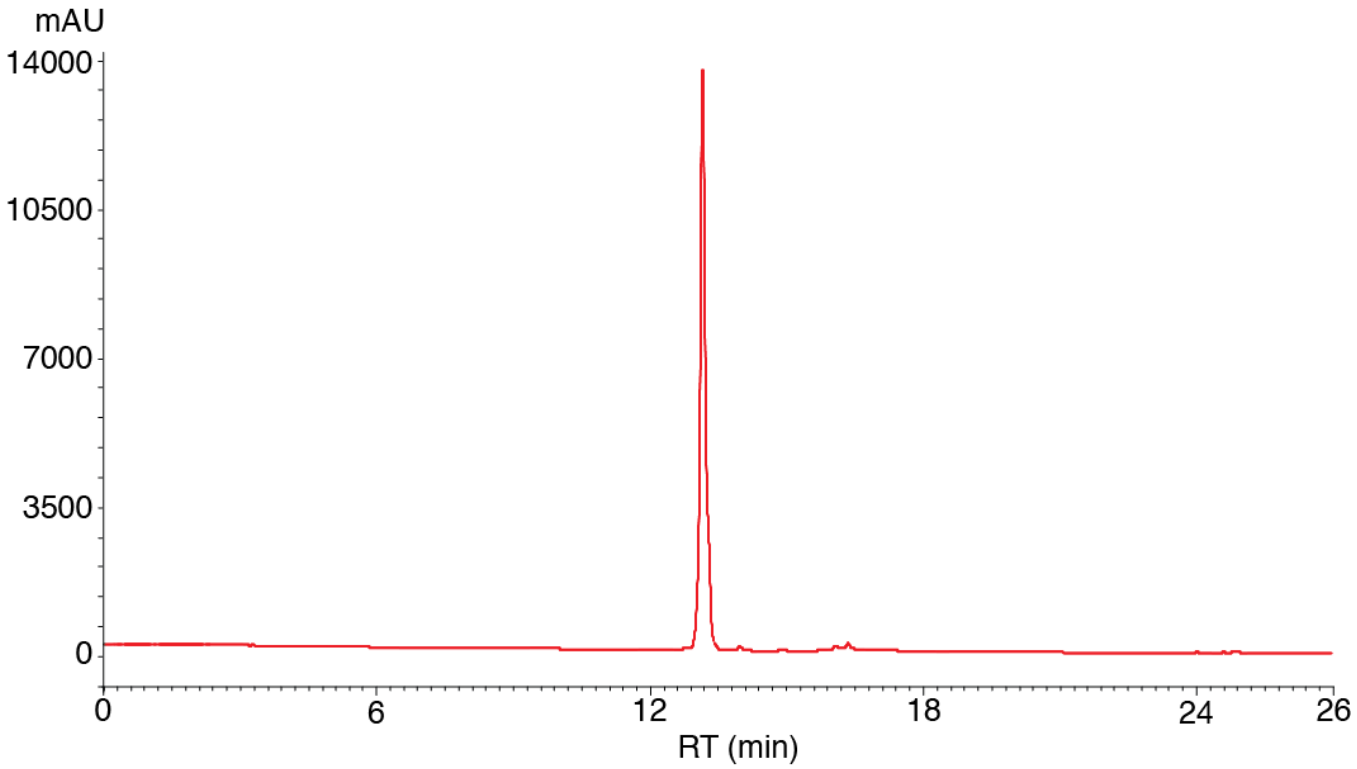


MH^{+1}(av)	MH^{+1}(mono)
8505.1516	8499.8590

Mass calculated

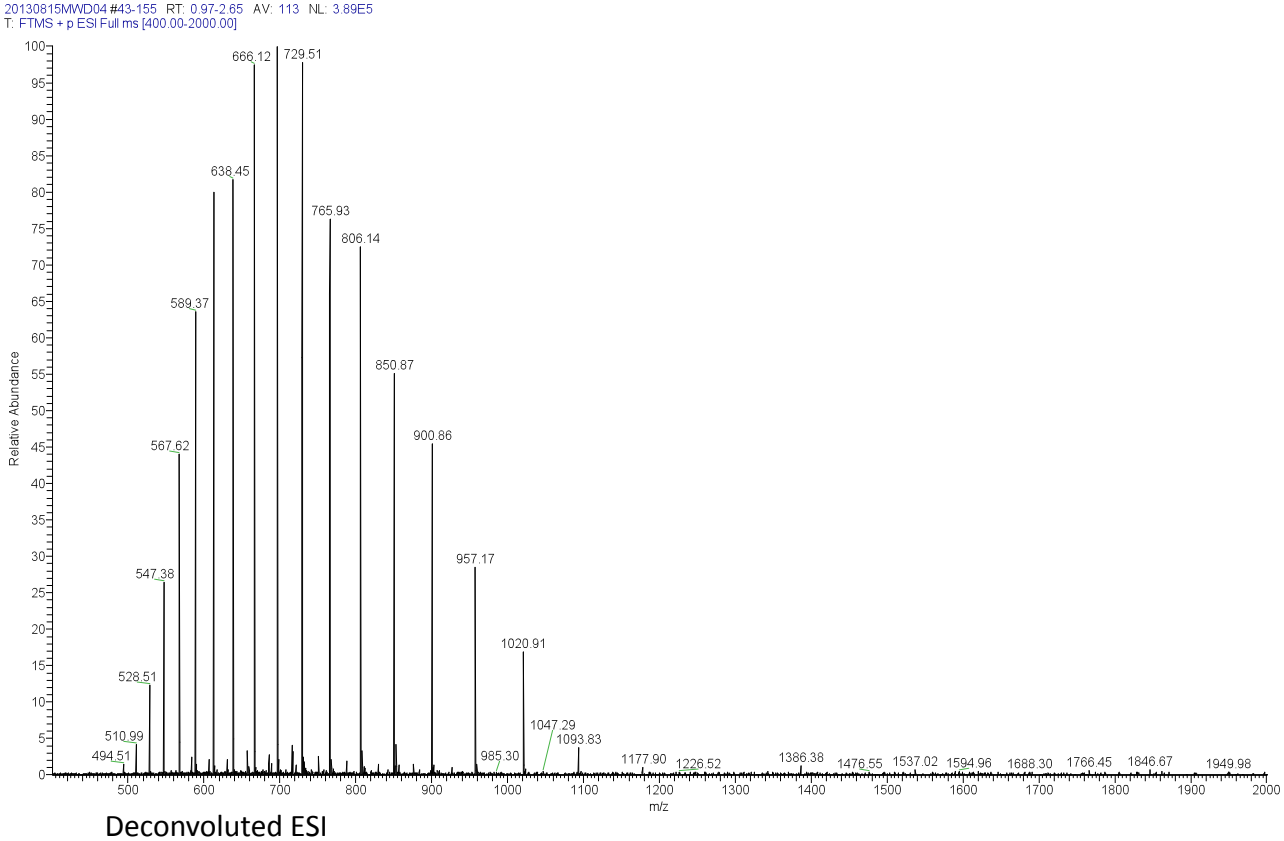
H3(1-74)-MES (21)

Analytical HPLC trace

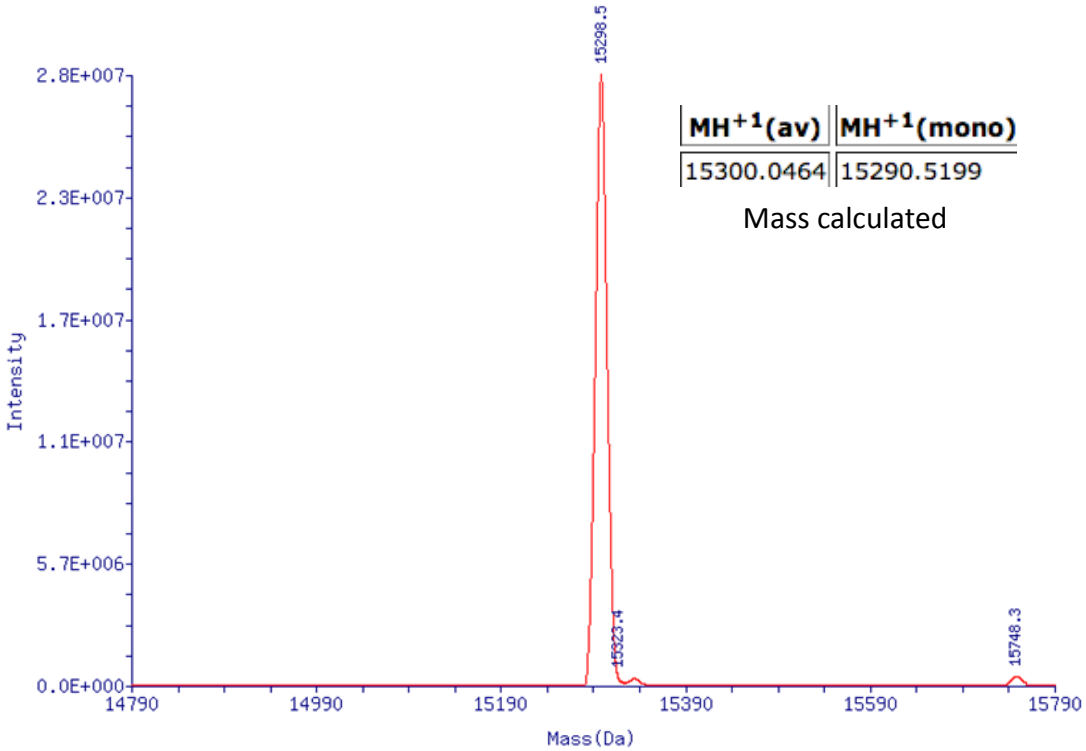


H3K4me3-A21C

ESI before deconvolution



Deconvoluted ESI

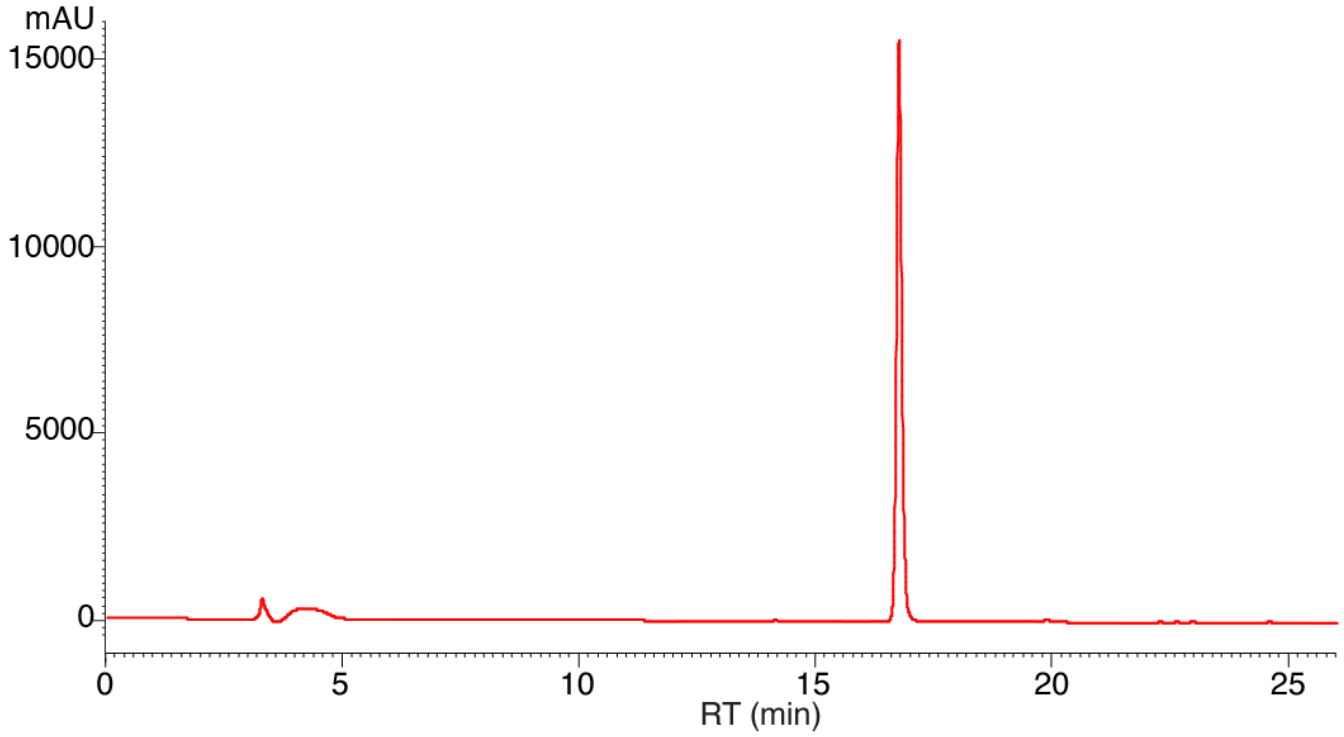


MH^{+1}(av)	MH^{+1}(mono)
15300.0464	15290.5199

Mass calculated

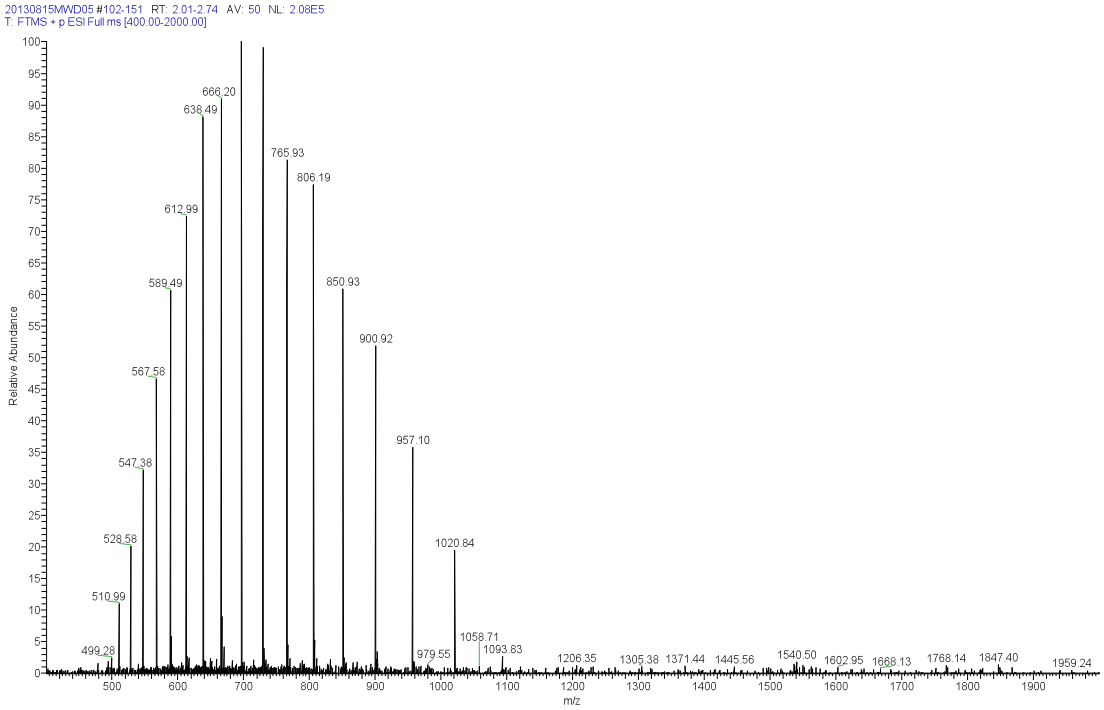
H3K4me3-A21C

Analytical HPLC trace

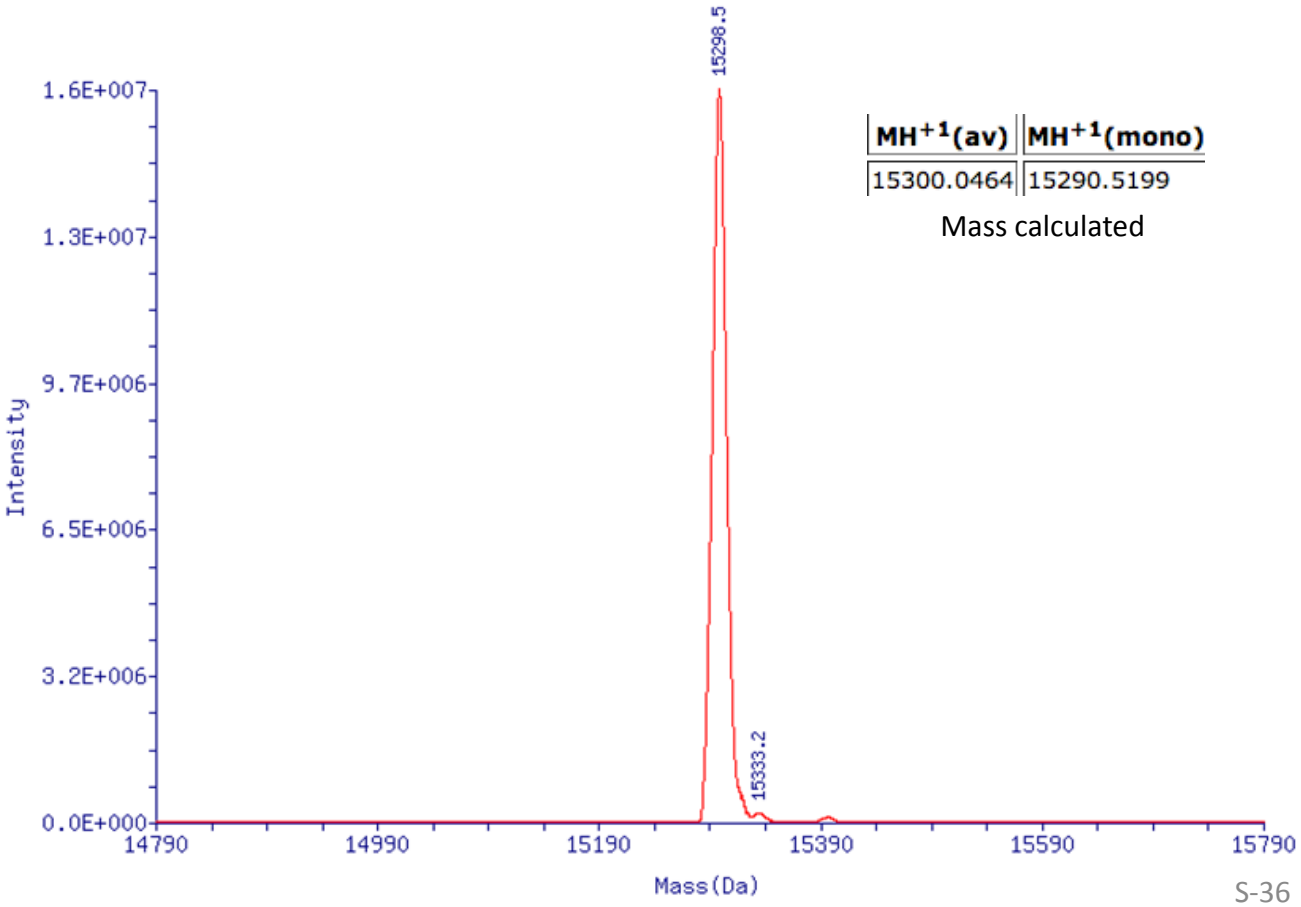


H3K9me3-A21C

ESI-MS before deconvolution



Deconvoluted ESI-MS

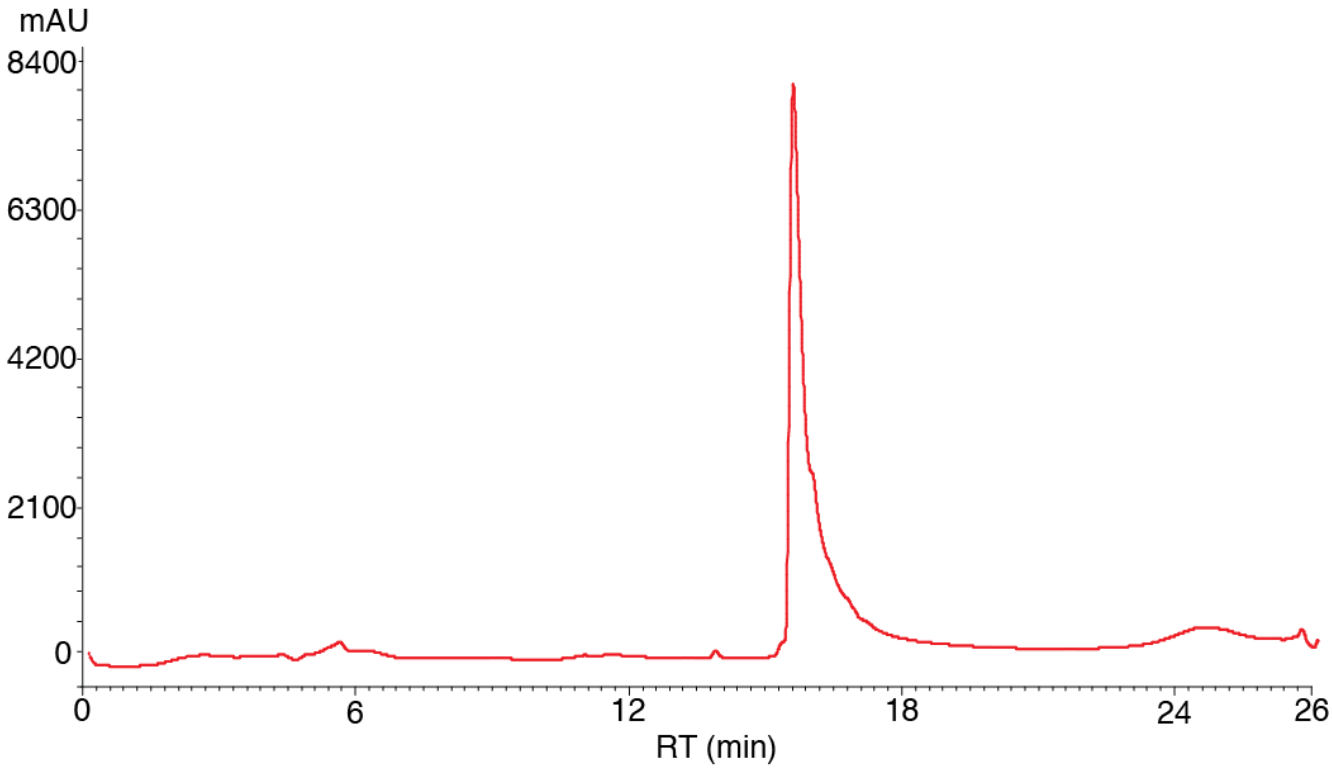


MH^{+1}(av)	MH^{+1}(mono)
15300.0464	15290.5199

Mass calculated

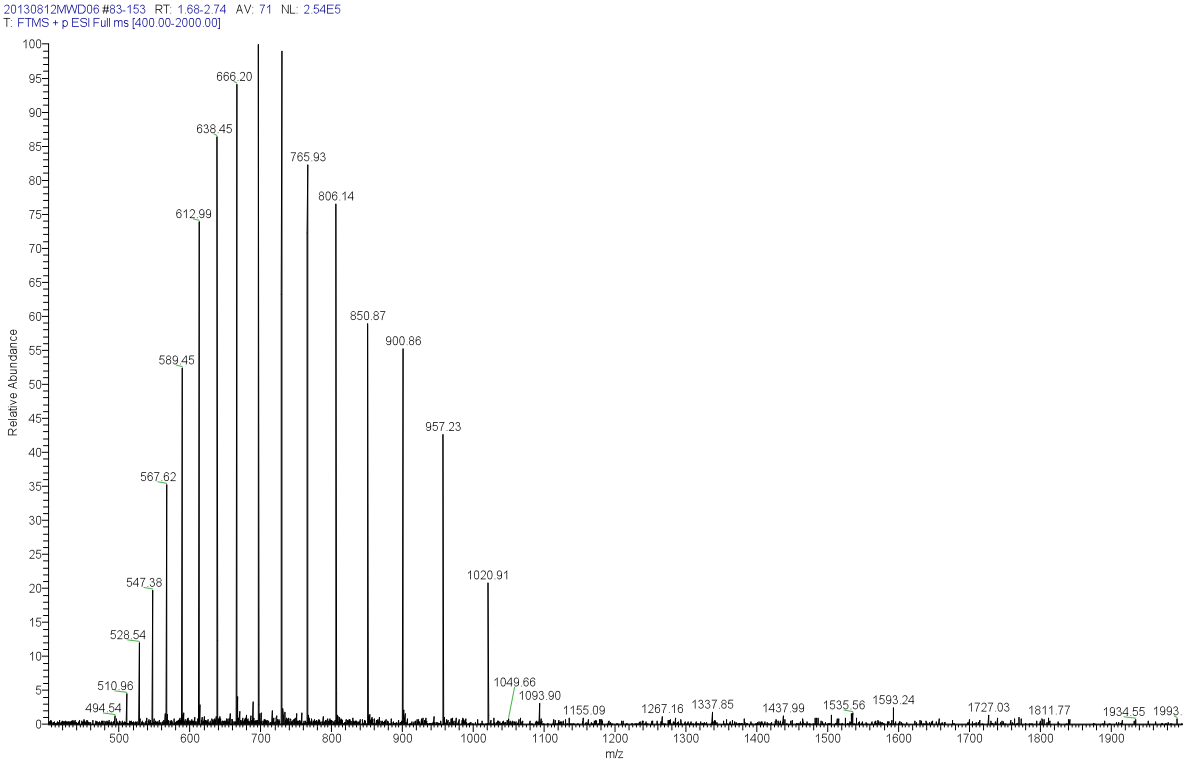
H3K9me3-A21C

Analytical HPLC trace

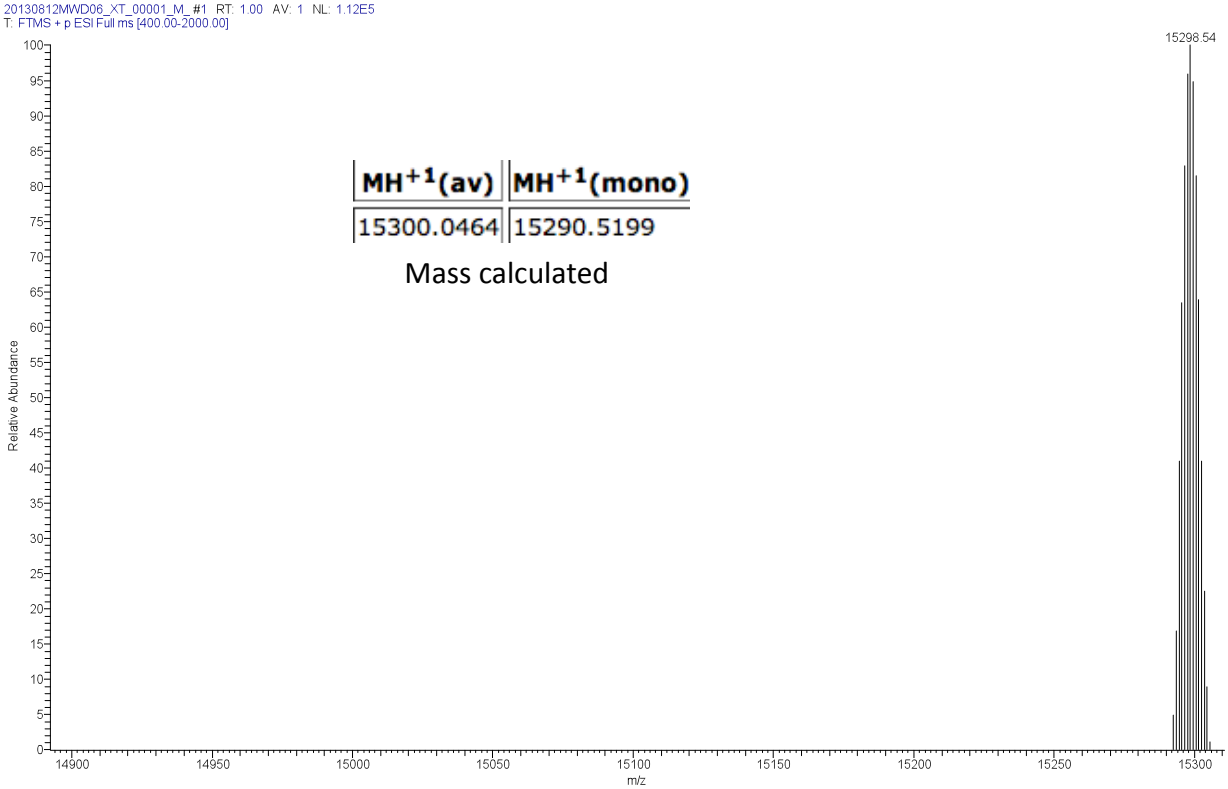


H3K27me3-A29C (5)

ESI-MS before deconvolution



Deconvoluted ESI-MS

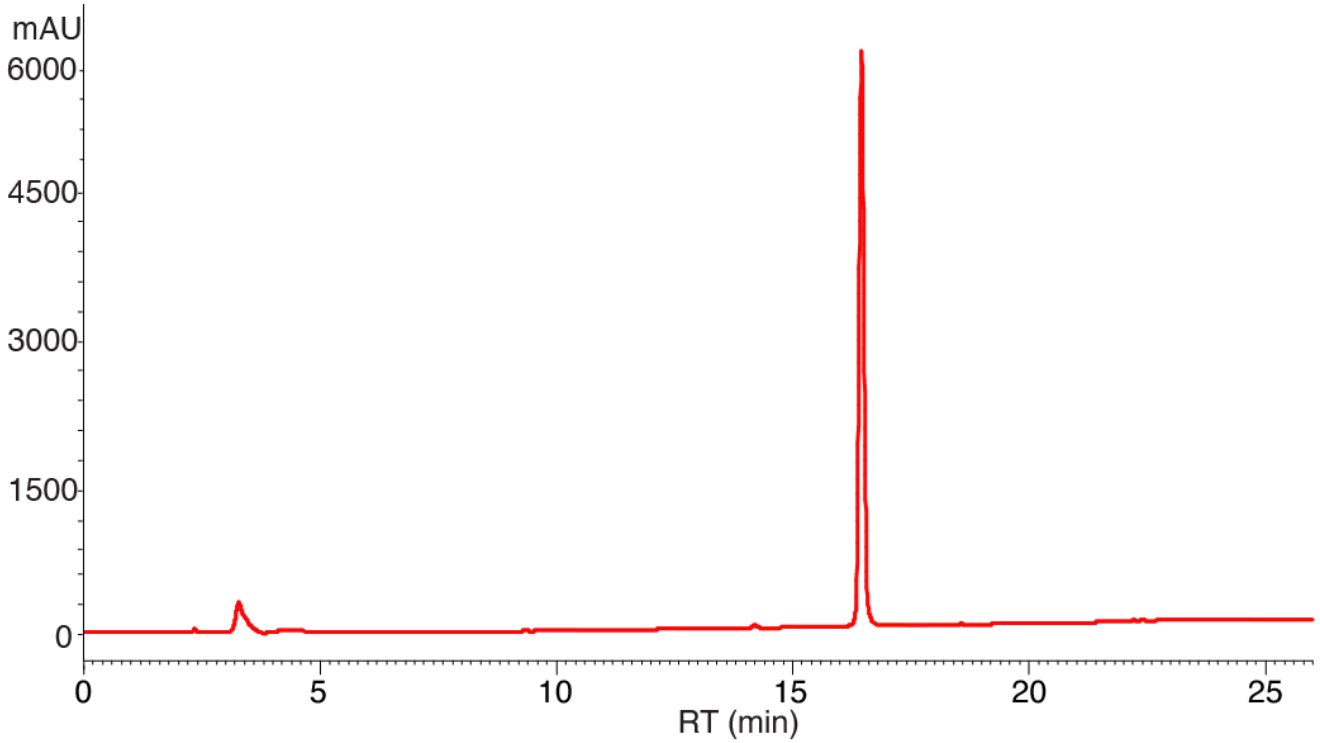


MH^{+1}(av)	MH^{+1}(mono)
15300.0464	15290.5199

Mass calculated

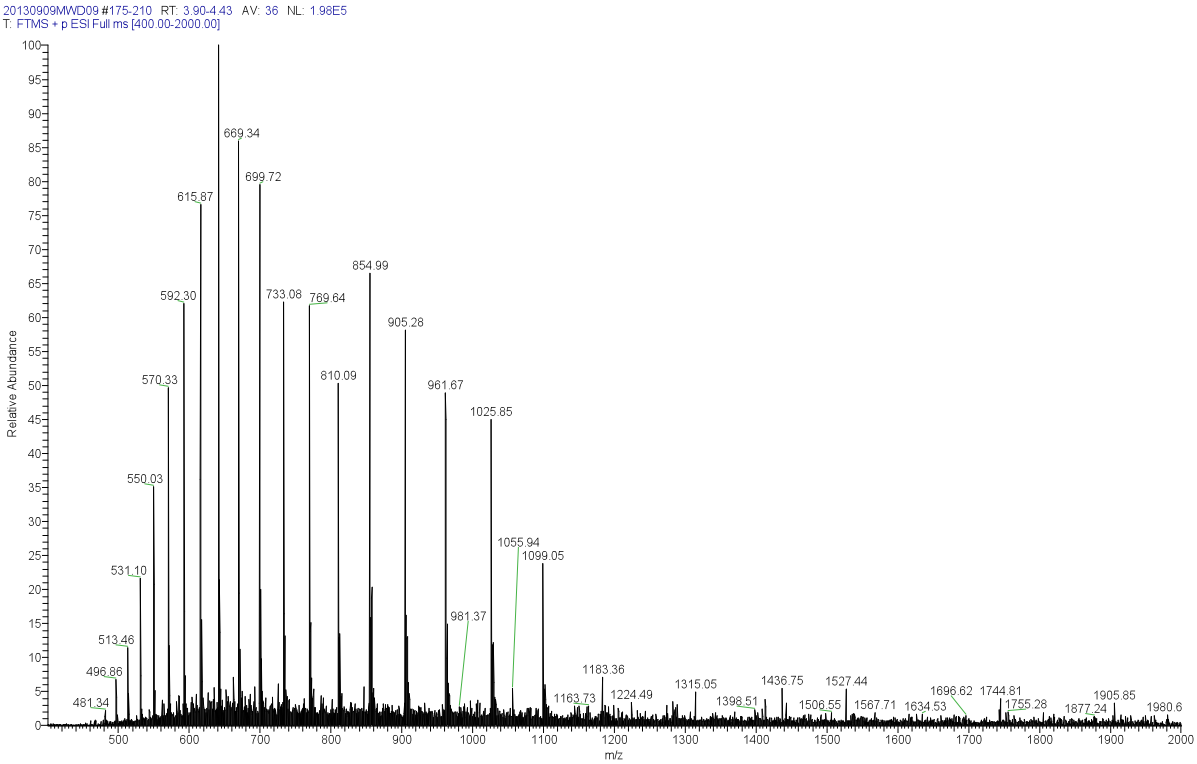
H3K27me3-A29C (5)

Analytical HPLC trace

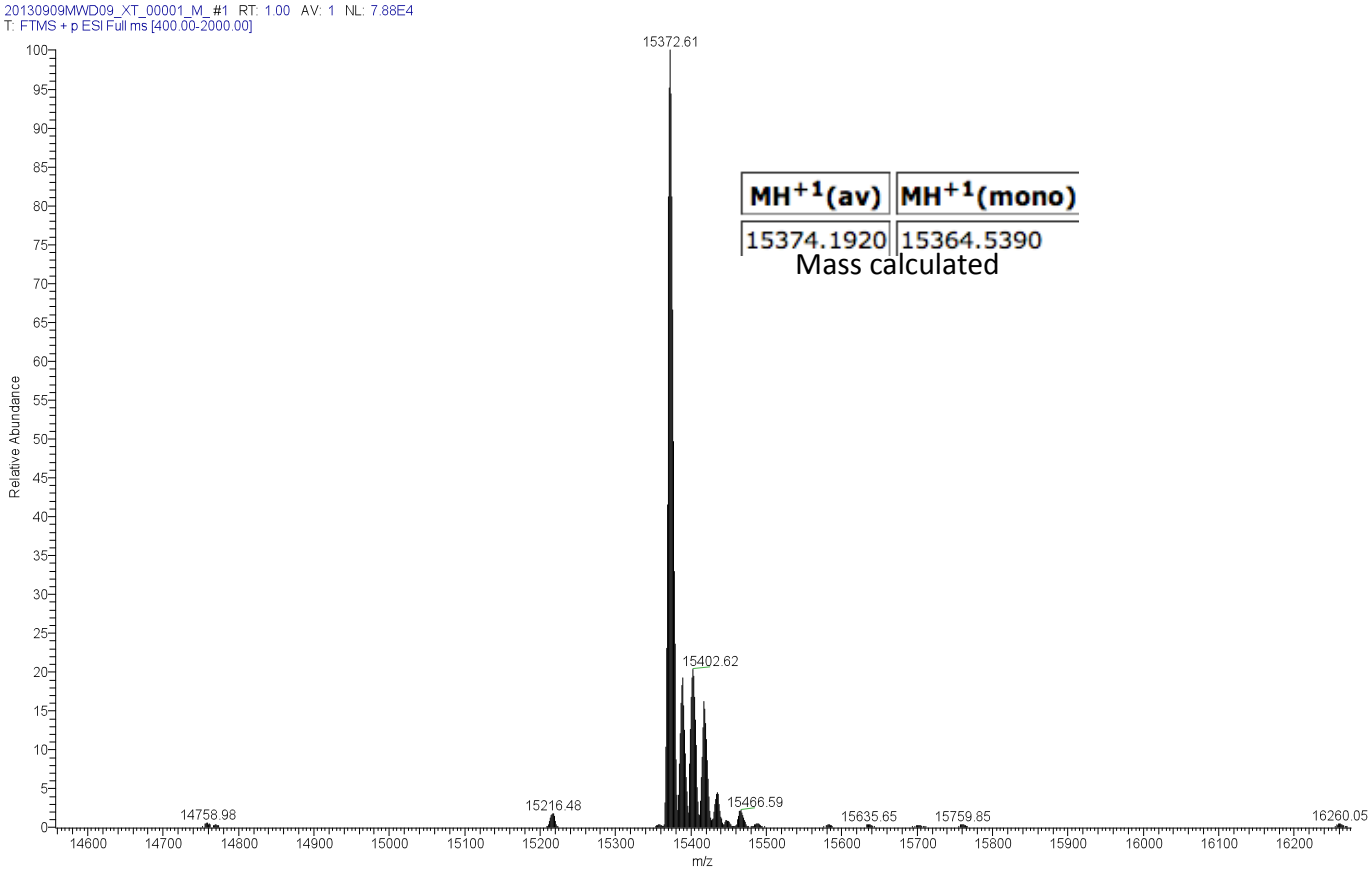


H3K4me3K27me3-A21C-A29C (6)

ESI-MS before deconvolution



Deconvoluted ESI-MS

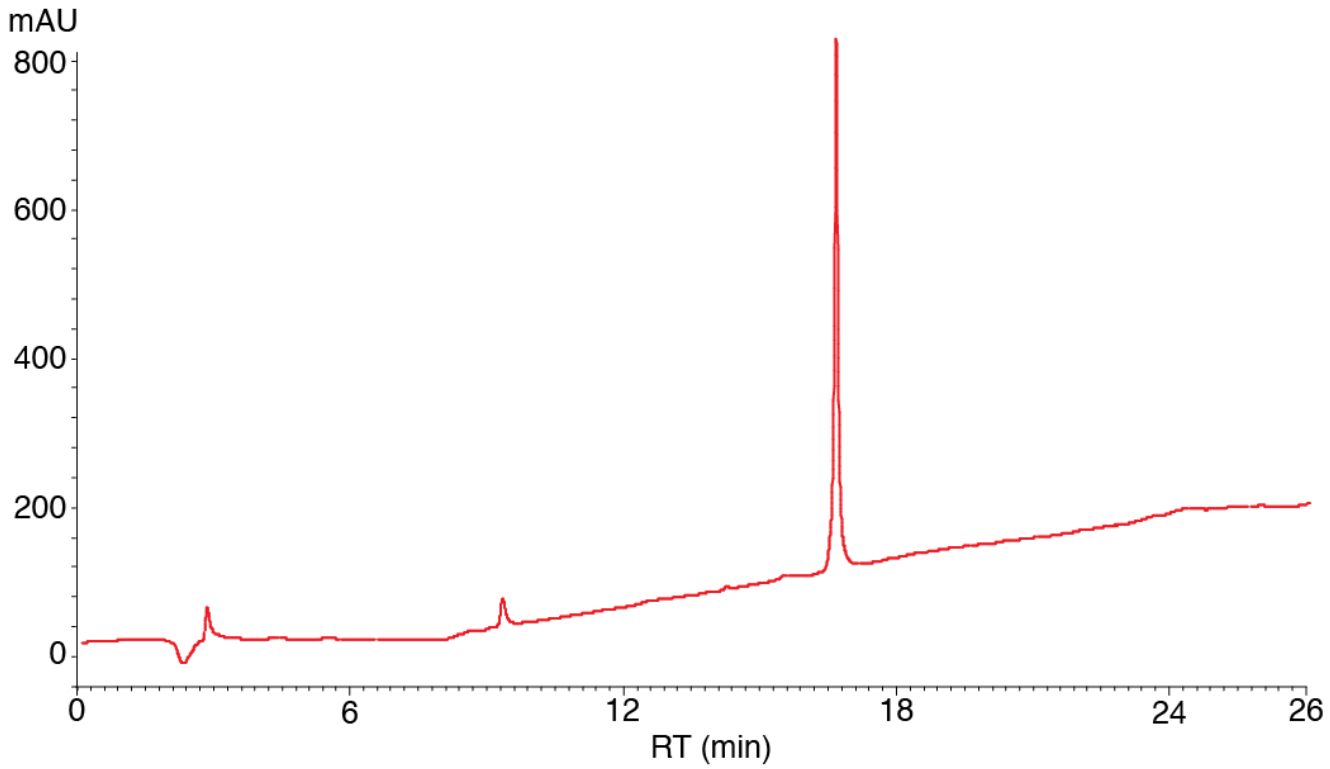


MH^{+1}(av)	MH^{+1}(mono)
15374.1920	15364.5390

Mass calculated

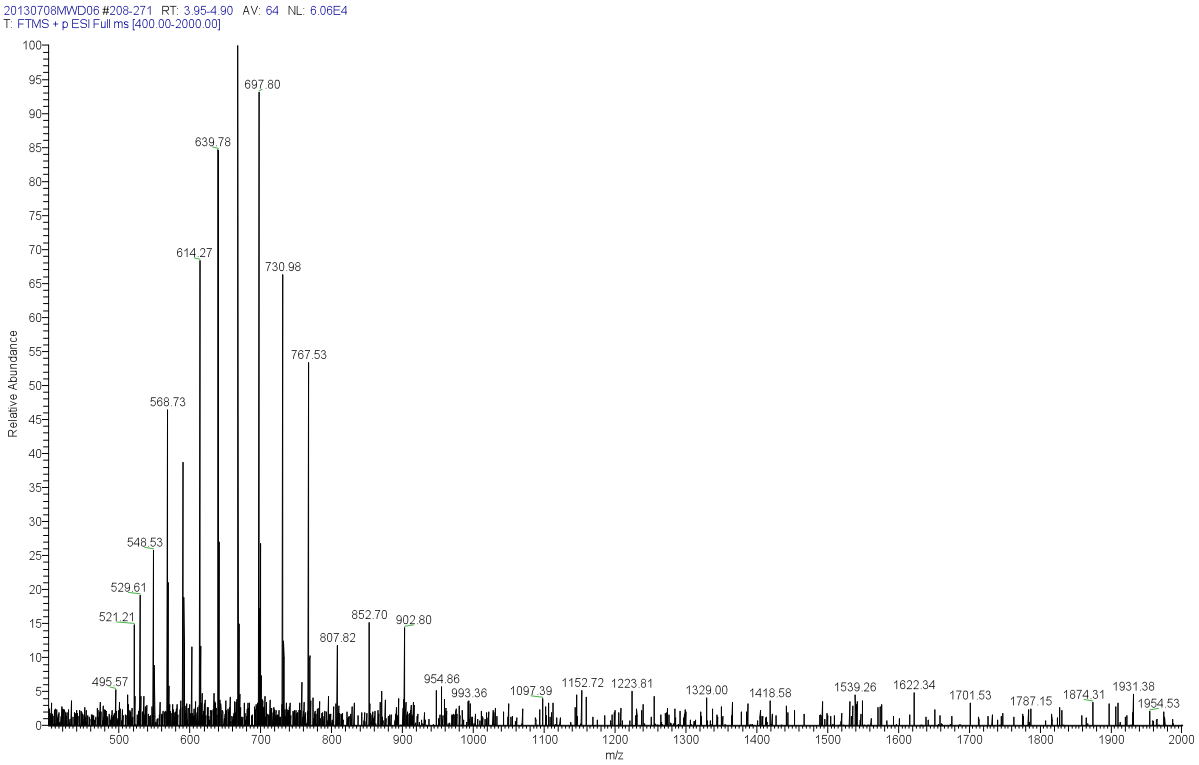
H3K4me3K27me3-A21C-A29C (6)

Analytical HPLC trace

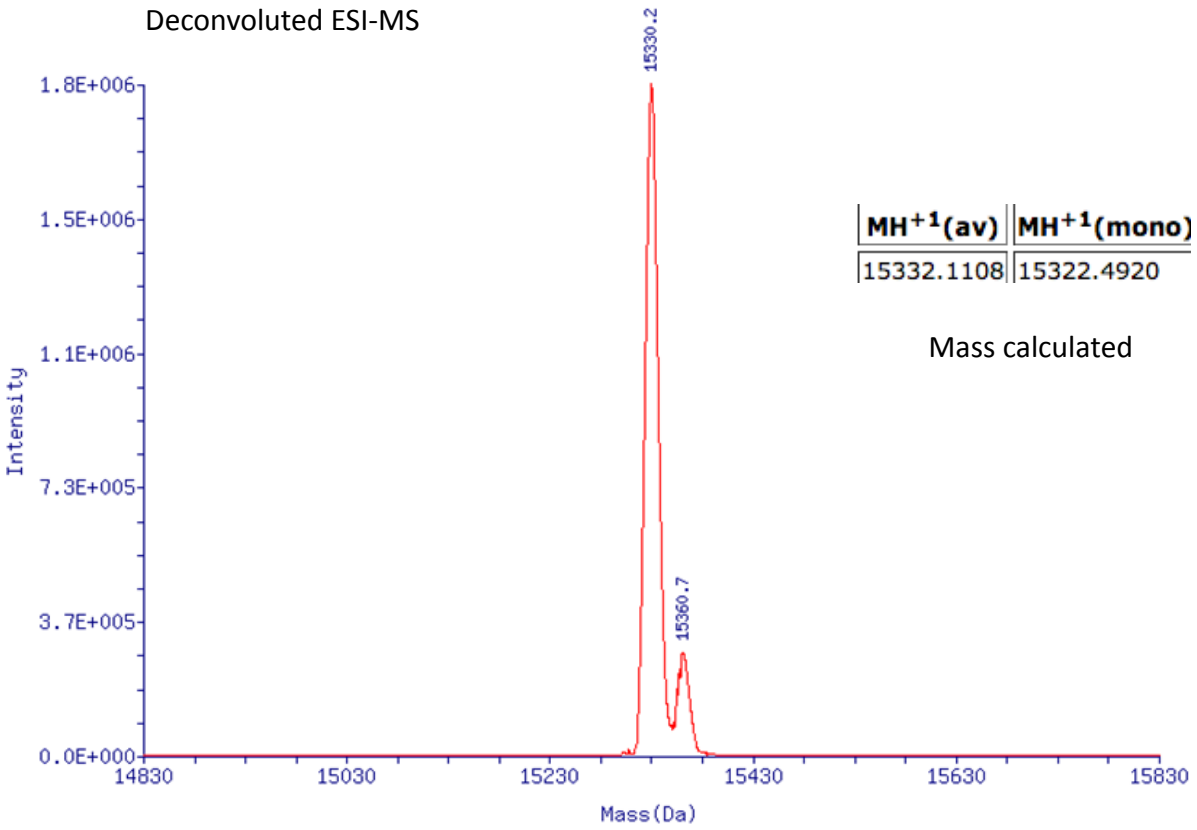


H3K36me3-A29C-A47C (28)

ESI-MS before deconvolution



Deconvoluted ESI-MS

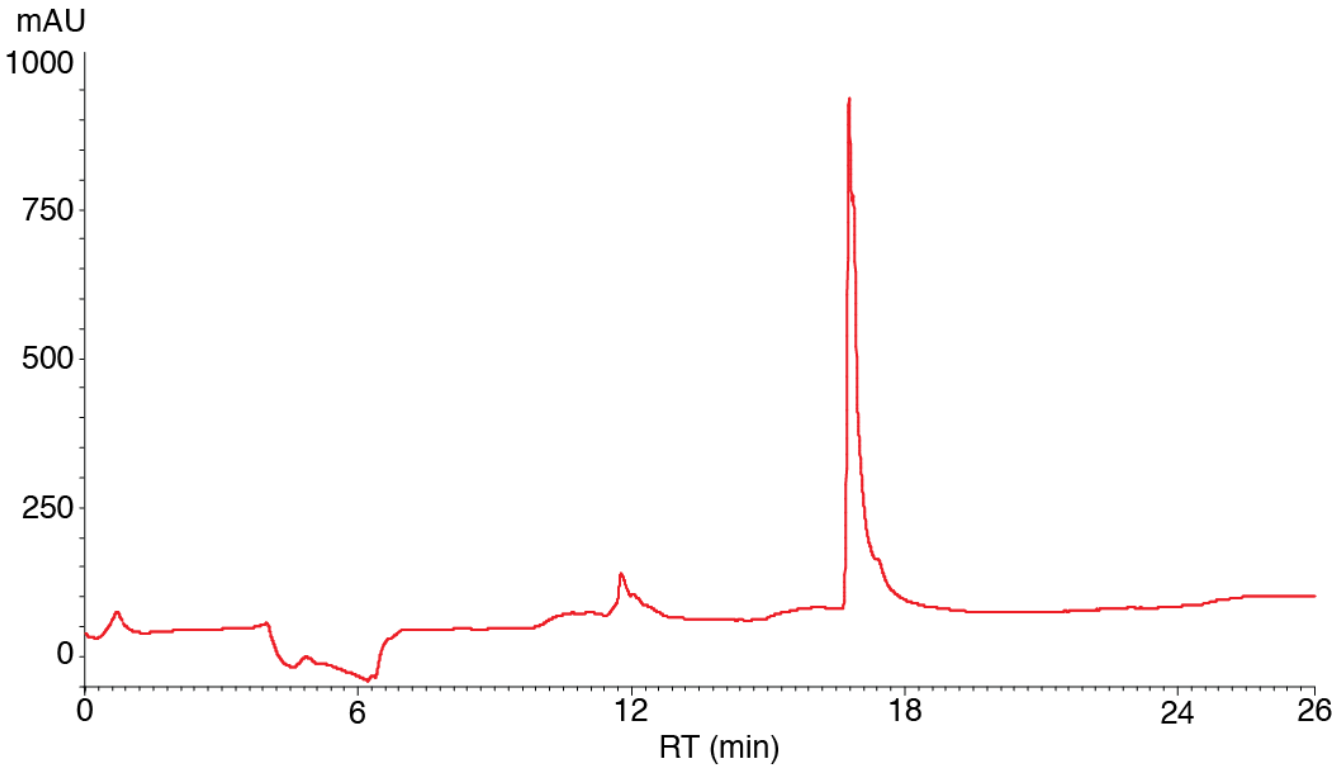


MH^{+1}(av)	MH^{+1}(mono)
15332.1108	15322.4920

Mass calculated

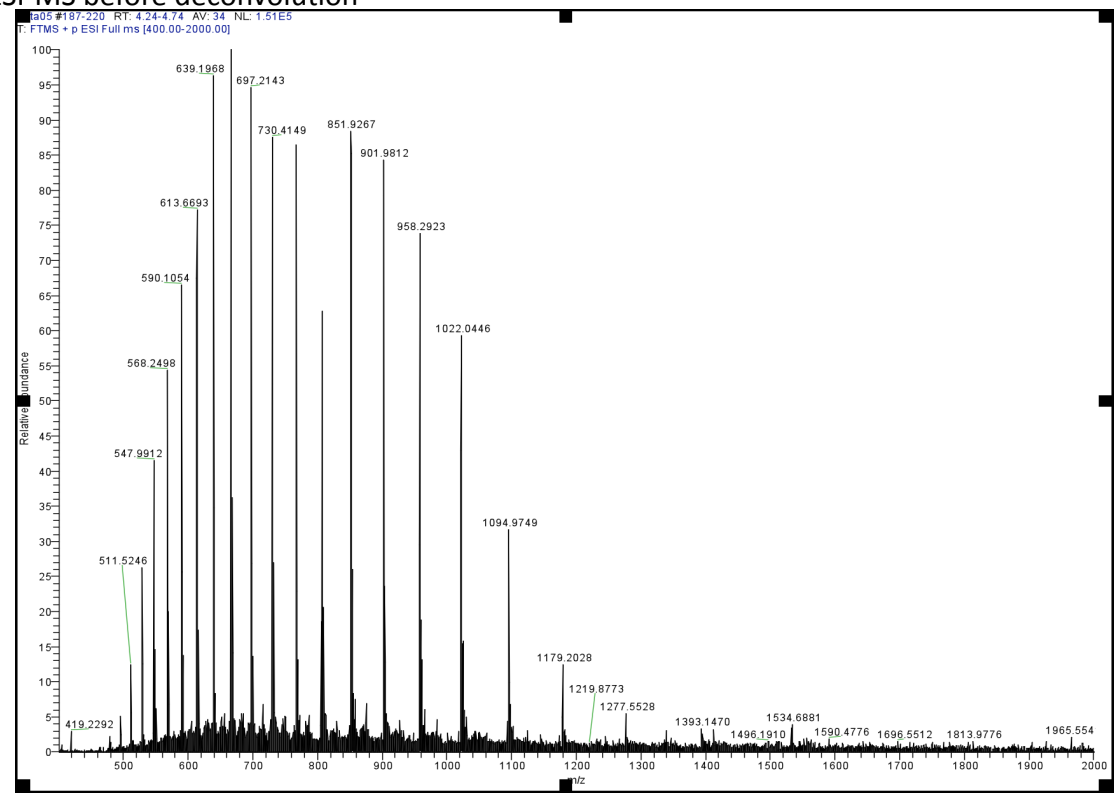
H3K36me3-A29C-A47C (30)

Analytical HPLC trace

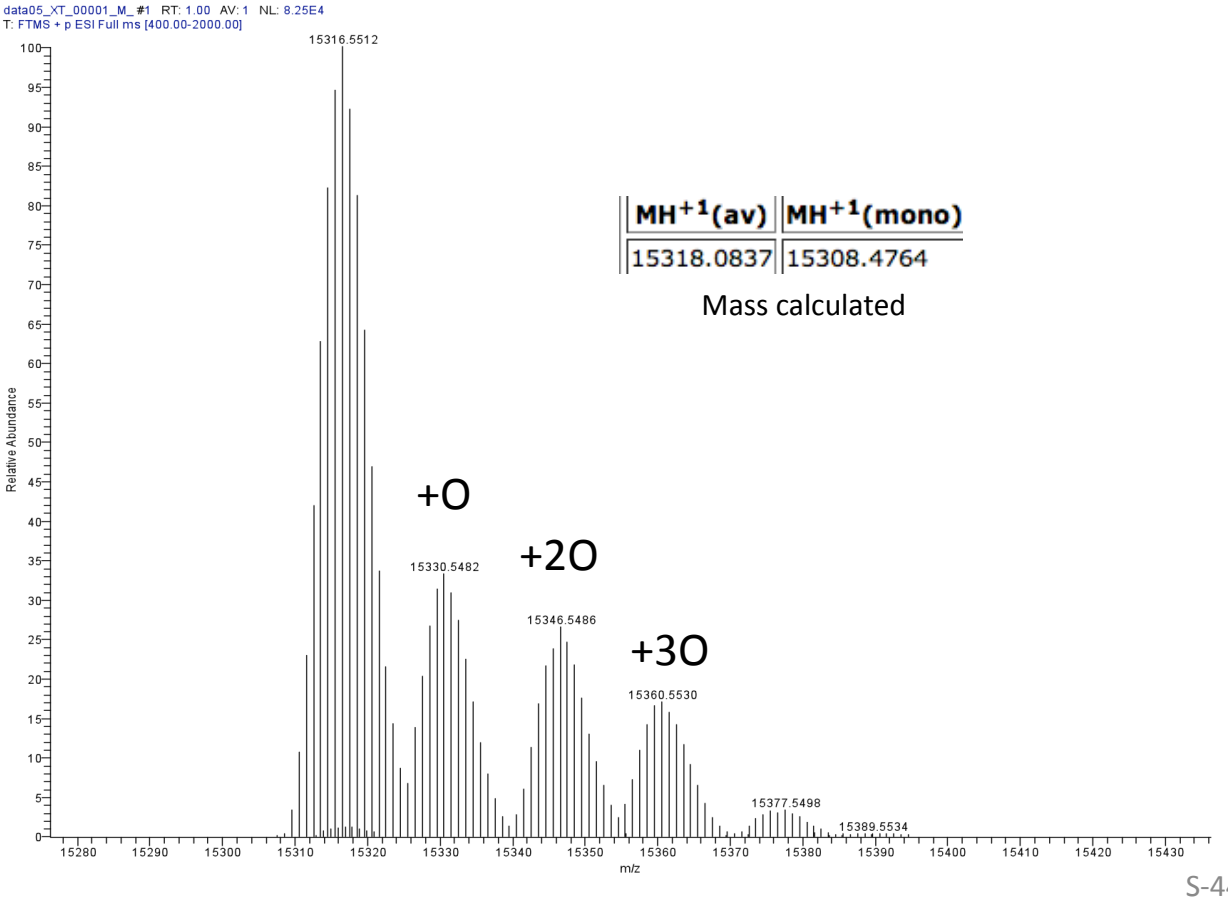


H3K79me2-A75C-A88C

ESI-MS before deconvolution



Deconvoluted ESI-MS

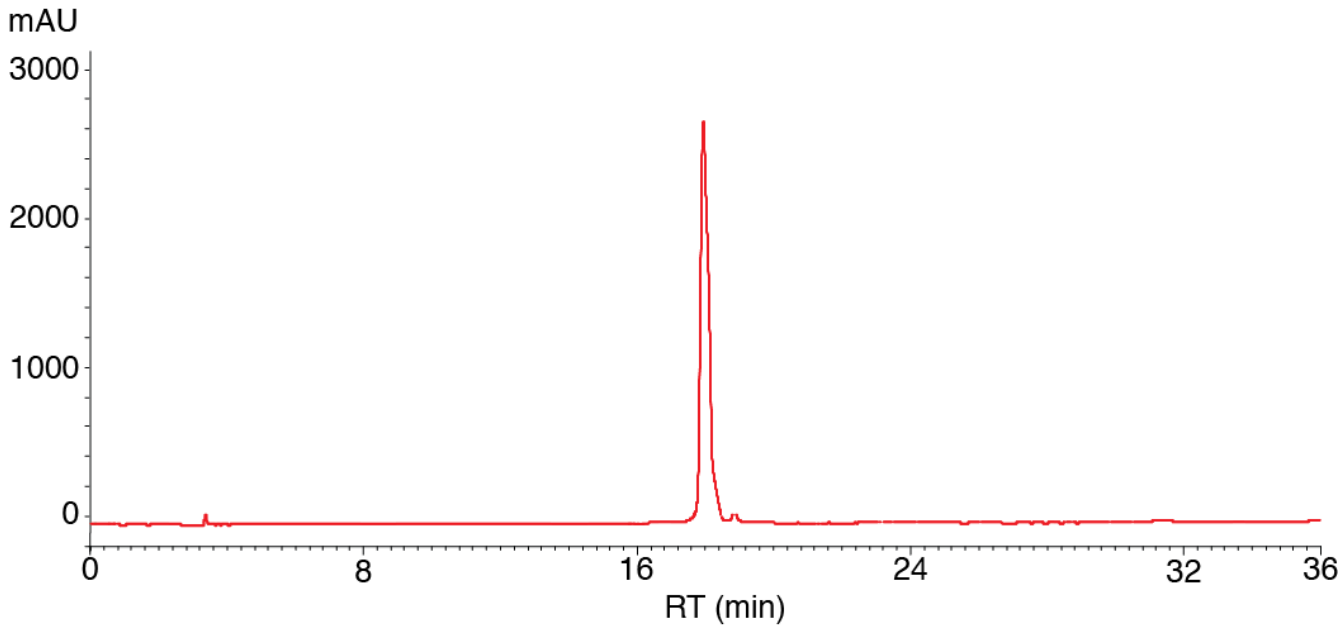


MH^{+1}(av)	MH^{+1}(mono)
15318.0837	15308.4764

Mass calculated

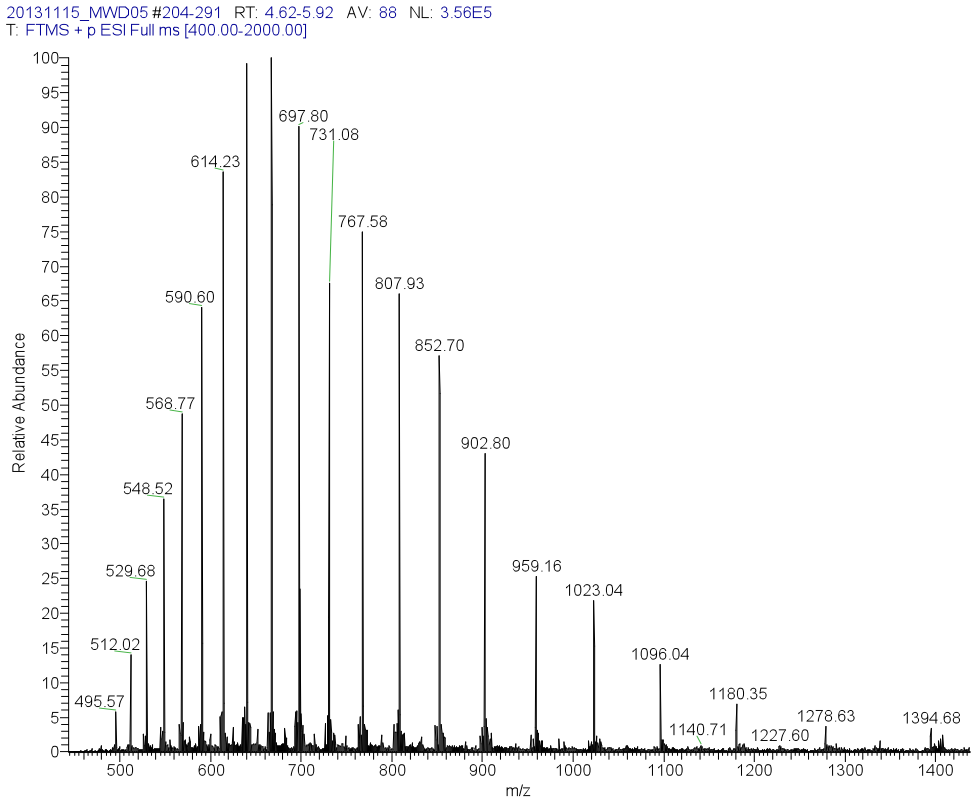
H3K79me2-A75C-A88C

Analytical HPLC

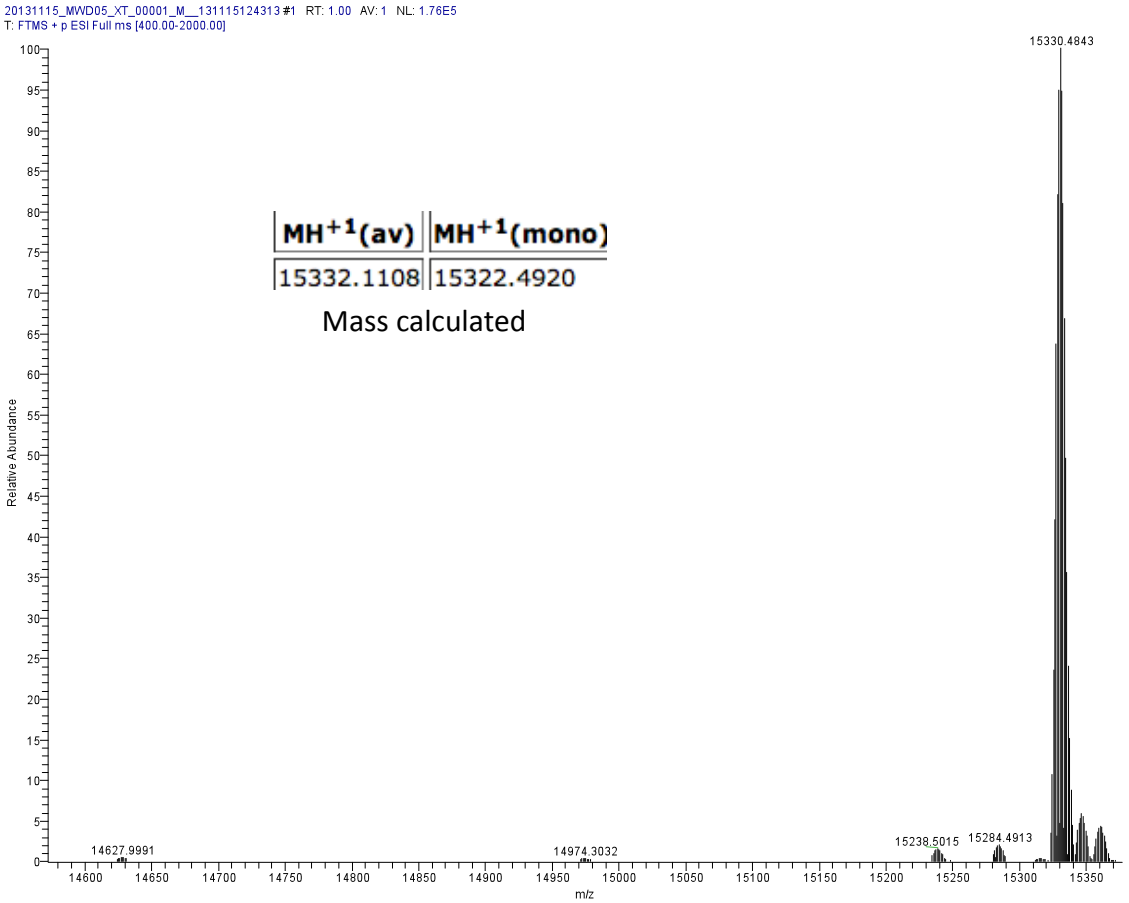


H3K79me3-A75C-A88C

ESI-MS before deconvolution



Deconvoluted ESI-MS

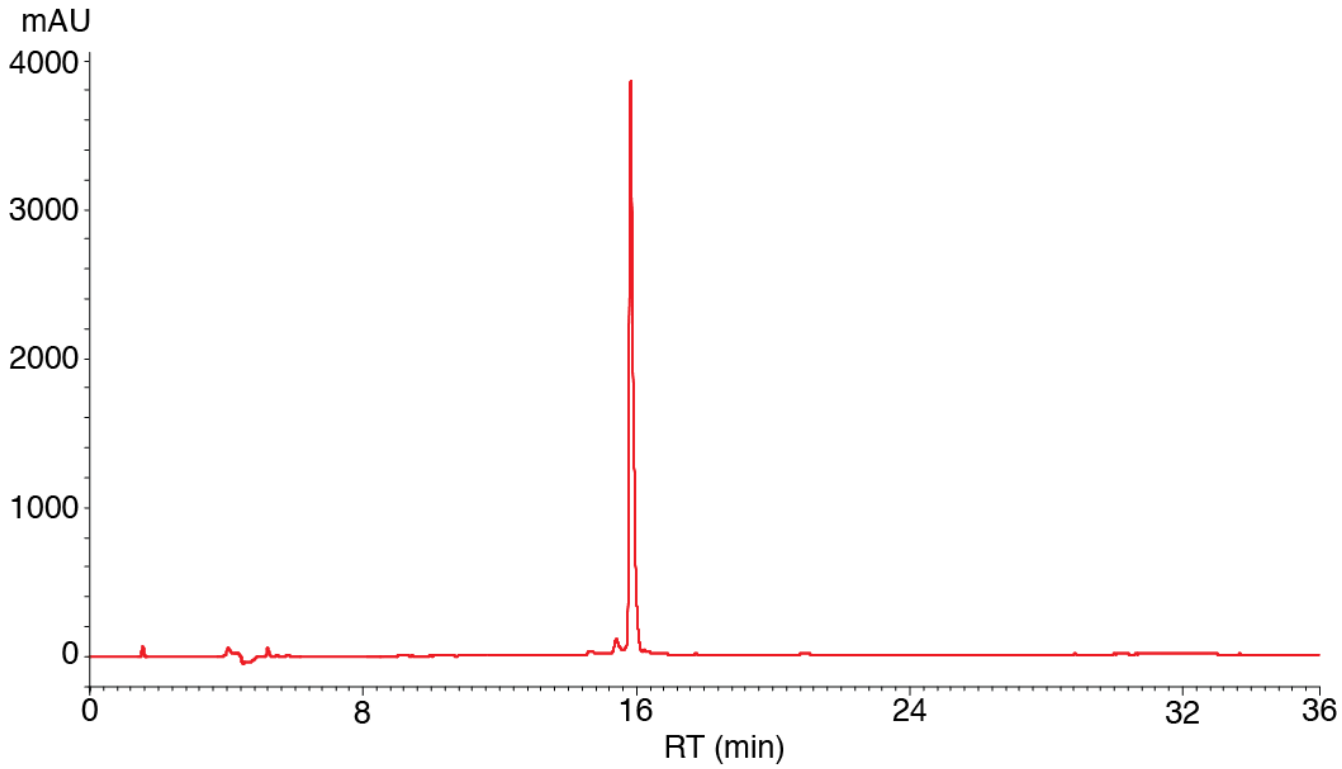


MH^{+1}(av)	MH^{+1}(mono)
15332.1108	15322.4920

Mass calculated

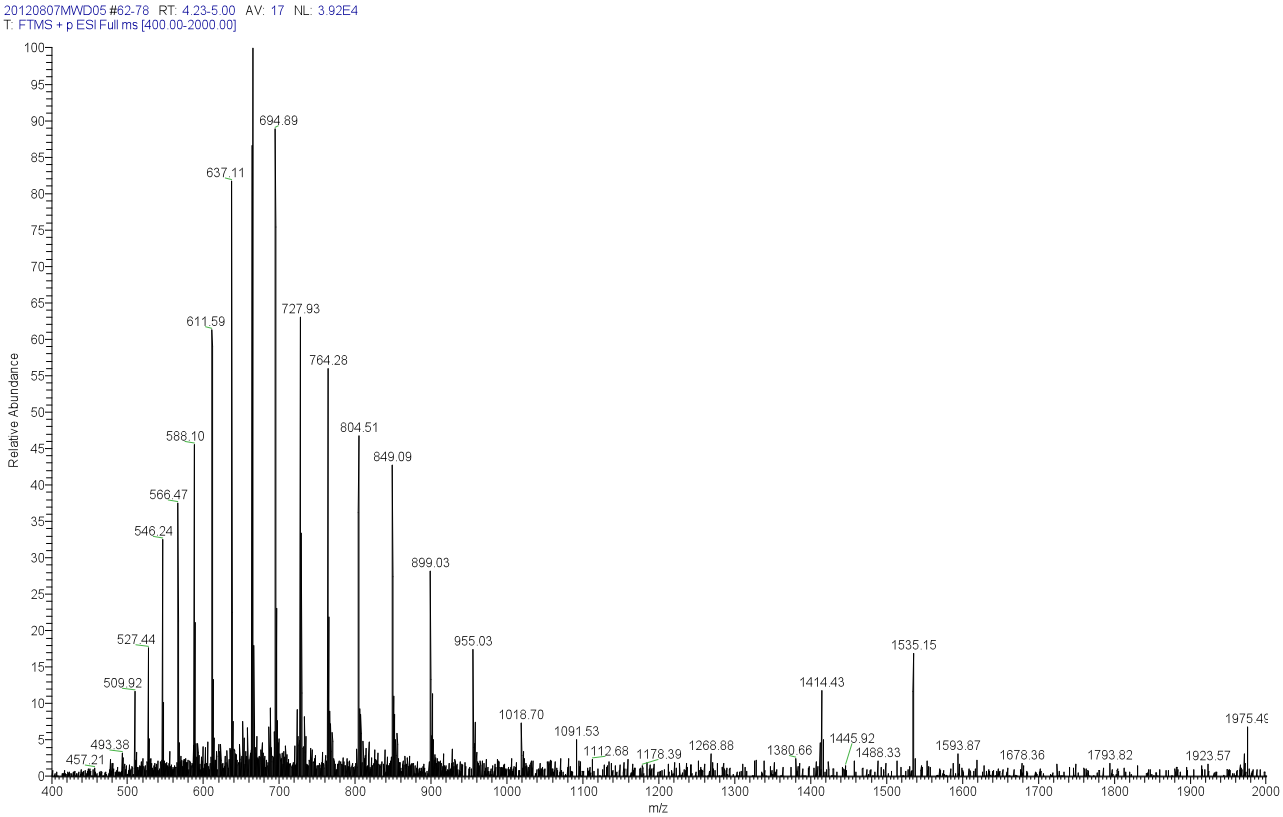
H3K79me3-A75C-A88C

Analytical HPLC

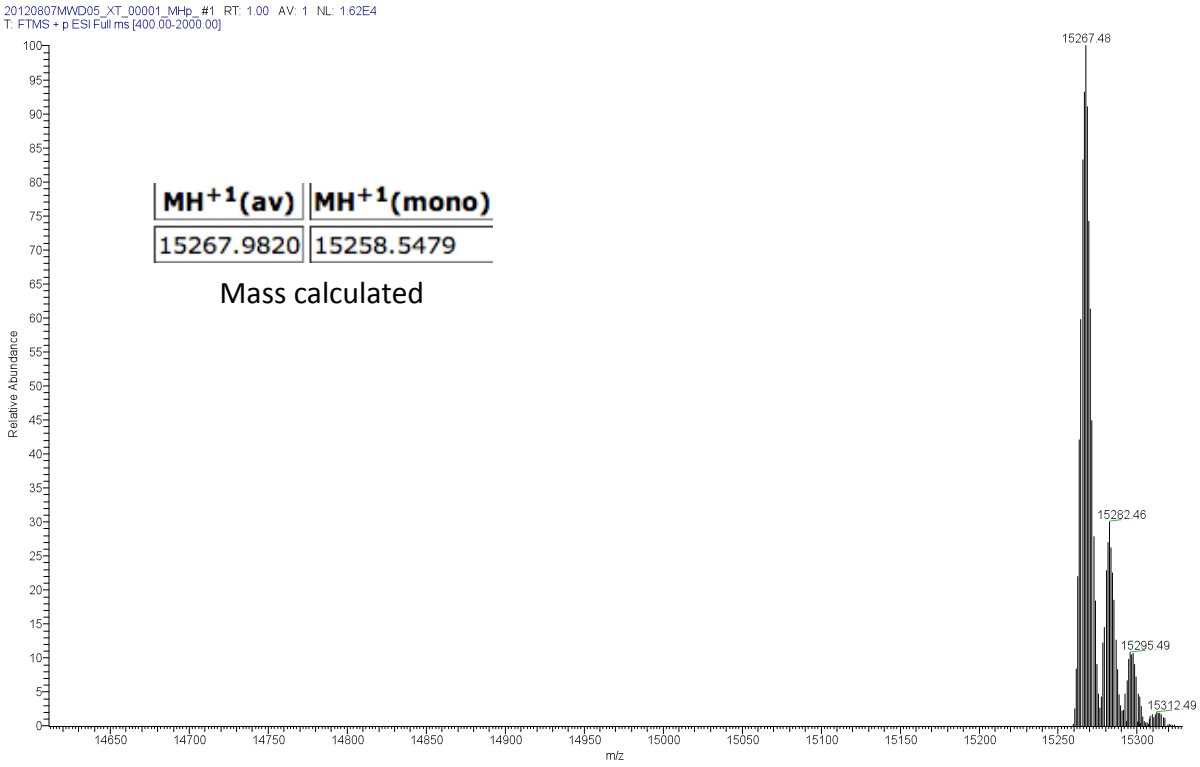


H3K9me3 (3)

ESI-MS before deconvolution



ESI-MS after deconvolution

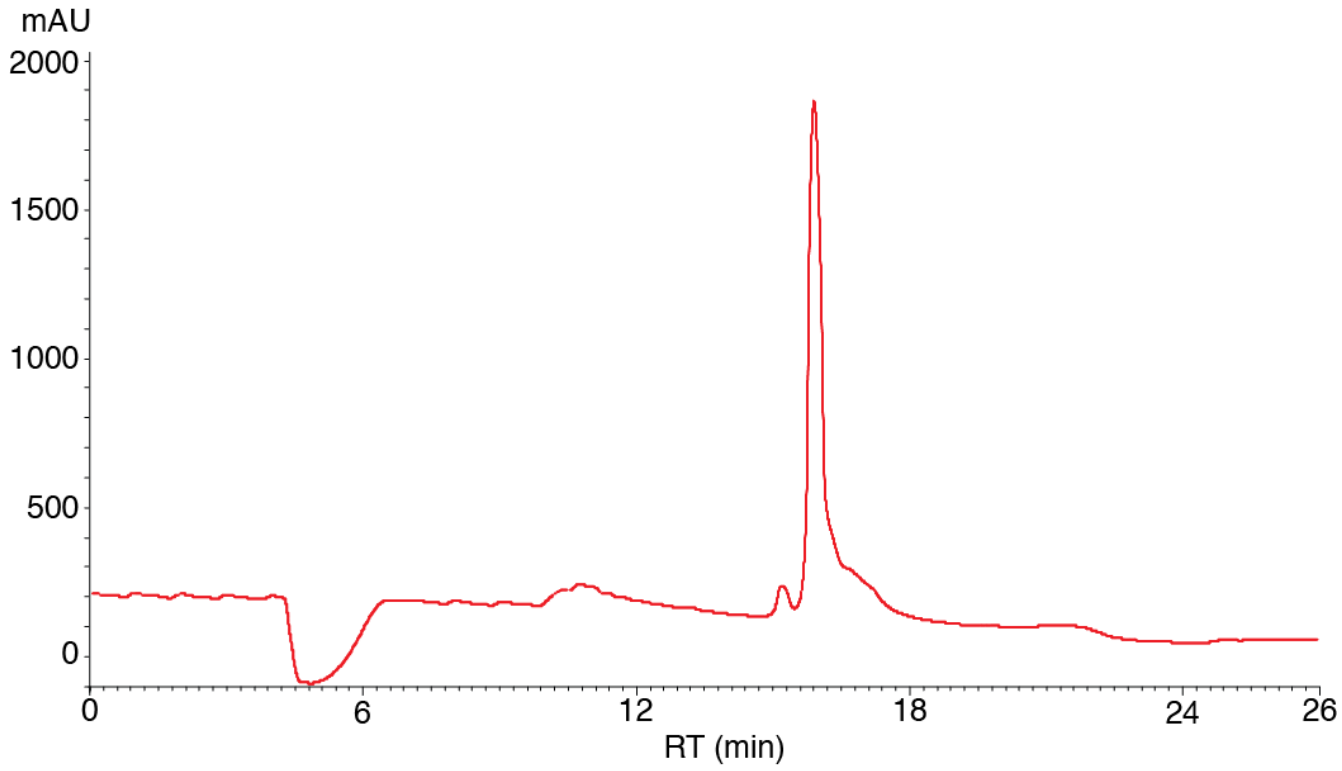


MH^{+1}(av)	MH^{+1}(mono)
15267.9820	15258.5479

Mass calculated

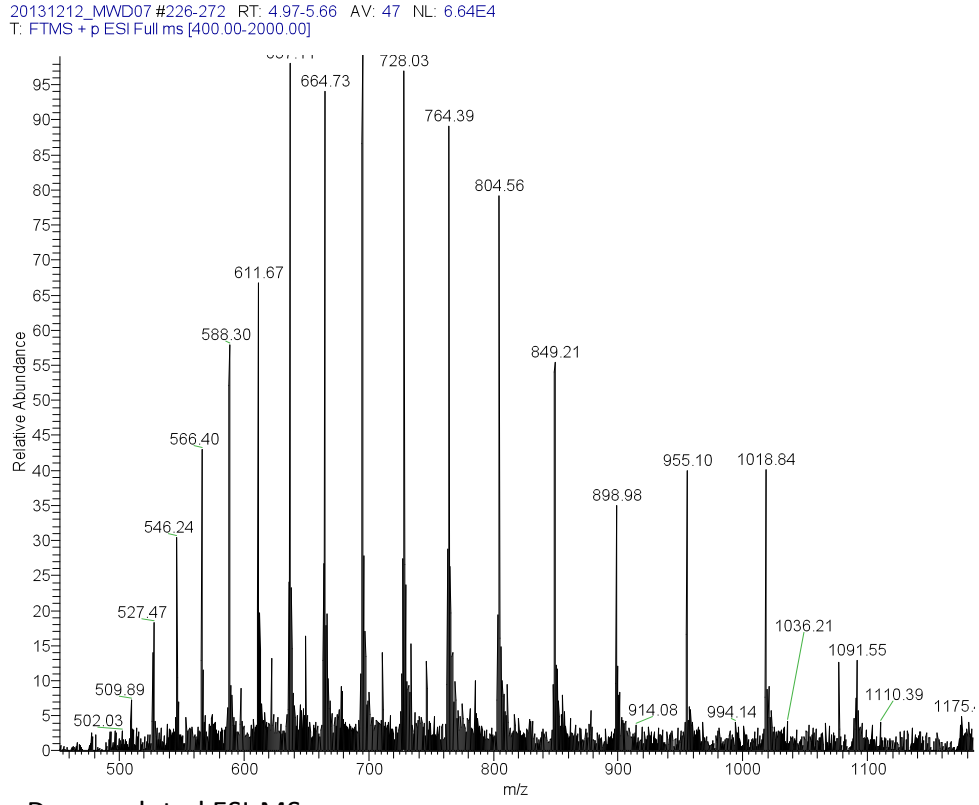
H3K9me3 (3)

Analytical HPLC

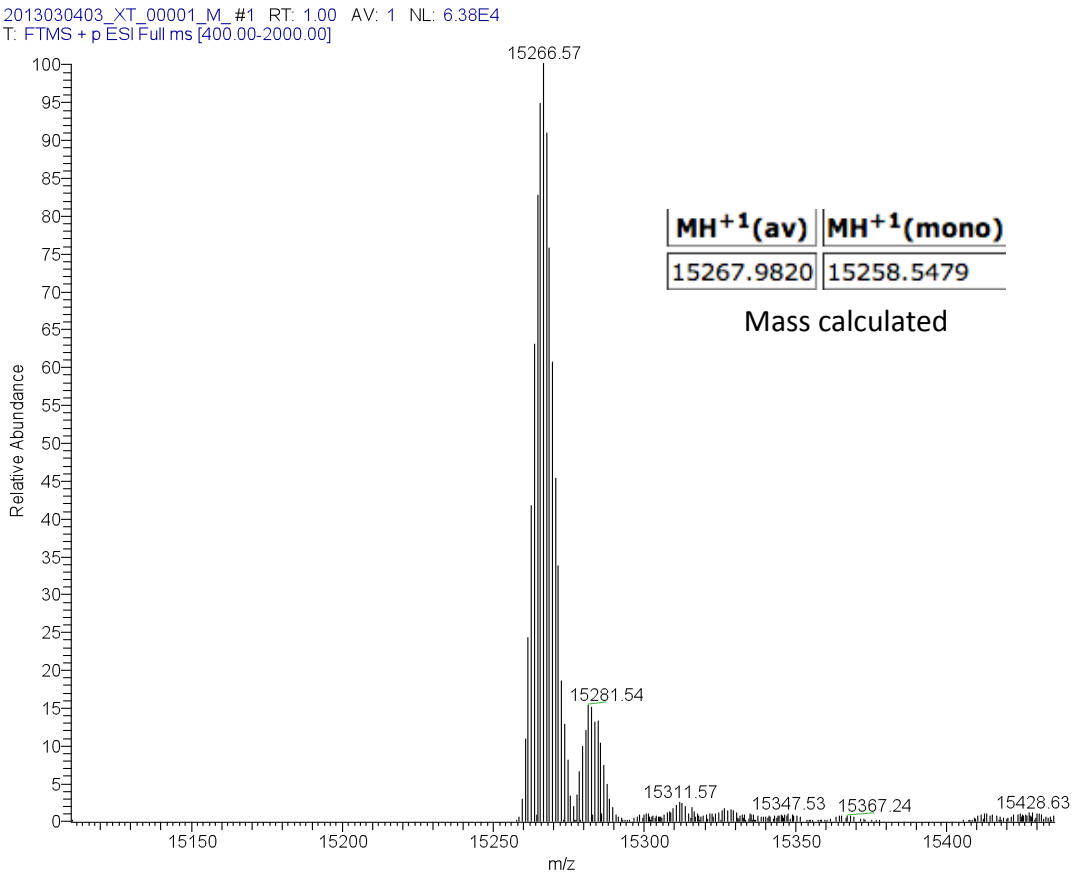


H3K27me3 (7)

ESI-MS before deconvolution



Deconvoluted ESI-MS

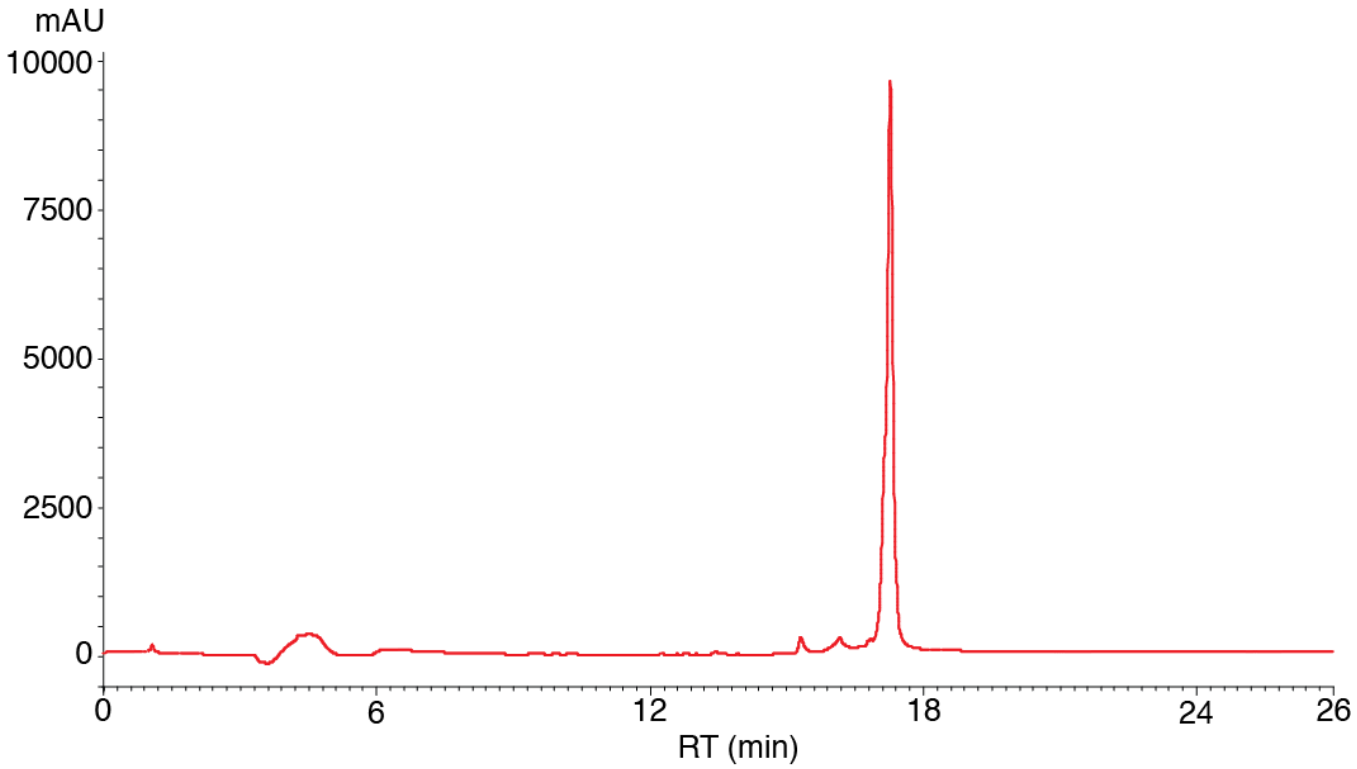


MH^{+1}(av)	MH^{+1}(mono)
15267.9820	15258.5479

Mass calculated

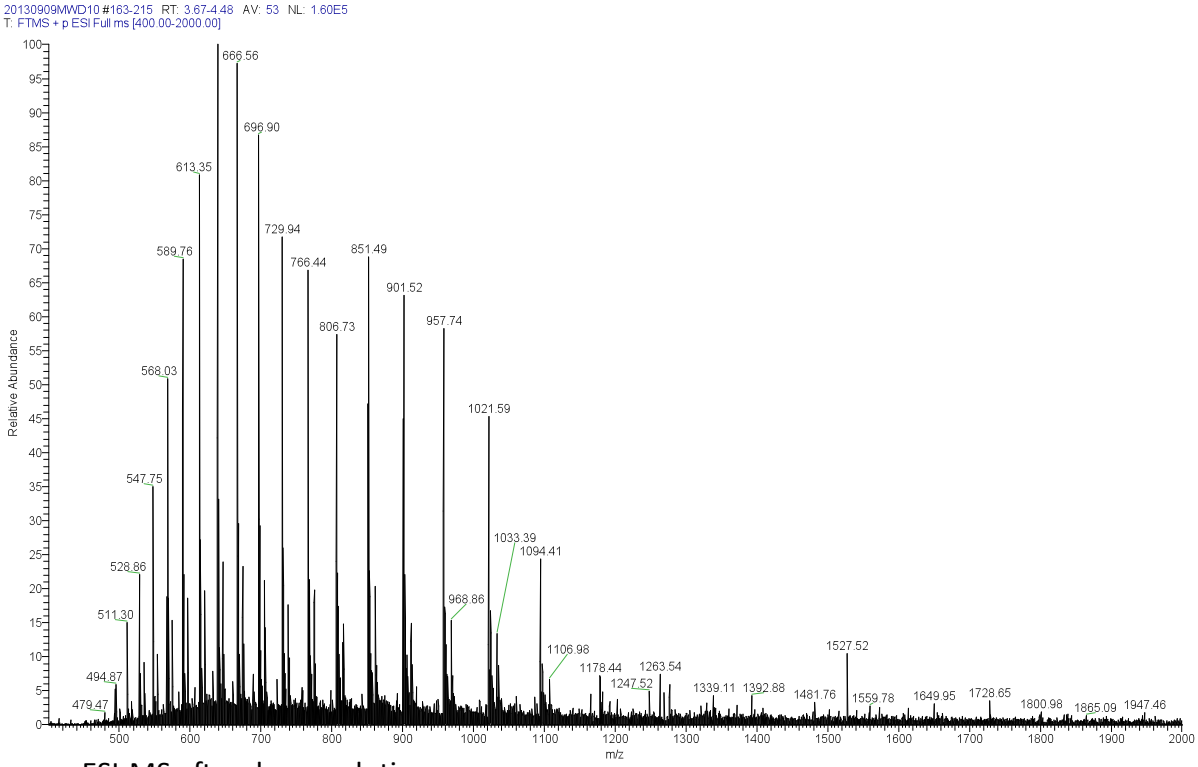
H3K27me3 (9)

Analytical HPLC trace

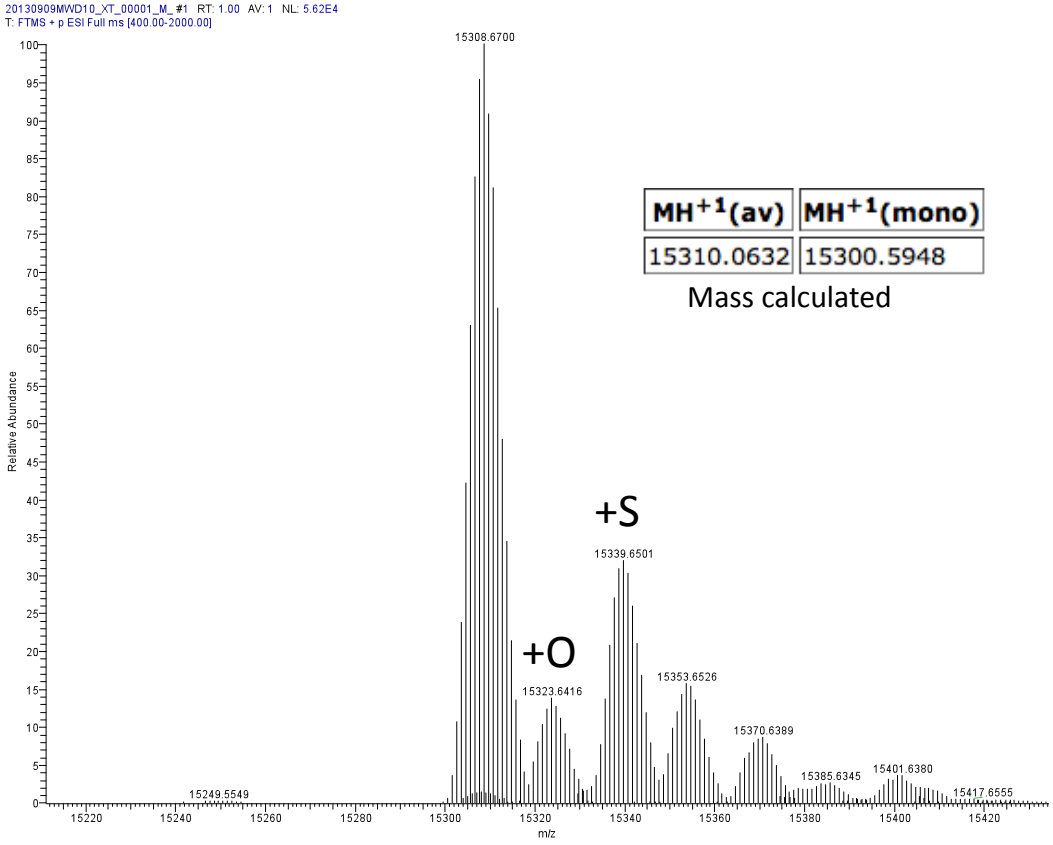


H3K4me3K27me3 (8)

ESI-MS after deconvolution



ESI-MS after deconvolution

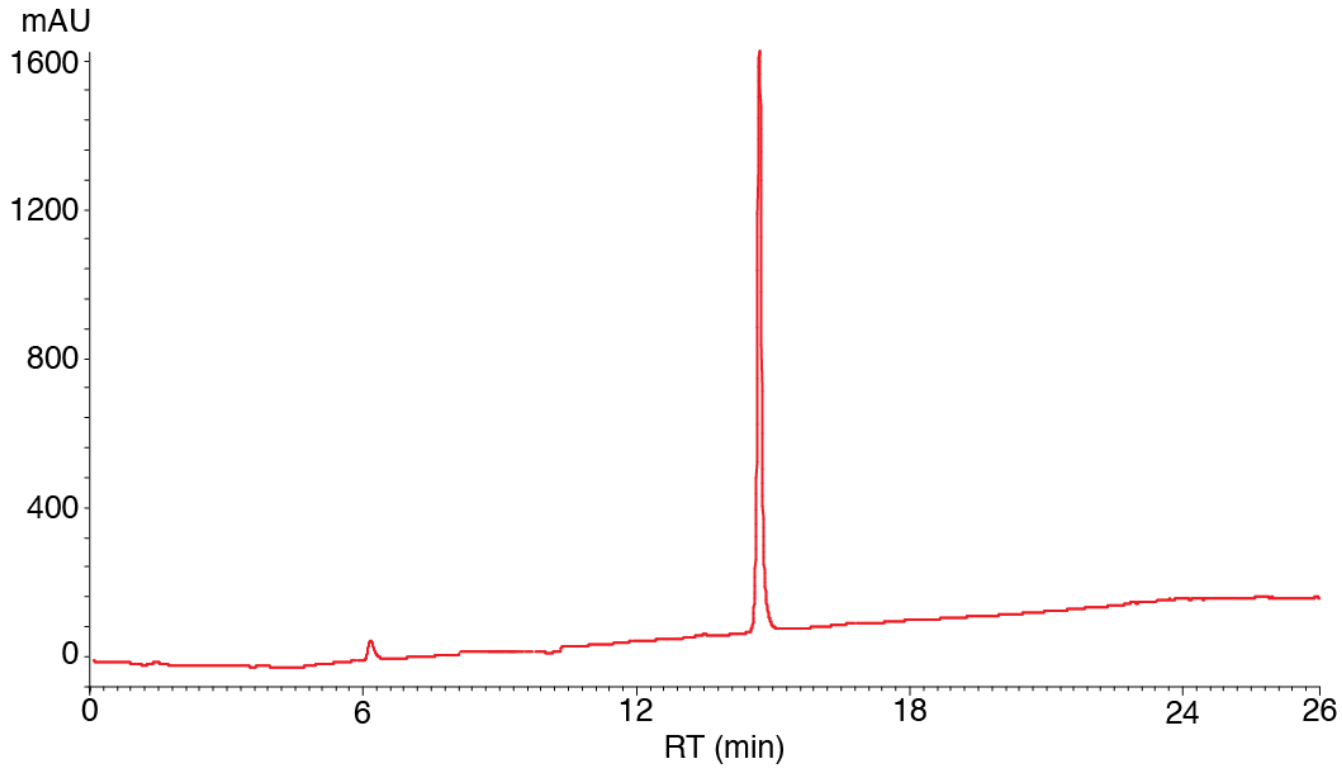


MH^{+1}(av)	MH^{+1}(mono)
15310.0632	15300.5948

Mass calculated

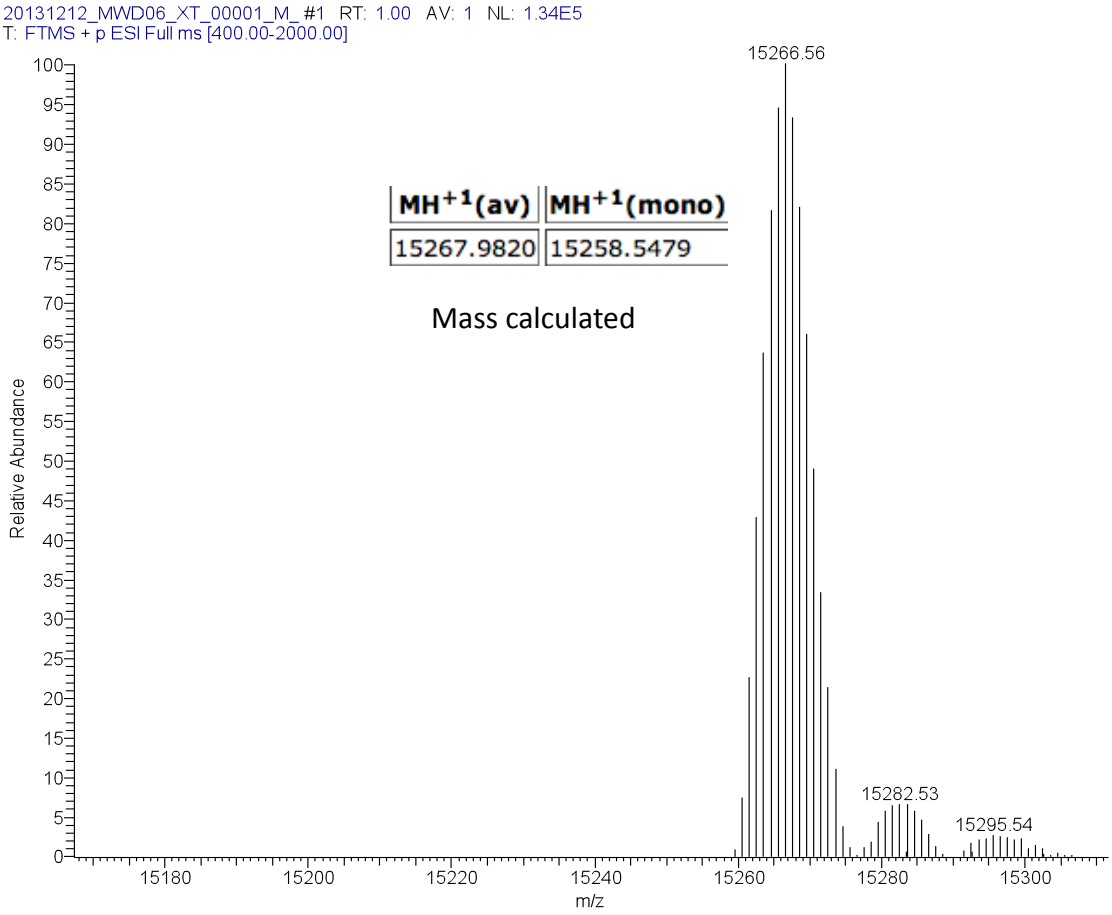
H3K4me3K27me3 (8)

Analytical HPLC trace



H3K36me3 (15)

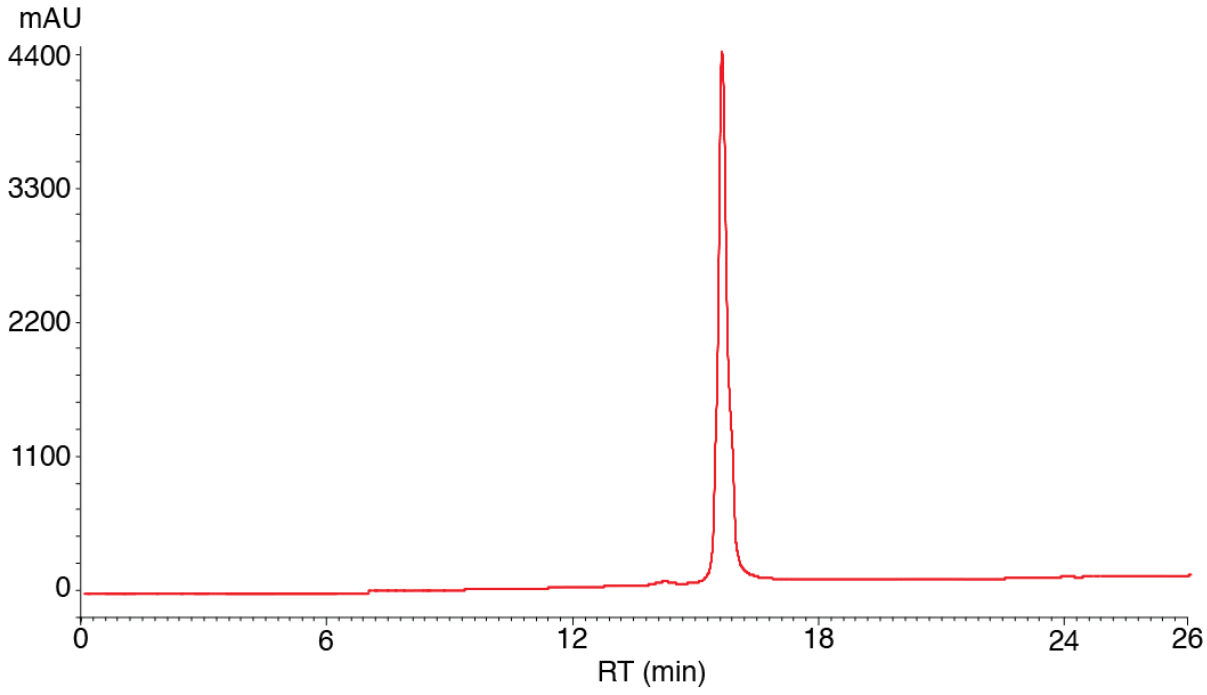
ESI-MS after deconvolution, see monoisotopic analysis for ESI-MS before deconvolution



MH^{+1}(av)	MH^{+1}(mono)
15267.9820	15258.5479

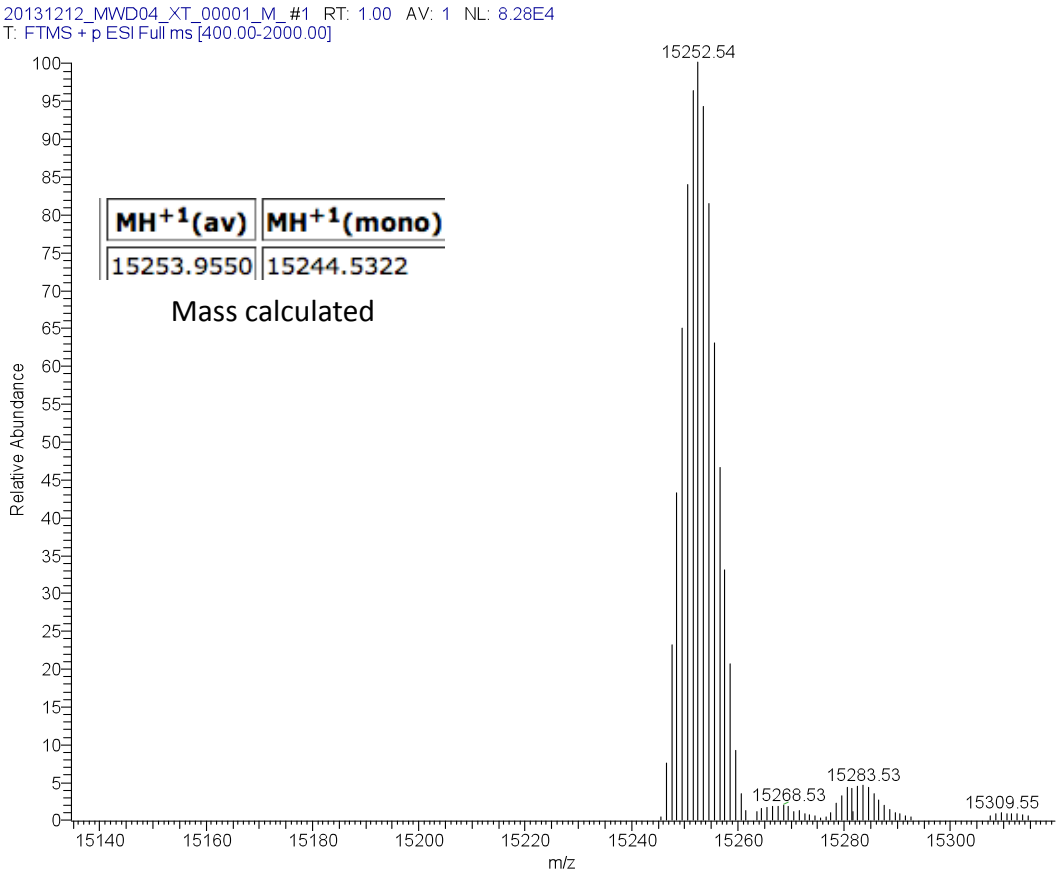
Mass calculated

Analytical HPLC trace



H3K79me2 (22a)

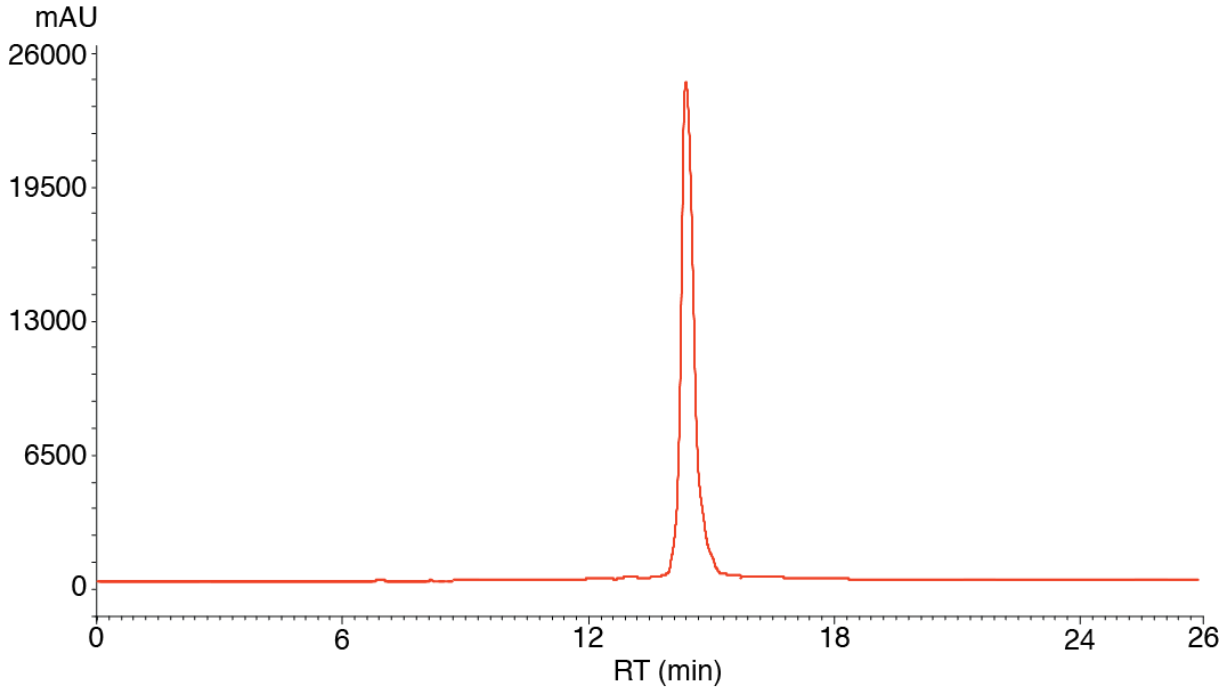
ESI-MS after desonvolution, see monoisotopic analysis for ESI-MS before deconvolution



MH^{+1}(av)	MH^{+1}(mono)
15253.9550	15244.5322

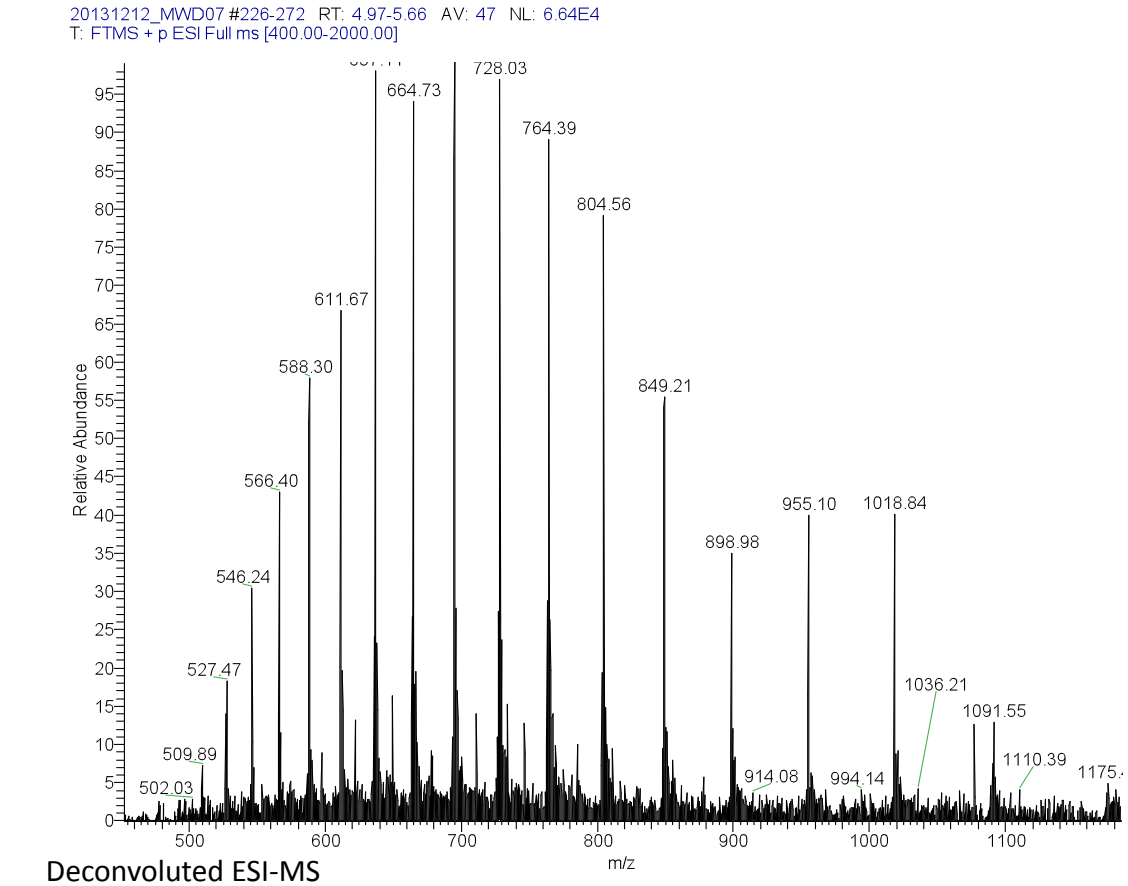
Mass calculated

Analytical HPLC trace

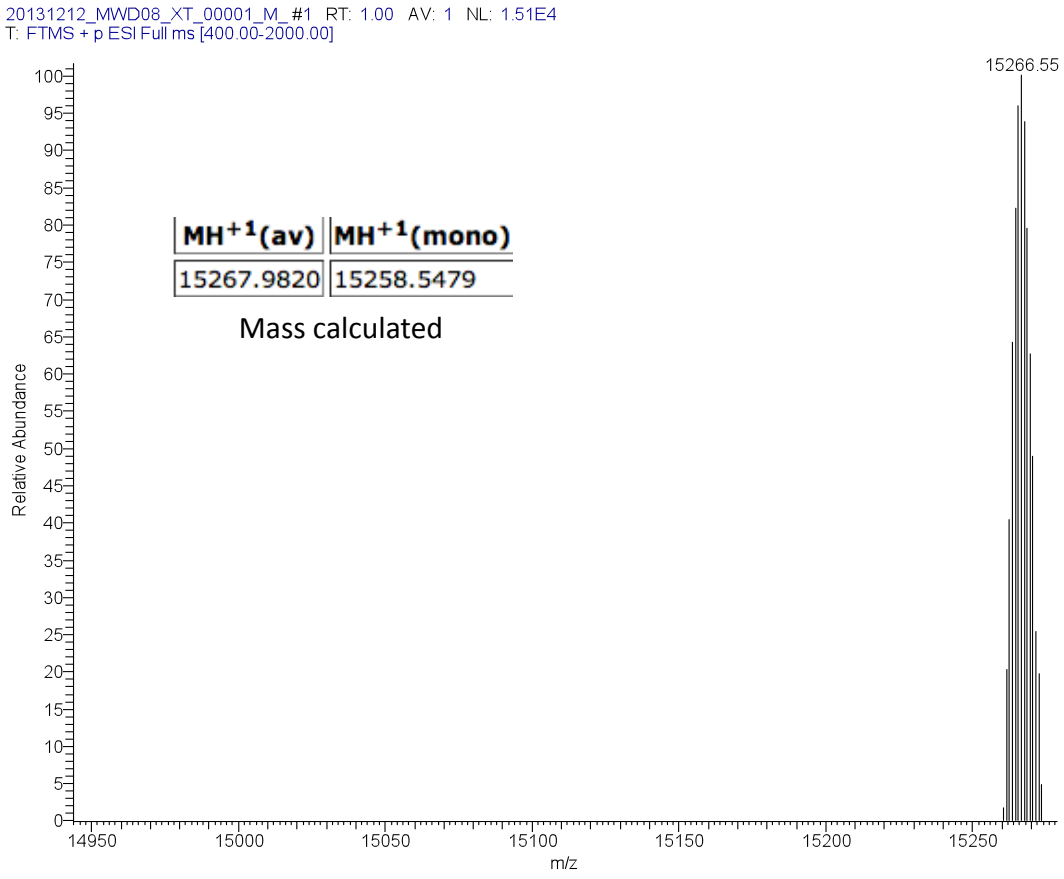


H3K79me3 (22b)

ESI-MS before deconvolution



Deconvoluted ESI-MS

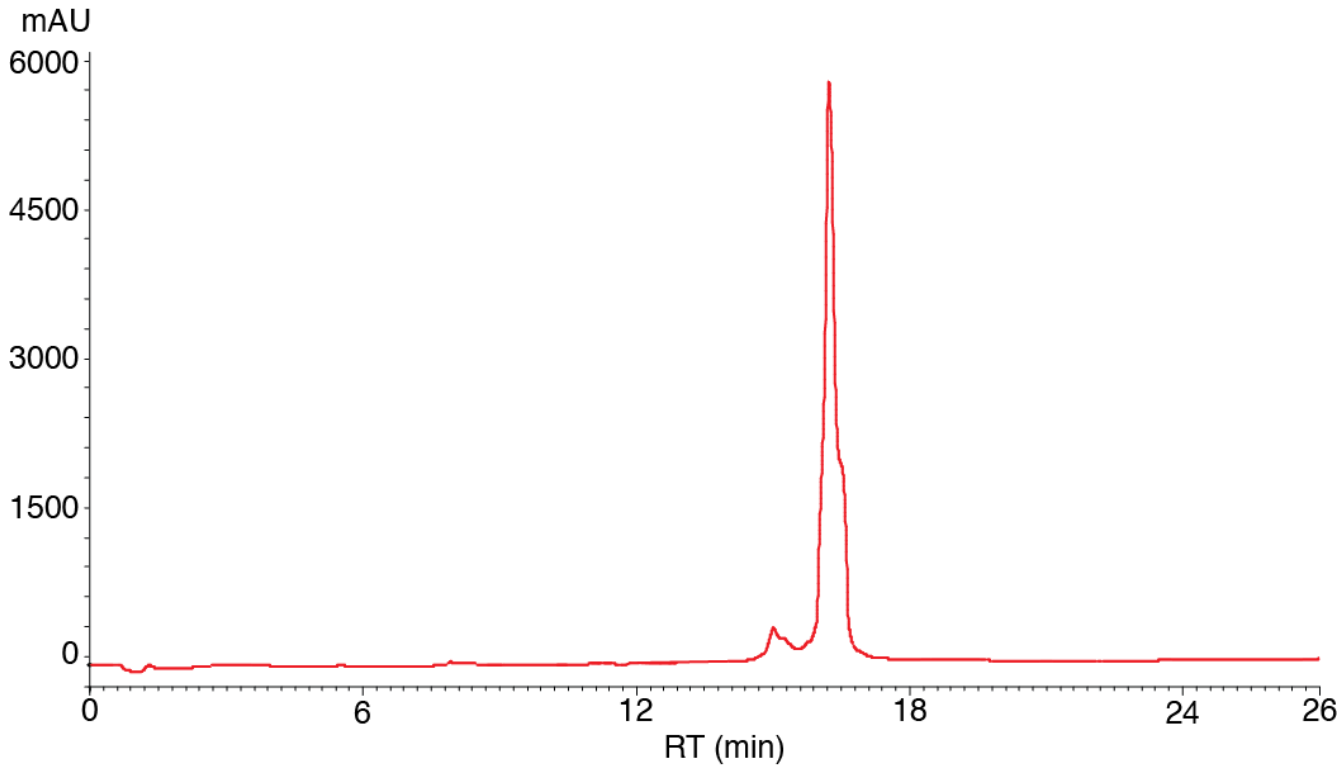


MH^{+1}(av)	MH^{+1}(mono)
15267.9820	15258.5479

Mass calculated

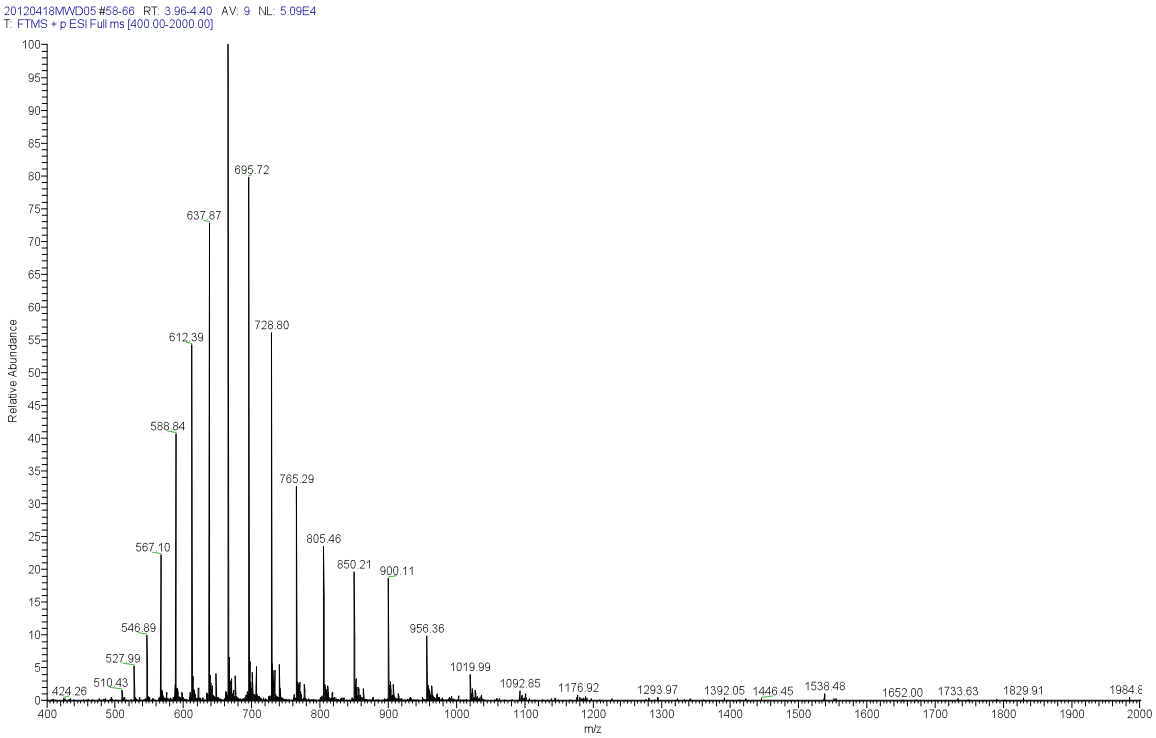
H3K79me3 (22b)

Analytical HPLC trace

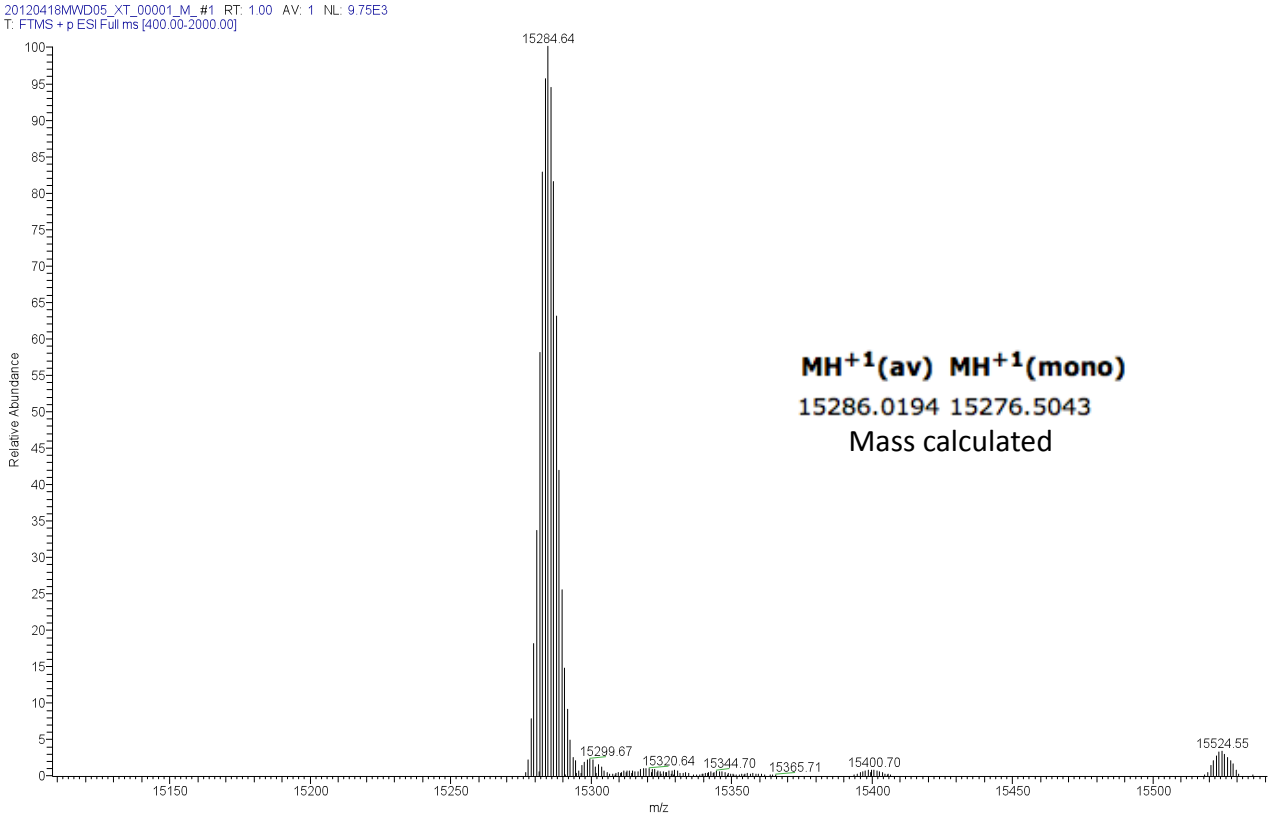


$H3K_c36me3$

ESI-MS before deconvolution



Deconvoluted ESI-MS



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