Protein Molecular Weight Determination

By UV-LC ESI MS

Order 12345

John Doe

ABCDE Pharma Inc.

Analysis start date: January 13, 2020

Analysis reporting date: January 20, 2020

Principal Investigator: Alphalyse

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n.a.

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Samples received

The following sample was received at Alphalyse for protein analysis.

Sample 1

Objective

Determination of the Molecular Weight (MW) of intact proteins by Liquid Chromatography coupled to Electrospray Ionization Mass Spectrometry (LC-ESI-MS). The proteins are separated by hydrophobicity in standard HPLC gradient and the MW of each peak analyzed by ESI-MS. The determined MW is compared to the theoretical MW calculated for the protein amino acid sequence.

Results Summary

Molecular Weight Determination

Sample name	Measured Mass [Da]	Intensity [%]
Sample 1	5932.16	4.9
	33628.53	3.0
	50457.59	5.1
	50521.12	3.0
	98956.01	3.5
	99130.33	3.2
	100775.09	9.2
	100823.22	50.1
	100861.57	37.8
	100902.29	100.0
	100942.49	61.5
	100975.70	50.1
	101017.93	39.5
	101083.23	28.1
	101134.31	19.3
	101189.62	10.9

Showing only the most abundant mass

Quality control standard included in the analysis

Sample name	Measured Mass [Da]	Theoretical Mass [Da]	Intensity [%]	Delta Mass [Da]
Myoglobin	16951.26	16951.48	100	-0.22

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Analytical Procedure

Introduction

LC-MS (or LC-ESI-MS) is a method to determine the accurate mass of biomolecules. The proteins in liquid phase are separated by a reversed phase chromatography gradient and sprayed through a capillary at high voltage (Electrospray Ionization - ESI) into the mass spectrometer (MS) where the mass over charge ratio (m/z) is measured. For large biomolecules the electrospray process results in multiply protonated molecules with a distribution of ion species at m/z range from 1-3000. The mass of the molecule is determined using a deconvolution algorithm that calculates the mass of the intact non-protonated protein. The ESI process requires that the protein is quite pure without interfering salts or detergents and is therefore cleaned up by Liquid Chromatography (LC) before the MS analysis.

Sample preparation

4µl sample was loaded directly onto the column without further dilution

Mass Spectrometry Analysis

LIQUID CHROMATOGRAPHY (Agilent 1200):
Column: Poroshell 300SB-C8 2.1x75mm, 5µm
Eluent A: 0.1% Trifluoroacetic acid in 10% acetonitrile
Eluent B: 0.1% Trifluoroacetic acid in 90% acetonitrile
Column temp: 80°C
Gradient:

Time [min]	A [%]	B [%]	Flow [mL/min]
0.0	95	5	0.6
6.0	95	5	0.6
6.3	95	5	0.3
7.0	95	5	0.3
12.0	25	75	0.3
13.0	5	95	0.3
13.5	5	95	0.3
14.0	95	5	0.3
15.0	95	5	0.3

ELECTROSPRAY MASS SPECTROMETRY (Bruker Maxis Inpact):
Mass range: 500-4500 m/z
End Plate Offset: 500V
Capillary: 4500V
Nebulizer: 1.2 Bar
Dry gas: 8.0 l/min
Dry temp: 200°C

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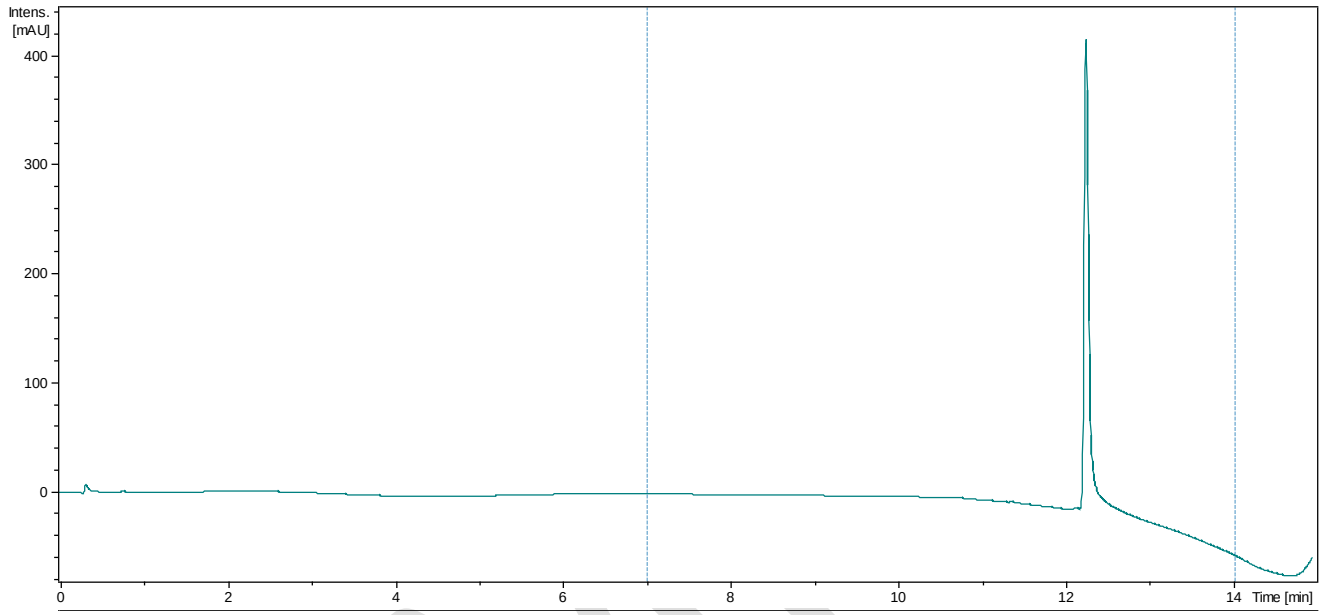
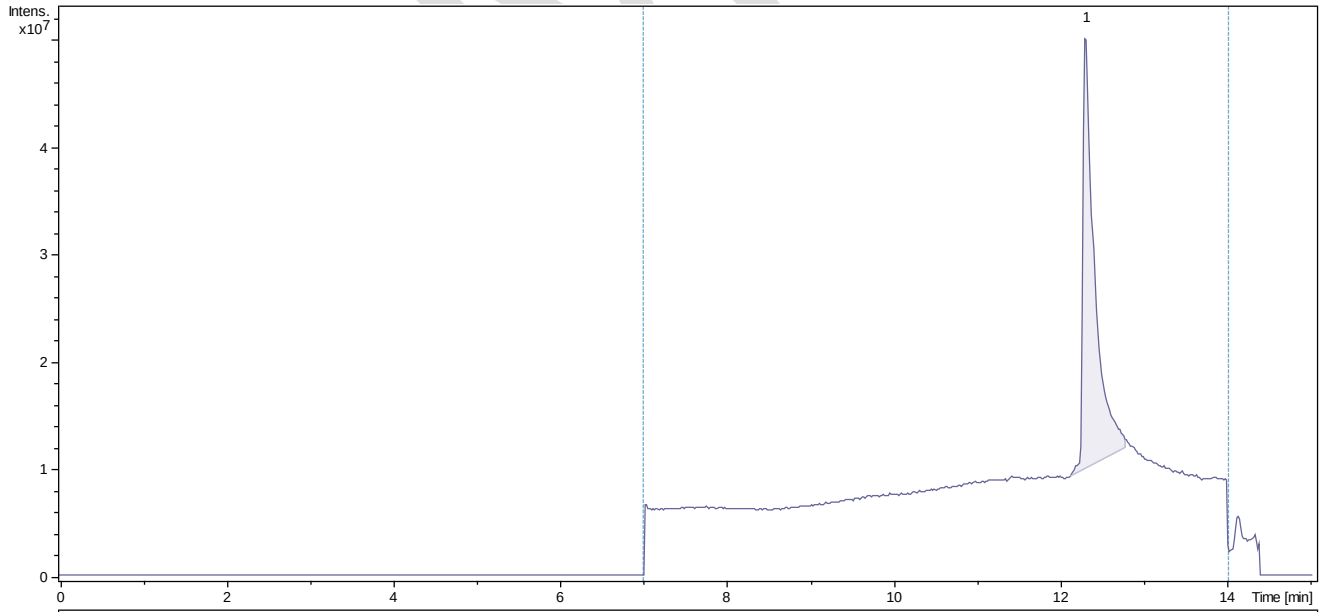
Results

Sample name: Sample 1

UV-Chromatogram



Total ion chromatogram



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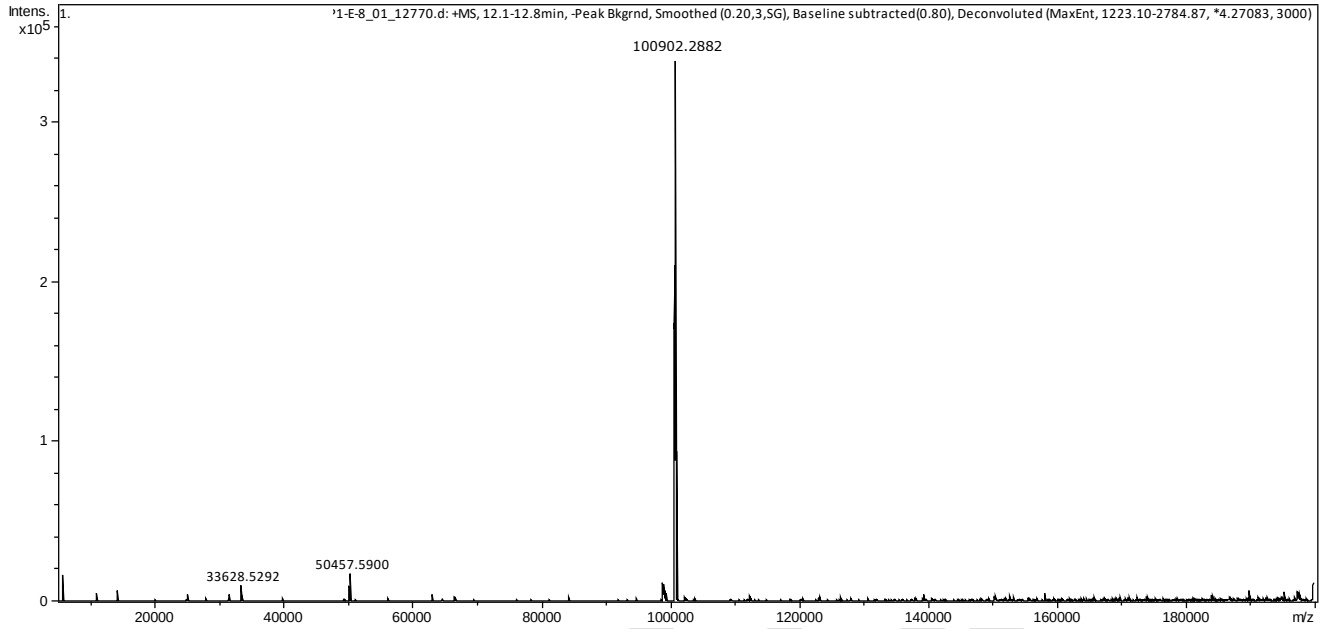
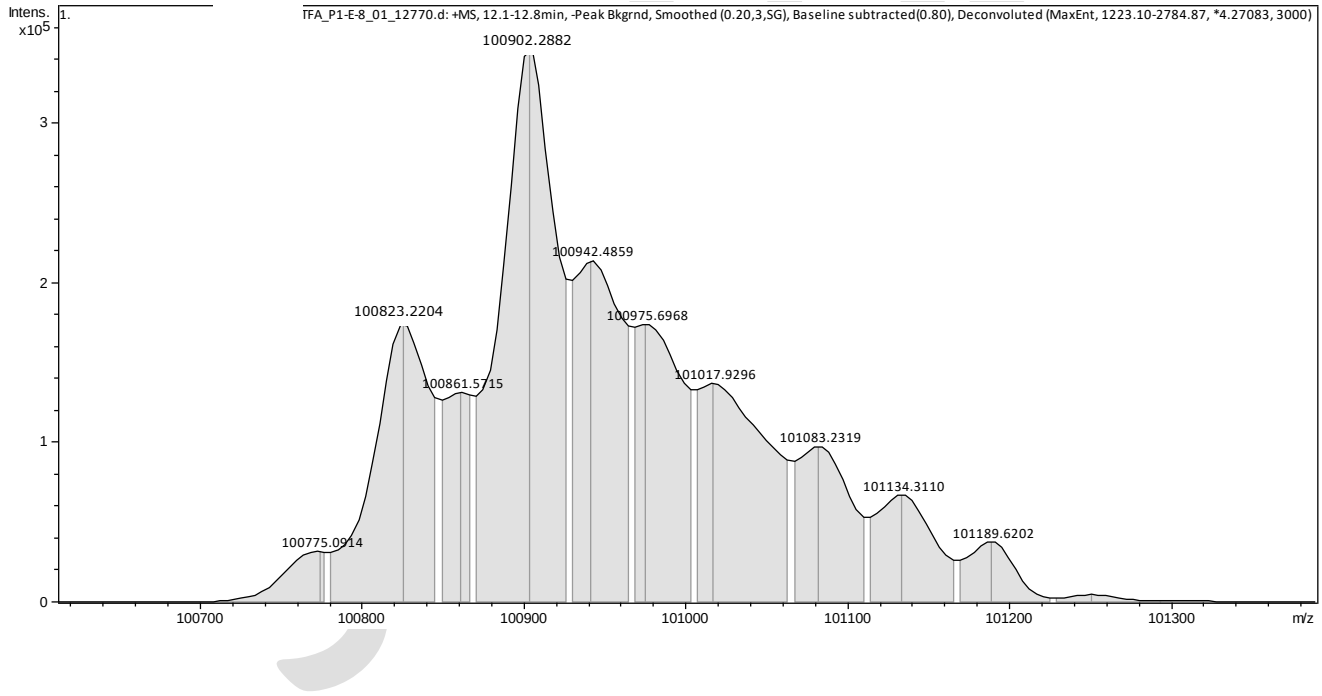
Deconvoluted data





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Protein Molecular Weight Determination

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Mass list

Sample name	Measured Mass [Da]	Intensity [%]
23006	5932.16	4.8
	33628.53	2.9
	50457.59	5.0
	50521.12	2.9
	98956.01	3.4
	99130.33	3.3
	100775.09	9.3
	100826.80	49.8
	100861.57	38.1
	100904.42	100.0
	100942.49	60.9
	100975.70	49.6
	101017.93	39.8
	101083.23	27.8
	101134.31	20.3
	101189.62	9.9

Intensity cutoff value: 3%

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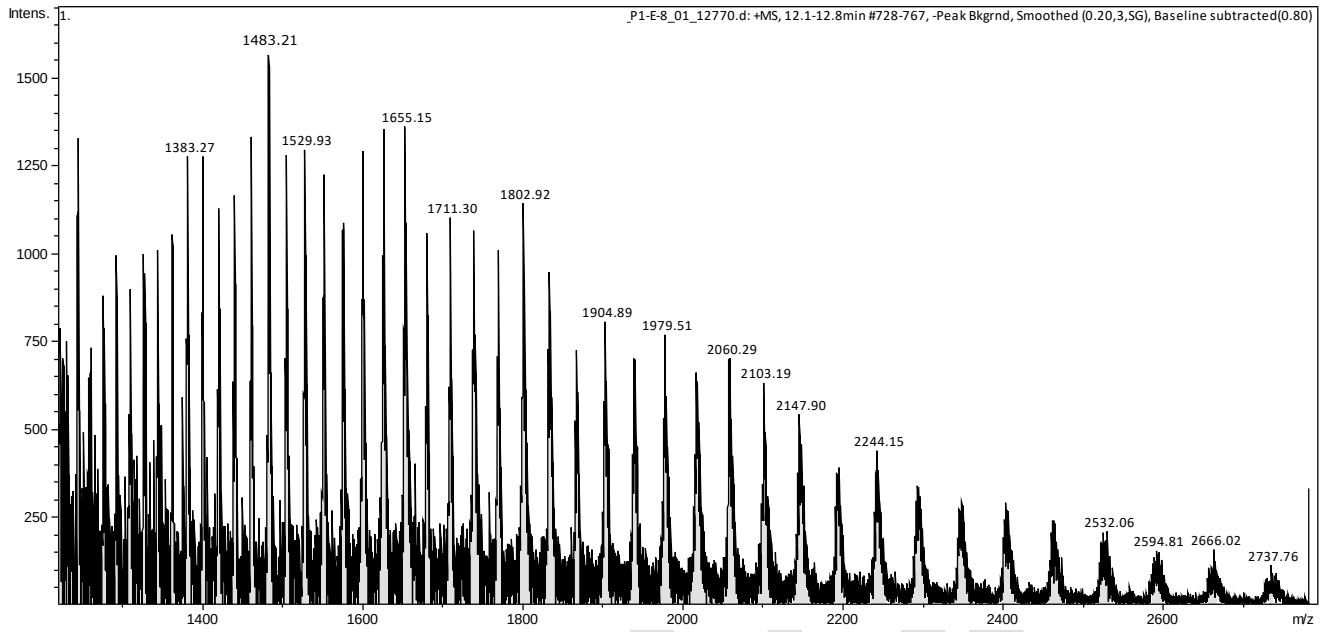
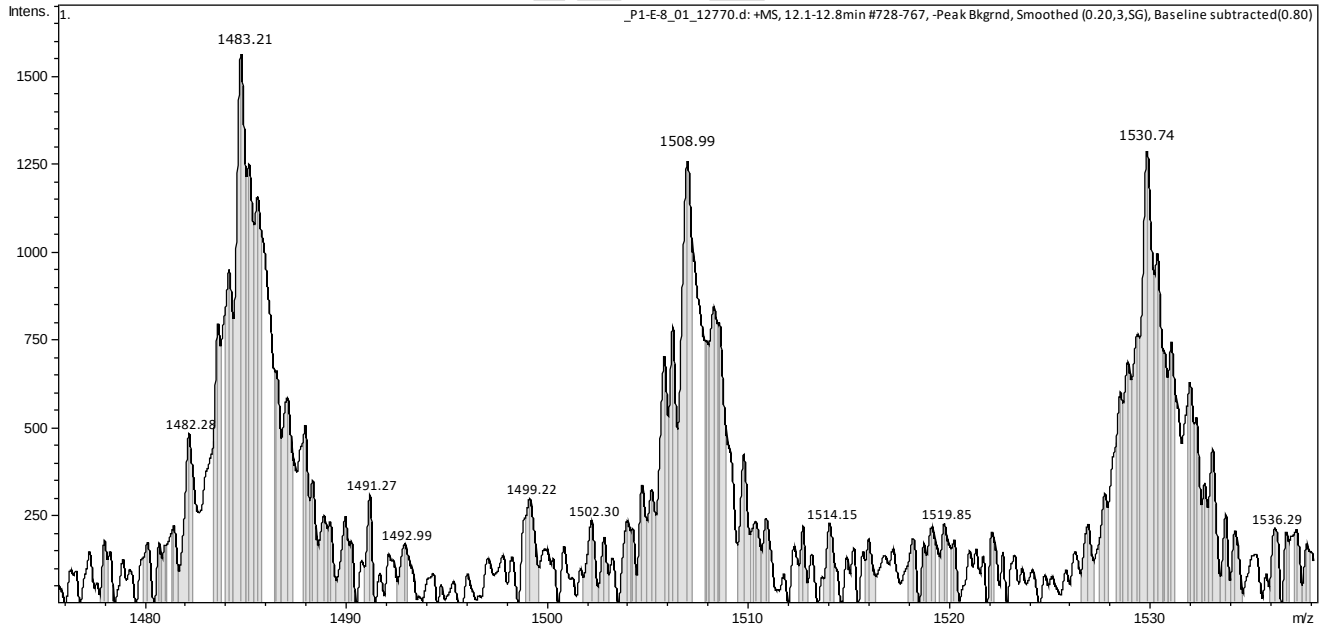
Raw MS data



Raw MS data, zoom



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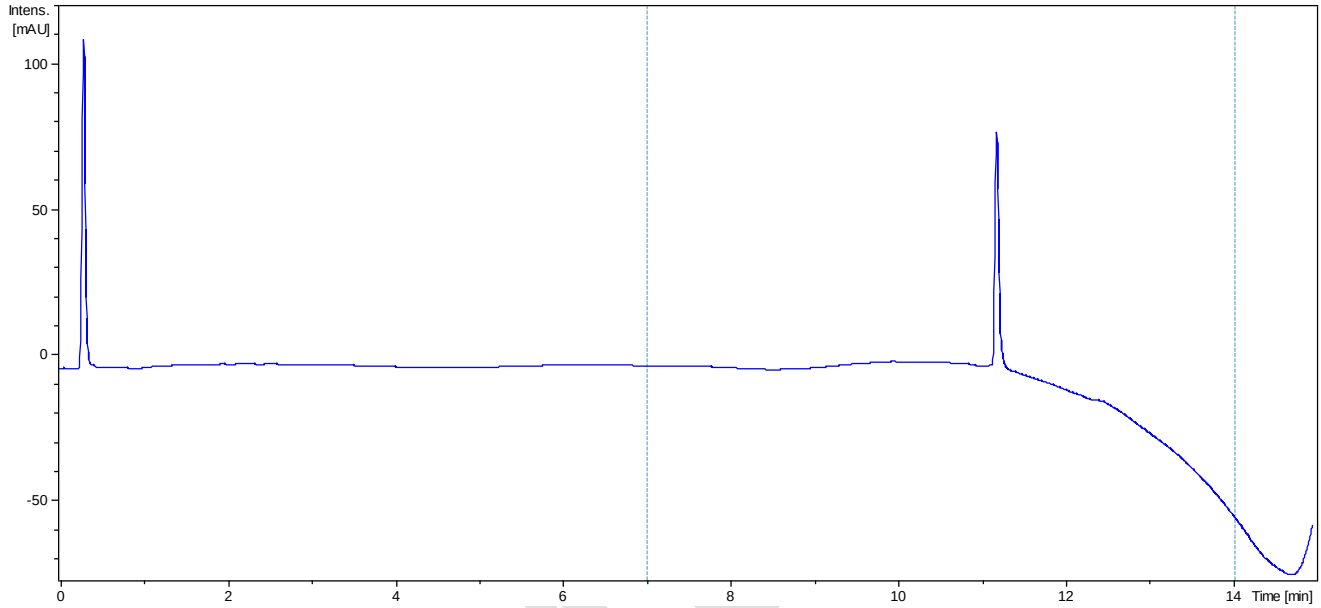
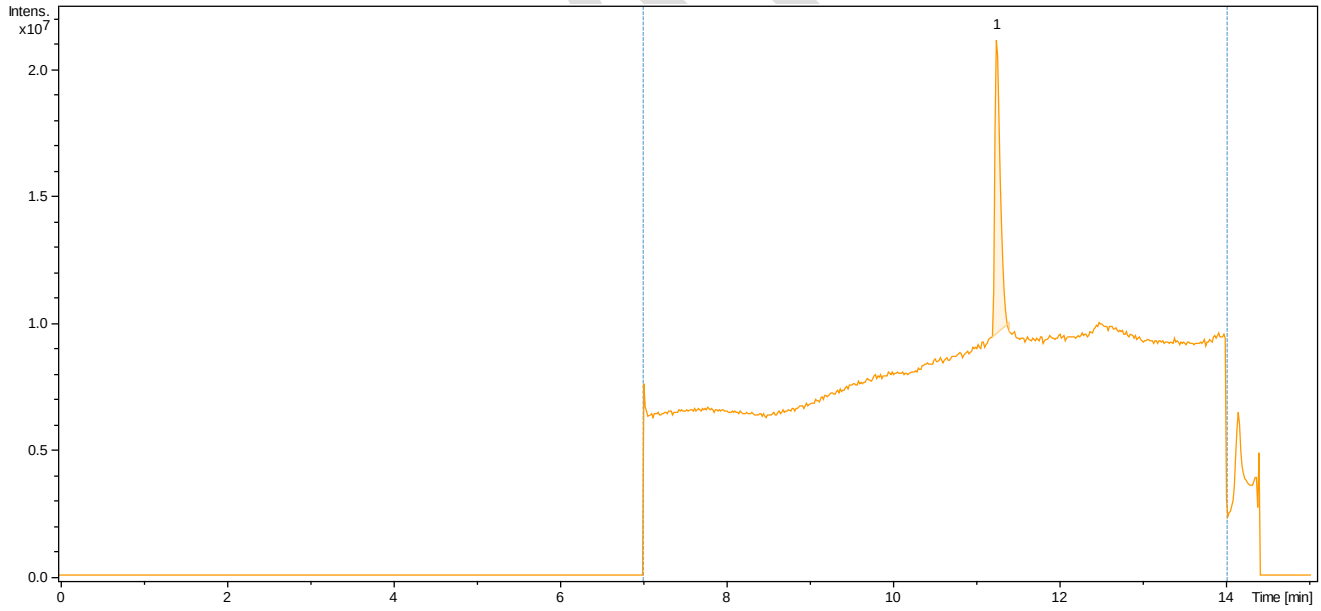
Quality control standard - Myoglobin

UV-Chromatogram



Total ion chromatogram



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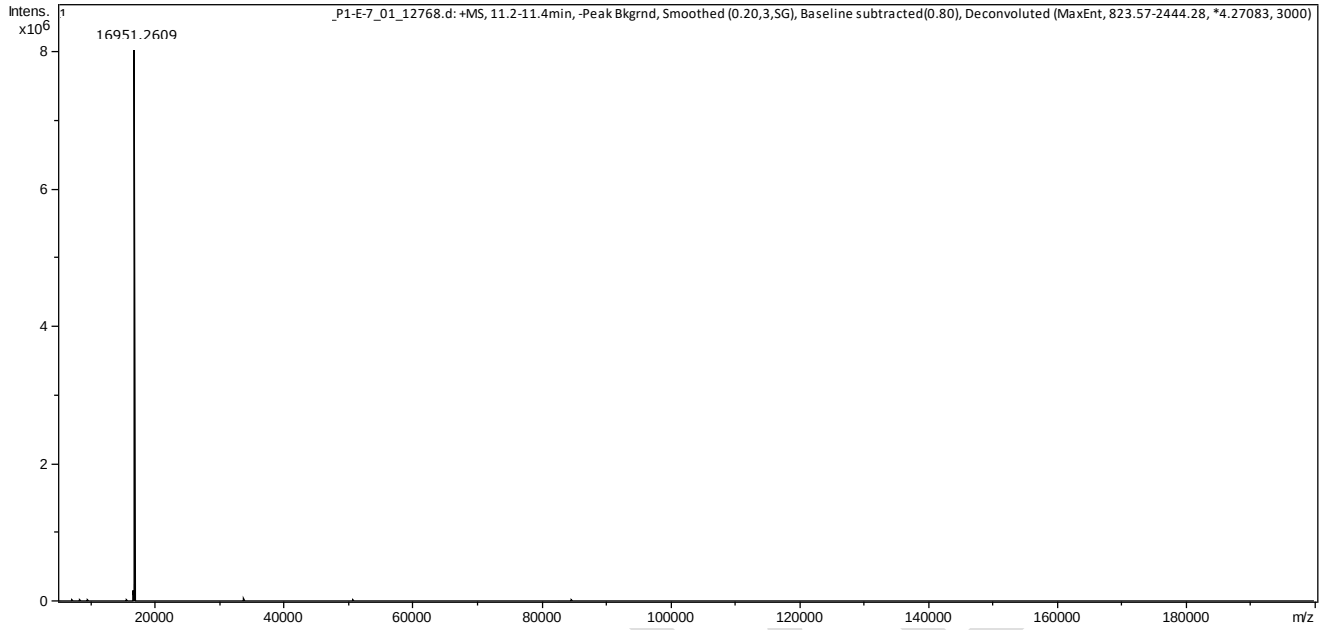
Deconvoluted data



Mass list

Sample name	Measured Mass [Da]	Intensity [%]
Myoglobin	16951.26	100

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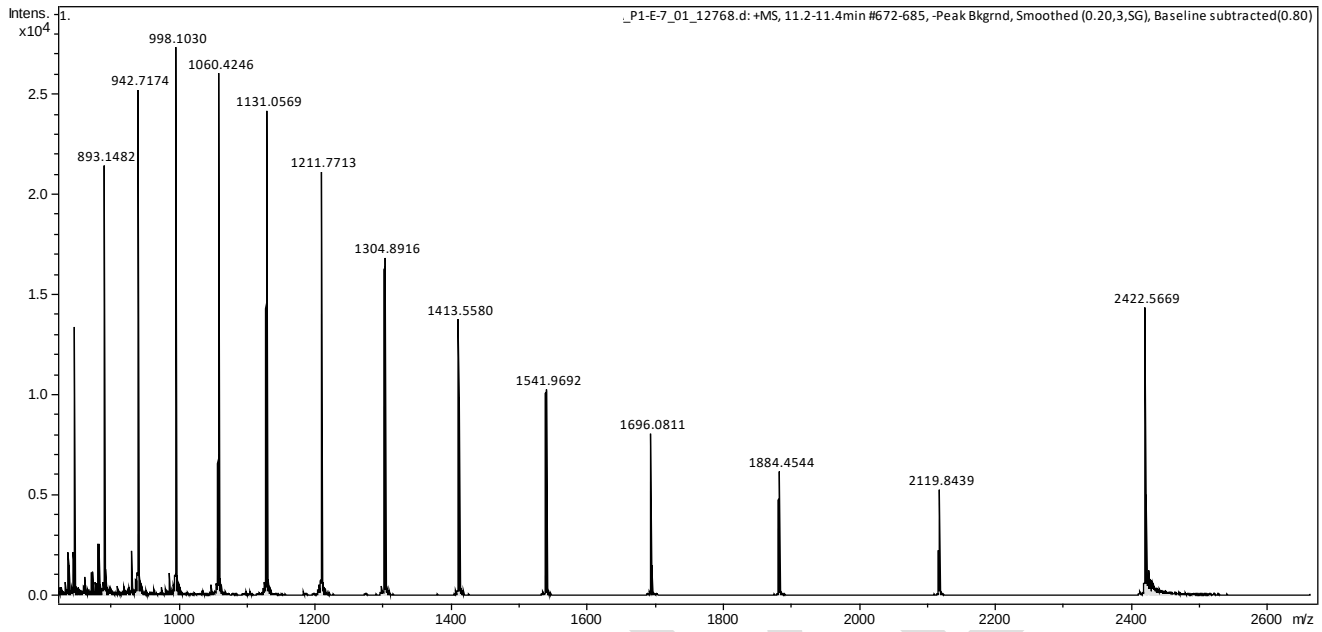
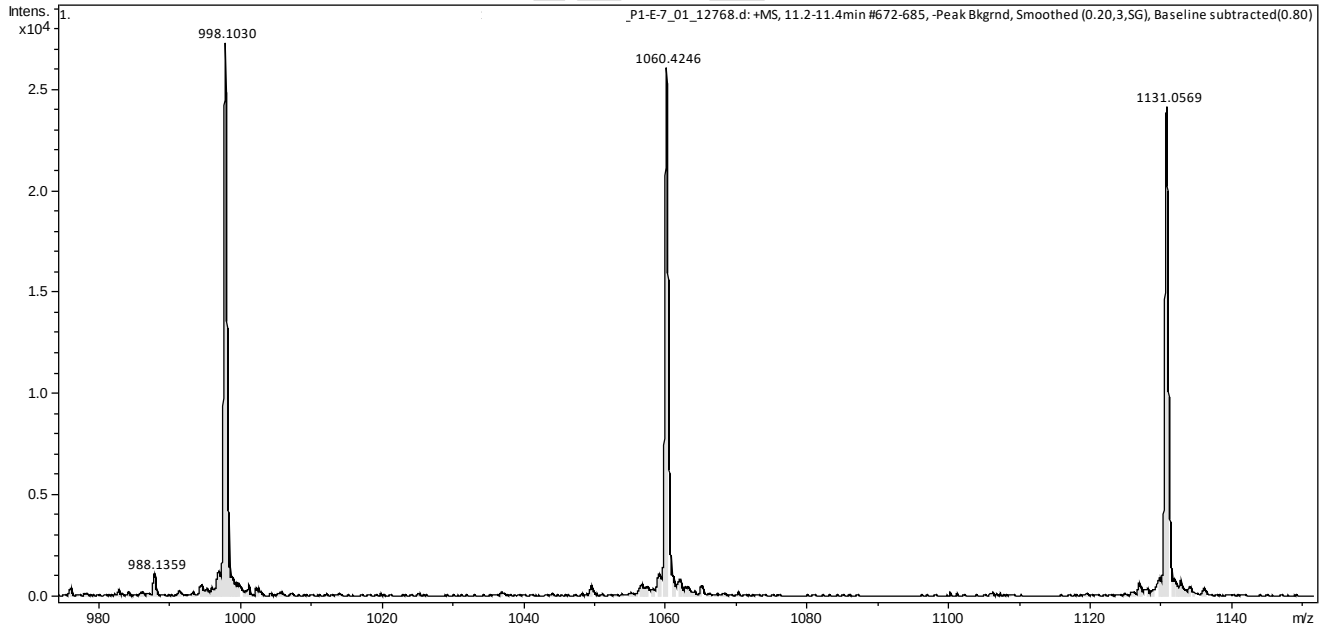


Raw MS data, zoom



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