European Network of Fourier-Transform Ion-Cyclotron-Resonance Mass Spectrometry Centers

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Deliverable D4.6 Round-robin test results – Iteration #2

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Document Abstract

The deliverable D4.1 “Round-robin test results” is part of WP4, which include several joint research actions performed between the EU_FT-ICR_MS centers. This deliverable reports the results of the second Round-robin test performed within the network. This Round-robin aimed to provide information on the different equipment performance among the EU_FT-ICR_MS network, as well as the consistency of data including mass accuracy, sensitivity and detection of isotope fine structure. The chosen sample for this second Round-robin was the glutathione, purchased by each EU_FT-ICR_MS center.

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Abbreviations (if any)

1. Introduction

FT-ICR mass spectrometers are the most powerful MS instruments available. They offer mass resolving power and mass accuracy up to 100 times higher than any other commercially available mass spectrometer. FT-ICR resolution, mass accuracy as well as dynamic range mainly depend on magnetic field strength, but other factors are also very important, namely the FT-ICR cell used and FT processing. In the EU_FT-ICR_MS network, different generation of mass spectrometers are in operations, with different characteristics and magnetic field strength ranging from 4.7 to 15 Tesla. All instruments available in the EU_FT-ICR_MS network were manufactured by Bruker, but belong to different generations, are controlled by different software platforms, and have different signal processing capabilities. Moreover, two different FT-ICR cell types are installed, the Infinity cell o older instruments and the new Paracel on more recent ones. Since all these differences have an impact on the performance of each instrument that goes beyond magnetic field strenght, a Round Robin test was set to assess their analytical capabilities.

2. Round-robin test

The main purpose of the second Round-robin was evaluate each site for instrument performance concerning resolution, mass accuracy and the ability do detect the most abundant isotopologues of a given molecule, thus being able to characterize the isotope fine structure, of paramount importance to establish unequivocal molecular formulas. The test molecule had to meet criteria such as being amenable to analysis on every site and available at each site, be easily ionisable by ESI of nanoESI and stable during analysis and containing another chemical element besides C, H, N and O.

2.1. Sample

The peptide glutathione was be analysed. This is a small tripeptide comprised of three amino acids, cysteine, glutamic acid, and glycine and containing sulphur (chemical formula: $C_{10}H_{17}N_3O_6S$, Figure 1) with a monoisotopic molecular mass of 307.08380644 Da.
Glutathione was purchased by each EU_FT-ICR_MS network site or was already available on each site. Purity and provenance have no effect on the test.

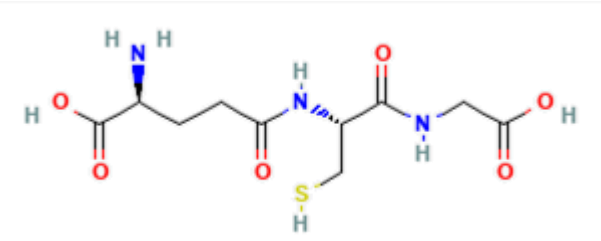

Figure 1. Structure of glutathione (image from PubChem).

3. Results

Table 1. Analysis of glutathione by the different EU_FT-ICR_MS centers.

EU_FT-ICR_MS center	CNRS	LIEG	PRAG	UHRO	UEF	ROMA	FC-LISB	MOSC	WARW	ROUEN	ORSAY
Mass spectrometer	SolariX XR	SolariX XR	SolariX XR	SolariX	SolariX XR	BioApex	SolariX XR	Apex Ultra	SolariX XR	SolariX XR	Apex Q
ICR cell tyoe	Paracell	Paracell	Paracell	Infinity	Paracell	Infinity	Paracell	Paracell	Paracell	Paracell	Infinity
FT-ICR magnetic field	9.4T	9.4T	15T	7T	12T	4.7T	7T	7T	15T	12T	7T
Ionization mode	nanoESI+	ESI+	ESI+	ESI+	ESI+	ESI+	ESI+	ESI+	ESI+	ESI+	ESI+
Infusion	Direct infusion	Direct infusion	Direct infusion	Direct infusion	Direct infusion	Direct infusion	Direct infusion	Direct infusion	Direct infusion	Direct infusion	Direct infusion
Analysis mode	Magnitude	Magnitude	Magnitude	Magnitude	Magnitude	Magnitude	Magnitude	Magnitude	Magnitude	Magnitude	Magnitude
Accurate mass measured (m/z)	308.09102	308.0909	308.09107	308.09059	308.0909778	308.091245	308.09109	308.09107	308.091084	308.09108	308.091404
Deviation (ppm)	-0.194	-0.594	0.03	1.61	-0.341	0.53	-0.03	0.03	0.0032	0.01	1
Highest resolution achieved (monoisotopic peak), broad band	1 716 285	883 389	870 598	975 071	1 328 618	60 000	695 339	971 978	1 956 550	492 276	918 000
Isotopic distribution of GSH	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Nr. Isotopologues identified	12	12	9	11	12	5	12	12	12	12	9
Highest deviation (isotopologue number)	59.31% (10th)	67.19% (6th)	2.5% (4th)	58% (8th)	48% (11th)	17% (4th)	27% (11th)	37% (8th)	58% (2nd)	50% (5th)	125% (12th)

The analysis we made with the purpose achieving the best possible coverage of teh12th most abundant isotopologues of GSH while striving for the highest relative intensities match between the observed and predicted intensities for each isotopologue thus pushing the limits of the instruments towards isotope pattern accuracy. All centres were able to detect carbon isotopologues (100%), 6 were able to detect all 12 (55%), one detected 11 (9%), 2 centres detected 9 (18%) and 1 only detected 5 (9%). Instruments of higher magnetic field, newer generation and equipped with the paracell performed better, although any field strength between 7 and 15 T allows detection of all the 12^{th} isotopologues. Concerning the deviation between observed and estimated isotopologue intensities at the nth worst case, it varied between 2.5% and 125%. For half the centres, the worst deviation was between 37% and 67%. These results were irrespective of instrument generation, field strength or cell type. PRAG achieved a deviation of 2.5% while WARW stood at 58% (most recent instruments, both 15T) while ROMA achieved 17% (oldest generation, lowest field, at 4.7 T).
Concerning mass accuracy, all centres, except one were accurate to 1 ppm or better, 5 were accurate to 0.03 ppm or better and one was accurate to 0.003 ppm. Most of these results were again independent of instrument generation, cell type or magnetic field strength although the best result was achieved at 15 T.
Resolution was above 500000 for all centres, except one that reached 60000. Six centres were close or moderately above one million resolving power, while two centres were close to two million resolution to m/z 308. Achieving the highest possible resolution was not an objective of the test hence the reported data should be interpreted as the resolution required to achieve a certain purpose such as identifying all isotopologues with the best possible intensity accuracy.

TNA Center: LILLE	**Analysis date:** 25-06-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Sigma-Aldrich (Merck) ref G4251 batch SLCG8830
Concentration for analysis: 50pmol/uL in 100% acetonitrile + 0.1% formic acid

Analysis & instrument setup

Equipment used: Bruker SolariX XR 9.4T
Ionization mode: nanoESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: internal **Calibrant used: ESI-L Low concentration tuning mix**
Acquisition range: 144 to **1500** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
Data processing was performed using Data Analysis 5.0 build 203.2.3586

Results

Accurate mass measured: 308.09102 *m/z*
Deviation: -0.194 ppm

Highest resolution achieved (monoisotopic peak): FWMH@m/z 308.091=1716285
broadband magnitude 16M m/z: 144-1500

Isotopic distribution:

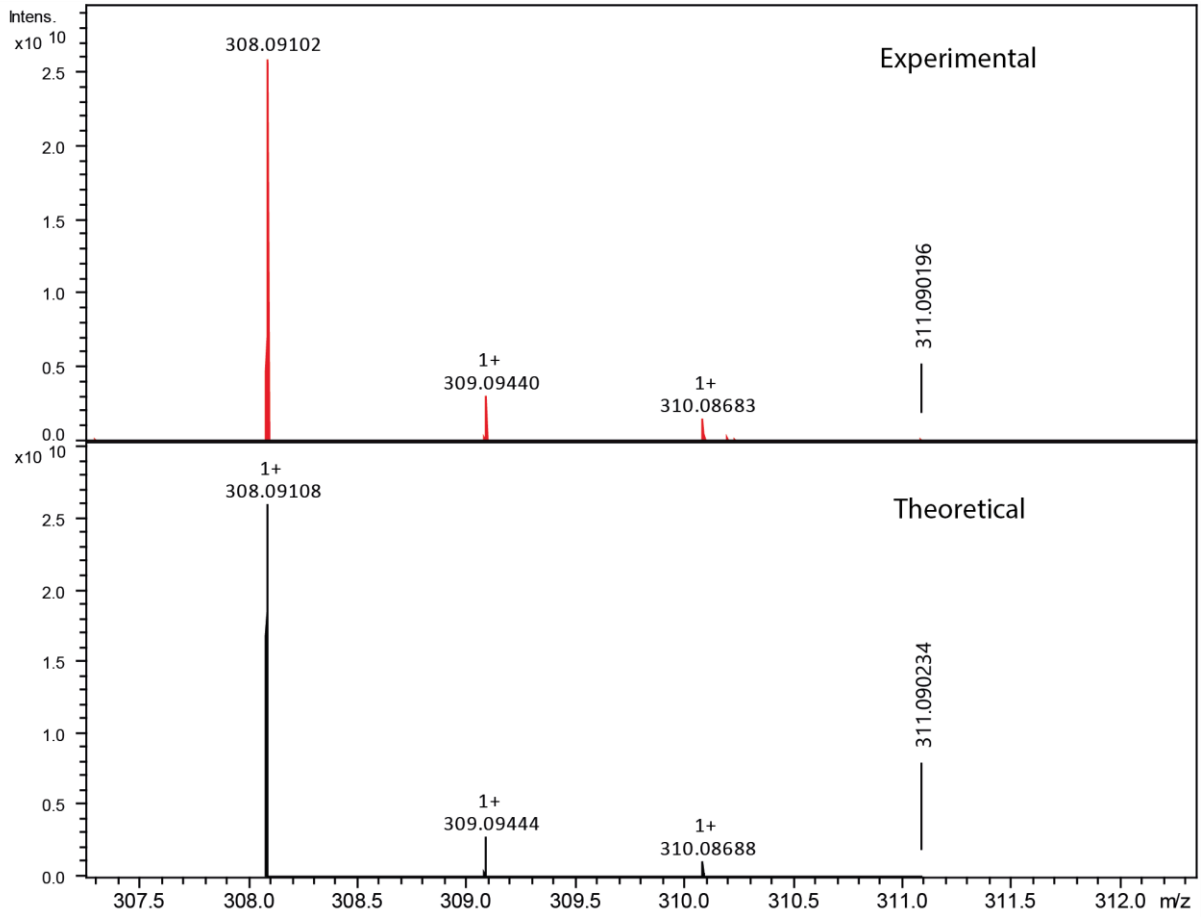

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100	0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	1.151	5.02
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	0.838	6.07
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	11.944	10.43
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	0.252	10.00
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.326	57.48
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	6.196	38.48
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	0.167	40.33
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	1.902	54.25
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.838	59.31
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.678	40.08
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.210	57.89

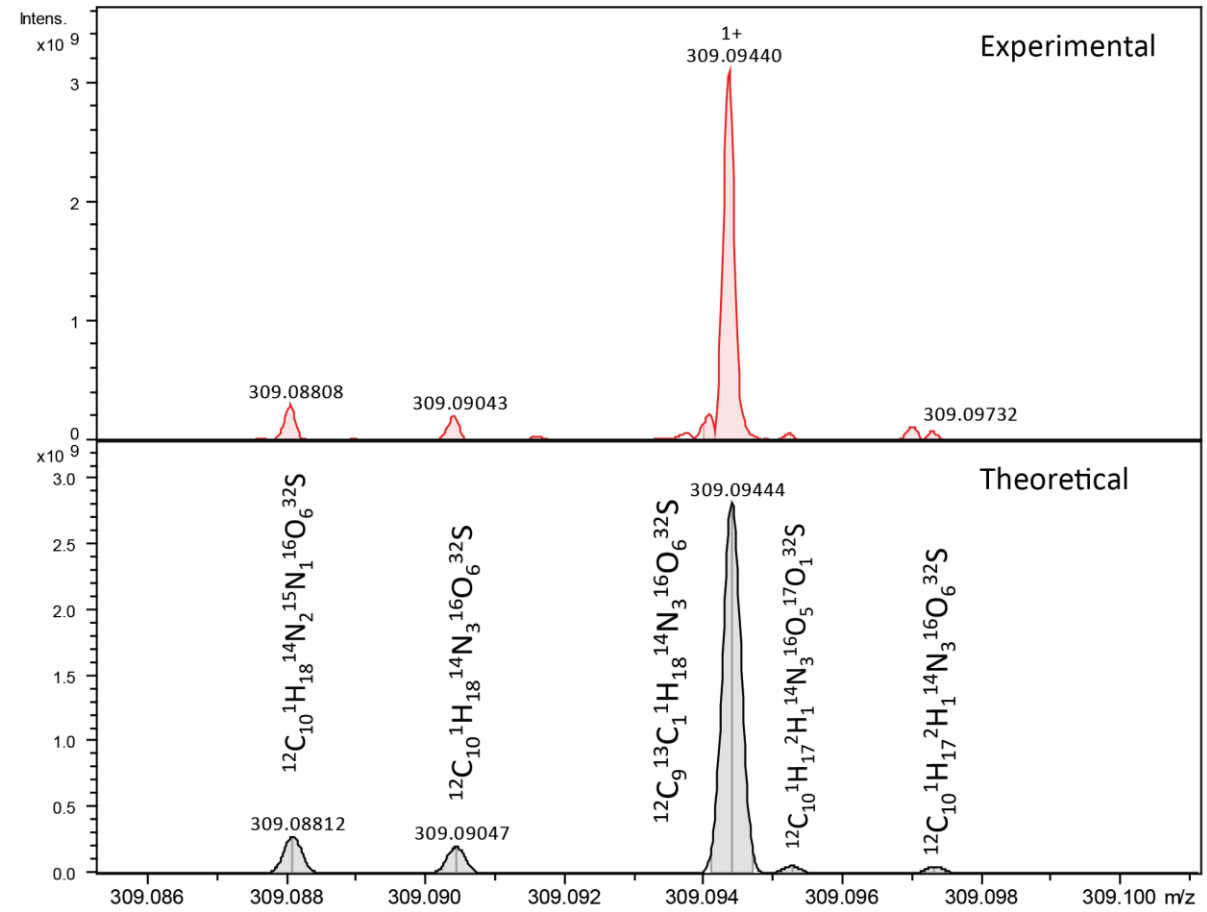

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

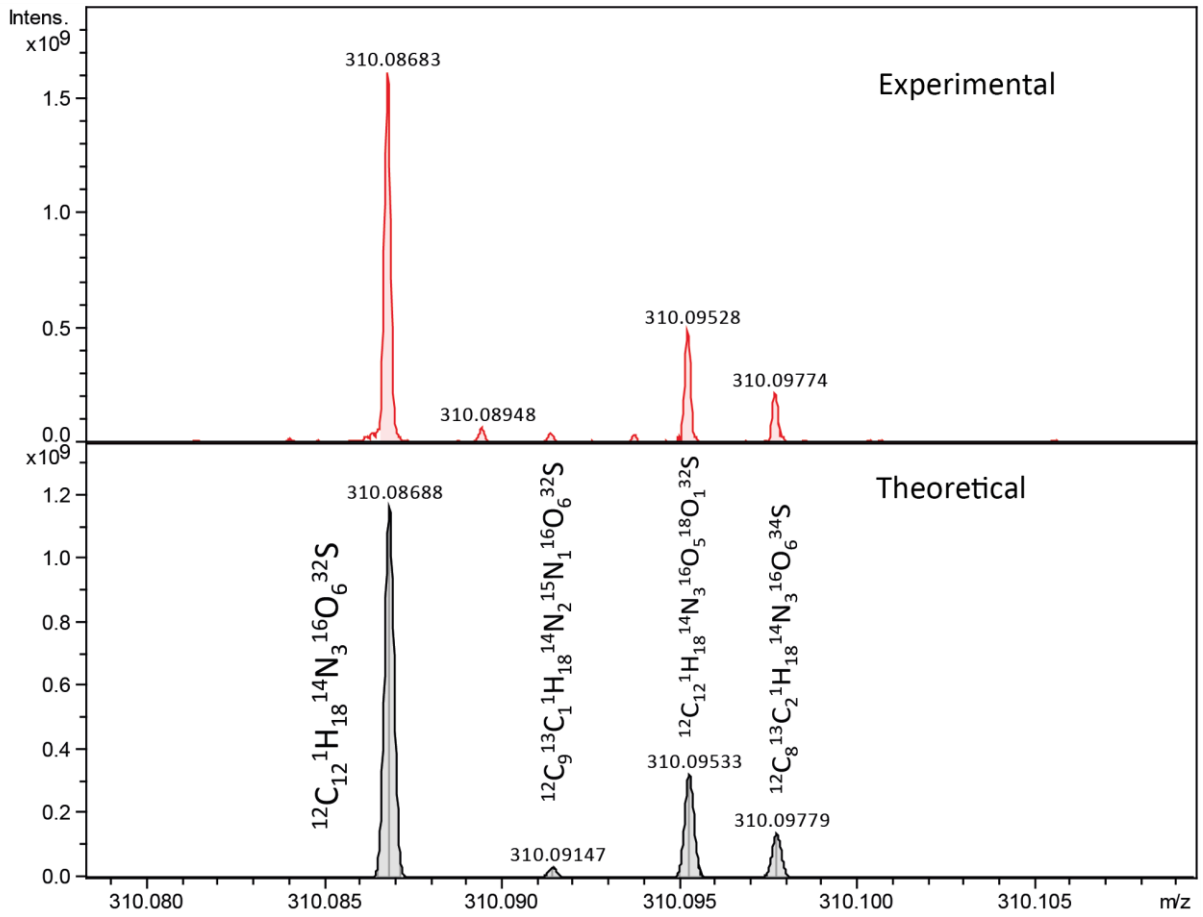

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

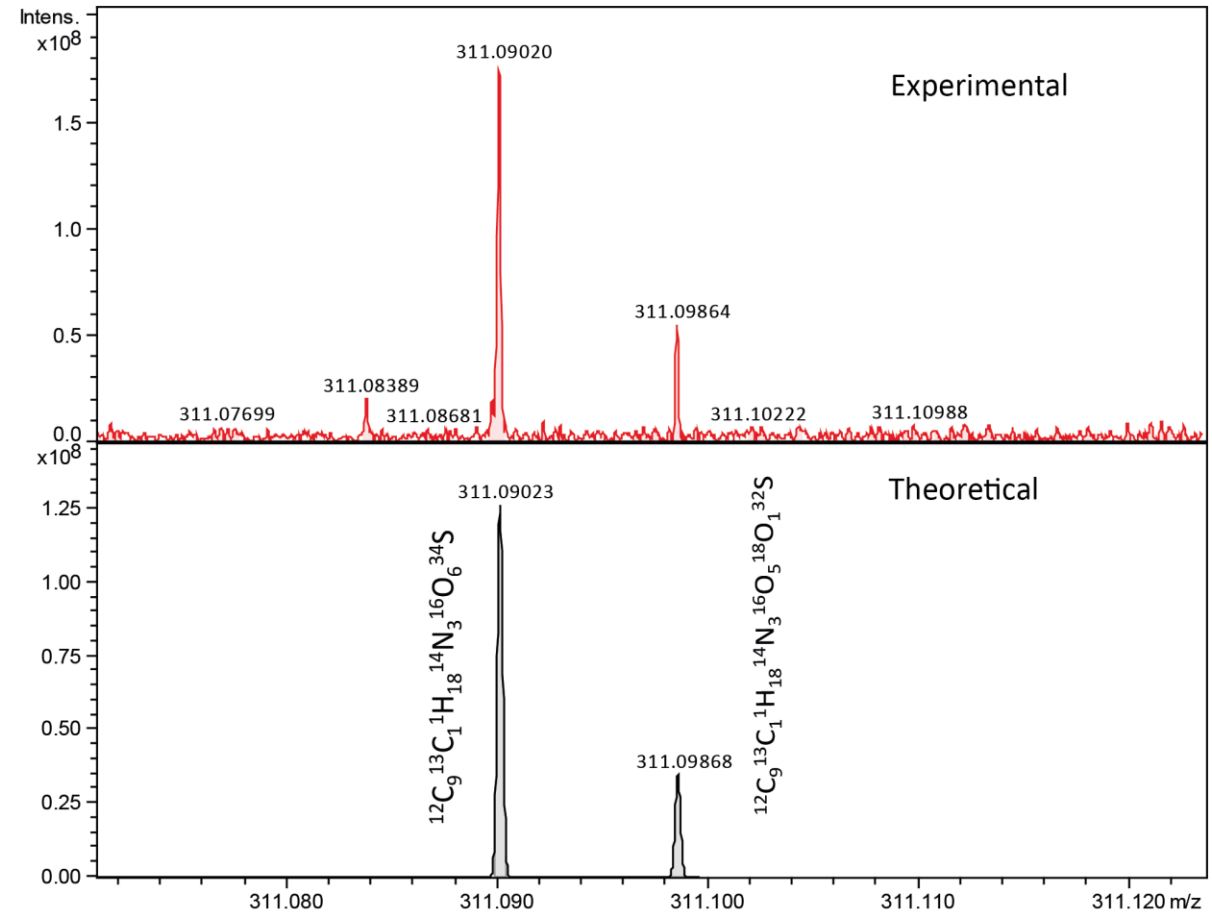

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: LIEG	**Analysis date:** 25-06-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: VWR Avantor (Acros Organics) ref 120000050 batch A0428230
Concentration for analysis: 10µmol/L in 50% acetonitrile + 0.1% formic acid @4µL/min

Analysis & instrument setup

Equipment used: SolariX XR 9.4T
Ionization mode: ESI positive
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: external **Calibrant used: phosphoric acid 0.4% in 50% ACN**
Acquisition range: 57 to **1000** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
Data processing was performed using Data Analysis 5.1 build 201.2.4019

Results

Accurate mass measured: 308.09090 *m/z*
Deviation: -0.594 ppm

Highest resolution achieved (monoisotopic peak): FWMH@m/z 308.09= 883389 broadband magnitude 16M m/z: 57-1000 (or 5,4 million narrowband magnitude m/z 309 +/- 3.4 /512k)

Isotopic distribution:

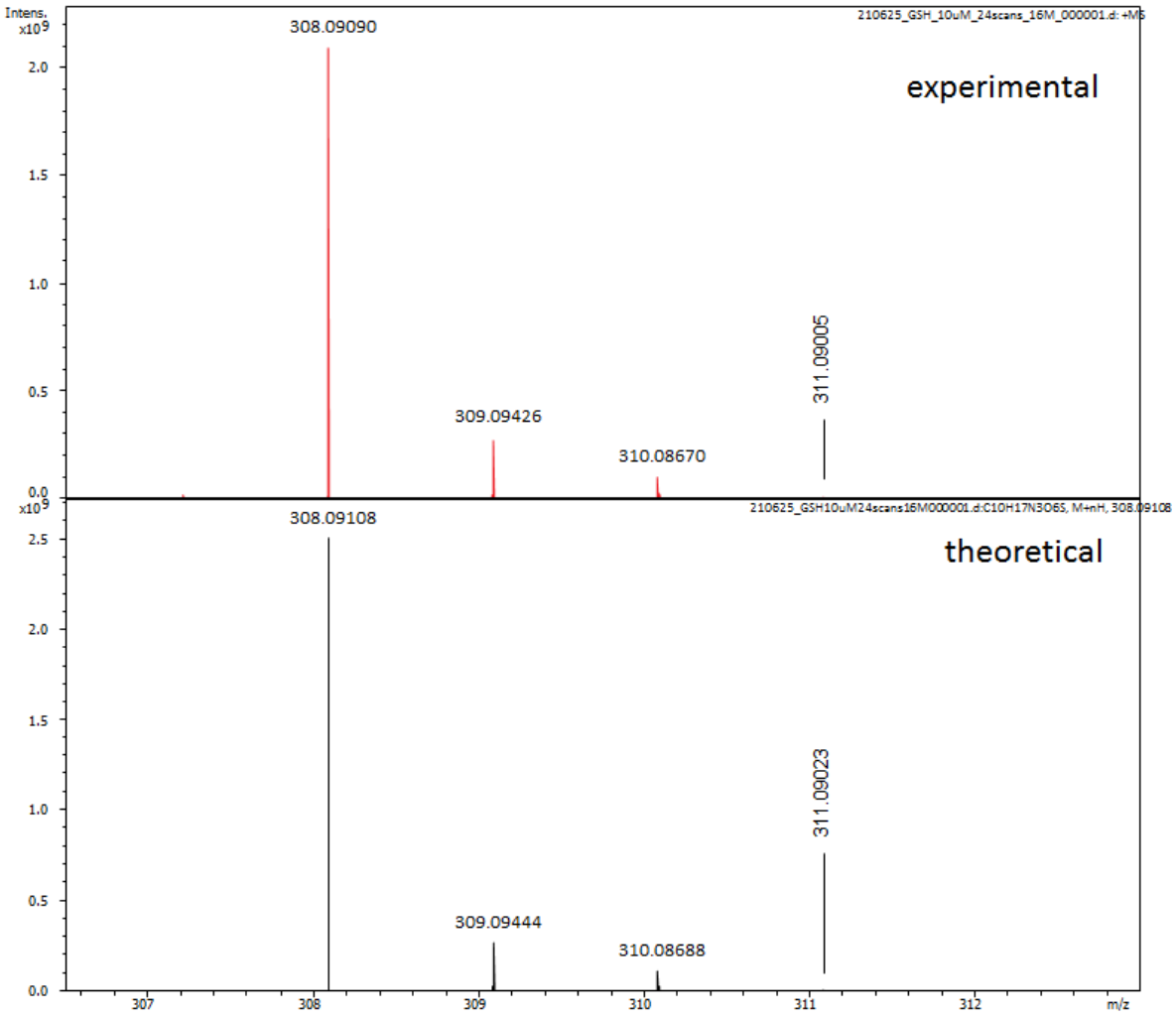

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100	0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	1.248	13.88
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	1.039	31.56
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	13.111	21.22
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	0.300	31.16
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.346	67.19
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	5.004	11.86
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	0.133	11.37
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	1.490	20.88
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.669	27.23
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.621	28.34
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.129	-2.85

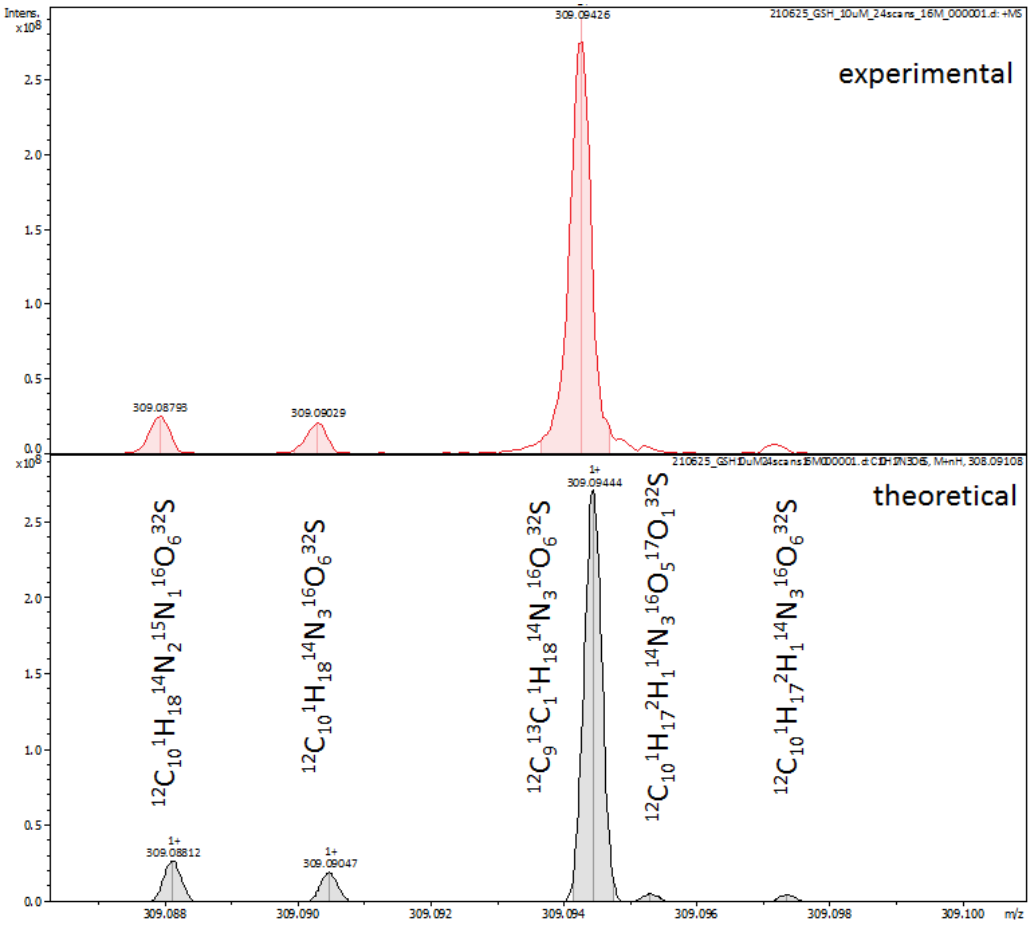

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

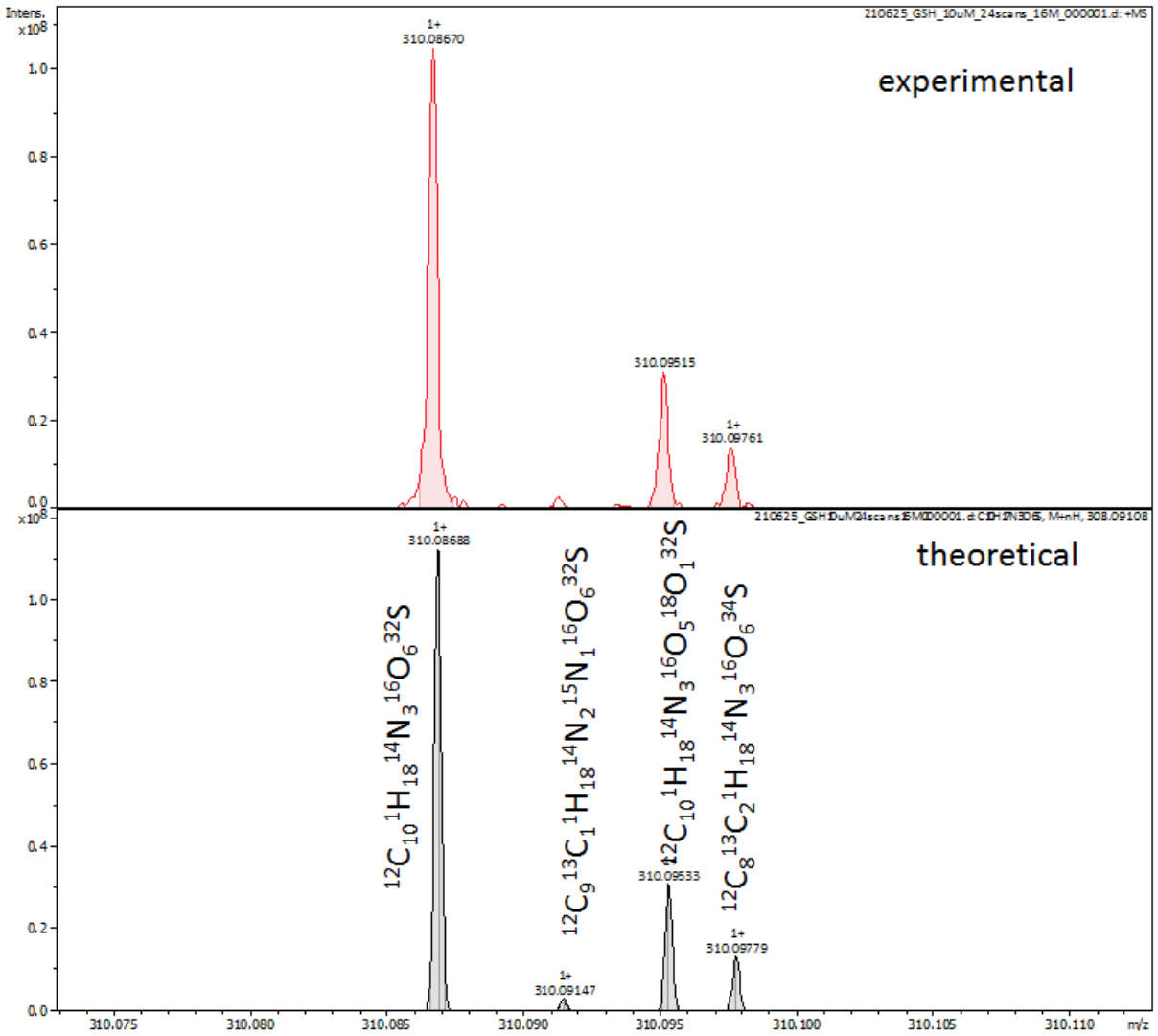

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

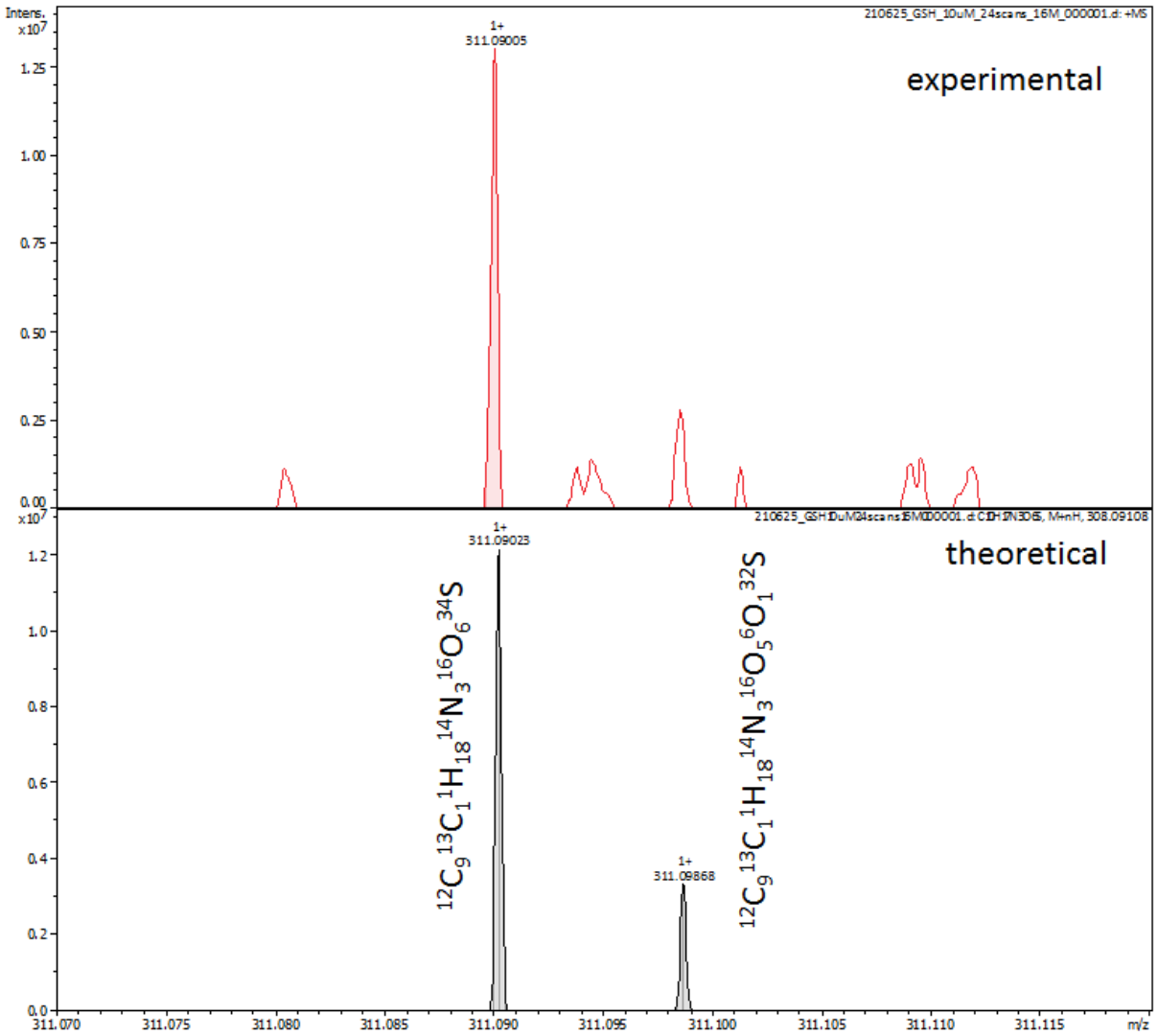

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: PRAG	**Analysis date:** 20-07-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Sigma - Aldrich
Concentration for analysis: 1mg/L

Analysis & instrument setup

Equipment used: solariX XT 15T
Ionization mode: ESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: external **Calibrant used: NaTFA**
Acquisition range: 153 to **3000** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
4M transient

Results

Accurate mass measured: 308.09107 *m/z*
Deviation: 0.03 ppm

Highest resolution achieved (monoisotopic peak): 870 598

Isotopic distribution:

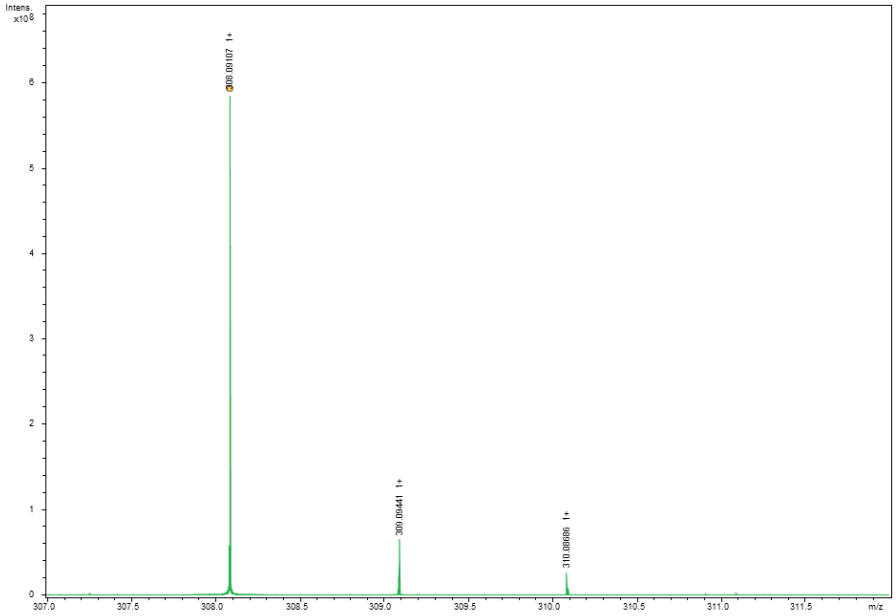

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100.00	0.00
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	1.2	0.104
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	0.6	-0.19%
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	11.1	0.284
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	n.o.	-
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	n.o.	-
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	4.5	0.026
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	n.o.	-
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	1.3	0.067
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.6	0.074
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.5	0.016
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.2	0.0670

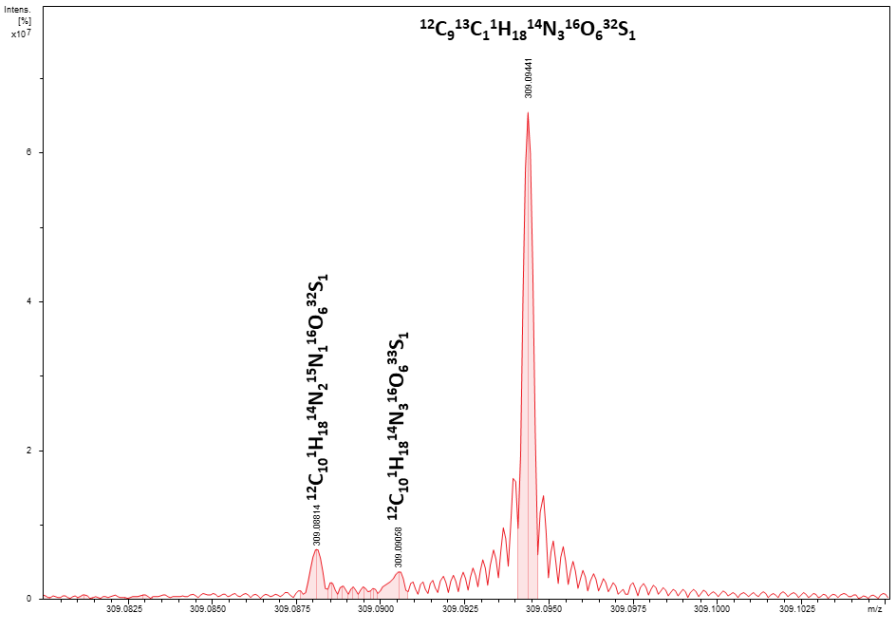

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

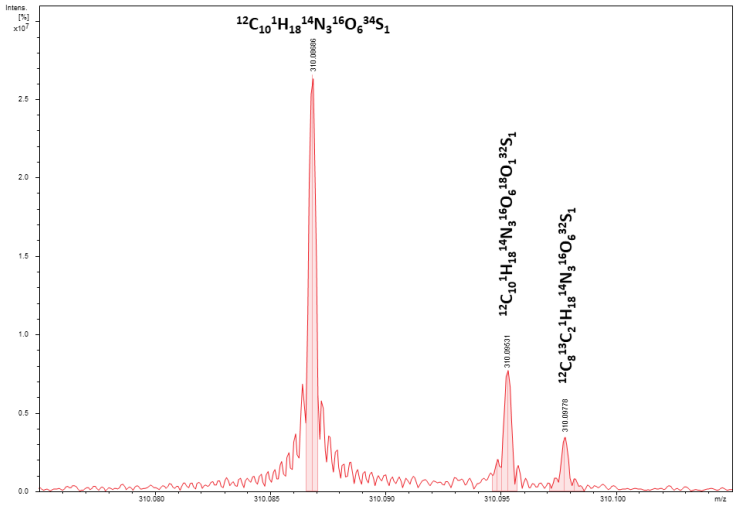

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

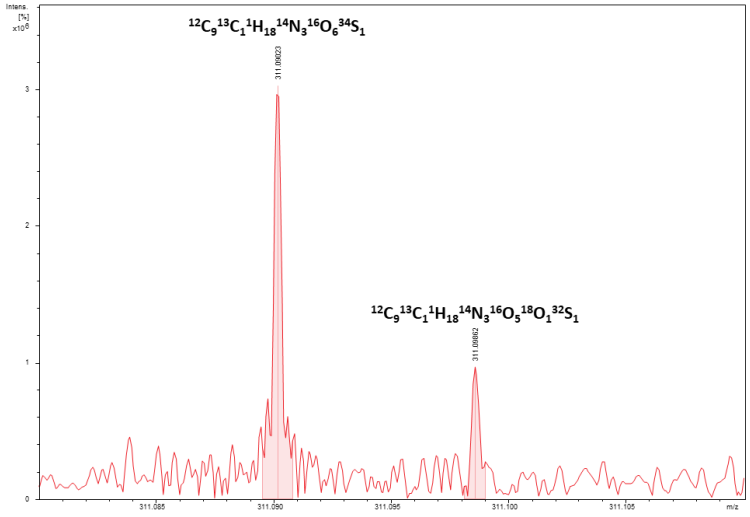

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: UHRO	**Analysis date:** 11-06-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Bruker ESI-Source
Concentration for analysis: 165 ppm; 0.165mg/ml

Analysis & instrument setup

Equipment used: solariX ESI Maldi-System; B-C 70/11 US/R - 7.05 T
Ionization mode: ESI(+)
Infusion: direct infusion
Analysis mode: magnitude R=650.000 (m/z 400)
Calibration: external **Calibrant used: Arginin Cluster**
Acquisition range: 247.2 to **1000** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
broadband 4 megaword transient length, quadrupol isolation center: 308 m/z; isolation window: 5 m/z

Results

Accurate mass measured: 308.09059 *m/z*
Deviation: 1.61 ppm

Highest resolution achieved (monoisotopic peak): 975071

Isotopic distribution:

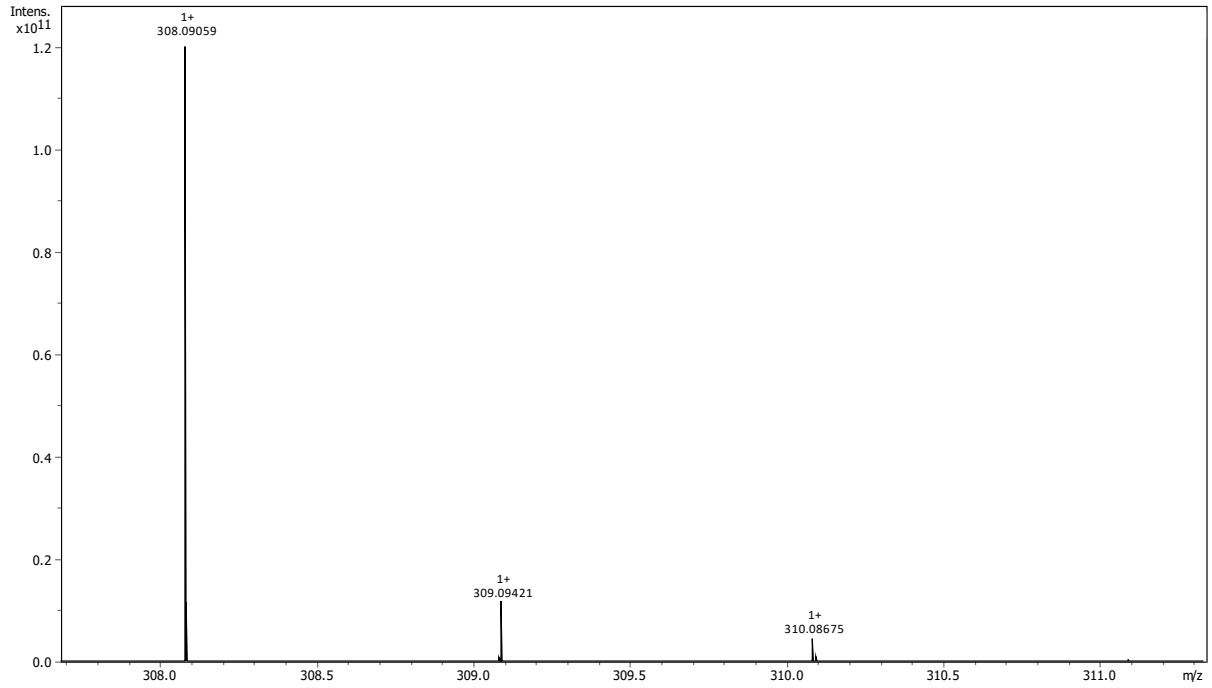

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100	0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	0.602	45.1
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	0.390	50.6
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	9.93	8.2
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	-	-
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.138	33.3
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	3.818	14.7
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	0.050	58.0
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	0.921	25.3
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.339	35.6
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.263	45.7
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.051	61.7

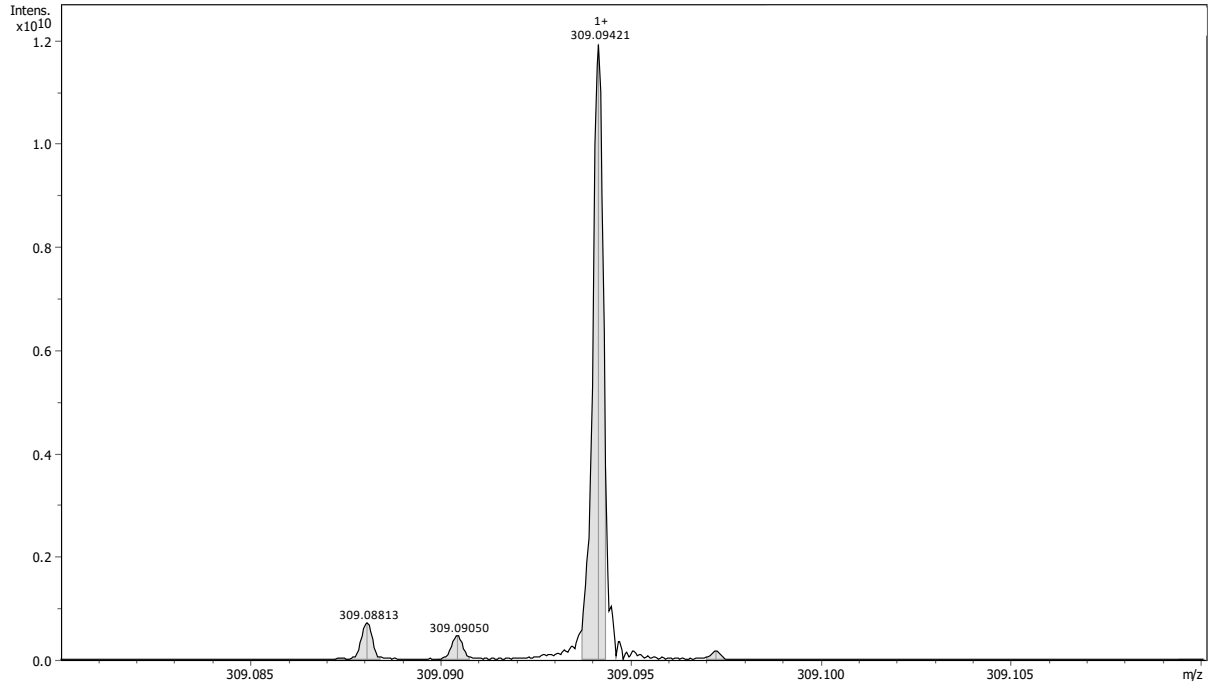

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

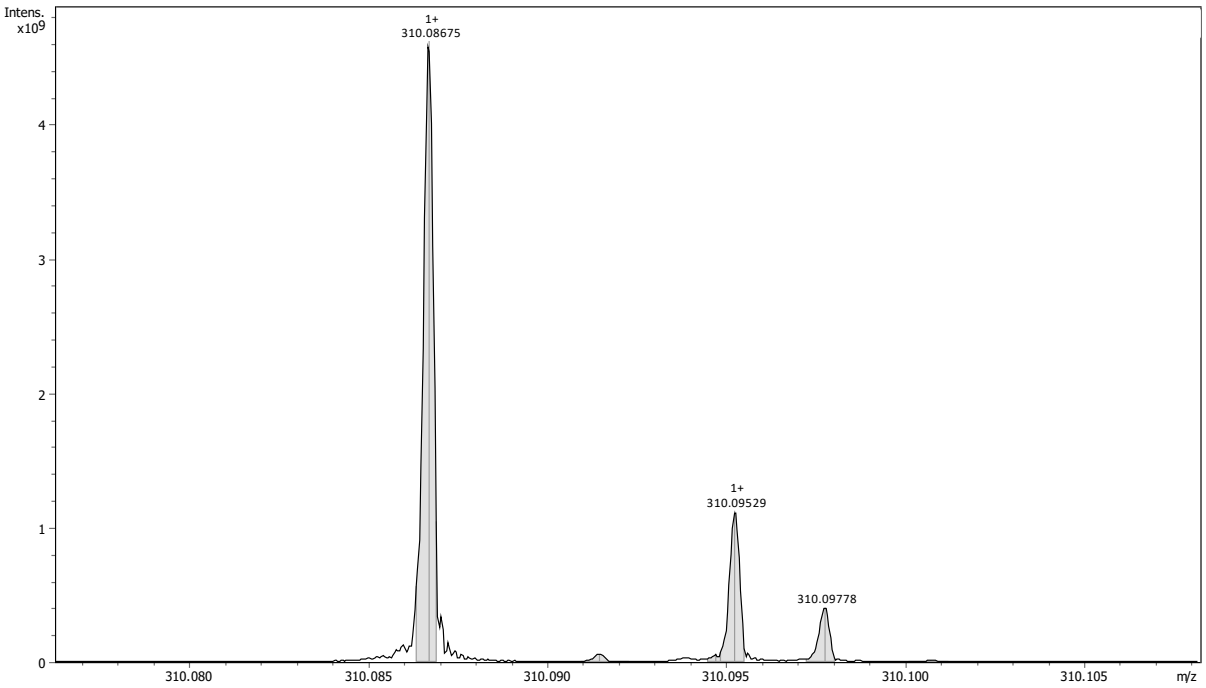

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

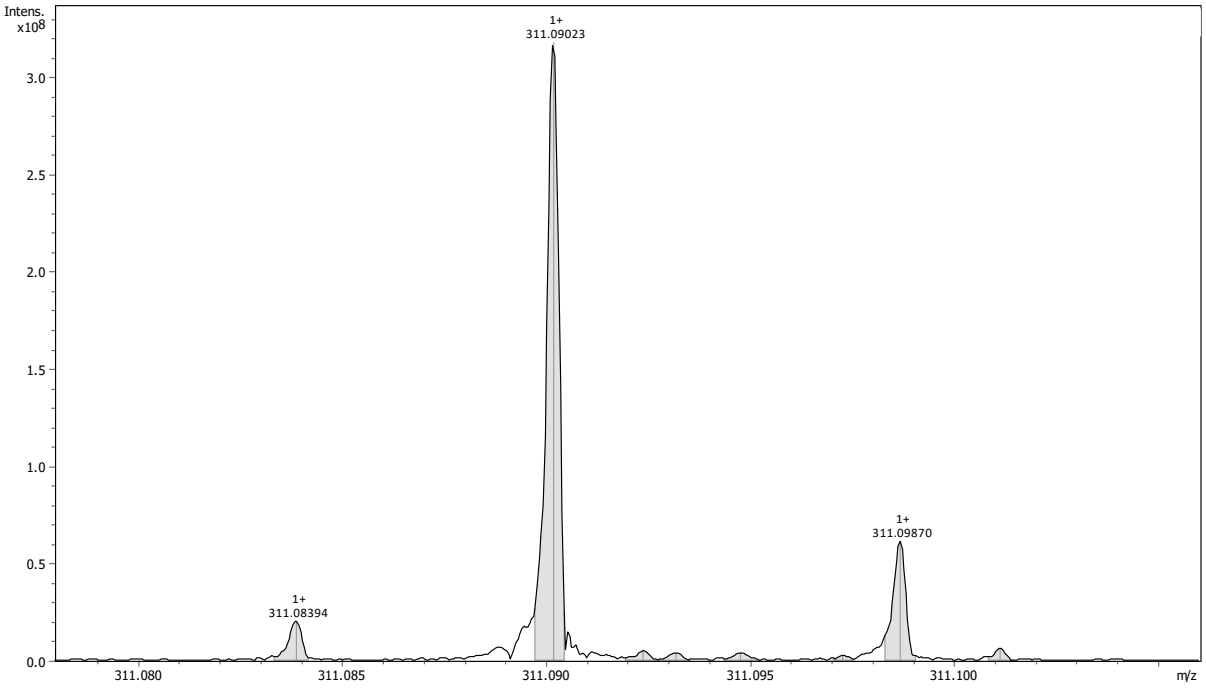

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: UEF	**Analysis date:** 06-07-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Sigma-Aldrich, Glutathione 98%, G-470-5, Lot# 03003JO-203
Concentration for analysis: 1 µM

Analysis & instrument setup

Equipment used: 12T solarix XR
Ionization mode: ESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: external **Calibrant used: Arginine 0.05 mg/ml**
Acquisition range: 172 to **5000** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
Click or tap here to enter text.

Results

Accurate mass measured: 308.0909778 *m/z*
Deviation: -0.341 ppm

Highest resolution achieved (monoisotopic peak): 1328618

Isotopic distribution:

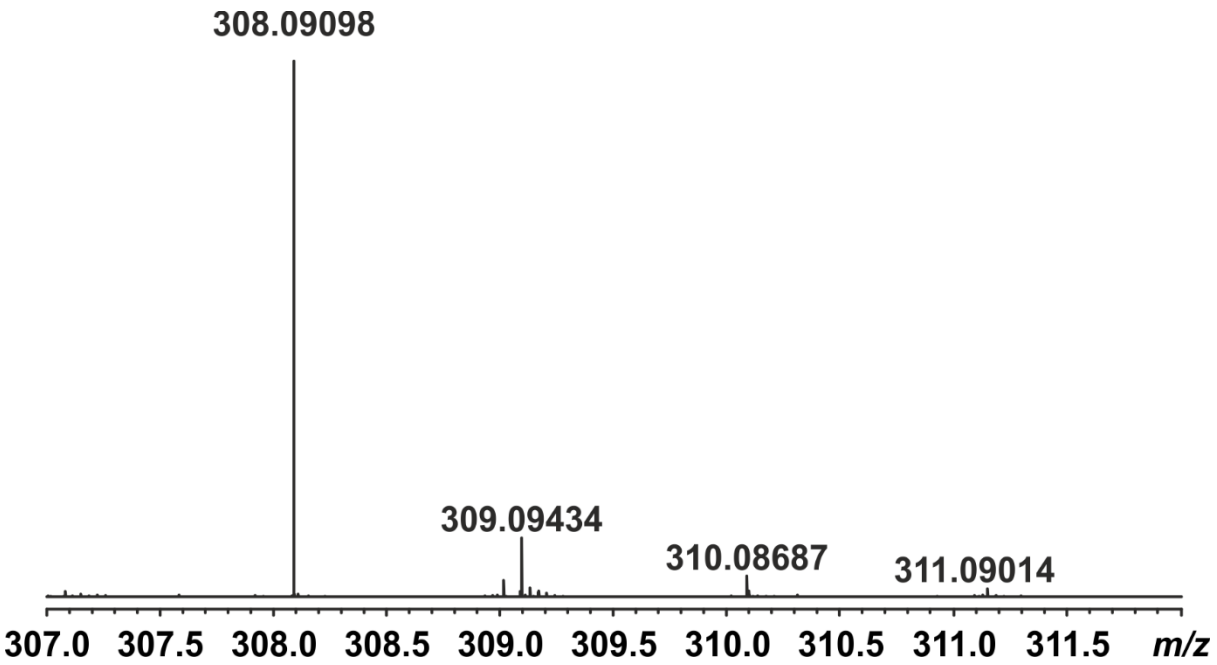

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100.00	0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	1.036	-5.43
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	0.785	-0.54
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	11.090	2.54
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	0.198	-13.17
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.291	40.53
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	3.842	-14.12
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	0.119	0.45
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	1.129	-8.47
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.488	-7.32
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.248	-48.83
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.068	-48.26

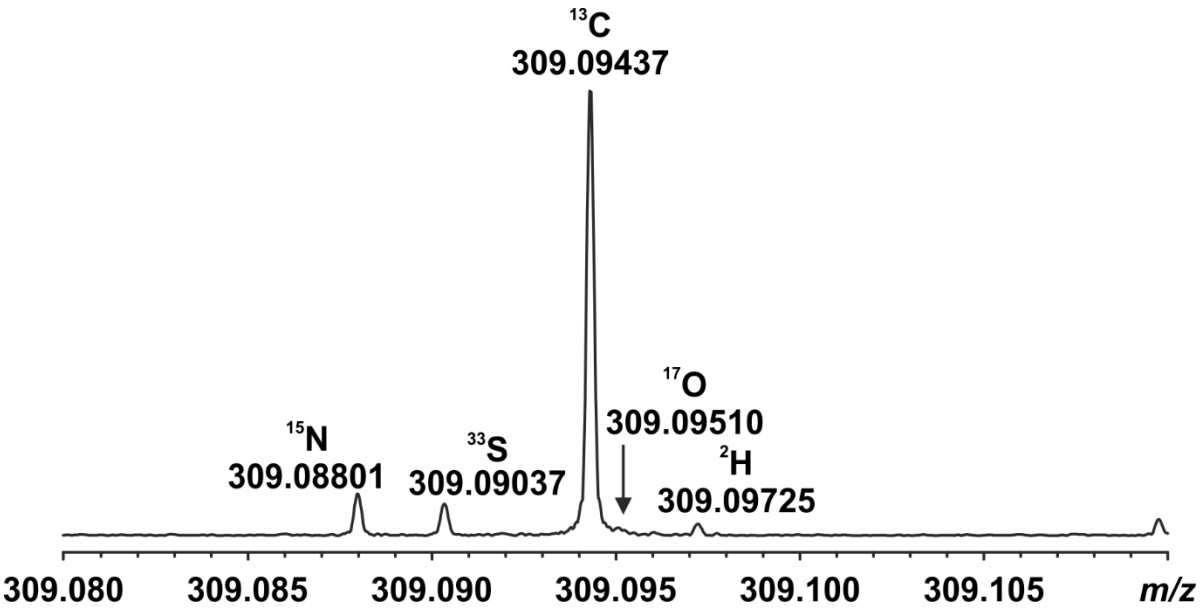

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

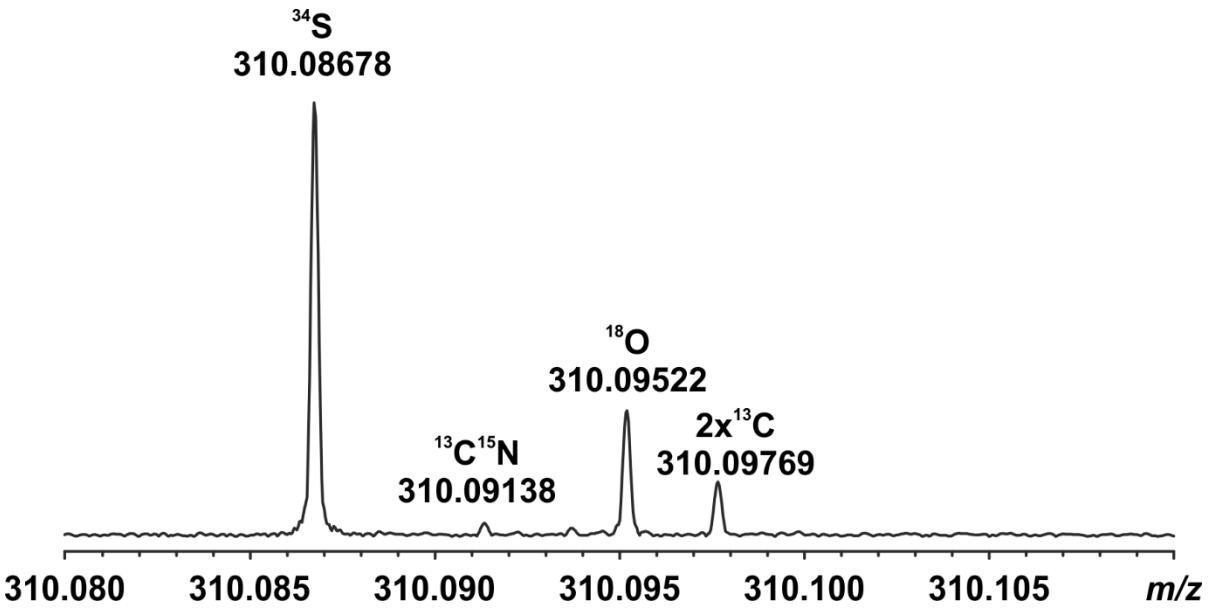

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

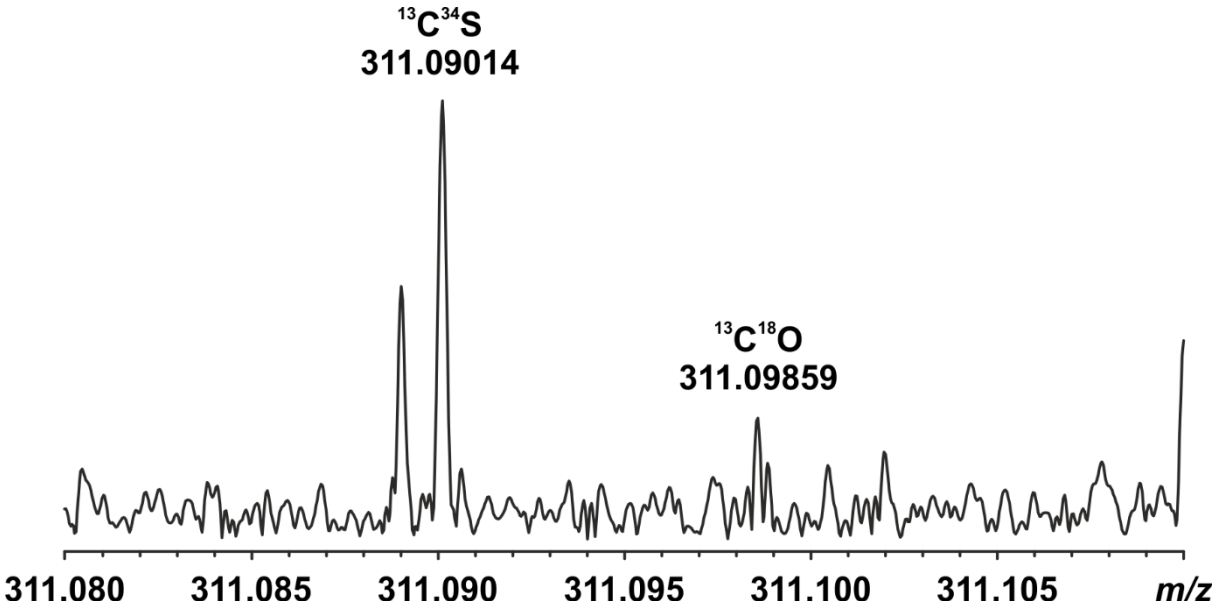

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: ROMA	**Analysis date:** 09-06-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Sigma Aldrich
Concentration for analysis: 3 microM

Analysis & instrument setup

Equipment used: *Bruker BioApex 4.7 T FT-ICR*
Ionization mode: ESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: external **Calibrant used: arginine**
Acquisition range: 200 to **400** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
Instrument installed in 1993 equipped with a cylindrical infinity cell

Results

Accurate mass measured: 308,091245 *m/z*
Deviation: +0.53 ppm

Highest resolution achieved (monoisotopic peak): 60000

Isotopic distribution:

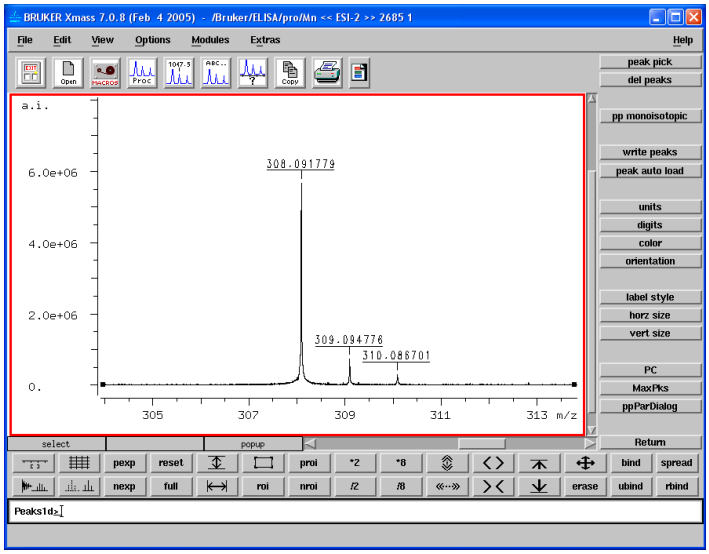

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100.00	0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	-	-
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	-	-
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	12.97	-
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	-	-
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	-	-
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	4.8	-
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	-	-
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	1.8	-
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	-	-
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.65	-
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	-	-

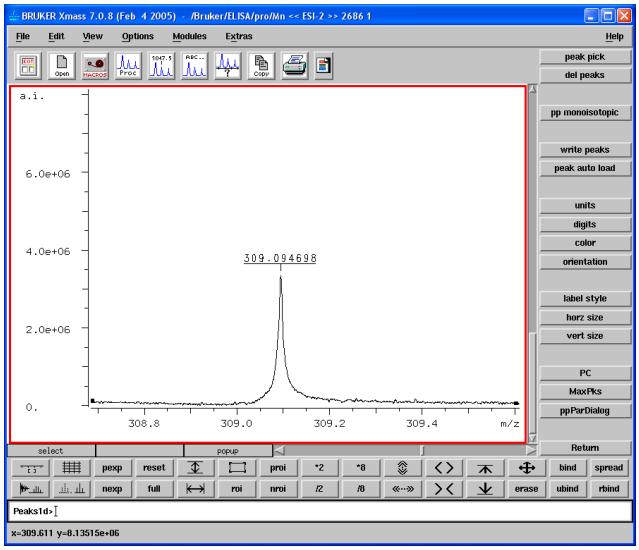

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

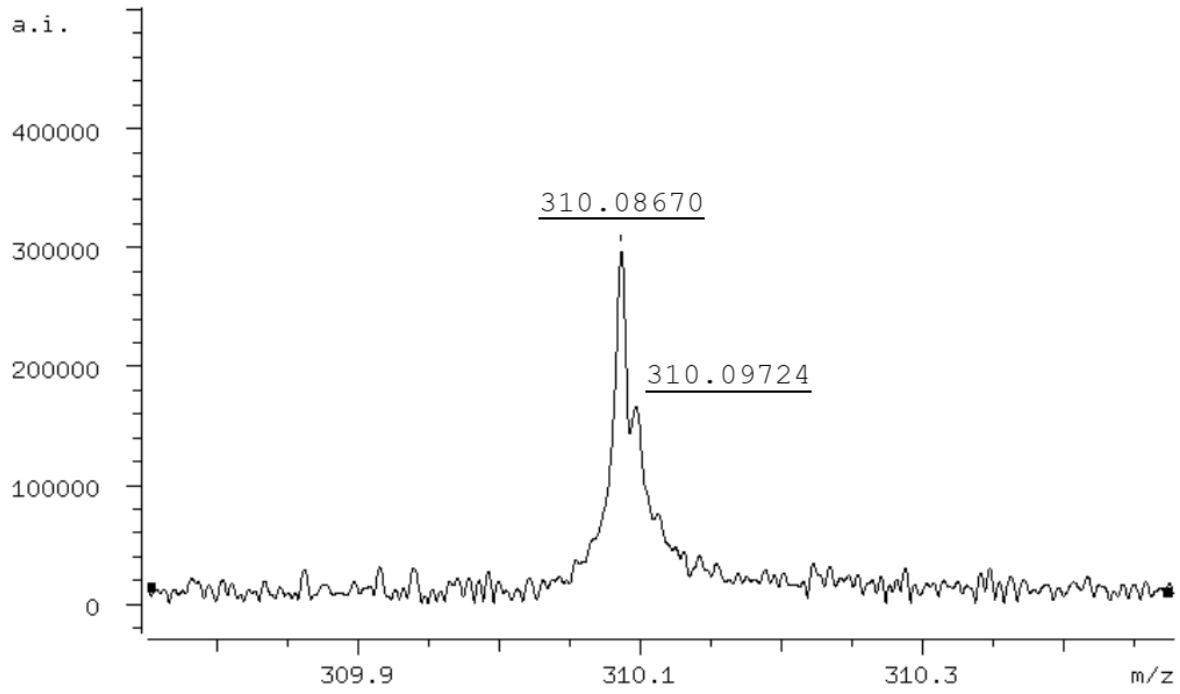

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

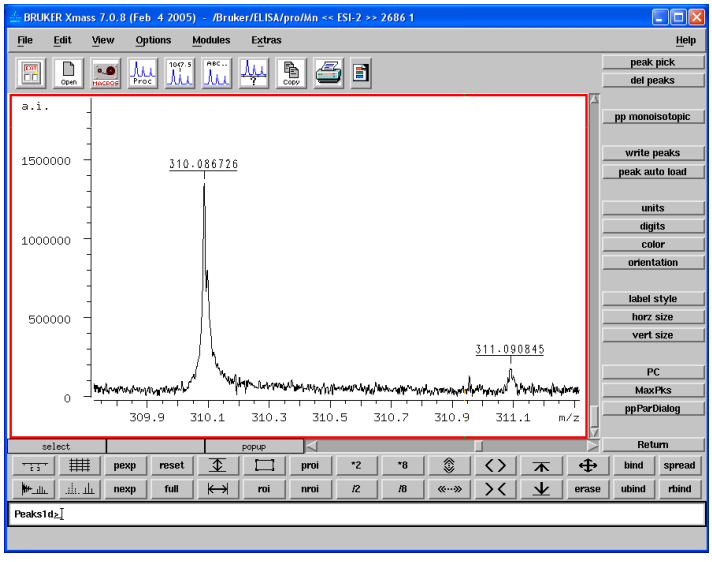

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: FC-LISB	**Analysis date:** 16-06-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Saccharomyces cerevisiae extract
Concentration for analysis: Click or tap here to enter text.

Analysis & instrument setup

Equipment used: FT-ICR Bruker Solarix XR 7T
Ionization mode: ESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: internal **Calibrant used: Leucine enkephalin (human)**
Acquisition range: 200 to **3000** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
Magnitude mode 4M acquisition size. DATA Analysis 5.0 SR1 build 203.2.3586

Results

Accurate mass measured: 308.09109 *m/z*
Deviation: -0.03 ppm

Highest resolution achieved (monoisotopic peak): 695339

Isotopic distribution:

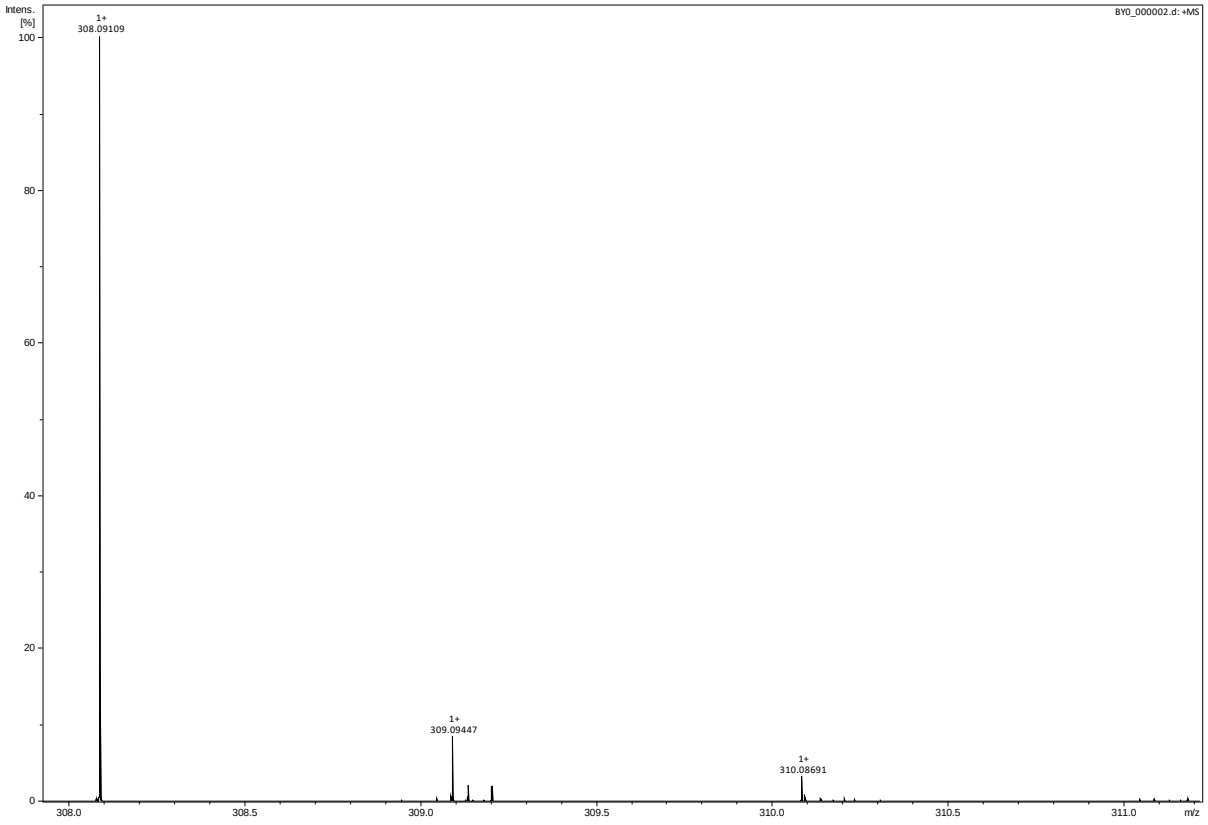

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100	0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	0.8254	-25
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	0.6072	-23
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	8.578	-20
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	0.2693	18
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.2033	-2
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	3.354	-25
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	0.07809	-34
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	0.9411	-24
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.4158	-21
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.3534	-27
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.1127	-15

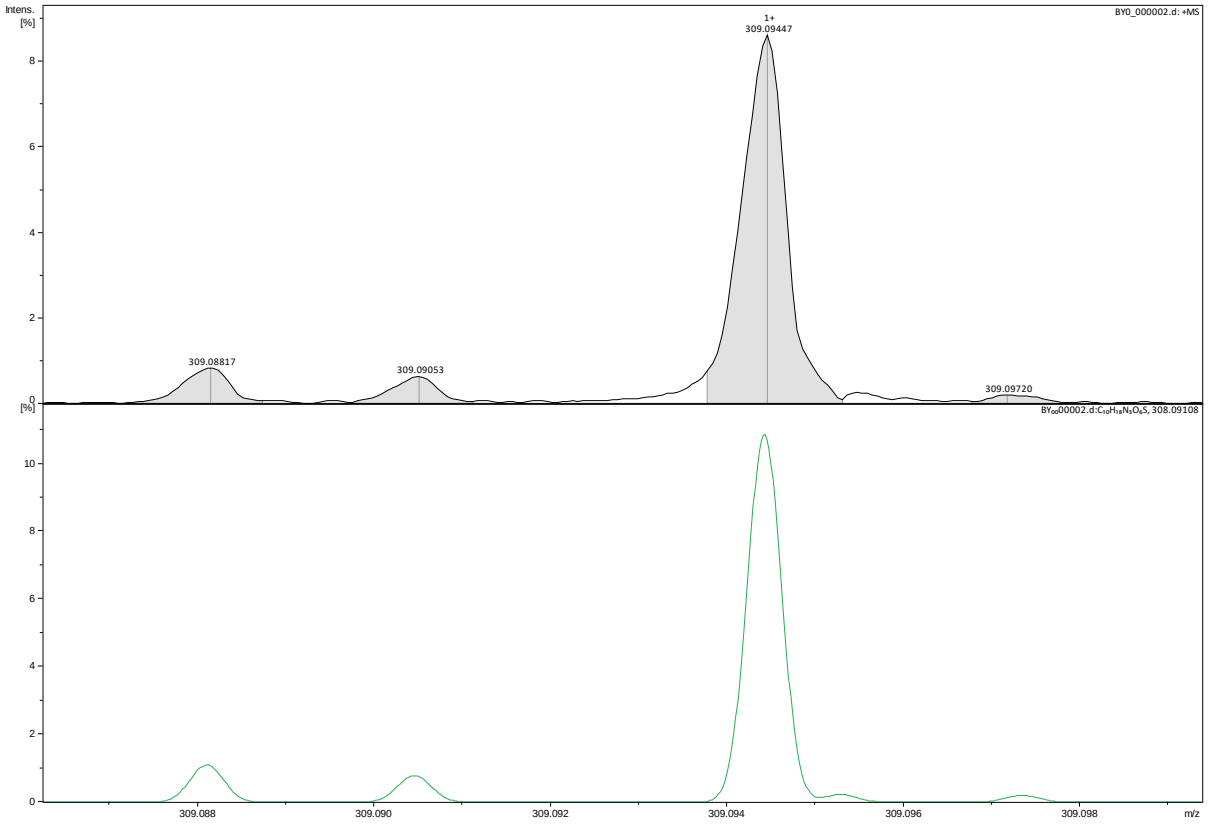

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

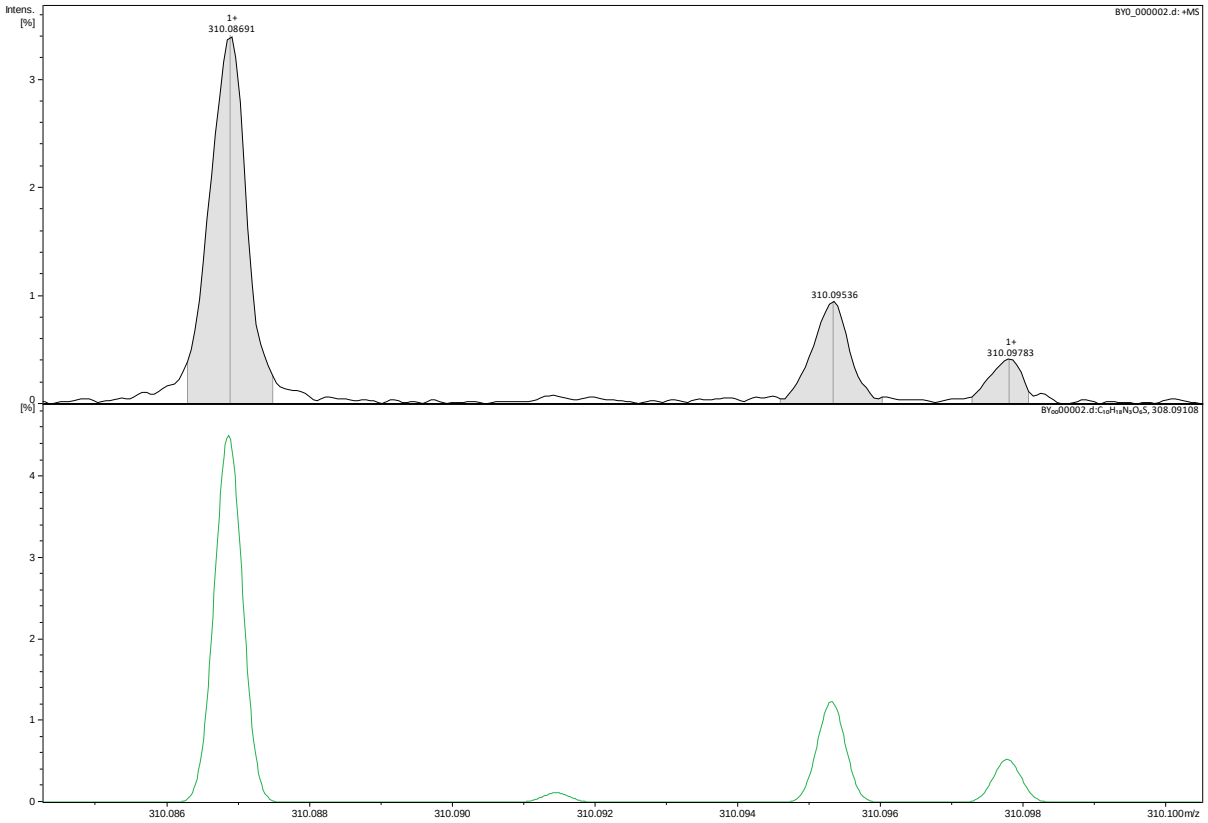

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

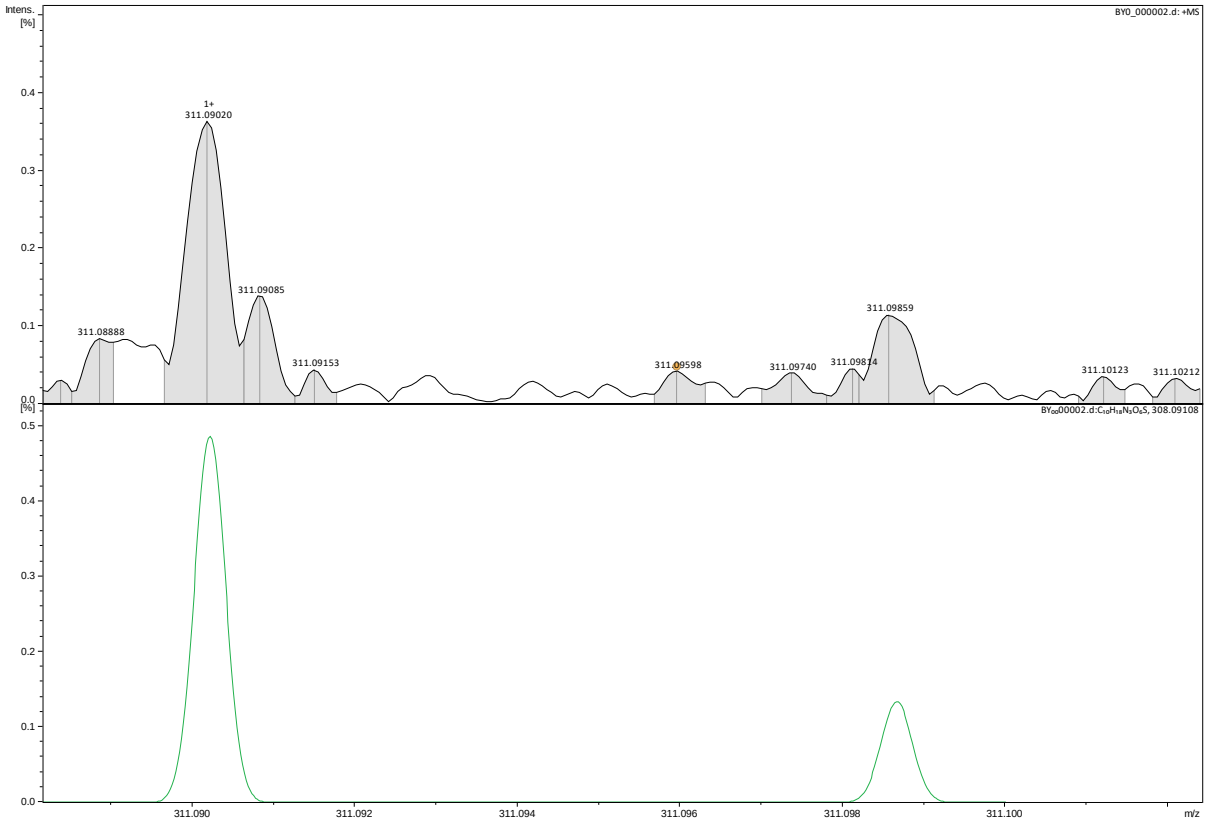

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: MOSC	**Analysis date:** 22-06-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Sigma
Concentration for analysis: 5 mg/L

Analysis & instrument setup

Equipment used: 7T Apex Ultra with DHC
Ionization mode: ESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: internal **Calibrant used: Arginine**
Acquisition range: 150 to **1500** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
Also there is a second file with Narrow Band accumulation

Results

Accurate mass measured: 308.09107 *m/z*
Deviation: 0.03 ppm

Highest resolution achieved (monoisotopic peak): 971978 in Bband and 1877698 in Nband

Isotopic distribution:

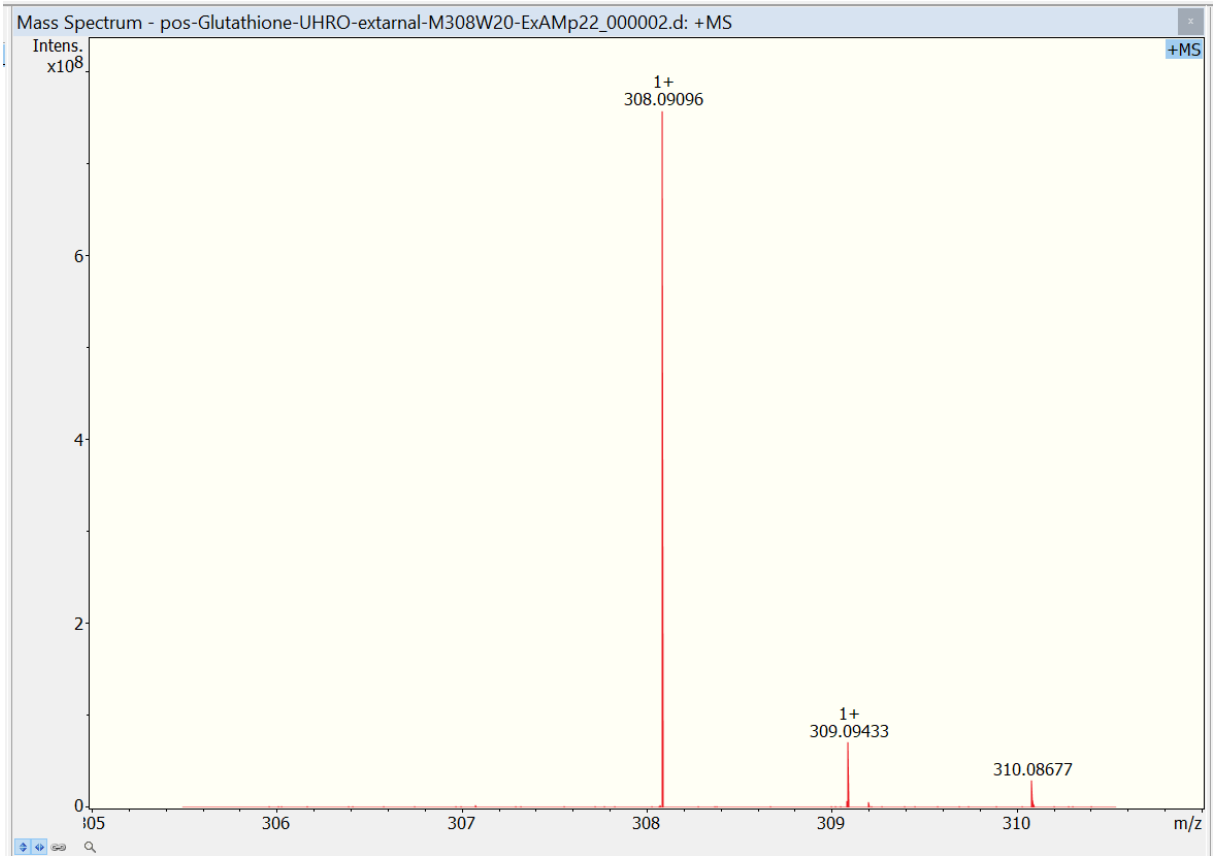

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100	0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	0.929	-0.181
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	0.709	-0.091
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	9.451	-0.136
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	0.178	-0.052
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.270	0.0.060
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	3.952	-0.568
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	0.191	0.071
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	1.170	-0.059
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.495	-0.035
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.606	0.116
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.192	0.062

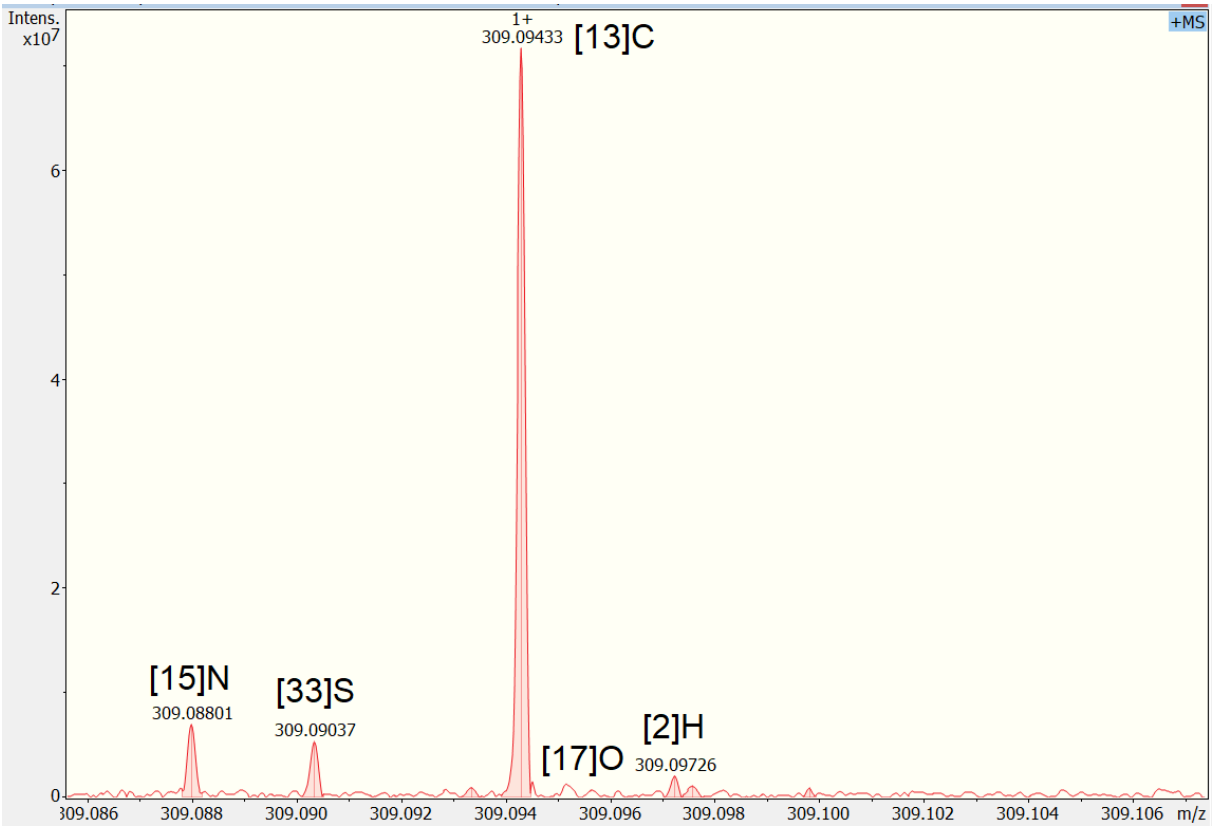

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

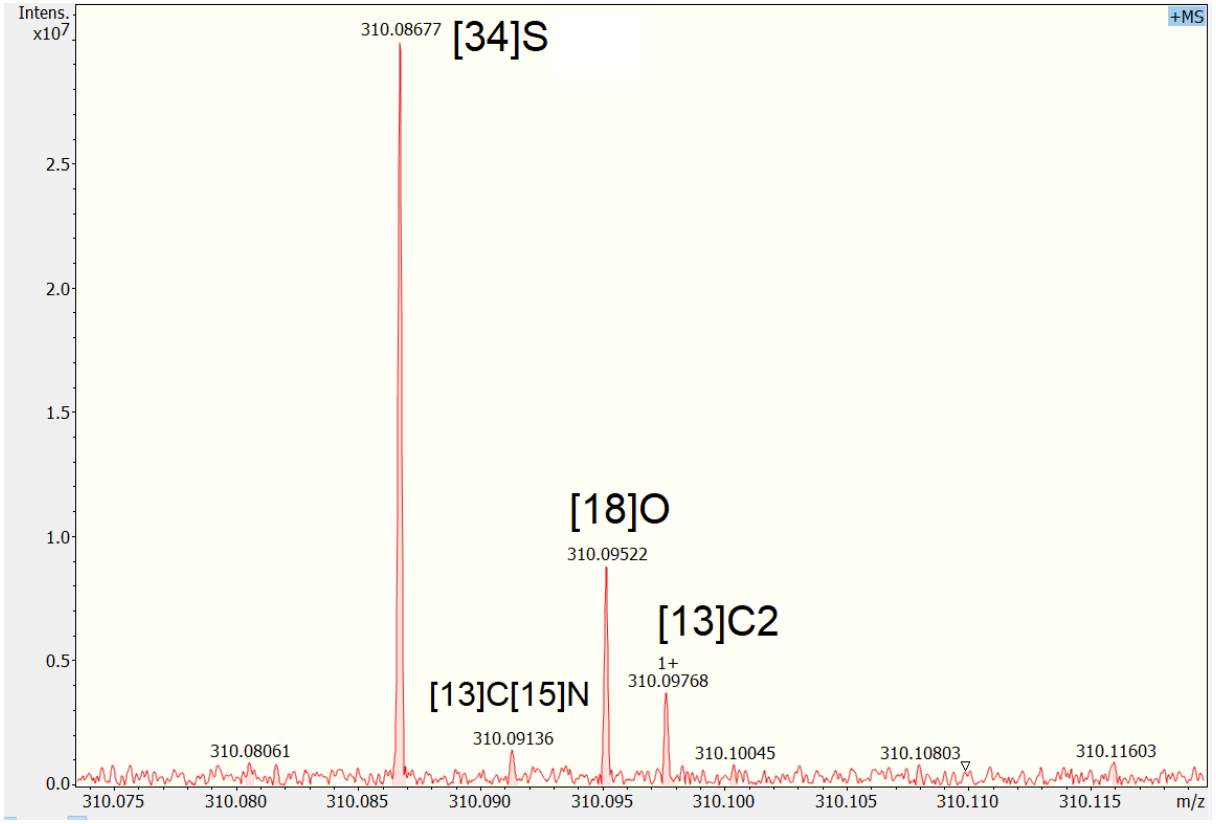

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

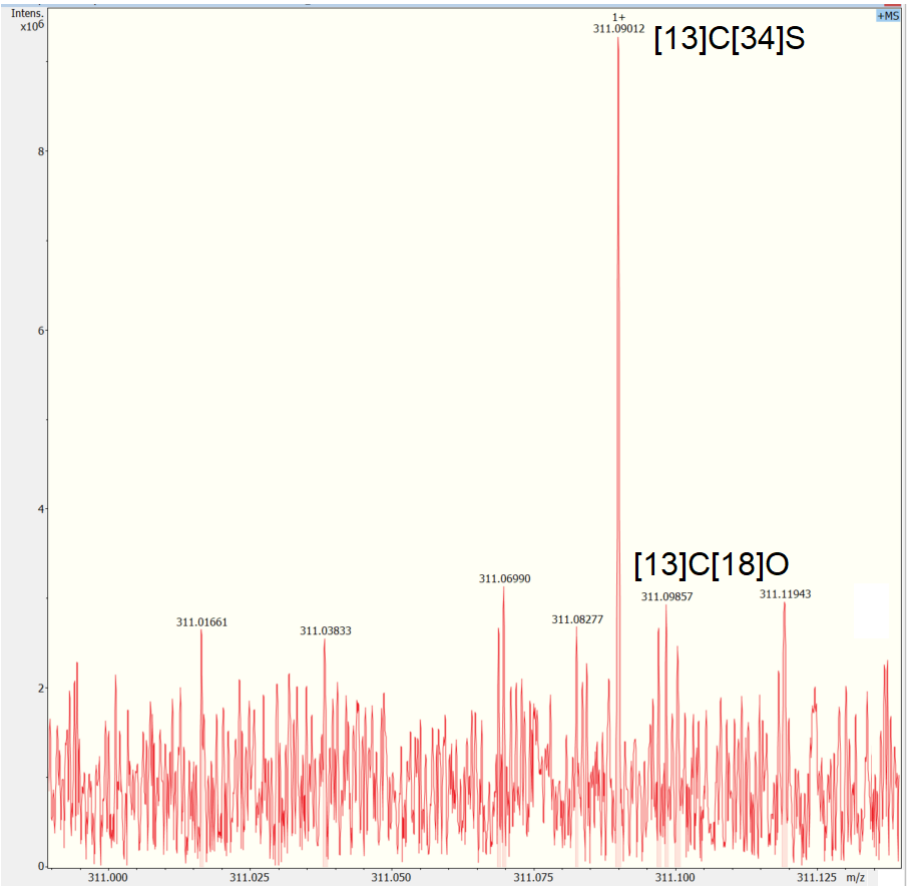

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: ROUEN	**Analysis date:** 24-06-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Sigma Aldrich
Concentration for analysis: 1x10-4mg/mL

Analysis & instrument setup

Equipment used: FTICR Solarix XR 12T
Ionization mode: ESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: internal **Calibrant used: Glutathion and isosopologues**
Acquisition range: 122.8 to **1000** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
Glutathion was isolated in the quadrupole with a isolation window of 15 Da. Spectrum was recorded in 4M, resulting in a 1.4s FID.

Results

Accurate mass measured: 308.09108 *m/z*
Deviation: 0.01 ppm

Highest resolution achieved (monoisotopic peak): 492 276

Isotopic distribution:

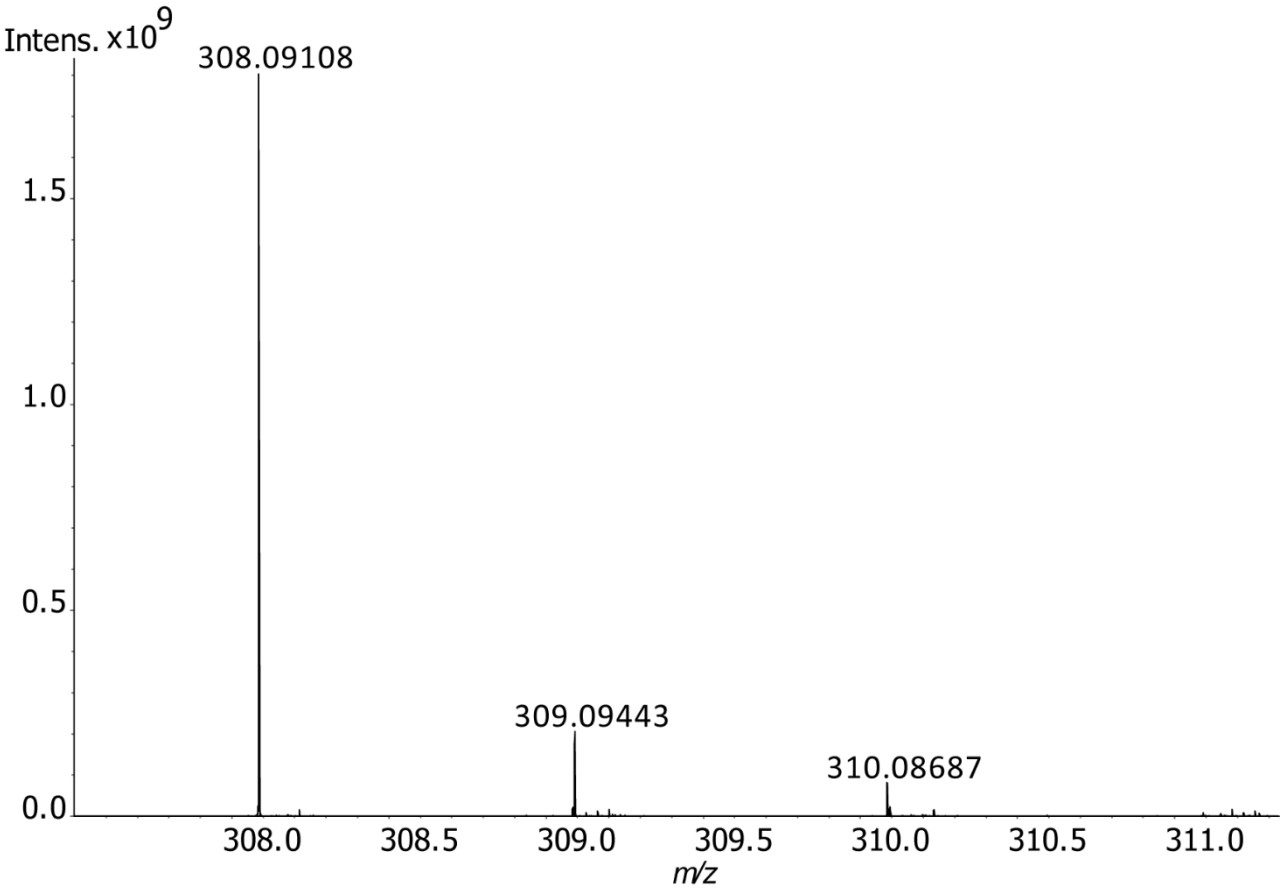

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100	0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	1.1765	0.080
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	0.8028	0.073
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	11.453	0.059
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	0.4603	1.010
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.3181	0.537
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	4.5280	0.012
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	0.1372	0.153
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	1.2207	-0.010
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.5563	0.058
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.4749	-0.019
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.1315	-0.011

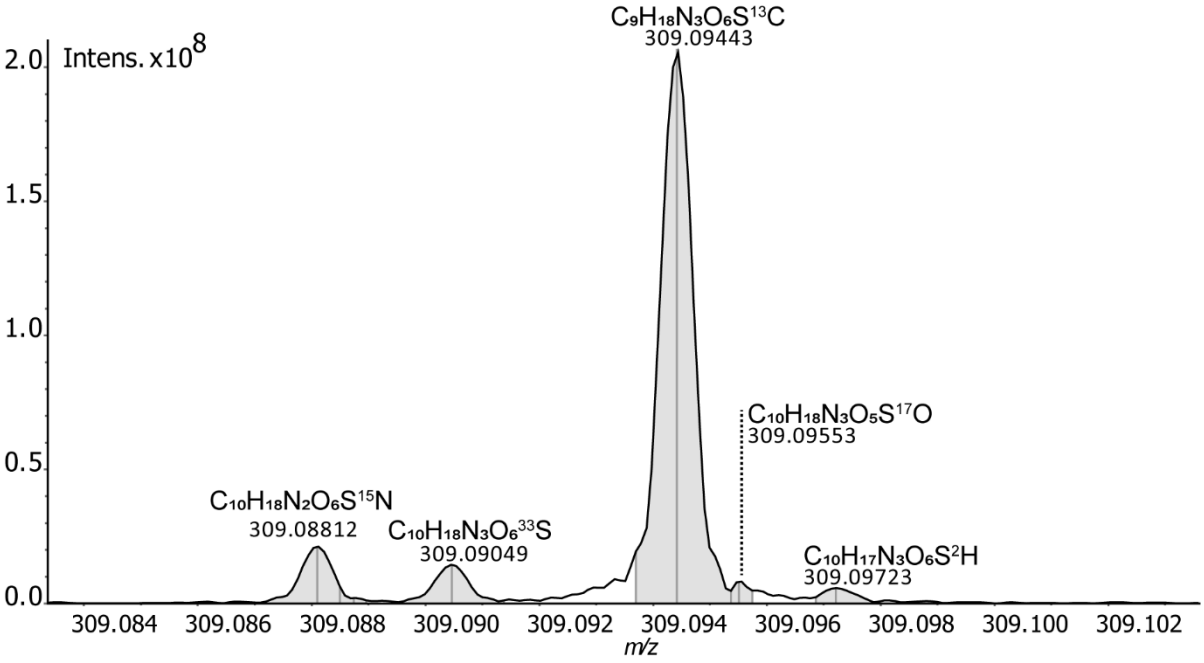

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

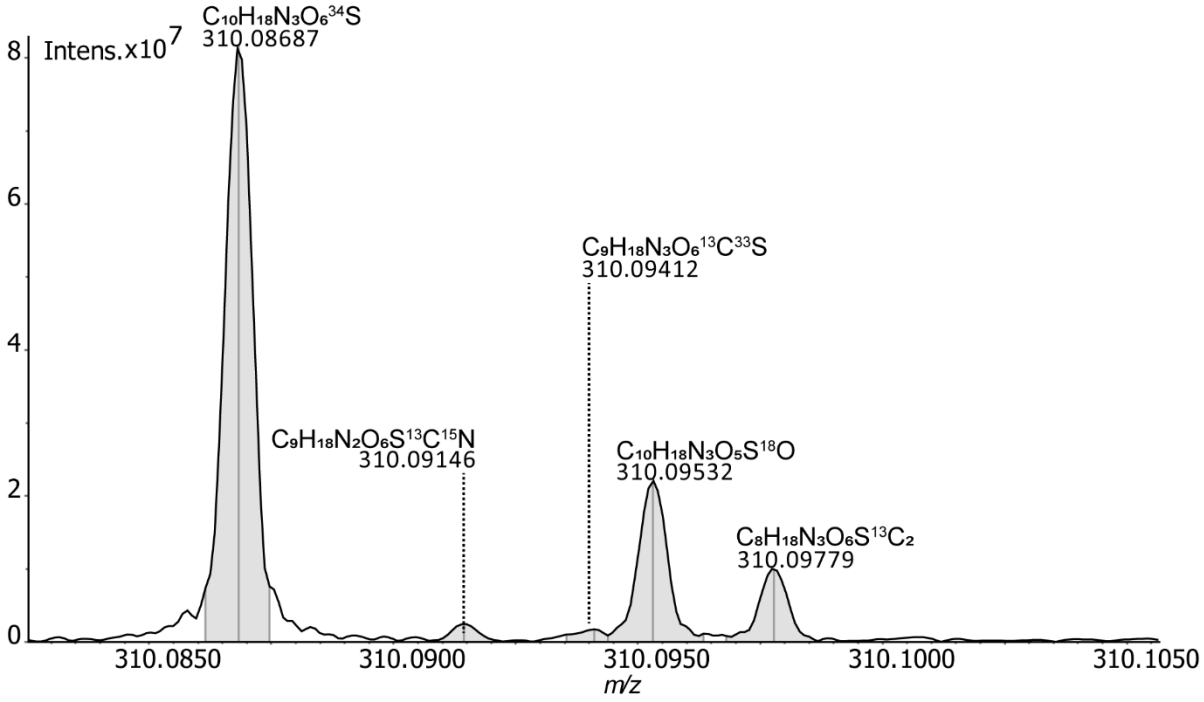

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

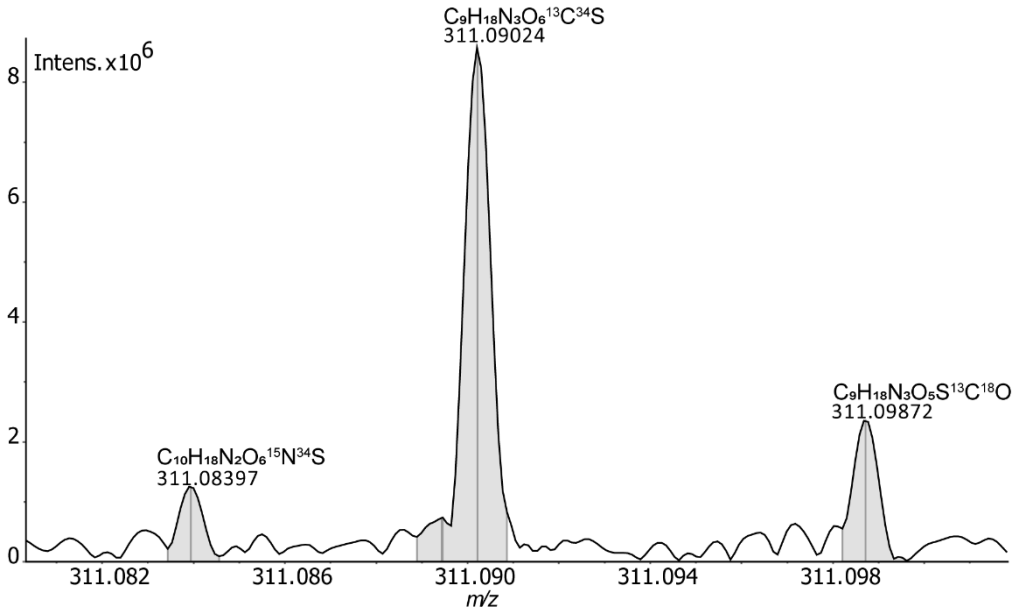

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: ORSAY	**Analysis date:** 16-06-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Click or tap here to enter text.
Concentration for analysis: 6.5 x 10-6 M

Analysis & instrument setup

Equipment used: FT-ICR 7T
Ionization mode: ESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: external **Calibrant used: TFANa**
Acquisition range: 250 to **1000** *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
Click or tap here to enter text.

Results

Accurate mass measured: 308.091404 *m/z*
Deviation: 1.0 ppm

Highest resolution achieved (monoisotopic peak): 918 000

Isotopic distribution:

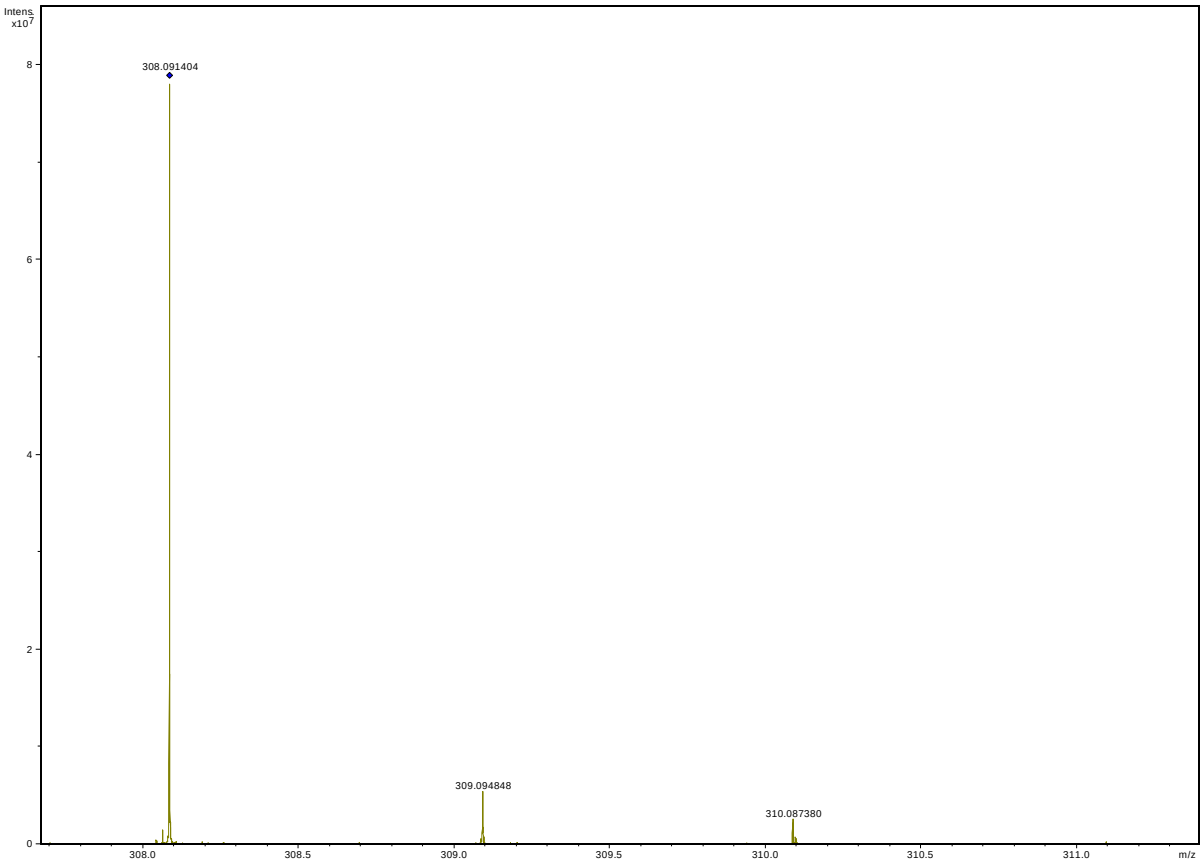

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (m/z)	Deviation (%)
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100	1.0 ppm	0.0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	0.610	1.4 ppm	-44.3
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	-	-	-
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	6.864	1.3 ppm	-36.5
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	-	-	-
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.221	3.6 ppm	6.8
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	3.236	1.6 ppm	-27.7
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	-	-	-
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	0.874	1.6 ppm	-29.1
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.363	1.6 ppm	-31.0
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.277	1.6 ppm	-42.8
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.059	1.3 ppm	-55.6

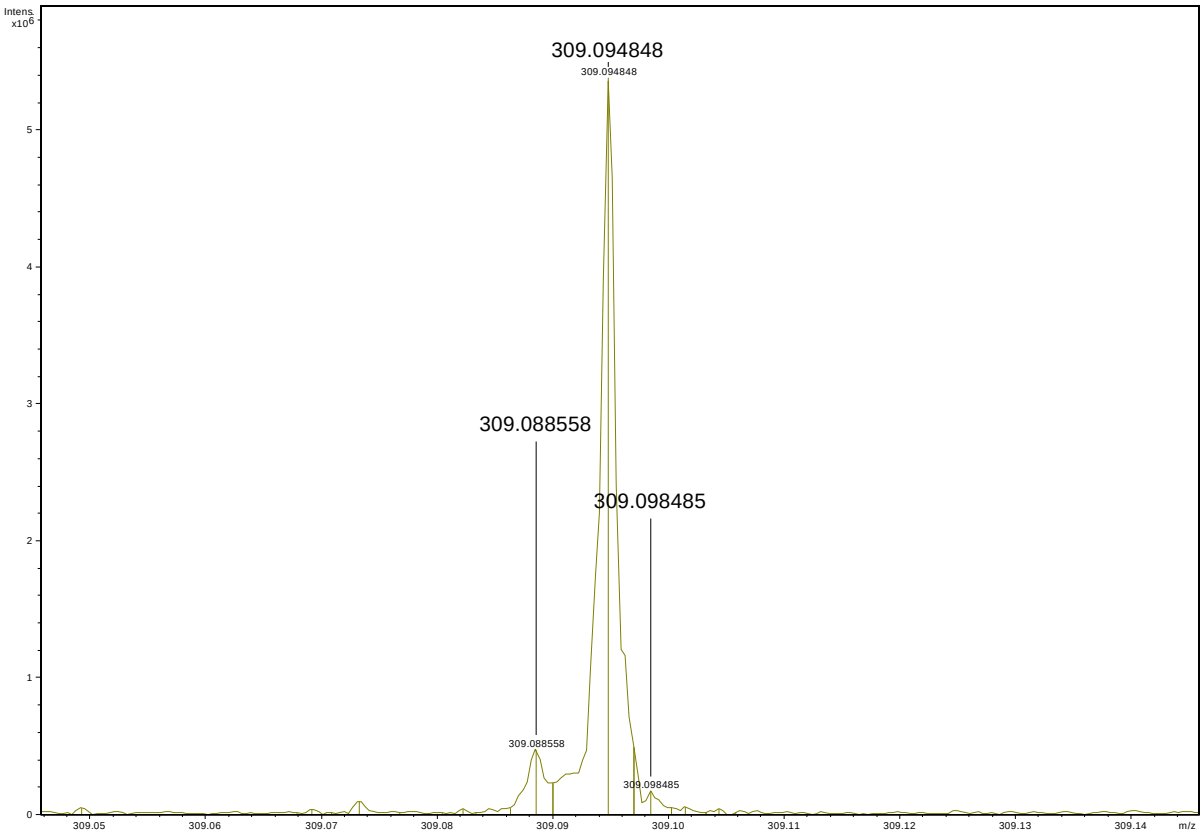

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

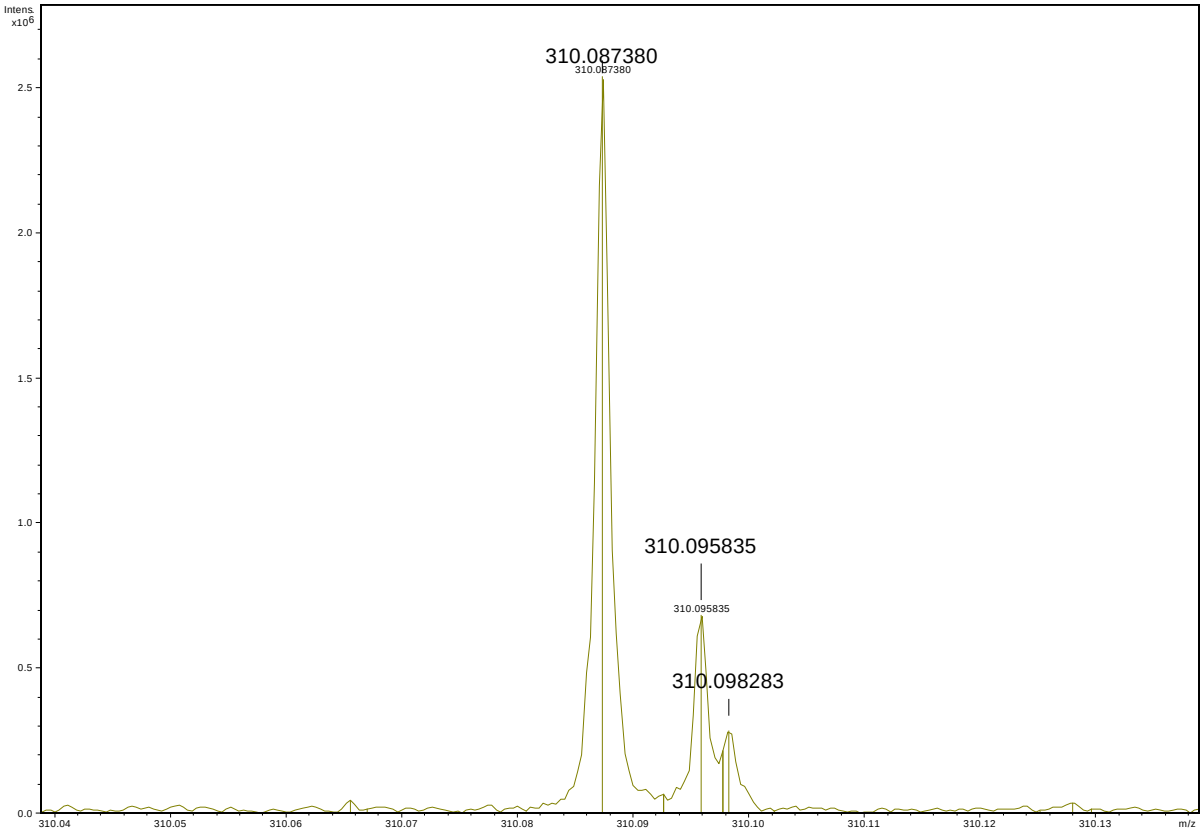

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

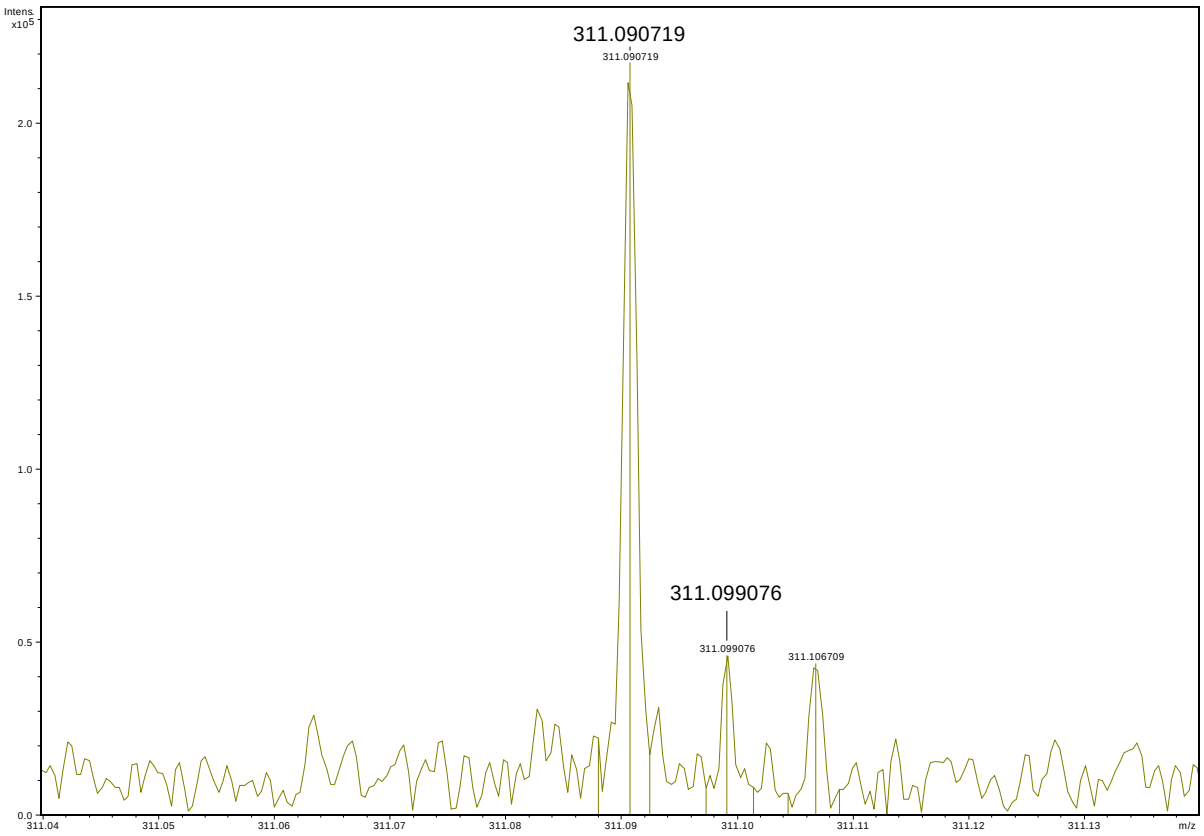

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

TNA Center: WARW	**Analysis date:** 06-12-2021

Sample: Glutathione $C_{10}H_{17}N_3O_6S$

Monoisotopic mass: 307.08380644 (Computed by PubChem 2.1; PubChem release 2021.05.07)
Product source: Merck (Product number: G4251, L-Glutathione reduced;)
Concentration for analysis: 10 uM

Analysis & instrument setup

Equipment used: Bruker Solarix XR 15T FT-ICR MS
Ionization mode: ESI+
Infusion: direct infusion
Analysis mode: magnitude broad band
Calibration: Quadratic mass calibration **Calibrant used: Arginine**
Acquisition range: 120 to 1000 *m/z*

Notes (indicate any other information related to instrument setup or relevant for the analysis):
The data shown in this report is based on 100 acquired scans, with accumulation time of 0.08s and dataset size of 16M in broadband mode.

Results

Accurate mass measured: 308.091084 *m/z*
Deviation: 0.0032 ppm

Highest resolution achieved (monoisotopic peak): 1,956,550

Isotopic distribution:

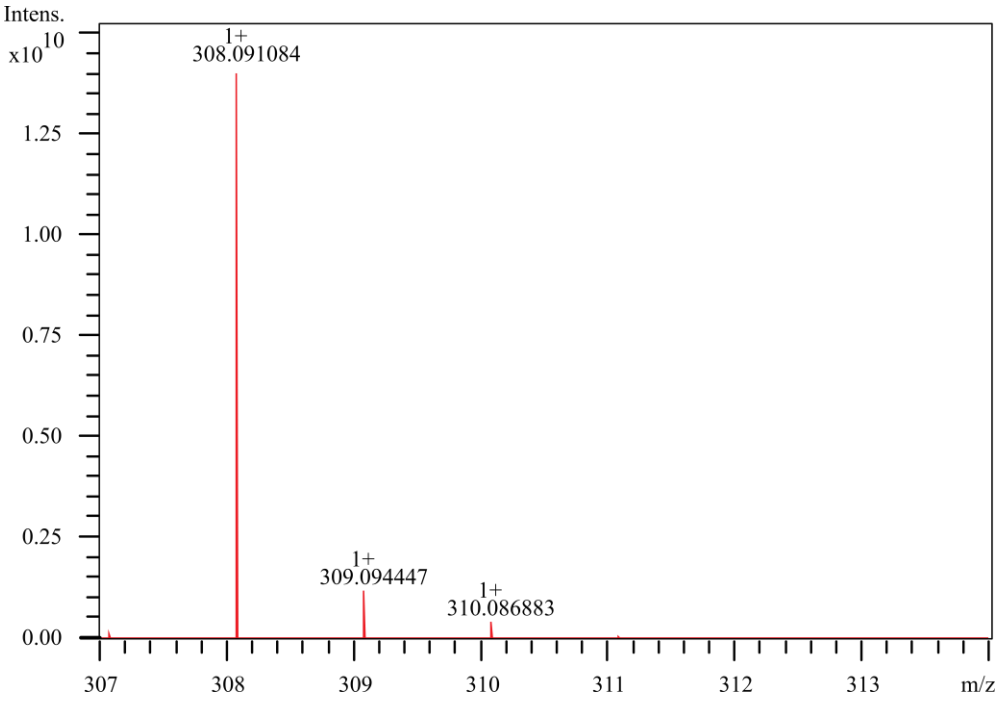

Figure 1. FT-ICR spectrum showing the isotopic distribution of glutathione.

Detected isotopologues:

Mass (*m/z*)	Relative intensity (%)	Isotopologues	Relative intensity measured (%)	Deviation (m/z)	Deviation (intensity) %
308.091083	100.00	^12C10^1H18^14N3^16O6^32S	100.000	0.0032 ppm	0.0
309.088118	1.096	^12C10^1H18^14N2^15N^16O6^32S	0.691	-0.0065 ppm	37.0
309.090471	0.790	^12C10^1H18^14N3^16O6^33S	0.511	-0.0032 ppm	35.3
309.094438	10.816	^12C9^13C^1H18^14N3^16O6^32S	8.665	0.0291 ppm	19.9
309.095300	0.229	^12C10^1H18^14N3^16O5^17O^32S	0.116	-0.0874 ppm	49.3
309.097360	0.207	^12C10^1H17^2H^14N3^16O6^32S	0.214	-0.0226 ppm	-3.4
310.086879	4.474	^12C10^1H18^14N3^16O6^34S	3.097	0.0129 ppm	30.8
310.091473	0.119	^12C9^13C^1H18^14N2^15N^16O6^32S	0.084	-0.0032 ppm	29.4
310.095328	1.233	^12C10^1H18^14N3^16O5^18O^32S	0.800	0.0000 ppm	35.1
310.097793	0.526	^12C8^13C2^1H18^14N3^16O6^32S	0.346	0.0064 ppm	34.2
311.090234	0.484	^12C9^13C^1H18^14N3^16O6^34S	0.331	0.0000 ppm	31.6
311.098683	0.133	^12C9^13C^1H18^14N3^16O5^18O^32S, ... (2)	0.104	-0.0096 ppm	21.8

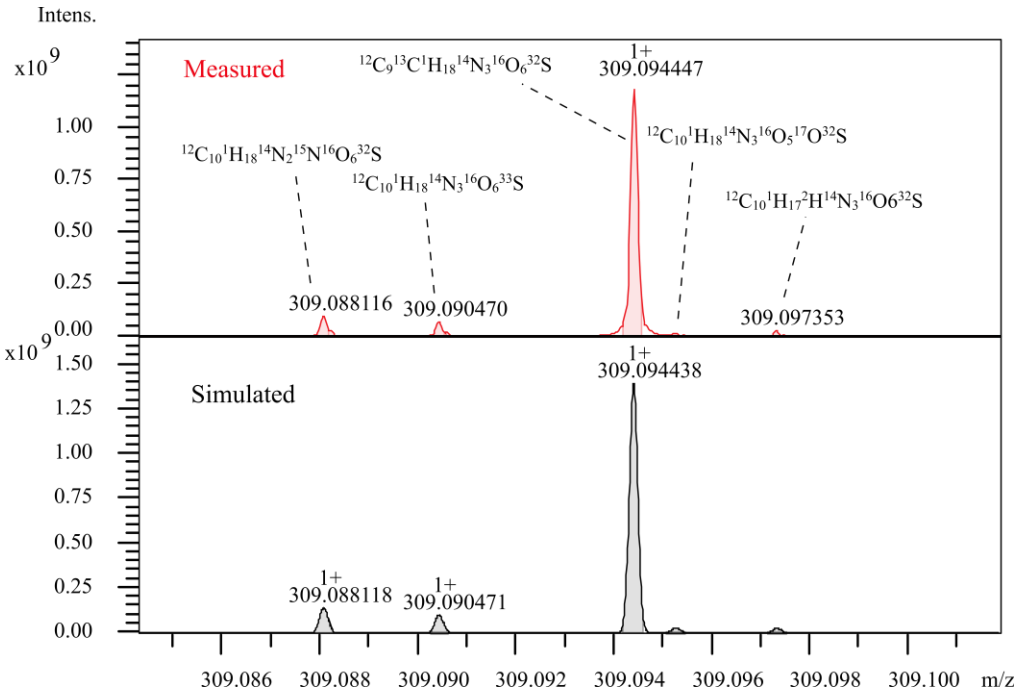

Figure 2. FT-ICR spectrum showing a magnification of the 2nd peak of the isotopic distribution of glutathione, with annotated isotopologues.

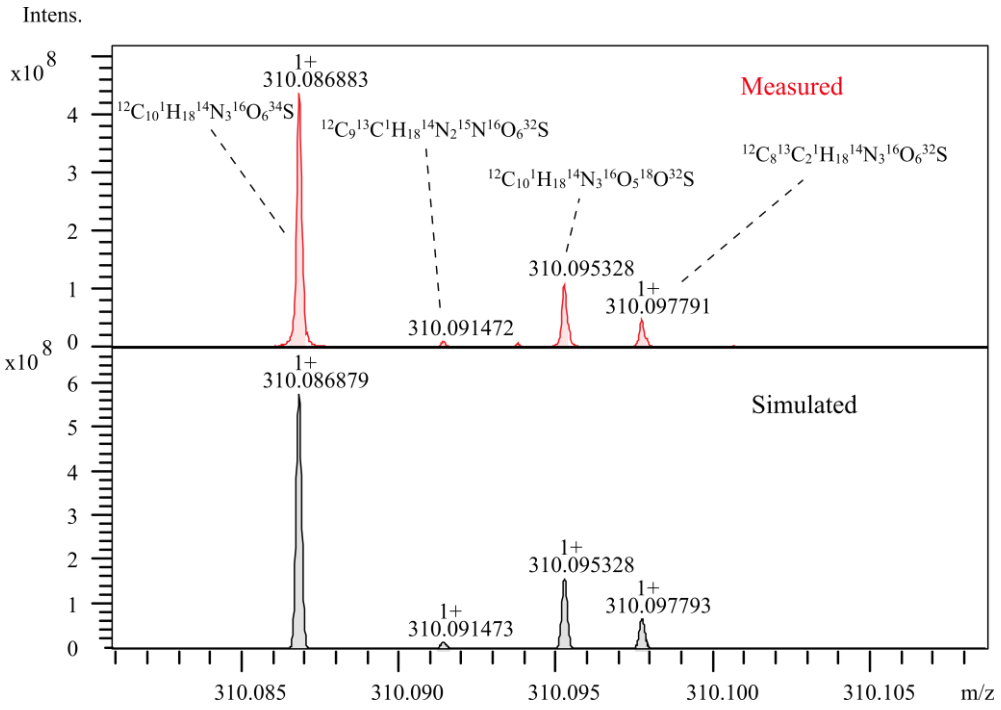

Figure 3. FT-ICR spectrum showing a magnification of the 3rd peak of the isotopic distribution of glutathione, with annotated isotopologues.

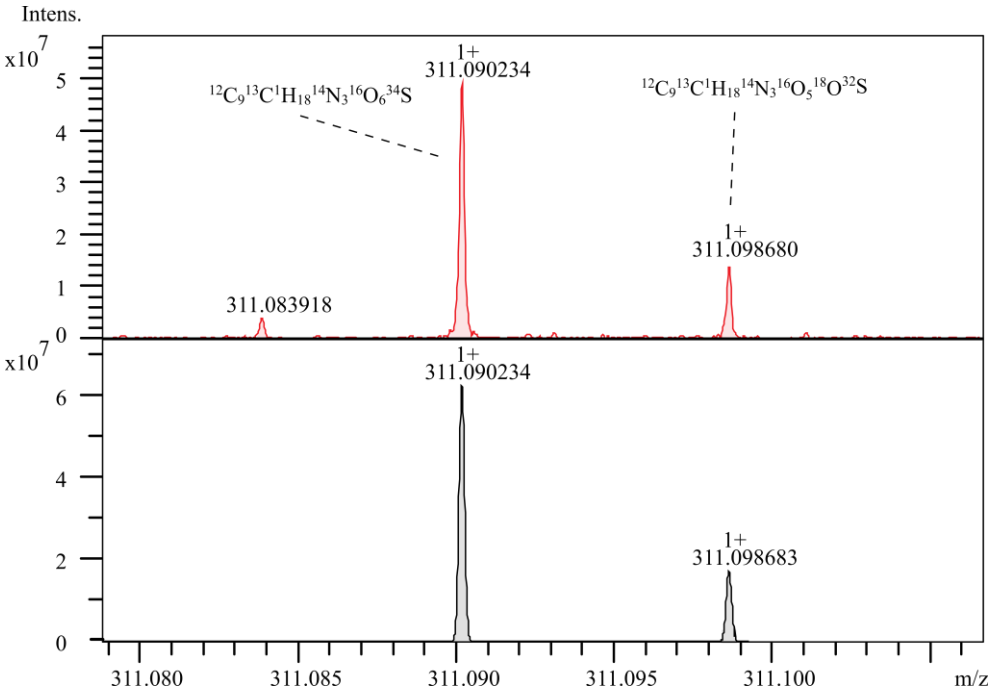

Figure 4. FT-ICR spectrum showing a magnification of the 4th peak of the isotopic distribution of glutathione, with annotated isotopologues.

4. Conclusion and perspectives

Accurate mass measurements at extremely high resolution are the main analytical capabilities of FT-ICR mass spectrometers and this 2nd round robin test showed that almost all center were capable to perform at an accuracy better than 1 ppm and five centers achieving an accuracy better than 0.03 ppm. Achieving the highest possible resolution was not a goal of the 2nd round robin all centers (except one) easily exceeded 500000 at 308 m/z while two where close to 2 million (at 9.4 and 15 T). The main conclusion is that a resolution of at least 500000 is required to reveal all the isotopologues of GSH and that 6 centres (55%) could resolve all twelve most abundant isotopologues, irrespectively of magnetic field (from 7 to 15 T) although spectra quality improves with magnetic field and the highest figure of merit were achieved at 15 T. Accuracy for measuring the relative intensities of all isotopologues varied widely when considering the worst case scenario, from 2.5% to 125%, most centres achieving a variation between 37% and 67%. Still, for most algorithms used for ab initio molecular formula determination, mass accuracy on all isotopologues found may still be more heavily weighted.
Future work will be aimed at understanding the roots of these deviations while investigating a real world scenario where a complex mixture will be analysed to obtain the highest number of possible molecular formulas (3rd Round Robin).