Supdata3a- MO2VING-MS facility - QC analysis of the P0 ProteoGenix - MS report

Aim of the experiment:
To confirm the sequence of the PO 180-199 peptide.

Samples to be analyzed :
PO 180-199 ProteoGenix Bulk synthesis >98% purity

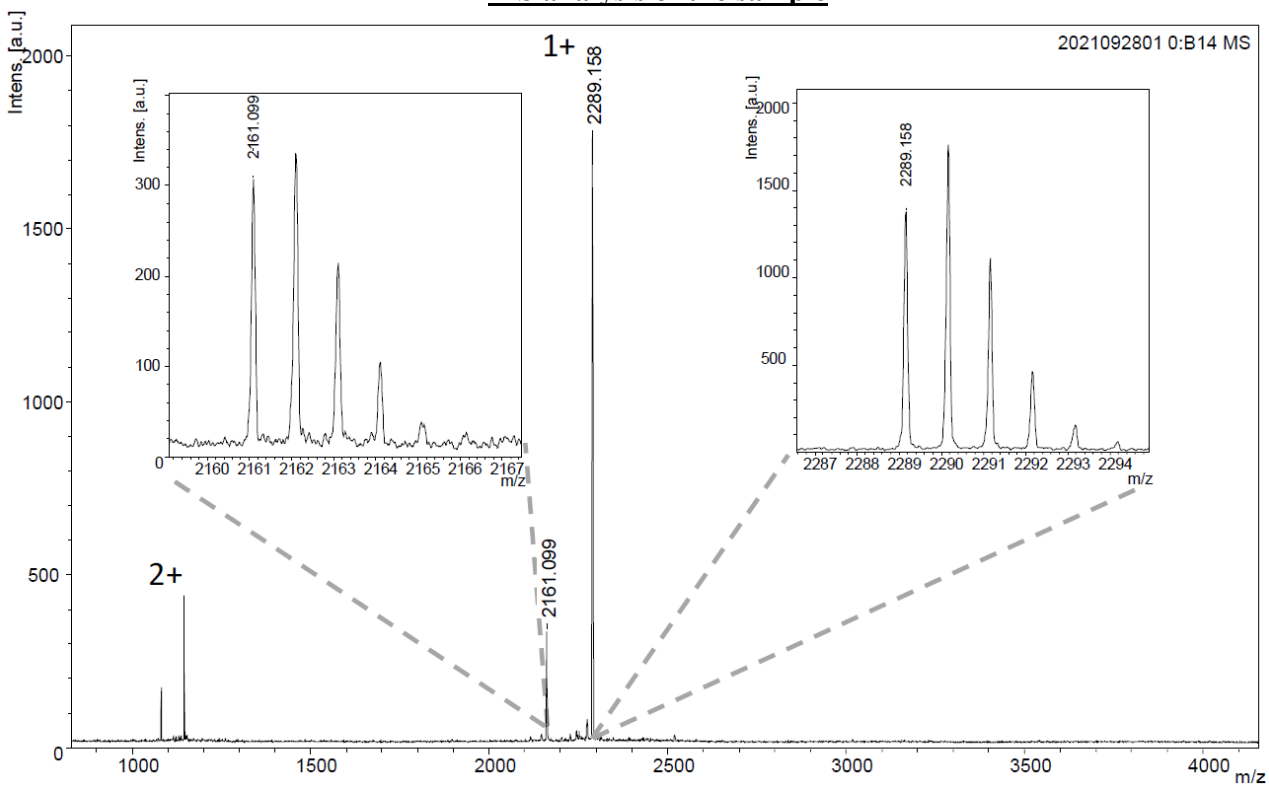
Material:
MS/MS analysis
- Mass spectrometer: ultrafleXtreme MALDI-TOF/TOF (Bruker)

Method:
Peptide sequencing by MALDI-TOF/TOF:
The peptide was diluted twenty thousand fold in a solution of 4-hydroxy-α-cyano-cinnamic acid (4-HCCA) saturated in a mixture of acetonitrile:water:trifluoroacetic acid 33.3:66.6:0.1 (TWA). Matrix-sample solutions were spotted onto a gold-plated sample probe using the ultrathin layer method (Cadene and Chait, 2000; Gabant and Cadene, 2008). Spectra were acquired in the reflectron positive ion mode in the 500 to 4500 *m/z* range for MS. Lift method was used for the sequencing experiment. Calibration of the instrument was performed externally using a neighboring spot with pepmix calibration kit consisting of angiotensin I, angiotensin II, substance P, bombesin, adrenocorticotropic hormones (clip 1–17 and clip18–39), and somatostatin 28 (Bruker Daltonics). MALDI-TOF-MS spectra were processed using FlexAnalysis 3.3 software from Bruker Daltonics

Annotation of MS/MS spectra
The MS/MS spectra were annotated with Biotools 3.2 software (Bruker).

Results

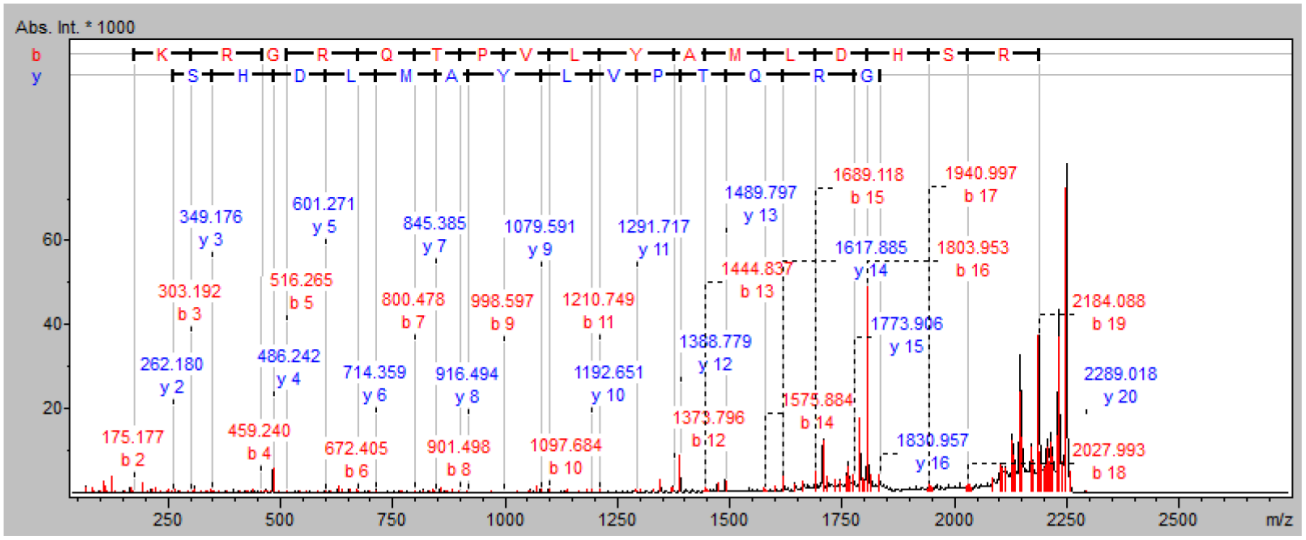
MS analysis of the sample



The MS analysis of the sample indicates that there are 2 peptides in the PO 180-199 sample. The peak at m/z 2289.158 corresponds to the peptide of interest (theoretical m/z 2289.188, error of 13 ppm).
The peak at m/z 2161.099, with a mass difference of -128 Da, appears to correspond to the peptide of interest with the loss of K or Q in the sequence.

Sequencing of the 2 peptides at m/z 2161 and 2289 and annotation of the spectra with Biotools software

Sequencing of the peptide at m/z 2289



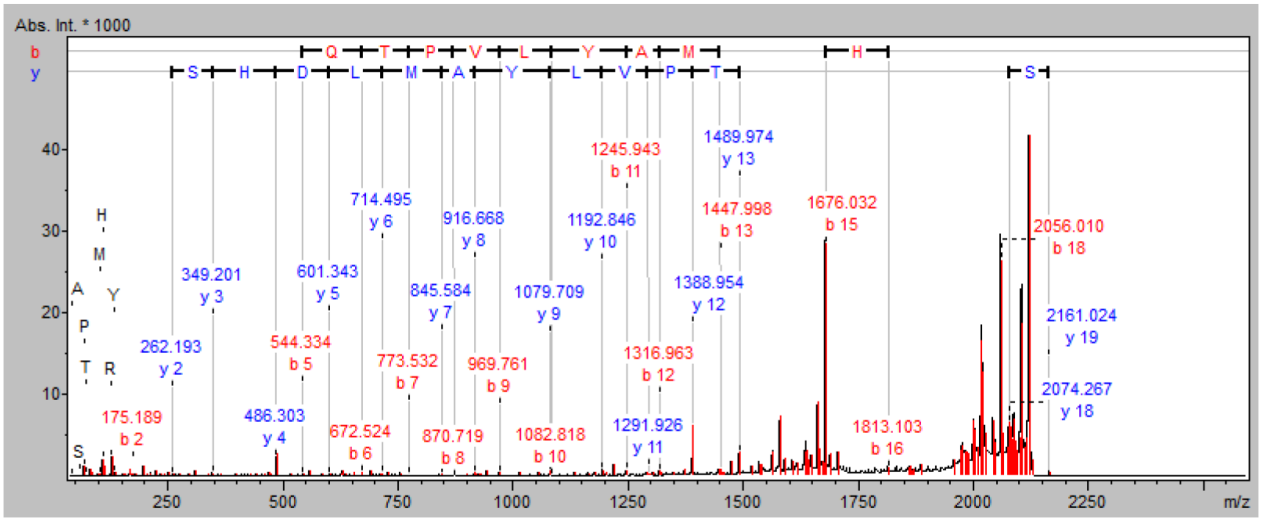
Biotools score = 294924

The sequence SSKRGRQTPVLYAMLDHSRS is confirmed for the m/z 2289.

Sequencing of the peptide at m/z 2161

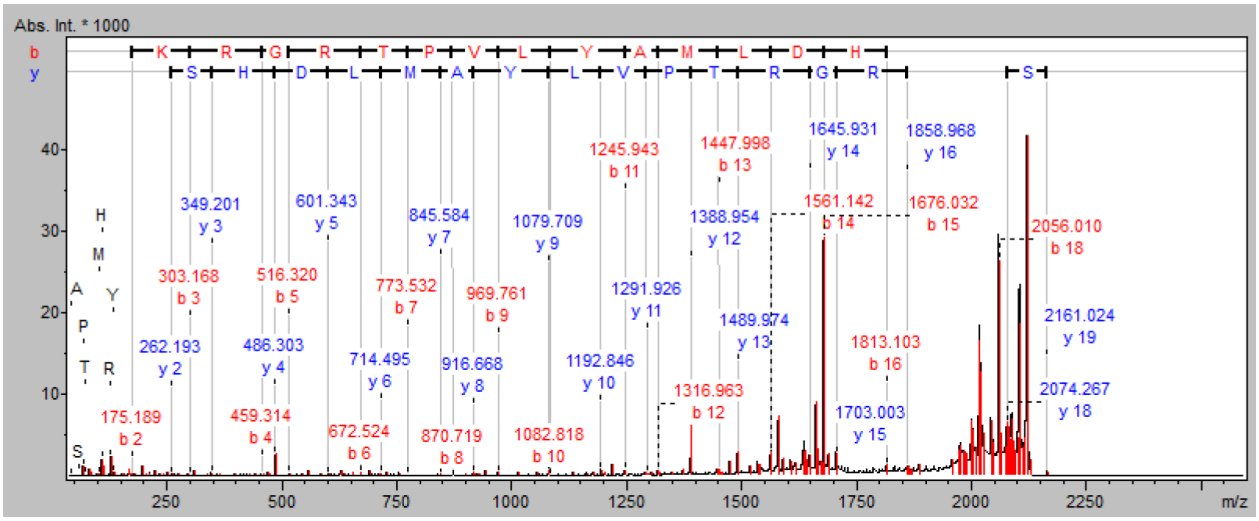
The following 2 sequences were considered for: SSKRGRQTPVLYAMLDHSRS

- SS_RGRQTPVLYAMLDHSRS (loss of K182)



Biotools score = 4620

- SSKRGR_TPVLYAMLDHSRS (loss of Q186)



Biotools score = 65612

The Biotools score is 4620 for the "loss of K182" hypothesis and 65612 for the "loss of Q186" hypothesis. This marked difference in score supports the "loss of Q186" hypothesis.

The peak at m/z 2161 corresponds to the peptide of interest without Q186: SSKRG_RTPVLYAMLDHSRS.

Conclusions

The PO 180-199 sample is composed of 2 peptides:

- The peptide of interest [180-199] at m/z = 2289 with the sequence SSKRGRQTPVLYAMLDHSRS

- The bad peptide [180-199]△Q186 at m/z = 2161 with the sequence SSKRGR_TPVLYAMLDHSRS

Supdata3b- MO2VING-MS facility – QC analysis P0 ProteoGenix - LC-UV-MS report

Aim of the experiment:
Determine the relative quantity of peptides [180-199] and [180-199]△Q186.

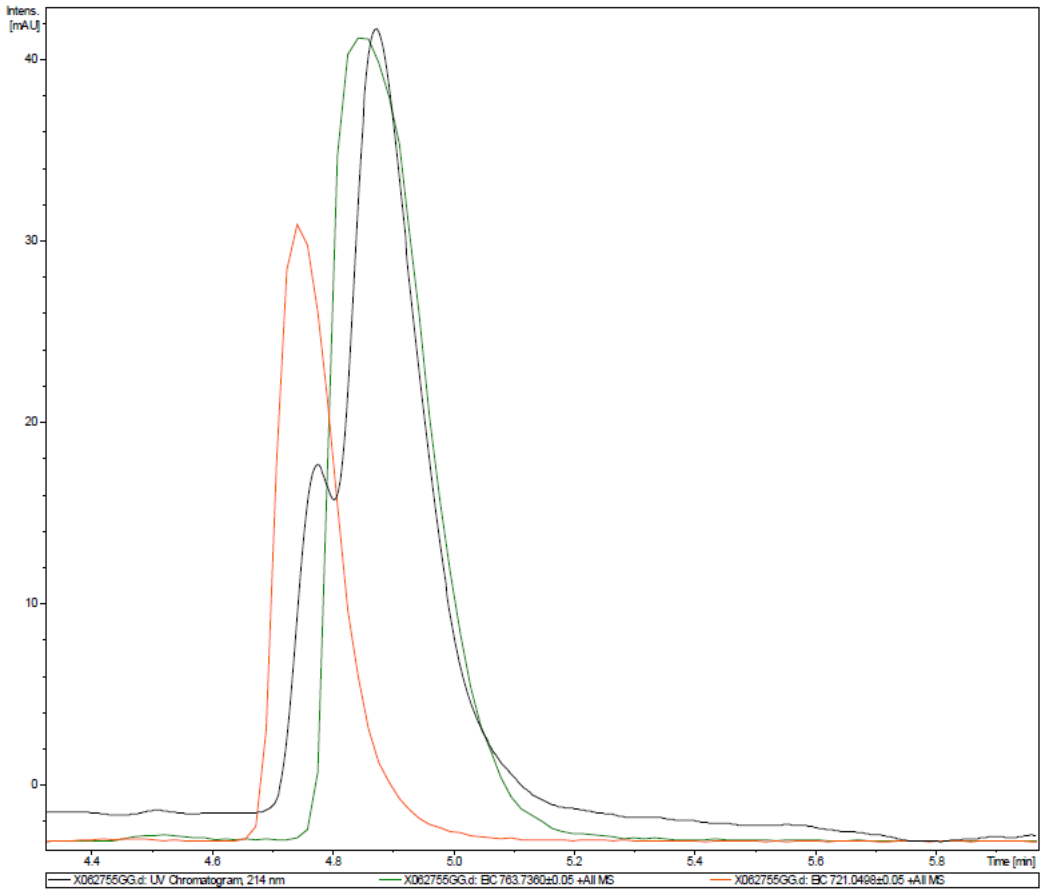
Sample to analyze
P0 180-199

Instrumental equipment:
- Ultra high-performance LC: U3000 RSLC (Thermo)
- Column: Aeris WidePore XB-C18 (2.1 x 150 mm; 3.6 µm) (Phenomenex)
- Mobile phases: A = H2O + 0.1% FA; B = ACN + 0.08% FA
- Gradient: 0.6-90% acetonitrile
- Temperature: 40°C
- Flow rate: 500 µL/min
- Source: ESI online - Mass spectrometer: MaXis HR high-resolution QTOF (Bruker)

Method
LC-UV-MS analysis
The peptide was diluted to 1 mg/mL in a solution of water and 0.5% formic acid. 200 µg were injected.

Results

LC-UV-MS analysis of the sample



The UV chromatogram is shown in black. The specific extracted ion chromatogram (EIC) of the peptide [180-199]△Q186 3+ is indicated in orange. The EIC of the peptide [180-199] 3+ is shown in green. These results show an initial separation of the 2 peptides. The peptide [180-199]△Q186 is eluted first with a UV intensity of 18 mAU and the peptide [180-199] is eluted second with a UV intensity of 42 mAU. These initial results indicate that the P0 180-199 sample is composed at 30% (18/(18+42)) of the peptide [180-199]△Q186 and 70% (42/60) of the peptide [180-199].

Supdata4- MO2VING-MS facility – QC analysis P0 MCE - MS+LC-UV-MS report

Aim of the experiment:
Evaluate the purity of the peptide P0 180-199 from MCE and confirm its sequence.

Samples to be analyzed
P0 180-199 from MCE >95% purity

Instrumental materials:
MS/MS analysis
- Mass spectrometers: ultrafleXtreme MALDI-TOF/TOF (Bruker)

LC-UV-MS
- Ultra-high-performance LC: U3000 RSLC (Thermo)
- Column: Aeris WidePore XB-C18 (2.1 x 150 mm; 3.6 µm) (Phenomenex)
- Mobile phases: A = H2O + 0.1% FA; B = ACN + 0.08% FA
- Gradient: 0.6-90% acetonitrile
- Temperature: 40°C
- Flow rate: 500 µL/min
- Source: ESI online
- Mass spectrometer: MaXis HR high-resolution QTOF (Bruker)

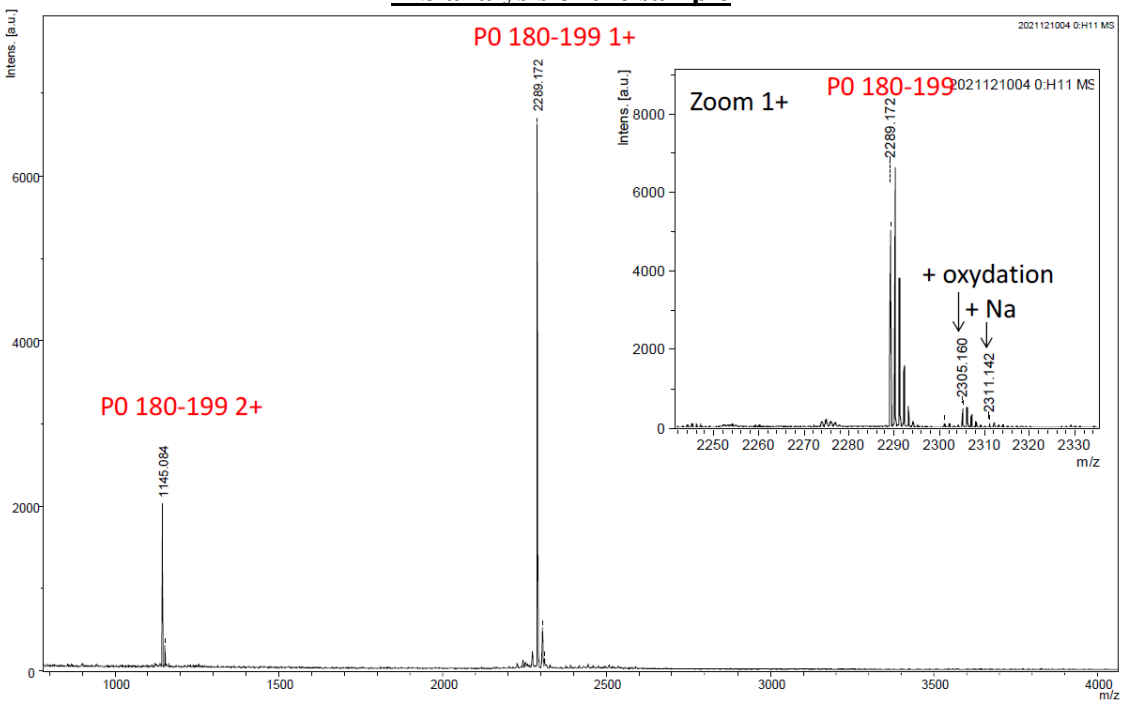
Method
Peptide Sequencing Method by MALDI-TOF/TOF:
The peptide at 1 mg/mL in PBS was diluted 1/4000 in a solution of 4-hydroxy-α-cyano-cinnamic acid (4-HCCA) saturated in a mixture of acetonitrile: water: trifluoroacetic acid 33.3:66.6:0.1 (TWA).
The sample-matrix mixture was then deposited on a gold-plated target using the ultra-thin layer method (Cadene and Chait, 2000; Gabant and Cadene, 2008).
The spectra were acquired using the RP 500-45000 method for MS and the LIFT method for MS/MS.

Annotation of MS/MS spectra
The MS/MS spectra were annotated using the Biotools software (Bruker).

LC-UV-MS Analysis
The peptide was diluted to 0.1 mg/mL in a solution of water and 0.5% formic acid. 20 µg were injected.

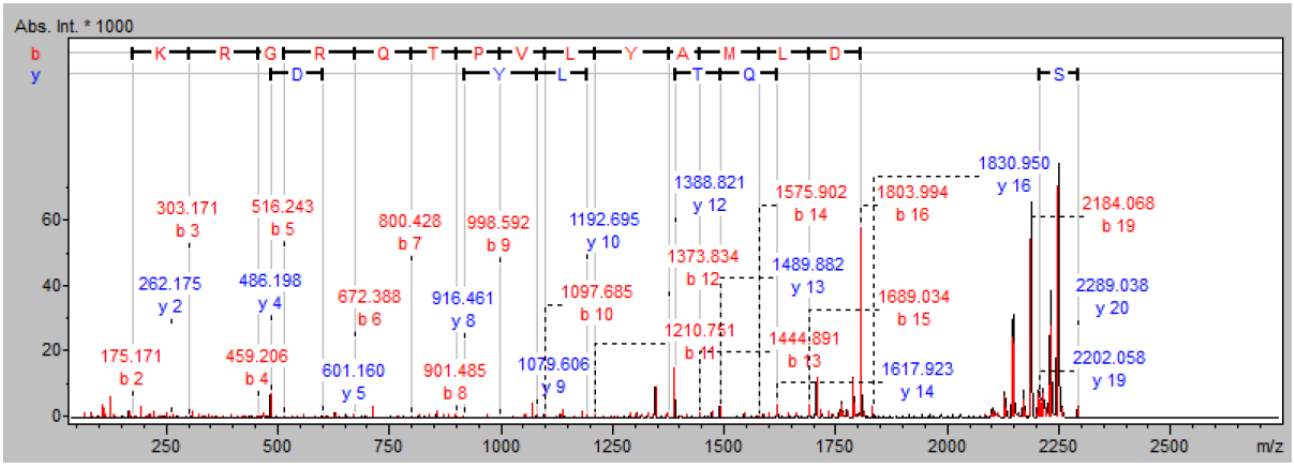
Results

MS analysis of the sample



The MS analysis indicates that there is one peptide in the PO 180-199 sample. The peak at m/z 2289.172 corresponds to the peptide of interest (theoretical m/z 2289.188, error of -7 ppm). A slight oxidation is observed.

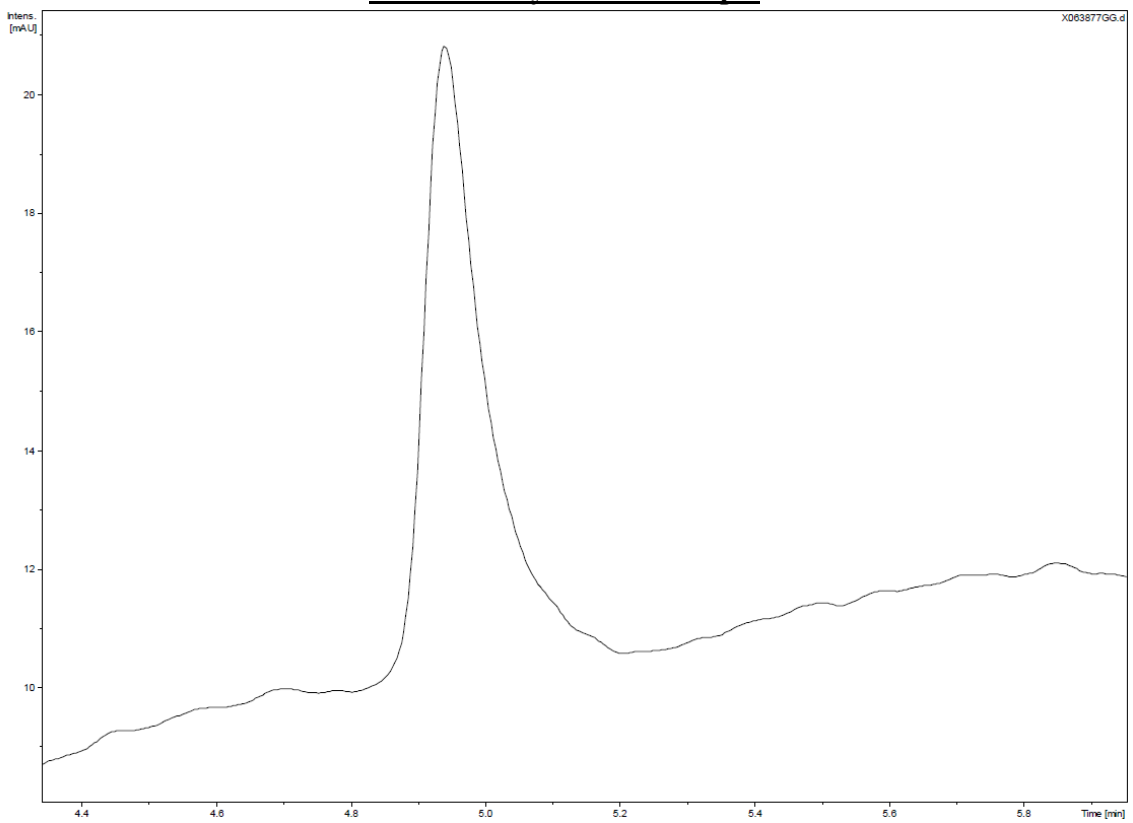
Sequencing of the peptide at m/z 2289 and annotation of the spectrum with Biotools software



Biotools score = 32807

The sequence SSKRGRQTPVLYAMLDHSRS is confirmed for the m/z 2289.

LC-UV analysis of the sample



No shoulder is observed, indicating a high level of purity (>95%).

Conclusions

The P0 180-199 sample is composed of the peptide of interest [180-199] with a high level of purity (>95%). Its sequence SSKRGRQTPVLYAMLDHSRS has been confirmed.