
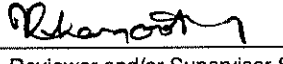
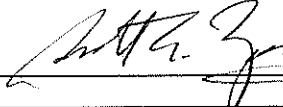


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A batch of purified recombinant aryloxyalkanoate dioxygenase (AAD-12) (batch TSN030732-002) was submitted by Barry Schafer of Dow AgroSciences for characterization. In conjunction with Dow AgroSciences characterization, Analytical Sciences Laboratory was requested to provide analytical data on the intact molecular weight, peptide mass fingerprinting, and N-terminal and C-terminal sequencing. Intact molecular weight analyses were accomplished by electrospray ionization/liquid chromatography/mass spectrometry (ESI/LC/MS). The mass spectrum revealed the presence of a principal mass component at 31,599.2 (*des*-Met¹). An earlier eluting peak, which accounts for ~14 % by peak area, primarily contained the protein with Met residue(s) oxidized: the base peak corresponds to *des*-Met¹ AAD-12 with two oxidized Met (or double oxidation of one Met) with a mass 31,631.2. The experimentally observed mass of *des*-Met¹ AAD-12 (31,599.2 Da) is within 0.004% of the calculated average mass of AAD-12 lacking a methionine, based on the expected amino acid sequence. Peptide mass fingerprinting was accomplished by in-solution trypsin, chymotrypsin, Arg-C, Asp-N, and Glu-C digests followed by ESI-LC/MS analysis. The peptide mass fingerprinting resulted in 100% overall mass coverage for the AAD-12 recombinant protein sample (batch TSN030732-002) (taking into account post-translational removal of Met¹). The N-terminal and C-terminal sequences for AAD-12 (batch TSN030732-002) were determined by a combination of in-solution digestion with endoproteases trypsin, Arg-C, and chymotrypsin, followed by tandem MS. The tandem MS data for both the N-terminal and C-terminal peptides confirmed the following sequences, and

²AQTTLQITPTGATLGATVTGVHLATLDDAGFAALHAAWLQHALLIFPGQHLSNDQQITFAK⁶² and

²⁸¹LAGRPETEGAALV²⁹³, respectively.

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INTRODUCTION

A sample of purified recombinant aryloxyalkanoate dioxygenase (AAD-12) (Batch TSN030732-002) was submitted by Barry Schafer of Dow AgroSciences for characterization. In conjunction with Dow AgroSciences characterization, Analytical Sciences Laboratory was requested to provide analytical data on peptide mass fingerprinting and N-terminal and C-terminal sequencing. Original experimental data are stored in the raw data packet ML-AL MD-2008-003833 ¹.

EXPERIMENTAL

Sample Preparation:

A sample of microbial recombinant purified AAD-12 (Batch TSN030732-002) (dark grey lyophilized material; several mg), was submitted for analysis by Barry Schafer (Dow AgroSciences, Indianapolis, IN). Prior to analyses by mass-spectrometry, the sample was prepared as follows:

AAD-12 (Batch TSN030732-002) material (1.16 mg) was resuspended in 1.16 mL of 25 mM ammonium bicarbonate/ 0.1M Gu:HCl, pH8, buffer to a final concentration of 1 mg/mL. An aliquot of the protein solution was stored at +4 °C prior to ESI/LC/MS analysis, and the rest of the solution was frozen at -20 °C. Preparation of enzymatic digests for peptide mass fingerprinting and sequencing is described separately below.

ESI/LC-MS for Intact Protein:

Reagents and Standards:

1. Acetonitrile (HPLC grade, 99.9%, Fisher Scientific), Lot no. 082100
2. Trifluoroacetic Acid (Aldrich, 99+%), Lot no. 00339JD
3. Deionized water, 18.2 MΩcm, MilliQ gradient A10, Millipore, freshly drawn
4. Poly-DL-Alanine, Sigma, Catalog no. P9003, Lot no. 97H5912
5. Ribonuclease A (RNase A), Sigma, Catalog no. R5000, Lot no. 122K1319
6. Bovine serum albumin (BSA), Sigma, Catalog no. A1900, Lot No. 036K7575
7. Lysozyme (from chicken egg white), Sigma, Catalog no. L7651, Lot no. 072K7062
8. Myoglobin (from horse heart), Sigma, Catalog no. M1882

Analytical Procedure:

ESI/LC/MS: The sample preparations were analyzed directly by mass spectrometry. All mass spectra were acquired on a Waters Q-ToF Micro MS system (S/N YA137). The mass spectrometer was calibrated prior to use in the mass range 500-1950 amu using 0.1 mg/mL Poly-DL-Alanine solution (in acetonitrile). A mixture of proteins with known molecular masses (RNase A, BSA, Lysozyme, Myoglobin; solutions in deionized water at 10 mg/mL were used) was run as a test standard. The following mass spectrometer settings were used.

LC : Acquity UPLC system
 Mobile Phase A : 0.01 % trifluoroacetic acid (TFA) in water
 Mobile Phase B : 0.01 % trifluoroacetic acid (TFA) in acetonitrile (ACN)
 Column : 2.1x150 mm Symmetry 300 C18 3.5 μ m 300 Å; S/N: 01283608610502 Part No: 186000188
 Flow rate : 100 μ L/min
 Column temperature : 50 °C
 Injection volume : 10 μ L
 Injection loop : 20 μ L
 UV detection : 214 nm, 40 pts/sec

Gradient table:

Time, min	Flow rate, mL/min	%A	%B
Initial	0.1	90	10
3	0.1	76	24
19	0.1	44	56
21	0.3	10	90
25	0.3	10	90
26	0.3	90	10
32	0.3	90	10
33	0.1	90	10
35	0.1	90	10

Q-ToF Micro with Micromass lock-spray interface: MS Parameters:

Capillary : 2800 V
 Desolvation Gas : 550 L/hr
 Desolvation Temperature: 345 °C
 Source Temperature : 90 °C
 Sample Cone : 15 V
 Extraction Cone : 0.9 V
 Collision Energy : 10.0 V
 MCP : 2350 V
 Mode : ESI-TOF-MS +
 Scan Range : 500 – 1950 amu
 Scan Rate : 0.98 sec/scan

The Micromass-supplied electrospray maximum entropy algorithm (MAXENT 1) was used to transform the spectra to a mass axis and to resolution enhance the transformed spectra. The maximum entropy algorithm was set to optimize the spectra with a resolution of 1 Da/channel. The resulting resolution-enhanced spectral peaks were centered and integrated to display the accurate mass for intact molecular mass analysis.

Table: Molecular weight of intact standard proteins determined by ESI/LC/MS:

Protein	Theoretical Mass, Da	Observed Mass, Da	Delta Mass, Da
RNase A	13681.3	13682.4	+ 1.1
Lysozyme	14303.9	14304.5	+ 0.6
BSA	66433.2	66432.3	- 1.2
Myoglobin	16951.5	16951.7	+ 0.2

In-solution Protein Processing and enzymatic digests:

Equipment:

- a) Mettler AE168 analytical balance serial no. F00518
- b) Eppendorf Centrifuge, Model 5415D, serial no. 5425 17645
- c) Eppendorf, Thermomixer R, serial no. 5355 20846
- d) Centrifugal evaporator (Centrivap), Labconco, cat. no. 7812013, S/N 051146935 A
- e) Eppendorf adjustable pipettes: 2.5µL serial no. 296447, 2-20µL serial no. 286820, 10-100µL serial no. 289560, and 1000µL serial no. 33165
- f) Fisher Vortex Genie 2, serial no. 2-156856
- g) Siliconized microcentrifuge tubes, 1.5mL, Fisher, cat no. 02-681-320
- h) Parafilm
- i) Eppendorf pipette tips (epTips) 10µL
- j) Fisher brand Reditip General Purpose, 200µL and 1000µL

Reagents and Standards:

- 1. Fisher, acetonitrile, cat no. A998-1
- 2. Sigma, ammonium bicarbonate, cat no. A-6141
- 3. Pierce, Dithiothreitol (DTT), cat no. 20290

4. Sigma, Iodoacetamide (IAA), Sigma, cat no. I-1149
5. Roche, Trypsin, cat no. 11-418-025-001 (Lot no. 13556621)
6. Roche, Chymotrypsin, cat no. 11-418-467-001 (Lot no. 13998020)
7. Roche, Asp-N, cat no. 11-054-589-001 (Lot no. 13883820)
8. Roche, Arg-C, cat no. 11-370-529-001 (Lot no. 11377132(13))
9. Roche, Glu-C, cat no. 11-047-817-001 (Lot no. 13390420)
10. Fluka, 98% Formic Acid (FA), Lot no. 1255194
11. Fisher, Trifluoroacetic acid (TFA), cat no. 04902-100
12. Milli-Q deionized water

Reagent Solution Preparation:

- a. 25 mM Ammonium Bicarbonate buffer: dissolved 98.83 mg NH_4HCO_3 in 50 mL of Milli-Q water; filtered through 0.22 μm sterile syringe filter.
- b. 100 mM Tris buffer: dissolved 121.1 mg Tris in 10 mL of Milli-Q water; adjusted pH to 8.11 with HCl; filtered through 0.22 μm sterile syringe filter.
- c. Protein dissolution buffer (6M guanidine hydrochloride (Gu:HCl)/ 400 mM ammonium bicarbonate, pH 7.8): to 316 mg of ammonium bicarbonate, 7.5 mL of 8M Gu:HCl solution and 2.5 mL of water were added. pH was adjusted to 7.8 with NaOH. Buffer was filtered through 0.22 μm sterile syringe filter.
- d. DTT solution (100 mM; prepared fresh): dissolved 15.4 mg DTT in 1 mL of water.
- e. Alkylating reagent (IAA) (200 mM; prepared fresh): dissolved 37 mg IAA in 1 mL of water.
- f. Trypsin solution. Step 1: Dissolved 25 μg of dried trypsin in 320 μL of 100 mM Tris buffer immediately prior to digestion procedure. Step 2: Dissolved 50 μg of dried trypsin in 320 μL of 100 mM Tris buffer immediately prior to digestion procedure.
- g. Chymotrypsin solution. Step 1: Dissolved 25 μg of dried chymotrypsin in 66 μL of 1 mM HCl immediately prior to digestion procedure. Step 2: Dissolved 50 μg of dried chymotrypsin in 160 μL of 1 mM HCl immediately prior to digestion procedure.
- h. Asp-N solution: Dissolved 2 μg of dried Asp-N in 50 μL of Milli-Q water immediately prior to digestion procedure.
- i. Glu-C solution. Step 1: Dissolved 50 μg of dried Glu-C in 65 μL of Milli-Q water immediately prior to digestion procedure. Step 2: Dissolved 50 μg of dried Glu-C in 160 μL of Milli-Q water immediately prior to digestion procedure.
- j. Arg-C solution. Step 1: Immediately prior to digestion procedure, dissolved 5 μg of dried Arg-C in 30 μL of Milli-Q water, and combine with 50 μL of activation solution (reconstituted in 100 μL of Milli-Q water, as per manufacturer's procedure). Step 2: Immediately prior to digestion procedure,

dissolved 10 µg of dried Arg-C in 30 µL of Milli-Q water, and combine with 50 µL of activation solution (reconstituted in 100 µL of Milli-Q water, as per manufacturer's procedure).

In-solution Protein Processing (Reduction/ alkylation/ digestion):

- a. Five 180-µL aliquots of 1 mg/mL AAD-12 (Batch TSN030732-002) protein solution were dried in the centrifugal evaporator to completeness.
- b. Reduction and carboxyamidomethylation (alkylation) of protein: approximately 180-µL of protein dissolution buffer, 6M Gu:HCl/ 0.4M ammonium bicarbonate, pH 7.8, was added to the dry AAD-12 [Batch TSN030732-002] samples, and samples were mixed by pipette action. Twenty microliters of 100 mM DTT (reducing reagent) solution was added to each tube. Tubes were sealed, vortexed, and incubated at 65 °C for 40 min in a thermomixer at 1100 rpm. Tubes were then cooled to room temperature, centrifuged for 30 sec. and 40 µL of 200 mM IAA (alkylating reagent) solution was added to each tube. Tubes were incubated in the dark at room temperature for 1 hour. Eighty microliters of DTT solution was added to consume unreacted IAA and the tubes were allowed to stand for 20 min at room temperature. The total reaction volume was approximately 320 µL in each tube.
- c. Desalting of the reduced/alkylated protein samples was performed using NAP-5 gravity cartridges (Sephadex G-25) as per the manufacturer's procedure. NAP-5 cartridges were pre-equilibrated with the corresponding digestion buffer (100 mM Tris buffer, pH 8.11, for tryptic and Arg-C digests; 25 mM ammonium bicarbonate, pH 7.8, for chymotryptic, Asp-N, and Glu-C digests), and protein elution was performed with the same buffer (final volume 1-mL for each sample).
- d. Tryptic digestion of reduced/alkylated protein: Step 1: 100-µL of trypsin solution (25 µg in 320 µL of 100 mM Tris buffer, pH8.11) was added to the 1-mL of reduced/alkylated protein AAD-12 [Batch TSN030732-002] sample in 100 mM Tris buffer, pH8.11. The digest was incubated for 2 hours at 37 °C in a thermomixer at 900 rpm. Step 2: 100-µL of trypsin solution (50 µg in 320 µL of 100 mM Tris buffer) was added to the digestion reaction. The digest was incubated for 16 hours at 37 °C in a thermomixer at 900 rpm. Sample was frozen at -20 °C until ready for analysis by mass-spectrometry.
- e. Arg-C digestion of reduced/alkylated protein: Step 1: 25-µL of Arg-C solution (5 µg in 30 µL of Milli-Q deionized water, combined with 50 µL of activation solution) was added to the 1-mL of reduced/alkylated protein AAD-12 [Batch TSN030732-002] sample in 100 mM Tris buffer, pH8.11. The digest was incubated for 2 hours at 37 °C in a thermomixer at 900 rpm. Step 2: 25-µL of Arg-C solution (10 µg in 30 µL of Milli-Q deionized water, combined with 50 µL of activation solution) was added to the digestion reaction. The

digest was incubated for 16 hours at 37 °C in a thermomixer at 900 rpm. Sample was frozen at -20 °C until ready for analysis by mass-spectrometry.

- f. Chymotryptic digestion of reduced/alkylated protein: Step 1: 20-μL of chymotrypsin solution (25 μg in 66 μL of 1 mM HCl) was added to the 1-mL of reduced/alkylated protein AAD-12 [Batch TSN030732-002] sample in 25 mM ammonium bicarbonate buffer, pH7.8. The digest was incubated for 2 hours at 22 °C in a shaker. Step 2: 50-μL of chymotrypsin solution (50 μg in 160 μL of 1 mM HCl) was added to the digestion reaction. The digest was incubated for 16 hours at 22 °C in a shaker. Sample was frozen at -20 °C until ready for analysis by mass-spectrometry.
- g. Asp-N digestion of reduced/alkylated protein: 50-μL of Asp-N solution (2 μg in 50 μL of Milli-Q deionized water) was added to the 1-mL of reduced/alkylated protein AAD-12 [Batch TSN030732-002] sample in 25 mM ammonium bicarbonate buffer, pH7.8. The digest was incubated for 16 hours at 37 °C in a thermomixer at 900 rpm. Sample was frozen at -20 °C until ready for analysis by mass-spectrometry.
- h. Glu-C digestion of reduced/alkylated protein: Step 1: 20-μL of Arg-C solution (50 μg in 65 μL of Milli-Q deionized water) was added to the 1-mL of reduced/alkylated protein AAD-12 [Batch TSN030732-002] sample in 25 mM ammonium bicarbonate buffer, pH7.8. The digest was incubated for 2 hours at 22 °C in a shaker. Step 2: 50-μL of Glu-C solution (50 μg in 160 μL of Milli-Q deionized water) was added to the digestion reaction. The digest was incubated for 16 hours at 22 °C in a shaker. Sample was frozen at -20 °C until ready for analysis by mass-spectrometry.

ESI-LC/MS and MS/MS of proteolytic digests

Reagents and Materials:

1. Acetonitrile (Baker analyzed HPLC solvent, JT Baker), Lot no. C10827
2. Milli-Q water
3. 98% Formic Acid (Fluka), Lot no. 1255194
4. Poly-DL-Alanine, Sigma, cat. no. P9003, Lot no. 97H5912
5. Leucine Enkephalin acetate salt, Sigma, cat. no. L-9133, Lot no. 095K5109
6. Waters total recovery HPLC vials, P/N 186000384c, lot no. 0384661180

Analytical Procedure:

ESI-LC/MS: The samples (digests) were dried to completeness in a centrifugal evaporator, resuspended in deionized water (180 µL; to a final concentration of approximately 1 mg/mL) and analyzed by LC/MS. All mass spectra were acquired on a Waters Q-ToF Micro MS system (S/N YA137). The mass spectrometer was calibrated prior to use in the mass range 350 – 1900 amu (MS for peptide mass fingerprinting) or 80 – 1900 amu (tandem MS) using 0.1 mg/mL Poly-DL-Alanine solution in acetonitrile. The following liquid chromatography and mass spectrometer settings were used:

LC : Acquity UPLC system
 Mobile Phase A : 0.1 % formic acid (FA) in water
 Mobile Phase B : 0.1 % formic acid (FA) in acetonitrile
 Column : 2.1x150 mm Acquity BEH C18 1.7 µm 135 Å; S/N: 01245523640B05 Part No: 186002353
 Flow rate : 100 µL/min
 Column temperature : 50 °C
 Injection volume : 10 µL
 Injection loop : 20 µL
 UV detection : 214 nm, 40 pts/sec

Gradient table:

Time, min	Flow rate, mL/min	%A	%B
Initial	0.1	95	5
5	0.1	95	5
63	0.1	60	40
63.5	0.3	60	40
69	0.3	10	90
70	0.3	10	90
71	0.3	95	5
79	0.3	95	5
80	0.1	95	5
85	0.1	95	5

MS : QTOF-micro mass spectrometer (S/N YA137)
 ESI : Micromass lock-spray electrospray interface
 Mode : +TOFMS

MS Parameters (peptide mass fingerprinting):

Capillary : 2850 V
 Desolvation Gas : 650 L/hr
 Desolvation Temperature : 300 °C
 Source Temperature : 110 °C
 Sample Cone : 15 V
 Extraction Cone : 0.9 V
 Collision Energy : 10.0 V
 MCP : 2350 V
 Mode : ESI-TOF-MS +

Scan Range : 350 – 1900 amu (PMF) or 80 – 1900 amu (tandem MS)
Scan Cycle Time : 0.98 sec/scan

MS/MS Parameters:

Capillary : 2850 V
Desolvation Gas : 650 L/hr
Desolvation Temperature : 300 °C
Source Temperature : 110 °C
Sample Cone : 15 V
Extraction Cone : 0.9 V
MCP : 2350 V
Mode : ESI-TOF-MS +
Scan Range : 80 – 1900 amu

Survey Scan

Collision Energy : 10.0 V
Scan Cycle Time : 0.98 sec/scan
Precursor Selection : Included Masses only
Include Window : +/- 300 mDa
Include Retention Time : 240 sec
Peak Detection Window : 1 Da

MS/MS Scan

MS to MSMS Switch Criteria : Intensity
MS to MSMS Switch Threshold : 10 counts/sec
MSMS to MS Switch Criteria : Intensity falling below threshold
Switchback Threshold : 3 counts/sec
MSMS Switch After Time : 12 sec
Scan Cycle Time : 1.98 sec/scan

Methods:

The samples were injected using a partial loop configuration. After sample injection, the column was held at 5 % MPB for 5 minutes. The gradient from 5 % MPB to 40 % MPB was then employed. At the end of the gradient, the MPB concentration was increased to 90% to allow removal of any hydrophobic components. The column was then re-equilibrated to the initial conditions.

The Time of Flight (ToF) analyzer was calibrated daily using a 0.1 mg/mL (100 ppm) solution (in acetonitrile) of Poly-Alanine at 20 µL/min flow rate. The same instrument parameter file (with the calibration parameters) was used for MS data acquisitions. Data acquisition was performed with cycle times of 1 sec/scan (scan acquisition time: 0.88 sec; interscan delay: 0.1sec) and 2 sec/scan (scan acquisition time: 1.88 sec; interscan delay: 0.1sec) in the MS mode and MSMS mode, respectively. The lock mass data was acquired using 2.5 µM Leucine-Enkephalin peptide solution (0.1 % formic acid in 50 % acetonitrile was used as the solvent) flowing at 3 – 5 µL/min. The lock mass channel was sampled every 7 sec during MS analysis and 10 sec during MS/MS analysis. The reference ion used was the singly charged Leucine-Enkephalin ion at m/z 556.2771. The tandem MS experimental parameters used

in the analyses of N- and C- terminal peptides from both tryptic and chymotryptic digests of AAD-12 (TSN030732-002) are listed above.

Peptide mass fingerprinting of the UPLC-MS data was performed manually. The spectrum of each chromatographic peak was summed, smoothed (SG, 2x3 channels), centroided (4 channels, top 80 %, by height) and m/z error corrected (lock mass channel: 10 scans, m/z 556.2271 \pm 0.5 Da). In-source fragmentation observed was used to further confirm the identity of the peptides. For some peptides eluting later in the gradient MaxEnt1 was used. The Micromass-supplied electrospray maximum entropy algorithm (MAXENT 1) was used to transform the spectra to a mass axis and to resolution-enhance the transformed spectra. The maximum entropy algorithm was set to optimize the spectra with a resolution of 1 Da/channel. The resulting resolution-enhanced spectral peaks were centered and integrated to display the accurate mass.

The spectra from tandem MS experiments were also summed, smoothed (SG, 2x3 channels), centroided (4 channels, top 80 %, by height) and m/z error corrected (lock mass channel: 10 scans, m/z 556.2271 \pm 0.5 Da). The fragments were assigned using a theoretical fragmentation ion table generated using either a local copy of Protein Prospector (v 3.2.1) or using Micromass BioLynx software.

RESULTS AND DISCUSSION

In this study, the numbering of the amino acid residues is in accordance with the theoretical sequence of the recombinant AAD-12 protein starting with Met¹ and containing a total of 293 residues (**Figure 1**).

ESI Intact Mass Spectral Characterization:

The purified AAD-12 (Batch TSN030732-002) was processed by directly solubilizing the dry protein material in PBS buffer supplemented with 0.1M Gu:HCl (to ease solubilization and to prevent immediate protein precipitation).

The solubilized proteins were then analyzed by ESI-LC/MS using a Symmetry C18 column for separation. The chromatography for AAD-12 (Batch TSN030732-002) revealed the presence of one major peak at retention time of 12.35 min, and two small satellite peaks at 11.86 min (~14% by LC-UV peak area) and 13.61 min (~4% by LC-UV peak area) (**Figure 2**). Unique features of the corresponding mass spectra were the broad charge distribution and partial resolution of the peaks. Ions related to the monomer form of AAD-12 were observed. The transformed and integrated maximum entropy spectra revealed the presence of a principal mass component at m/z 31,599.2 (LC peak II, 12.35 min). This mass is consistent with the calculated molecular weight for *des*-Met¹ AAD-12 (theor. average mass: 31,598 Da, see **Table I**).

Ions related to the monomer form of AAD-12 (*des*-Met¹) were observed under the non-reducing conditions of the described ESI-LC/MS experiment. Thus the observed mass of the major sample component is within 0.004% of the theoretical molecular weight of AAD-12 (*des*-Met¹) (**Table I**). Peak I, which accounts for approximately 14% by peak area, primarily contains oxidized Met residue(s) (peak I, 11.86 min; **Figure 2**). The majority of the oxidized AAD-12 protein contained two oxidation sites (either on two separate Met, or a single doubly-oxidized Met; **Table I**).

Peptide Mass Fingerprinting:

ESI-LC/MS analysis was used to generate peptide coverage maps, N-terminal and C-terminal sequences, and to determine post-translational processing sites. For that purpose, in-solution trypsin, chymotrypsin, Arg-C, Asp-N, and Glu-C digests of reduced and alkylated AAD-12 were generated and analyzed by ESI-LC/MS. **Figures 3** through **7** show the corresponding LC chromatograms of the digested AAD-12 (Batch TSN030732-002). The corresponding mass spectral data with assignments from ESI-LC/MS analyses are presented in **Tables II** through **VI** for the tryptic, Arg-C, chymotryptic, Asp-N, and Glu-C digests, respectively (in that order). The combined sequence coverage is full (100%), given the expected post-translational removal of N-terminal Met¹ (**Figure 1**). Most peptides observed in the proteolytic digests of AAD-12 (Batch TSN030732-002) exhibited in-source fragmentation patterns consistent with their expected theoretical amino acid sequences (**Tables II** through **VI**). The N- and C-terminal peptides were further analyzed by LC tandem MS to confirm their amino acid sequences.

LC Tandem MS:

N- and C-terminal peptides observed by LC-MS analyses were further analyzed by tandem MS to confirm their amino acid sequences. The results are presented in **Tables VII** and **VIII**. Sequence tags were generated from the tryptic fragments with m/z 642.36 (C-terminal peptide, $[M+2H]^{2+}$) and m/z 1063.22 (N-terminal peptide, $[M+6H]^{6+}$), and chymotryptic fragments with m/z 585.82 (C-terminal peptide, $[M+2H]^{2+}$) and m/z 533.29 (N-terminal peptide, $[M+H]^{1+}$). LC tandem MS ion spectra were acquired for each individual peptide at specific retention time obtained in the preceding peptide mass fingerprinting study. Tandem MS experiments were performed with multiple collision energies for each peptide. The tandem MS fragments observed for N-terminal peptides from both tryptic and chymotryptic digests were consistent with the N-terminal peptide sequence, AQTTLQITPTGATLGATVTGVHLATLDDAGFAALHAAWLQHALLIFPGQHLSNDQQITFAK (**Table VIII**). The tandem MS fragments observed for C-terminal peptides from both tryptic and chymotryptic digests were consistent with the C-terminal peptide sequence, LAGRPETEGAALV (**Table VII**).

REFERENCES

1. Raw data packet ML-AL MD-2008-003833

Table I: Molecular weight of intact AAD-12 (Batch TSN030732-002) determined by ESI-LC/MS

Sample Lot # / Peak #	Residues	Theoretical Average Mass	Observed	Comment
TSN030732-002/ I	2-293 (des-Met ¹)	31598.0	31594.6	
	2-293 (des-Met ¹)		31612.3	One oxidation site
	2-293 (des-Met ¹)		31631.2*	Two oxidation sites
	2-293 (des-Met ¹)		31645.7	Three oxidation sites
	2-293 (des-Met ¹)		31661.7	Four oxidation sites
TSN030732-002/ II	2-293 (des-Met ¹)	31598.0	31599.2*	
	2-293 (des-Met ¹)	31398.8	31615.1	One oxidation site
	4-293 (des-Met ¹)		31399.1	
			31579.8	unidentified
TSN030732-002/ III	2-293 (des-Met ¹)	31598.0	31596.1	
	2-293 (des-Met ¹)		31612.1	One oxidation site
			31714.3	unidentified
			31720.9	unidentified
			31733.4	unidentified

* Primary mass component within the LC peak

Table II: Tryptic digest peptide mass fingerprinting of AAD-12 (Batch TSN030732-002).

Peptide	# of missed cleavages	a.a. ##	Sequence	m/z theor.							m/z obs. (ESI-LC/MS)	UPLC elution time, min	In-source fragments observed (matching amino-acid sequence)	Comment
				(M+H) ⁺	(M+2H) ²⁺	(M+3H) ³⁺	(M+4H) ⁴⁺	(M+5H) ⁵⁺	(M+6H) ⁶⁺	(M+7H) ⁷⁺				
T1	0	2-62	(-) AQTTLQITPTGATLGATVGVHLATLDDAGFAALHAALQHALLIFFPGQHLSDNQITFAK (R)	6366.22	3183.62	2122.75	1592.31	1274.05	1061.88	910.32	1532.09 (4+), 1273.90 (5+), 1061.84 (6+), 910.28 (7+)	63.42		processed N-terminus; no oxidation observed; average masses
T3	0	64-69	(R) FGAIER (I)	692.37	346.69	231.46	173.85	139.28			692.37 (1+)	21.69	545.31 (1+, y5), 488.28 (1+, y4), 417.25 (1+, y3)	
T4	0	70-82	(R) IGGGDVAISNVK (A)	1242.71	621.86	414.91	311.43	249.35			1242.70 (1+), 621.86 (2+)	34.84	1129.64 (1+, y12), 1072.39 (1+, y11), 843.55 (1+, y8), 796.45 (1+, b8), 631.38 (1+, y6), 612.33 (1+, b7), 560.33 (1+, y5), 513.27 (1+, b6), 447.26 (1+, b4), 400.19 (1+, b5), 360.23 (1+, y3)	
T5	0	83-88	(K) ADGTVR (Q)	618.32	309.66	206.78	155.34	124.47			618.32 (1+)	4.70		
T6	0	89-100	(R) QHSPAEWDDMMK (V)	1474.61	737.81	492.21	369.41	295.73			737.81 (2+), 492.21 (3+)	31.31	954.37 (1+, y7), 825.33 (1+, y6), 650.29 (1+, b6), 639.25 (1+, y5), 524.23 (1+, y4), 521.24 (1+, b5), 504.24 (2+, y8-H2O)	
T6-Met-ox1	0	89-100	(R) QHSPAEWDDMMK (V)	1490.61	745.81	497.54	373.40	298.92			ND	ND		
T6-Met-ox2	0	89-100	(R) QHSPAEWDDMMK (V)	1506.61	753.81	502.87	377.40	302.12			ND	ND		
T7	0	101-135	(K) VIVGNMAWHADSTYMPVMAQGAVFSAEVVPAVGGR (T)	3617.77	1809.39	1206.60	905.20	724.36			1206.60 (3+), 905.22 (4+)	53.51	838.87 (2+, b15), 655.39 (1+, y7), 556.32 (1+, y6)	
T7-Met-ox1	0	101-135	(K) VIVGNMAWHADSTYMPVMAQGAVFSAEVVPAVGGR (T)	3633.77	1817.39	1211.92	909.19	727.55			ND	ND		
T7-Met-ox2	0	101-135	(K) VIVGNMAWHADSTYMPVMAQGAVFSAEVVPAVGGR (T)	3649.77	1825.39	1217.26	913.19	730.75			ND	ND		
T8	0	136-142	(R) TCFADMR (A)	900.37	450.69	300.80	225.85	180.88			900.37 (1+), 450.69 (2+)	24.42	639.29 (1+, y5), 595.22 (1+, b5), 492.22 (1+, y4), 421.19 (1+, y3)	
T8-Met-ox1	0	136-142	(R) TCFADMR (A)	916.37	458.69	306.12	229.84	184.07			ND	ND		
T9	0	143-153	(R) AAYDALDEATR (A)	1195.56	598.28	399.19	299.65	239.92			1195.67 (1+), 598.29 (2+)	26.67	1053.49 (1+, y9), 1021.45 (1+, b10), 920.44 (1+, b9), 890.44 (1+, y8), 849.33 (1+, b8), 775.40 (1+, y7), 720.33 (1+, b7), 704.34 (1+, y6), 591.27 (1+, y5), 527.25 (2+, y9), 492.22 (1+, b5), 476.24 (1+, y4), 421.18 (1+, b4)	
T10	0	154-159	(R) ALVHQR (S)	723.43	362.22	241.81	181.61	145.49			723.43 (1+)	7.82	440.24 (1+, y3), 539.30 (1+, y4)	
T12	0	163-171	(R) HSLVYSQSK (L)	1048.54	524.78	350.19	262.89	210.51			1048.54 (1+), 524.78 (2+)	15.99		
T13	0	172-199	(K) LGHVQQAGSAYIGYGMDDTTATPLRPLVK (V)	2944.54	1472.77	982.18	736.89	589.71			1472.64 (2+), 982.19 (3+), 736.90 (4+)	40.74	919.89 (2+, y17), 860.46 (2+, y16), 831.96 (2+, y15), 790.41 (2+, y14), 723.39 (2+, b14), 641.82 (2+, b13), 613.33 (2+, b12), 556.77 (2+, b11), 475.25 (2+, b10)	
T13-Met-ox1	0	172-199	(K) LGHVQQAGSAYIGYGMDDTTATPLRPLVK (V)	2960.54	1480.77	987.51	740.89	592.91			ND	ND		
T14	0	200-213	(K) VHPETGRPSLLIGR (H)	1531.87	766.44	511.30	383.72	307.18			766.45 (2+), 511.30 (3+)	29.31	777.41 (1+, b7), 648.37 (2+, y12), 594.34 (2+, b11), 537.80 (2+, b10), 481.26 (2+, b9), 458.32 (1+, y4)	
T15	0	214-228	(R) HAHAI PGMDAAESER (F)	1591.73	796.37	531.25	398.69	319.15			796.38 (2+), 531.26 (3+)	21.95	1062.50 (1+, y10), 965.41 (1+, y9), 908.38 (1+, y8), 777.35 (1+, y7), 684.37 (1+, b7), 662.32 (1+, y6), 591.28 (1+, y5), 530.28 (1+, b5), 520.24 (1+, y4), 465.72 (2+, b9), 417.20 (1+, b4), 391.20 (1+, y3)	
T15-Met-ox1	0	214-228	(R) HAHAI PGMDAAESER (F)	1607.73	804.37	536.58	402.68	322.35			ND	ND		
T16	0	229-242	(R) FLEGLVDWACQAPR (V)	1661.81	831.41	554.61	416.21	333.17			831.41 (2+), 554.61 (3+)	49.66	774.41 (1+, b7), 702.34 (1+, y6), 695.83 (2+, b12), 659.39 (1+, b6), 631.30 (1+, y5), 560.30 (1+, b5), 502.23 (2+, y8), 471.27 (1+, y4), 390.21 (1+, b3)	
T17	0	243-259	(R) VHAHQWAAGDVVVDNR (C)	1959.96	980.48	653.99	490.75	392.80			980.49 (2+), 654.00 (3+)	34.84	1172.54 (1+, b11), 1073.49 (1+, b10), 788.41 (1+, y6), 788.89 (2+, b14), 689.34 (1+, y5), 685.85 (2+, b13), 636.33 (2+, b12), 590.27 (1+, y4), 586.78 (2+, b11), 572.80 (2+, a11), 537.26 (2+, b10), 404.19 (1+, y3), 394.72 (2+, y6)	
T18	0	260-264	(R) CLLHR (A)	698.38	349.69	233.46	175.35	140.48			698.37 (1+)	17.57	425.27 (1+, y3)	
T19	0	265-271	(R) AEPWDFK (L)	892.42	446.71	298.15	223.86	179.29			892.42 (1+), 446.72 (2+)	34.41	892.34 (1+, y5), 595.30 (1+, y4), 571.26 (1+, a5), 409.21 (1+, y3)	
T20	0	272-274	(K) LPR (V)	385.26	193.13	129.09	97.07	77.86			385.26 (1+)	8.57		
T21	0	275-280	(R) VMWHSR (L)	815.40	408.20	272.47	204.61	163.89			815.39 (1+), 408.21 (2+)	18.07	716.32 (1+, y5), 585.29 (1+, y4), 399.22 (1+, y3)	
T21-Met-ox1	0	275-280	(R) VMWHSR (L)	831.40	416.20	277.80	208.60	167.08			ND	ND		
T22	0	281-293	(R) LAGRPETEGAAVL (-)	1283.70	642.35	428.57	321.68	257.55			1283.71 (1+), 642.36 (2+)	30.86	1053.54 (1+, b11), 982.50 (1+, b10), 886.46 (1+, y9), 854.43 (1+, b8), 583.82 (2+, b12), 527.27 (2+, b11), 491.77 (2+, b10), 398.25 (1+, b4)	C-terminus
T1-2	1	2-63	(-) AQTTLQITPTGATLGATVGVHLATLDDAGFAALHAALQHALLIFFPGQHLSDNQITFAK (F)	6522.41	3261.71	2174.81	1631.36	1305.29	1087.91	932.64	1304.92 (5+), 1087.71 (6+), 932.46 (7+)	62.27		Processed N-term.; missed cleavage
T2-3	1	63-69	(K) RFGAIER (I)	848.47	424.74	283.50	212.87	170.50			424.74 (2+)	19.93		missed cleavage
non-spec1		106-135	(N) MAWHADSTYMPVMAQGAVFSAEVVPAVGGR (T)	3135.49	1568.25	1045.83	784.63	627.90			1568.19 (2+), 761.34 (3+), 784.68 (4+)	51.04	1290.64 (2+, b24), 860.72 (3+, b24), 761.34 (2+, b13), 754.44 (1+, y8), 655.38 (1+, y7), 597.74 (2+, b10), 556.32 (1+, y6)	non-specific cleavage
non-spec1-Met-ox1		106-135	(N) MAWHADSTYMPVMAQGAVFSAEVVPAVGGR (T)	3151.49	1576.25	1051.16	788.62	631.10			ND	ND		
non-spec1-Met-ox2		106-135	(N) MAWHADSTYMPVMAQGAVFSAEVVPAVGGR (T)	3167.49	1584.25	1056.50	792.62	634.30			ND	ND		
non-spec1-Met-ox3		106-135	(N) MAWHADSTYMPVMAQGAVFSAEVVPAVGGR (T)	3183.49	1592.25	1061.83	796.62	637.50			ND	ND		

"Met-ox#" = number of possible oxidations of Met residue(s) in a peptide

Cys residues are reduced and carboxamidomethylated

Table III: Arg-C digest peptide mass fingerprinting of AAD-12 (Batch TSN030732-002).

Peptide	# of missed cleavages	a.a. ##	Sequence	m/z theor.							m/z obs. (ESI-LC/MS)	UPLC-MS elution time (min)	In-source fragments observed (matching amino-acid sequence)	Comment	
				(M+H) ⁺	(M+2H) ²⁺	(M+3H) ³⁺	(M+4H) ⁴⁺	(M+5H) ⁵⁺	(M+6H) ⁶⁺	(M+7H) ⁷⁺					
1	0	2-63	(-) AQTLLQITPTGATLGATVTVGHLATLDDAGFAALHAWLQHALLIFPGQHLSDQOITFAKR (F)	6522.41 6521.40 [0 charge]	3261.71	2174.71	1631.36	1305.29	1087.91	932.64	6523.47 (± 0.03) (2+)	61.96		average mass, transformed	
2	0	64- 69	(R) FGAIER (I)	692.37	346.69	231.46	173.85	139.28			692.37 (1+)	21.60	545.30 (1+, y5), 488.28 (1+, y4), 417.25 (1+, y3)		
3	0	70- 88	(R) IGGGDIVAISNVKADGTVR (T)	1842.01	921.51	614.67	461.26	369.21			921.51 (2+), 614.68 (3+)	33.83	836.45 (2+, y17), 807.93 (2+, y16), 779.45 (2+, y15), 721.91 (2+, y14), 683.37 (1+, b8), 665.38 (2+, y13), 580.32 (2+, y11), 523.78 (2+, y10), 513.26 (1+, b6), 432.26 (1+, y4), 400.18 (1+, b5)		
5	0	136-142	(R) TCFADMR (A)	900.37	450.69	300.79	225.85	180.88			900.37 (1+), 450.70 (2+)	24.41	639.29 (1+, y5), 595.22 (1+, b5), 492.23 (1+, y4), 421.18 (1+, y3), 409.16 (1+, b3), 400.17 (2+, y6)		
6	0	143-153	(R) AAYDALDEATR (A)	1195.56	598.28	399.19	299.65	239.92			1195.56 (1+), 598.28 (2+)	26.68	1053.47 (1+, y9), 1021.48 (1+, b1), 920.35 (1+, b9), 890.42 (1+, y8), 849.35 (1+, b8), 775.39 (1+, y7), 720.33 (1+, b7), 704.38 (1+, y8), 591.27 (1+, y5), 527.25 (2+, y9), 492.21 (1+, b5), 476.25 (1+, y4), 421.17 (1+, b4)		
7	0	154-159	(R) ALVHQR (H)	723.43	362.22	241.81	181.61	145.49			723.42 (1+), 362.22 (2+)	6.71	539.30 (1+, y4), 440.24 (1+, y3)		
10	0	214-228	(R) HAHAIPIGMDAAESER (F)	1591.73	796.37	531.25	398.69	319.15			796.37 (2+), 531.25 (3+)	21.93	1062.44 (1+, y10), 965.40 (1+, y9), 777.34 (1+, y7), 684.35 (1+, b7), 662.32 (1+, y6), 601.27 (2+, b12), 591.26 (1+, y5), 530.28 (1+, b5), 520.24 (1+, y4), 501.24 (2+, b10), 465.72 (2+, b9), 417.20 (1+, b4), 408.20 (2+, b8), 391.20 (1+, y3)		
11	0	229-242	(R) FLEGLVDWACQAPR (V)	1661.81	831.41	554.61	416.21	333.17			1661.80 (1+), 831.41 (2+), 554.61 (3+)	49.58	1390.66 (1+, b12), 1319.57 (1+, b11), 1191.54 (1+, b10), 1031.52 (1+, b9), 1003.42 (1+, y8), 960.51 (1+, b8), 774.40 (1+, b7), 702.34 (1+, y6), 701.34 (2+, y12), 695.82 (2+, b12), 669.37 (1+, b8), 631.30 (1+, y5), 596.27 (2+, b10), 560.30 (1+, b5), 551.76 (2+, y8), 516.27 (2+, b9), 502.22 (2+, y8), 471.26 (1+, y4), 444.71 (2+, y7), 390.21 (1+, b3)		
12	0	243-259	(R) VHAHQWAAAGDVVVWDNR (C)	1959.96	980.48	653.99	490.74	392.80			980.49 (2+), 653.99 (3+)	34.78	1172.57 (1+, b11), 1073.44 (1+, b10), 901.45 (1+, b8), 887.50 (1+, y7), 830.41 (1+, b7), 788.40 (1+, y6), 778.89 (2+, b14), 689.34 (1+, y5), 686.86 (2+, b13), 636.32 (2+, b12), 590.27 (1+, y4), 586.79 (2+, b11), 572.78 (2+, a11), 537.25 (2+, b10), 451.23 (2+, b8), 424.55 (3+, b12), 417.71 (2+, b7), 404.19 (1+, y3), 394.71 (2+, y6)		
13	0	260-264	(R) CLLHR (A)	698.38	349.69	233.46	175.35	140.48			698.37 (1+), 349.69 (2+)	17.33	425.26 (1+, y3)		
14	0	265-274	(R) AEPWDFKLPR (V)	1258.66	629.83	420.22	315.42	252.54			629.83 (2+), 420.22 (3+)	40.60	861.54 (1+, y7), 775.43 (1+, y6), 660.41 (1+, y5), 529.79 (2+, y8), 513.34 (1+, y4), 481.26 (2+, y7), 385.26 (1+, y3), 399.16 (1+, y10)		
15	0	275-280	(R) VMWHSR (L)	815.40	408.20	272.47	204.60	163.89			815.40 (1+), 408.21 (2+)	17.92	716.34 (1+, y5), 585.29 (1+, y4), 399.21 (1+, b3+H2O), 358.58 (2+, y5)		
16	0	281-293	(R) LAGRPETEGAALV (-)	1283.70	642.35	428.57	321.68	257.54			1283.69 (1+), 642.35 (2+)	30.75	1166.62 (1+, b12), 1053.52 (1+, b11), 982.47 (1+, b10), 911.44 (1+, y9), 886.44 (1+, y8), 854.42 (1+, b8), 624.35 (1+, b6), 583.81 (2+, b12), 569.80 (2+, a12), 574.80 (2+, b12+H2O), 527.27 (2+, b11), 513.27 (2+, a11), 518.26 (2+, b11+H2O), 491.75 (2+, b10), 398.25 (1+, b4)		
8-16	8	160-293	(R) SARHSLVYSGSKLGHVQAGSAYIGYGMDDTTATPLRPLVKVHPETGRPSLLIGRHAHAIPGMDAAESERFLEGLVDWACQAPRVHAHQWAAAGDVVVWDNRCLLHRAEPWDFKLPRVMWHSRLAGRPETEGAALV (-)	14946.03 [0 charge]								14946.03 (± 0.02) (2+)	52.73		Incomplete C-term. cleavage; average masses, transformed; ave. 17+ charge; traces of Na & K-salts are observed
non-spec1		196-206	(R) PLVKVHPETGR (P)	1232.71	616.86	411.58	308.93	247.35			616.86 (2+), 411.58 (3+)	19.11	795.44 (1+, y7), 696.32 (1+, y6), 674.43 (1+, b5), 598.34 (2+, y10), 559.26 (1+, y5), 511.78 (2+, y8), 462.25 (2+, y8)	unusual R-P cleavages	
non-spec2		163-171	(R) HSLVYSQSK (L)	1048.54	524.78	350.19	262.89	210.51			1048.53 (1+), 524.77 (2+)	15.75	612.29 (1+, y5), 449.23 (1+, y4)	tryptic-like cleavage	
non-spec3		172-195	(K) LGHVQAGSAYIGYGMDDTTATPLR (P)	2507.24	1254.12	836.42	627.56	502.25			1254.11 (2+), 836.42 (3+)	36.51	1282.63 (1+, y12), 1225.64 (1+, y11), 1167.05 (2+, b23), 1112.50 (1+, b11), 1061.89 (2+, b21), 1011.48 (2+, b20), 976.00 (2+, b19), 925.42 (2+, b18), 817.43 (2+, b16), 759.43 (1+, y7), 751.90 (2+, b15), 723.37 (2+, b14), 668.37 (1+, y6), 641.81 (2+, b13), 613.31 (2+, b12), 556.79 (2+, b11), 486.30 (1+, y4), 385.26 (1+, y3)	tryptic-like & R-P cleavage; trace of K-salt also observed	

Cys residues are reduced and carboxyamidomethylated

Table IV: Chymotrypsin digest peptide mass fingerprinting of AAD-12 (Batch TSN030732-002).

Peptide	# of missed cleavages	a.a. ##	Sequence	m/z theor.					m/z obs. (ESI-LC/MS)	UPLC-MS elution time (min)	In-source fragments observed (matching amino-acid sequence)	Comment
				(M+H) ⁺	(M+2H) ²⁺	(M+3H) ³⁺	(M+4H) ⁴⁺	(M+5H) ⁵⁺				
Y1	0	2-6	(-) AQTTL (Q)	533.29	267.15	178.44	134.08	107.46	533.29 (1+)	18.93	515.29 (1+, pep - H ₂ O), 402.20 (1+, b4), 384.19 (1+, b4+H ₂ O)	
Y2	0	7-15	(L) QITPTGATL (G)	901.50	451.25	301.17	226.13	181.11	901.50 (1+)	31.96	770.39 (1+, b8), 752.38 (1+, b8+H ₂ O), 669.36 (1+, b7), 651.35 (1+, b7+H ₂ O), 598.30 (1+, b6), 559.31 (1+, y6), 385.70 (2+, b8), 376.70 (2+, b8+H ₂ O)	
Y3	0	16- 24	(L) GATVTGVHL (A)	854.47	427.74	285.50	214.37	171.70	854.48 (1+), 427.74 (2+)	31.73	726.41 (1+, y7), 625.37 (1+, y6), 586.32 (1+, b7), 526.30 (1+, y5), 390.20 (2+, y8-H ₂ O), 363.72 (2+, y7), 354.71 (2+, y7-H ₂ O)	
Y5	0	28- 32	(L) DDAGF (A)	524.20	262.60	175.40	131.81	105.65	524.20 (1+)	19.39	506.19 (1+, pep - H ₂ O), 359.13 (1+, b4)	
Y7	0	36- 39	(L) HAAW (L)	484.23	242.62	162.08	121.81	97.65	484.23 (1+)	18.67	466.22 (1+, pep - H ₂ O)	
Y11	0	46- 52	(L) IFPGQHL (S)	811.45	406.23	271.15	203.62	163.10	811.44 (1+), 406.23 (2+)	31.29	698.37 (1+, y6), 551.30 (1+, y5), 533.28 (1+, y5+H ₂ O), 454.24 (1+, y4), 397.22 (1+, y3), 380.19 (1+, y3-17)	
Y12	0	53- 60	(L) SNDQKITF (A)	952.44	476.72	318.15	238.86	191.29	952.45 (1+)	31.96	787.37 (1+, b7), 769.34 (1+, b7+H ₂ O), 686.31 (1+, b6), 573.22 (1+, b5), 433.25 (2+, y7), 394.18 (2+, b7), 380.22 (2+, a7)	
Y13	0	61- 64	(F) AKRF (G)	521.32	261.16	174.44	131.09	105.07	521.32 (1+)	6.00		
Y24	0	168-172	(Y) SQSKL (G)	562.32	281.66	188.11	141.34	113.27	562.32 (1+)	9.15		
Y27	0	186-194	(Y) GMDTTATPL (R)	906.42	453.72	302.81	227.36	182.09	906.43 (1+)	30.58	718.37 (1+, y7), 700.34 (1+, y7+H ₂ O), 678.28 (1+, b7), 660.27 (1+, b7+H ₂ O), 577.23 (1+, b6), 559.21 (1+, b6+H ₂ O), 506.20 (1+, b5), 416.15 (2+, y8+H ₂ O), 405.15 (1+, b4)	
Y27-Met-ox1	0	186-194	(Y) GMDTTATPL (R)	922.42	461.71	308.14	231.36	185.28	ND	ND		
Y28	0	195-197	(L) RPL (V)	385.26	193.13	129.09	97.07	77.86	385.26 (1+)	11.42		
Y29	0	198-209	(L) VKVHPETGRPSL (L)	1319.74	660.38	440.59	330.69	264.75	660.34 (2+), 440.57 (3+)	20.35		
Y36	0	249-256	(W) AAGDVVVW (D)	816.43	408.72	272.81	204.86	164.09	816.43 (1+)	41.12	698.37 (1+, x6), 657.36 (1+, y6-17), 612.34 (1+, b7), 594.33 (1+, b7+H ₂ O), 584.33 (1+, a7), 513.27 (1+, b5), 495.26 (1+, b6+H ₂ O), 485.27 (1+, a6), 414.21 (1+, b5), 403.25 (1+, y3), 396.20 (1+, b5+H ₂ O), 386.20 (1+, a5)	
Y39	0	263-268	(L) HRAEPW (D)	795.39	398.20	265.80	199.60	159.88	795.40 (1+), 398.20 (2+)	20.76	658.32 (1+, y5), 591.30 (1+, b5), 494.24 (1+, b4), 485.09 (1+, y4-17), 466.26 (1+, a4)	
Y41	0	271-277	(F) KLPRVMW (H)	929.54	465.27	310.52	233.14	186.71	929.53 (1+), 465.27 (2+)	37.65	688.36 (1+, y5)	
Y41-Met-ox1	0	271-277	(F) KLPRVMW (H)	945.54	473.27	315.85	237.14	189.91	ND	ND		
Y42	0	278-281	(W) HSRL (A)	512.29	256.65	171.44	128.83	103.26	512.29 (1+)	5.50		
Y43	0	282-292	(L) AGRPTEGAAL (V)	1071.54	536.28	357.85	268.64	215.11	1071.53 (1+), 536.28 (2+)	21.89	940.44 (1+, b10), 869.42 (1+, b9), 811.38 (1+, x8), 470.73 (2+, b10), 435.22 (2+, b9)	
Y23-24	1	166-172	(L) VYSQSKL (G)	824.45	412.73	275.49	206.87	165.70	824.46 (1+), 412.73 (2+)	26.92		
Y29-30	1	198-210	(L) VKVHPETGRPSLL (I)	1432.83	716.92	478.28	358.96	287.37	1432.80 (1+), 716.93 (2+), 478.28 (3+)	28.13	969.53 (1+, y9), 872.45 (1+, y8), 810.46 (1+, c7), 667.39 (2+, y12), 651.38 (2+, b12), 642.38 (2+, b12+H ₂ O), 603.34 (2+, y11), 553.32 (2+, y10), 485.28 (2+, y9), 464.30 (1+, b4), 434.59 (3+, b12), 425.25 (3+, a12)	
Y37-38	1	257-262	(W) DNRCLL (H)	790.39	395.70	264.13	198.35	158.88	790.39 (1+), 395.71 (2+)	28.80	659.30 (1+, b5), 561.32 (1+, y4), 546.20 (1+, b4)	
Y38-39	1	262-268	(L) LHRAEPW (D)	908.47	454.74	303.50	227.87	182.50	908.48 (1+), 454.75 (2+)	23.51	658.34 (1+, y5), 607.32 (1+, b5), 352.70 (2+, b6)	
Y43-44	1	282-293	(L) AGRPTEGAALV (-)	1170.61	585.81	390.88	293.41	234.93	1170.60 (1+), 585.82 (2+)	27.64	1053.54 (1+, b11), 940.45 (1+, b10), 886.46 (1+, y9), 869.41 (1+, b9), 741.37 (1+, b7), 527.27 (2+, b11), 518.27 (2+, b11+H ₂ O), 513.28 (2+, a11), 470.73 (2+, b10), 435.21 (2+, b9)	
Y12-14	2	53- 95	(L) SNDQKITFAKRFGAIERIGGGDIVAISNVKADGTVRQHSPEW (D)	4639.38	2320.19	1547.13	1160.60	928.68	1547.31 (3+), 1160.54 (4+), 928.64 (5+)	40.87		
Y32-34	2	230-236	(F) LEGLVDW (A)	831.42	416.22	277.81	208.61	167.09	831.43 (1+)	47.16	672.33 (1+, EGLVDW-28 int.fr.), 627.33 (1+, b6), 512.35 (1+, b5), 484.31 (1+, a5), 419.19 (1+, y3), 413.24 (1+, b4), 385.25 (1+, a4)	
non-spec1		125-135	(F) SAEVPPAVGGGR (T)	1041.57	521.29	347.86	261.15	209.12	1041.56 (1+), 521.29 (2+)	25.28	833.51 (1+, y9), 754.45 (1+, y8), 655.39 (1+, y7), 556.32 (1+, y6), 486.28 (1+, b5), 458.26 (1+, a5), 442.25 (2+, y9), 387.19 (1+, b4), 359.20 (1+, a4)	partially-tryptic cleavage
non-spec2		125-136	(F) SAEVPPAVGGRT (C)	1142.62	571.81	381.54	286.41	229.33	1142.57 (1+), 571.82 (2+)	25.98	855.49 (1+, y9), 756.44 (1+, y8), 657.37 (1+, y7), 486.25 (1+, b5), 390.21 (1+, y4), 387.20 (1+, b4)	
non-spec3		65-80	(F) GAIERIGGGDIVAISN (V)	1541.83	771.42	514.61	386.21	309.17	1541.78 (1+), 771.43 (2+)	36.01	705.40 (2+, b15), 661.88 (2+, b14), 605.34 (2+, b13), 404.22 (1+, y4)	
non-spec4		65-78	(F) GAIERIGGGDIVAI (S)	1340.75	670.88	447.59	335.94	268.96	1340.80 (1+), 670.89 (2+)	42.20	1138.64 (1+, b12), 1039.58 (1+, b11), 926.53 (1+, b10), 640.38 (1+, b6), 605.34 (2+, b13), 569.82 (2+, b12), 555.82 (2+, a12), 520.28 (2+, b11)	
non-spec5		269-275	(W) DFKLPRV (M)	874.52	437.76	292.18	219.38	175.71	874.51 (1+)	51.35	583.38 (1+, b5+H ₂ O), 484.31 (1+, y4), 466.30 (1+, y4+H ₂ O), 391.20 (1+, b3), 371.23 (1+, y3), 353.22 (1+, y3+H ₂ O)	

Cys residues are reduced and carboxyamidomethylated

Table V: Asp-N digest peptide mass fingerprinting of AAD-12 (Batch TSN030732-002).

Peptide	# of missed cleavages	a.a. ##	Sequence	m/z theor.					m/z obs. (ESI-LC/MS)	UPLC elution time, min	In-source fragments observed (matching amino-acid sequence)
				(M+H) ¹⁺	(M+2H) ²⁺	(M+3H) ³⁺	(M+4H) ⁴⁺	(M+5H) ⁵⁺			
D1	0	2-27	(-) AQTTLQITPTGATLGATVTGVHLATL (D)	2536.40	1268.70	846.14	634.85	508.09	1268.72 (2+), 846.15 (3+)	51.58	1152.63 (2+, b24), 802.47 (3+, b25),
D9	0	111-139	(A) DSTYMPVMAQGAVFSAEVVPAVGGRTCFA (D)	3018.42	1509.71	1006.81	755.36	604.49	1006.84 (3+)	52.53	598.24 (1+, b5), 518.28 (2+, y10)
D10	0	140-145	(A) DMRAAY (D)	726.32	363.67	242.78	182.34	146.07	726.34 (1+)	19.17	545.25 (1+, b5), 480.26 (1+, y4)
D17	0	257-268	(W) DNRCLLHRAEPW (D)	1566.76	783.88	522.92	392.45	314.16	783.88 (2+), 522.93 (3+)	30.73	633.32 (2+, b10)
D18	0	269-293	(W) DFKLPRVMWHSRLAGRPETEGAALV (-)	2836.50	1418.76	946.17	709.88	568.11	946.17 (3+), 709.89 (4+)	40.57	907.13 (3+, b24), 869.47 (3+, b23), 845.77 (3+, b22), 680.87 (4+, b24), 652.35 (4+, b23), 634.61 (4+, b22), 413.25 (1+, y5-17)
D4-5	1	55- 83	(N) DQKITFAKRFGAIERIGGGDIVAISNVKA (D)	3074.67	1537.84	1025.56	769.42	615.74	1025.57 (3+), 769.45 (4+)	44.31	
D14-15	1	222-251	(M) DAAESERFLEGLVDWACQAPRVHAHQWAAAG (D)	3377.59	1689.30	1126.53	845.15	676.32	845.19 (4+), 676.56 (5+)	54.79	
non-spec1		23-100	(V) HLATLDDAGFAALHAAWLQHALIFPGQHLSDQKITFAKRFGAIERIGGGDIVAISNVK ADGTVRQHSAPAEWDDMMK (V)	8492.61 [0 charge]					8492.65 [0 charge; transformed, average mass]	60.95	

Cys residues are reduced and carboxyamidomethylated

Table VI: Glu-C digest peptide mass fingerprinting of AAD-12 (Batch TSN030732-002).

Peptide	# of missed cleavages	a.a. ##	Sequence	m/z theor.					m/z obs. (ESI-LC/MS)	UPLC elution time, min	In-source fragments observed (matching amino-acid sequence)
				(M+H) ¹⁺	(M+2H) ²⁺	(M+3H) ³⁺	(M+4H) ⁴⁺	(M+5H) ⁵⁺			
A11	0	112-127	(D) STYMPVMAQGAVFSAE (V)	1688.77	844.89	563.59	422.95	338.56	844.89 (2+)	44.29	1206.57 (1+, y12), 810.35 (1+, b7), 679.34 (1+, b6), 483.20 (1+, b4)
A12	0	128-140	(E) VVPAVGGRTCFAD (M)	1348.67	674.84	450.23	337.92	270.54	674.84 (2+)	29.45	575.78 (2+, y11)
A13-14	1	141-150	(D) MRAAYDALDE (A)	1154.52	577.76	385.51	289.38	231.71	577.76 (2+)	28.09	892.43 (1+, b8)
A17-18	1	204-225	(E) TGRPSLLIGRHAHAIPGMDAE (S)	2270.18	1135.60	757.40	568.30	454.84	1135.63 (2+), 747.40 (3+), 568.30 (4+)	31.43	790.97 (2+, b15), 734.42 (2+, b14), 708.39 (3+, b21), 698.93 (2+, b13), 684.71 (3+, b20), 675.37 (3+, a20), 661.03 (3+, b19), 630.34 (2+, b12), 622.69 (3+, b18), 579.01 (3+, b17), 531.55 (4+, b21), 513.78 (4+, b20), 496.02 (4+, b19), 489.95 (3+, b14), 466.27 (3+, b13)
A1-4	3	2-68	(-) AQTTLQITPTGATLGATVTGVHLATLDDAGFAALHAAWLQHALIFPGQHLSDNDQQITFA KRFGAIE (R)	7039.99	3520.50	2347.33	1760.75	1408.80	1408.67 (5+), 1174.11 (6+), 1006.52 (7+), 880.81 (8+)	62.58	
				7038.99 [0 charge]					7040.57 [0 charge; transformed]		
A12-14	3	128-150	(E) VVPAVGGRTCFADMRAAYDALDE (A)	2484.17	1242.59	828.73	621.80	497.64	1245.55 (2+), 828.74 (3+), 621.82 (4+)	40.82	1169.07 (2+, b22), 1143.52 (2+, y21), 1111.55 (2+, b21), 1055.00 (2+, b20), 1019.50 (2+, b19), 1009.98 (2+, y18), 961.98 (2+, b18), 880.42 (2+, b17), 779.71 (3+, b22), 762.69 (3+, y21), 741.37 (3+, b21), 725.30 (1+, y6), 703.68 (3+, b20), 562.24 (1+, y5), 466.29 (1+, b5), 447.21 (1+, y4), 376.17 (1+, y3)

Cys residues are reduced and carboxyamidomethylated

Table VII:

(A) Amino acid sequence obtained by in-source fragmentation for the C-terminal peptide $^{281}\text{LAGRPETEGAALV}^{293}$ (m/z 642.35) from Arg-C digest of sample AAD-12 (Batch TSN030732-002) eluting at 30.7 min.

Sequence: LAGRPETEGAALV
 Fragment ion masses: monoisotopic
 Peptide mass $[\text{M}+2\text{H}]^{2+}$: 642.35

Ion		L	A	G	R	P	E	T	E	G	A	A	L	V
y	theoretical	-	1170.61	1099.57	1042.55	886.45	789.40	660.36	559.31	430.27	373.25	-	-	-
	experimental					886.41				430.26				
y²⁺	theoretical		585.81 ⁺²	550.29 ⁺²	521.78 ⁺²	443.73 ⁺²	395.20 ⁺²	-	-	-	-	-	-	-
	experimental													
b	theoretical	-	-	-	398.25	495.30	624.35	725.39	854.44	911.46	982.50	1053.53	1166.62	-
	experimental				398.25		624.35		854.40	911.44	982.47	1053.52	1166.61	
b²⁺	theoretical	-	-	-	-	-	-	363.20 ⁺²	427.72 ⁺²	456.23 ⁺²	491.75 ⁺²	527.27 ⁺²	583.81 ⁺²	-
	experimental										491.75	527.27	583.81	
a	theoretical	-	-	-	370.26	467.31	596.35	697.40	826.44	883.46	954.50	1025.54	1138.62	-
	experimental				467.22									
a²⁺	theoretical	-	-	-	-	-	-	-	413.73 ⁺²	442.24 ⁺²	477.75 ⁺²	513.27 ⁺²	569.81 ⁺²	
	experimental											513.27	569.80	

* only ions with m/z between 350 and 1900 were recorded in this experiment (LC/MS).

(B) Amino acid sequence obtained by tandem MS for the C-terminal peptide $^{281}\text{LAGRPETEGAALV}^{293}$ (m/z 642.36) from tryptic digest of sample AAD-12 (Batch TSN030732-002) eluting at 29.65 min.

Sequence: LAGRPETEGAALV
 Fragment ion masses: monoisotopic
 Theoretical monoisotopic peptide mass $[\text{M}+2\text{H}]^{2+}$: 642.35

Ion		L	A	G	R	P	E	T	E	G	A	A	L	V
y	theoretical	-	1170.61	1099.57	1042.55	886.45	789.40	660.36	559.31	430.27	373.25	302.21	231.17	118.09
	experimental												231.18	118.09
b	theoretical	114.09	185.13	242.15	398.25	495.30	624.35	725.39	854.44	911.46	982.50	1053.53	1166.62	-
	experimental		185.14	242.17	398.23		624.37	725.41	854.43	911.43	982.51	1053.55	1166.66	
b²⁺	theoretical	-	93.07 ⁺²	121.58 ⁺²	199.63 ⁺²	248.16 ⁺²	312.68 ⁺²	363.20 ⁺²	427.72 ⁺²	456.23 ⁺²	491.75 ⁺²	527.27 ⁺²	583.81 ⁺²	-
	experimental									456.25	491.77	527.29		
a	theoretical	86.10	157.13	214.16	370.26	467.31	596.35	697.40	826.44	883.46	954.50	1025.54	1138.62	-
	experimental	86.10	157.14	214.15	370.27		596.38		826.46		954.54	1025.55		
a²⁺	theoretical	-	-	107.58 ⁺²	185.63 ⁺²	234.16 ⁺²	298.68 ⁺²	349.20 ⁺²	413.73 ⁺²	442.24 ⁺²	477.75 ⁺²	513.27 ⁺²	569.81 ⁺²	
	experimental										477.76			

* only ions with m/z between 80 and 1900 were recorded in this experiment (MS/MS).

Table VII: (continued)

(C) Amino acid sequence obtained by tandem MS for the C-terminal peptide $^{282}\text{AGRPETEGAALV}^{293}$ (m/z 585.82) from chymotryptic digest of sample AAD-12 (Batch TSN030732-002) eluting at 26.6 min.

Sequence: AGRPETEGAALV
 Fragment ion masses: monoisotopic
 Theoretical monoisotopic peptide mass $[\text{M}+2\text{H}]^{2+}$: 585.81

Ion		A	G	R	P	E	T	E	G	A	A	L	V
y	theoretical	-	1099.57	1042.55	886.45	789.40	660.36	559.31	430.27	373.25	302.21	231.17	118.09
	experimental											231.18	118.09
b	theoretical	-	129.07	285.17	382.22	511.26	612.31	741.35	798.37	869.41	940.45	1053.53	-
	experimental			285.18	382.18	511.26	612.30	741.37	798.38	869.42	940.46	1053.56	
b²⁺	theoretical	-	-	143.09 ⁺²	191.61 ⁺²	256.14 ⁺²	306.66 ⁺²	371.18 ⁺²	399.69 ⁺²	435.21 ⁺²	470.73 ⁺²	527.27 ⁺²	-
	experimental								399.68	435.20	470.72		
a	theoretical	-	101.07	257.17	354.23	483.27	584.32	713.36	770.38	841.42	912.45	1025.54	-
	experimental		101.07	257.18		483.25		713.40	770.39	841.42	912.46	1025.54	
a²⁺	theoretical	-	-	129.09 ⁺²	177.62 ⁺²	242.14 ⁺²	292.66 ⁺²	357.18 ⁺²	385.69 ⁺²	421.21 ⁺²	456.73 ⁺²	513.27 ⁺²	
	experimental									421.20			

* only ions with m/z between 80 and 1900 were recorded in this experiment (MS/MS).

Table VIII:

(A) Amino acid sequence obtained by tandem MS for the N-terminal peptide $^2\text{AQTTLQITPTGATLGATVTGVHLATLDDAGFAALHAAWLQHALLIFPGQHLSNDQQITFAK}^{62}$ (m/z 1063.22) from tryptic digest of sample AAD-12 (Batch TSN030732-002) eluting at 59.93 min.

Sequence: AQTTLQITPTGATLGATVTGVHLATLDDAGFAALHAAWLQHALLIFPGQHLSNDQQITFAK
 Fragment ion masses: monoisotopic
 Theoretical peptide average mass $[M+6H]^{6+}$: 1061.88

Ion		A	Q	T	T	L	Q	I	T	P	T	G	A	T	L	G	A	T	V	T	G	V
y⁵⁺	theoretical	-	1259.06	1233.45	1213.24	1193.03	1170.41	1144.80	1122.19	1101.98	1082.56	1062.35	1050.95	1036.74	1016.53	993.92	982.51	968.30	948.09	928.28	908.07	896.67
	experimental								1102.02													
y⁴⁺	theoretical	-	1573.58	1546.57	1516.30	1491.04	1462.77	1430.76	1402.49	1377.22	1352.96	1327.70	1313.44	1295.68	1270.42	1242.15	1227.90	1210.14	1184.87	1160.11	1134.84	1120.59
	experimental															1242.18						
b	theoretical	-	200.10	301.15	402.20	515.28	643.34	756.43	857.47	954.53	1055.57	1112.60	1183.63	1284.68	1397.76	1454.79	1525.82	1626.87	1725.94	1826.99	1884.01	1938.08
	experimental		200.12	301.17		515.30		756.51														
b²⁺	theoretical	-	100.56	151.08	201.60	258.15	322.17	378.72	429.24	477.77	528.29	556.80	592.32	642.84	699.39	727.90	763.42	813.94	863.47	914.00	942.51	992.04
	experimental																					
a	theoretical	-	172.11	273.16	374.20	487.29	615.35	728.43	829.48	926.53	1027.58	1084.60	1155.64	1256.69	1369.77	1426.79	1497.83	1598.88	1697.94	1798.99	1856.01	1955.08
	experimental																					

Ion		H	L	A	T	L	D	D	A	G	F	A	A	L	H	A	A	W	L	Q	H
y²⁺	theoretical	2190.64	2122.11	2065.57	2030.05	1979.52	1922.98	1865.47	1807.96	1772.44	1743.93	1670.39	1634.87	1599.35	1542.81	1474.28	1438.76	1403.25	1310.21	1253.66	1189.63
	experimental																				
b²⁺	theoretical	1060.57	1117.11	1152.63	1203.16	1259.70	1317.21	1374.72	1410.24	1438.75	1512.29	1547.81	1583.33	1639.87	1708.40	1743.92	1779.43	1872.47	1929.02	1993.04	2061.57
	experimental																				

Ion		A	L	L	I	F	P	G	Q	H	L	S	N	D	Q	Q	I	T	F	A	K
y	theoretical	2241.20	2170.17	2057.08	1944.00	1830.91	1683.85	1586.79	1529.77	1401.71	1264.65	1151.57	1064.54	950.49	835.47	707.41	579.35	466.27	365.22	218.15	147.11
	experimental																				
y²⁺	theoretical	1121.11	1085.59	1029.04	972.50	915.96	842.43	793.90	765.39	701.36	632.83	576.29	532.77	475.75	418.24	354.21	290.18	233.64	183.11	109.58	-
	experimental																				

* only ions with m/z between 80 and 1900 were recorded in this experiment (MS/MS).

Table VIII: (continued)

(B) Amino acid sequence obtained by tandem MS for the N-terminal peptide $^2\text{AQTTL}^6$ (m/z 533.29) from chymotryptic digest of sample AAD-12 (Batch TSN030732-002) eluting at 18.52 min.

Sequence: AQTTL
 Fragment ion masses: monoisotopic
 Theoretical peptide average mass $[\text{M}+\text{H}]^{1+}$: 533.29

Ion		A	Q	T	T	L
y	theoretical	-	462.26	334.20	233.15	132.10
	experimental				233.16	132.11
y^o	theoretical	-	444.25	316.19	215.14	114.09
	experimental				215.13	
b	theoretical	-	200.10	301.15	402.20	-
	experimental		200.11	301.15		
b^o	theoretical	-	182.09	283.14	384.19	-
	experimental			283.13	384.16	
b*	theoretical	-	183.08	284.12	385.17	-
	experimental		183.08	284.11		
a	theoretical	-	172.11	273.16	374.20	-
	experimental					

* only ions with m/z between 80 and 1900 were recorded in this experiment (MS/MS).

Figure 1: Theoretical amino acid sequence of AAD-12 with sequence coverage for AAD-12 (Batch TSN030732-002) by ESI-LC/MS. Cys residues were alkylated with Iodoacetamide. Overall sequence coverage was 100%. Complete removal of N-terminal Met¹ was observed (shown with an arrow). No Asn deamidation was observed.

Theoretical average mass of processed (*des*-Met¹) AAD-12 is 31769.17 Da (Cys reduced and carboxyamidomethylated) [31598.02 Da for unmodified reduced Cys].

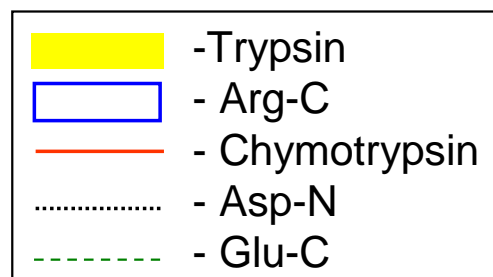
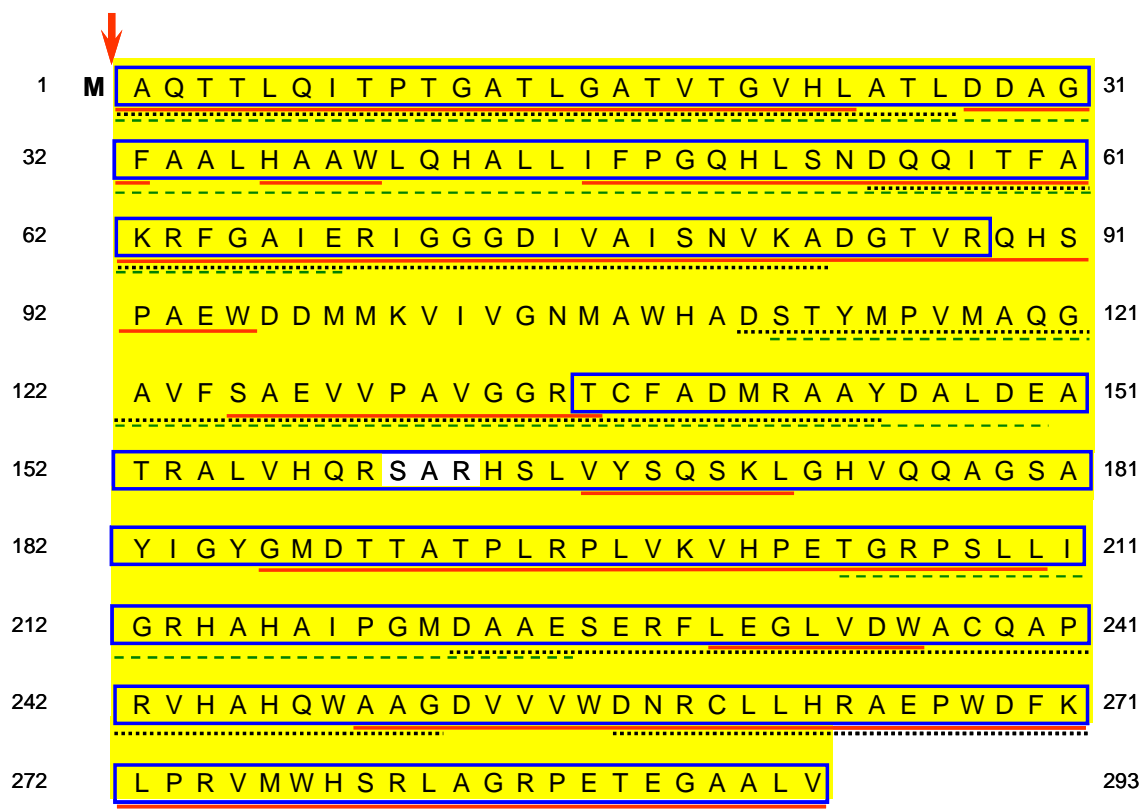


Figure 2: AAD-12 sample (Batch TSN030732-002) prepared in PBS/0.1M Gu:HCl buffer, analyzed by UPLC-MS. **(a)** Chromatograph (UV 214 nm trace); **(b)** multiple charge envelope mass spectrum (top; m/z axis) of component in Peak I, and the corresponding deconvoluted and centered mass spectrum (bottom; true mass axis); **(c)** multiple charge envelope mass spectrum (top; m/z axis) of component in Peak II, and the corresponding deconvoluted and centered mass spectrum (bottom; true mass axis); **(d)** multiple charge envelope mass spectrum (top; m/z axis) of component in Peak III, and the corresponding deconvoluted and centered mass spectrum (bottom; true mass axis). See **Table I** for peak assignments.

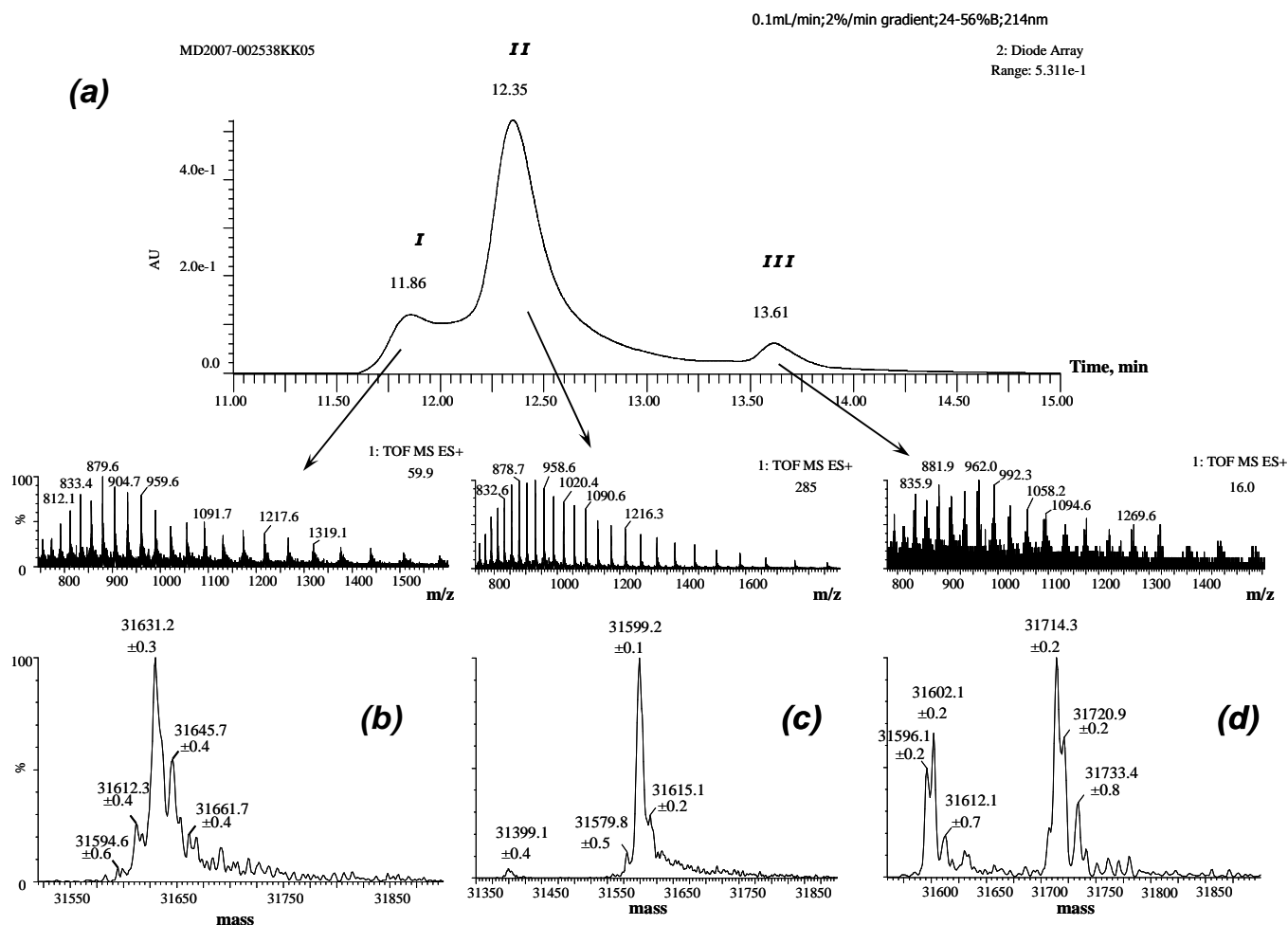


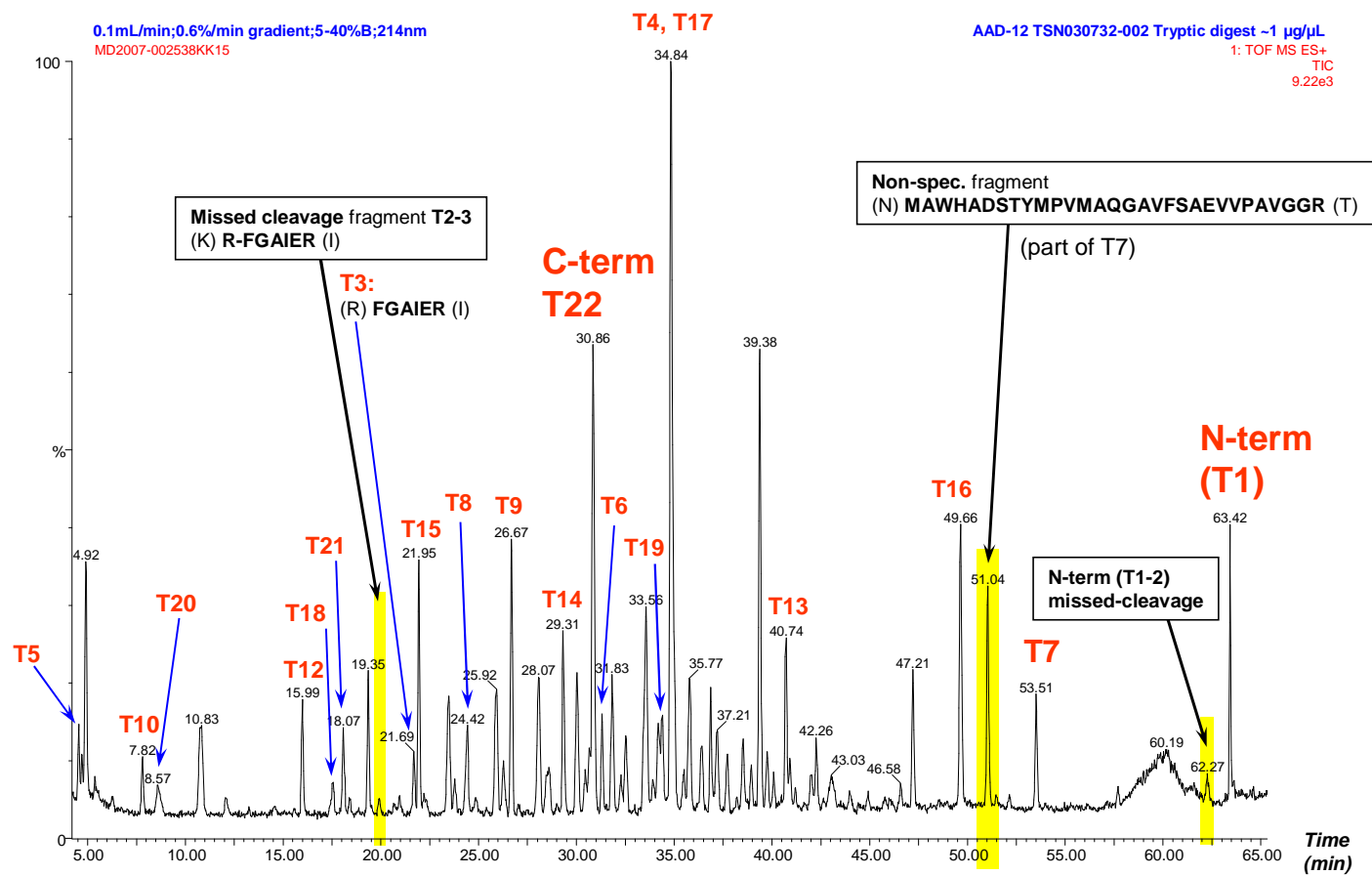
Figure 3: ESI-LC/MS chromatogram (MS TIC) for AAD-12 (Batch TSN030732-002) tryptic digest.

Figure 4: ESI-LC/MS chromatogram (MS TIC) for AAD-12 (Batch TSN030732-002) Arg-C digest.

0.1mL/min;0.6%/min gradient;5-40%B;214nm
MD2007-002538KK16

AAD-12 TSN030732-002 ArgC digest ~1 µg/µL
1: TOF MS ES+
TIC
1.08e4

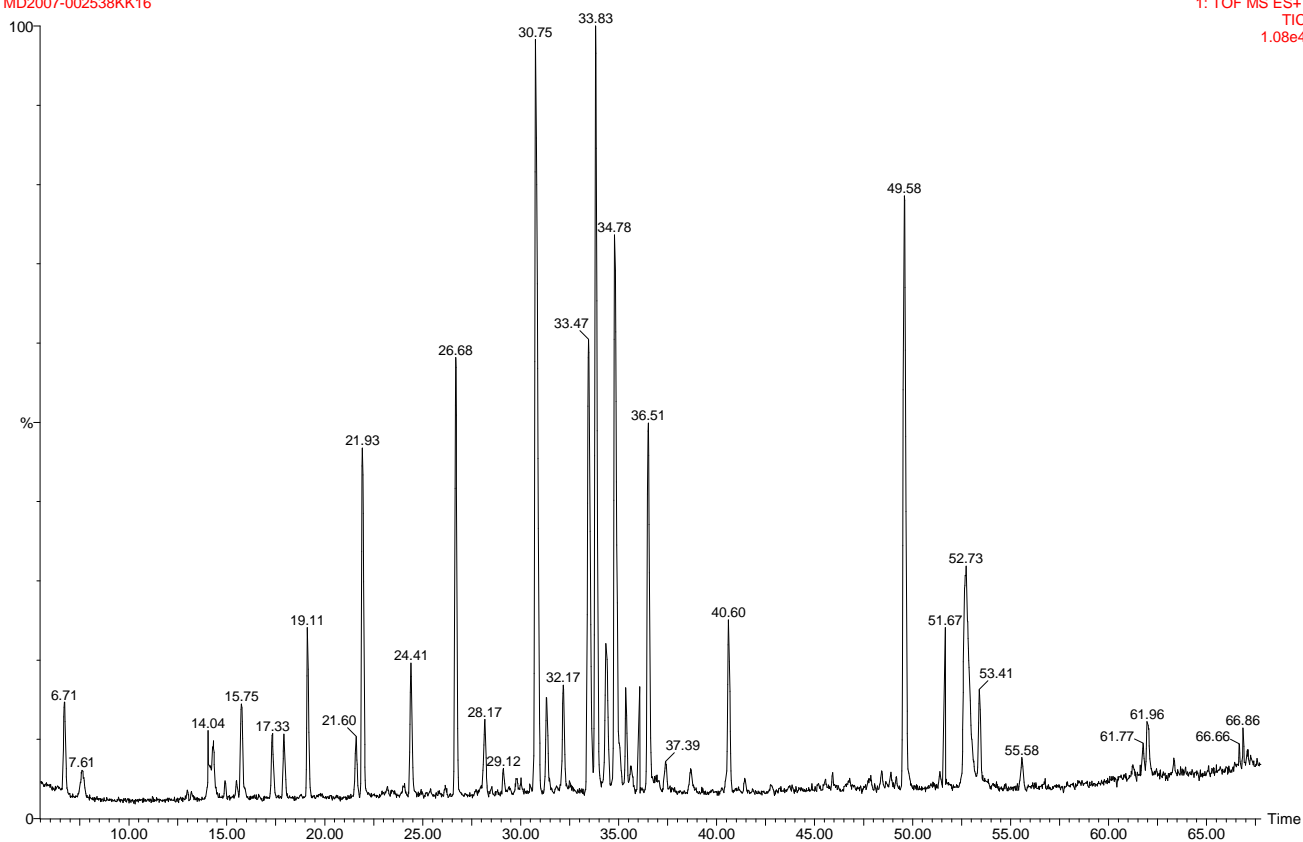


Figure 5: ESI-LC/MS chromatogram (MS TIC) for AAD-12 (Batch TSN030732-002) chymotryptic digest.

0.1mL/min;0.6%/min gradient;5-40%B;214nm
MD2007-002538KK18

AAD-12 TSN030732-002 Chymotryptic digest ~1 µg/µL
1: TOF MS ES+
TIC
8.13e3

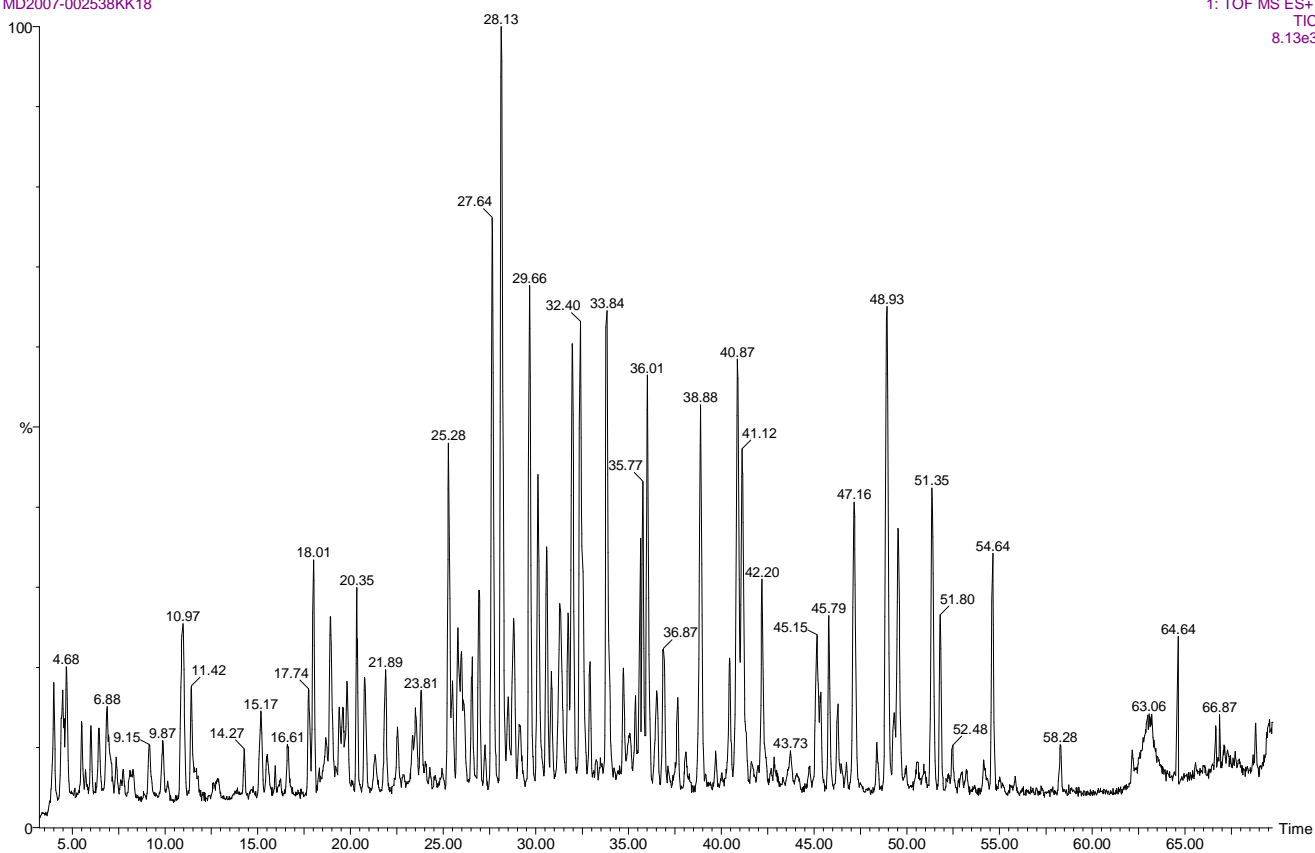


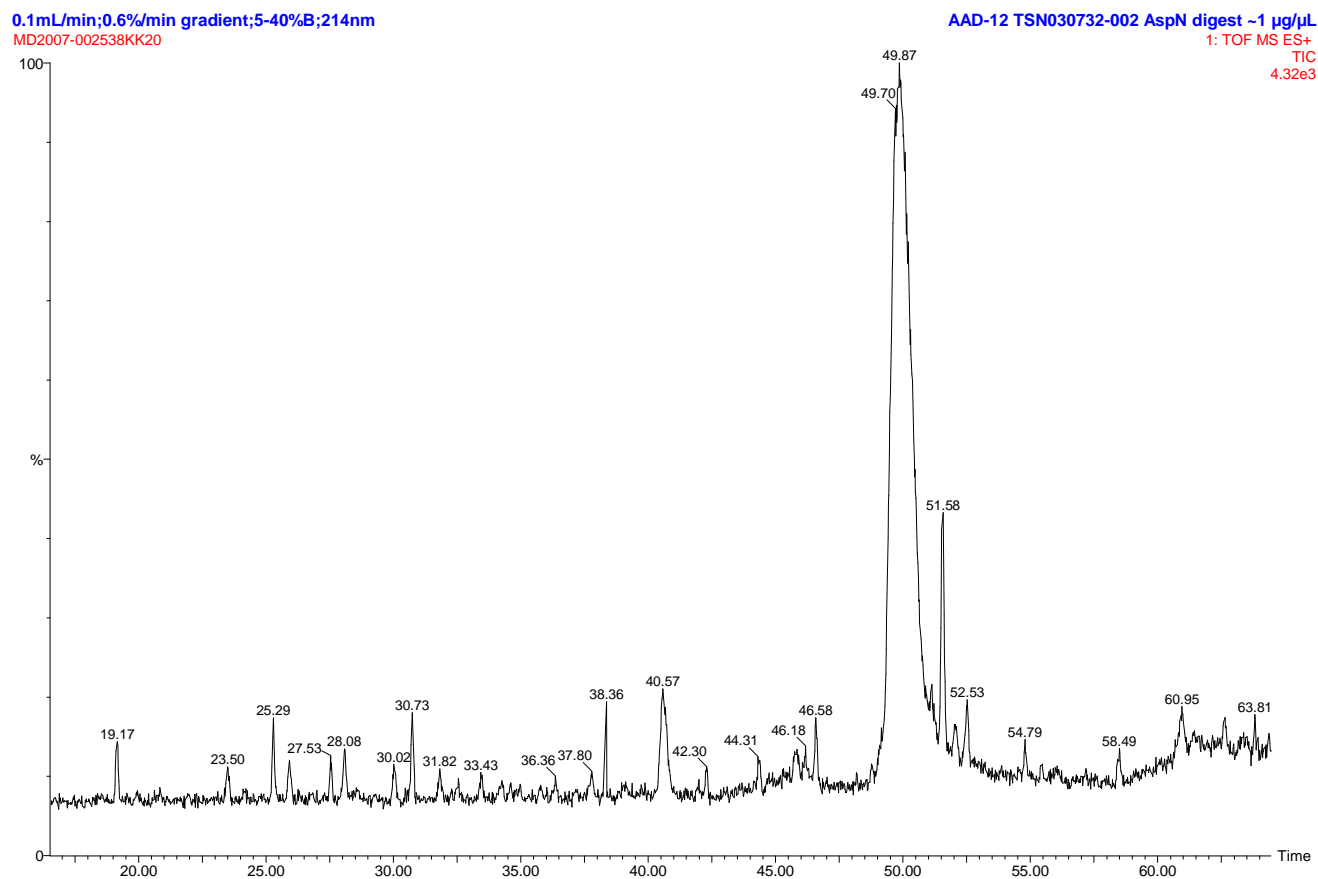
Figure 6: ESI-LC/MS chromatogram (MS TIC) for AAD-12 (Batch TSN030732-002) Asp-N digest.

Figure 7: ESI-LC/MS chromatogram (MS TIC) for AAD-12 (Batch TSN030732-002) Glu-C digest.

0.1mL/min;0.6%/min gradient;5-40%B;214nm
MD2007-002538KK19

AAD-12 TSN030732-002 GluC digest ~1 µg/µL

1: TOF MS ES+
TIC
1.93e4

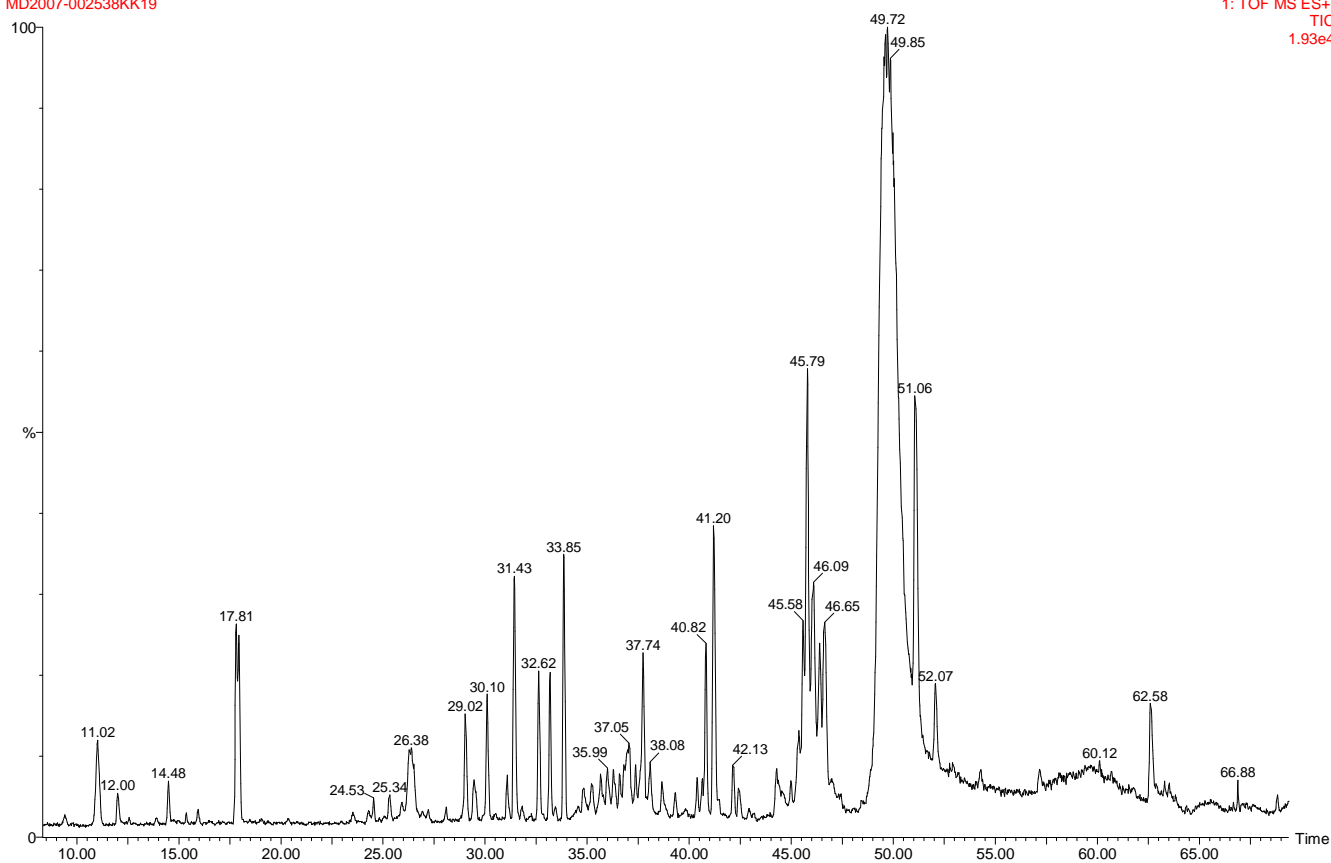


Figure 8: A representative ESI-MS spectrum of N-terminal tryptic fragment T1 (*des*-Met¹): ²AQTTTLQITPTGATLGATVTGVHLATLDDAGFAALHAAWLQHALLIFPGQHLSNDQQITFAK⁶². Charged states and m/z values are indicated above peaks (see **Figure 3**, and **Tables II** and **VIII** for details). Sample: AAD-12 (Batch TSN030732-002) tryptic digest. Similar spectra of N-terminal fragments were also observed in Arg-C and Glu-C digests (not shown; see **Tables III** and **VI**).

