ZOOARCHAEOLOGY BY MASS SPECTROMETRY (ZOOMS) REPORT

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'THE TIMES OF THEIR LIVES': BELO BRDO, SERBIA

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SUMMARY

1. Alex Bayliss supplied 48 bone powder samples for ZooMS analysis.

2. The samples were weighed out, rinsed, extracted and trypsinated, prior to MALDI-MS being carried out.

3. Of the 48 samples sent for analysis, 47 were identified to species level, while one could not be identified and has therefore been recorded as ‘No ID’.

4. Of the 47 samples identified, 18 were either cattle or bison, 14 were either fallow or red deer, 14 were roe deer, and 1 was goat.

5. There is remaining material for 10 of the 48 supplied samples. The remaining sample will be returned to the original sender (Alex Bayliss) in due course.

CONTENTS

INTRODUCTION

Collagen

Collagen is the main protein of connective tissue in animals and the most abundant protein in mammals, making up about 25% of the whole-body protein content. Its fundamental structural unit is a long (300-nm), thin (1.5-nm-diameter) protein that consists of three coiled subunits: two α1 chains and one α2 chain. Each chain contains precisely 1050 amino acids, wound around one another in a characteristic right-handed triple helix to form tropocollagen subunits that are in turn bound in super-helices with each other to form the collagen molecule.

There are, so far, 28 collagen types, with types 1-4 being the most predominant as follows;

Type 1: the most common collagen (bone and teeth is almost exclusively Type I). It is also found in tendons, skin, artery walls and fibrocartilage with other collagen types.

Type 2: cartilage, vitreous humour of the eye.

Type 3: reticular fibres, artery walls, skin, intestines and the uterus.

Type 4: Basal lamina, eye lens, kidney.

Like many proteins the amino acid sequence of collagen is unique to every species, although the uniqueness may only be represented by a single amino acid substitution in the whole protein molecule.

Zooarchaeology by Mass Spectrometry (ZooMS)

ZooMS is a cheap, fast method of analysis that allows us to identify species from skeletal remains through MALDI-TOF-MS and the subsequent analysis of collagen-peptides.

The specific preparation and processing method used is described in the ‘Materials and Method’ section on page 5.

ZooMS Specific to Deer, Sheep, Goats, Cattle and Bison

Of the five deer present in our current database, Fallow deer, Red deer and Roe deer were contextually relevant to the sample set in question. The collagen sequences for these three species are very similar; Fallow deer and Red deer cannot be told apart, and there are only two differences separating those species from Roe deer.

Similar to deer collagen sequences, the sequences for sheep and goats are incredibly similar; there is only one peptide difference that can be used to distinguish between the two.

Cattle and bison have exactly the same collagen peptides and therefore unfortunately cannot be distinguished using the ZooMS technique alone.

The table below provides details of the specific peptide differences and matches just mentioned. Red text highlights presumed but not confirmed peptides. A green background indicates a peptide that can be used to distinguish that species from others listed in the table (but which is not necessarily a truly 'unique' peptide for that species, as another species not listed here may also share it).

Common Name	Peptides											
Cattle	1105	1192	1208	1427	1580	1648	2131	2792	2853	2869	3017	3033
Bison	1105	1192	1208	1427	1580	1648	2131	2792	2853	2869	3017	3033
Sheep	1105	1180	1196	1427	1580	1648	2131	2792	2883	2899	3017	3033
Goat	1105	1180	1196	1427	1580	1648	2131	2792	2883	2899	3077	3093
Fallow deer	1105	1180	1196	1427	1550	1648	2131	2792	2883	2899	3017	3033
Red deer	1105	1180	1196	1427	1550	1648	2131	2792	2883	2899	3017	3033
Roe deer	1105	1180	1196	1427	1550	1648	2131	2792	2883	2899	3043	3059

MATERIALS & METHOD

Materials

- Hydrochloric acid
- Ammonium bicarbonate
- Acetonitrile
- Sequencing grade trypsin
- Trifluoroacetic acid
- α-cyano-4-hydroxycinnamic acid (matrix)
- C18 ZipTip® pipette tips
- Calibrant
- Conditioning solution (50% CAN / 0/1% TFA)
- Washing solution (0.1% TFA)

Method

1. The samples were weighed out into individual eppendorf tubes and the weights recorded.
2. 100ul of 50mM ammonium bicarbonate solution (NH4HCO3) pH 8.0 (AmBic) was added to each of the samples before all were placed at room temperature on a roller-rocker to be rinsed.
3. After five days, the samples were removed from the roller-rocker and spun down in a centrifuge. The initial AmBic was removed.
4. A new 100ul of AmBic was then added to the samples, followed by all being incubated for one hour at 65°C. After this, 50ul of supernatant was transferred from each of the original eppendorfs to a second eppendorf, and the remainder was transferred to the third eppendorf and frozen.
5. 1ul of trypsin solution was added to each of the 'second' eppendorfs. These were then incubated overnight at 37°C.
6. Following incubation, the samples were centrifuged and 1ul of 5% TFA solution was added to each to terminate trypsin activity.
7. Peptides were then extracted from the sample solution using C18 ZipTip® pipette tips and eluted with 50ul of conditioning solution.
8. 1ul of sample was then spotted on to a Bruker ground steel target plate, following which 1ul of matrix was added on top. Each sample was spotted in triplicate and the plate was then run on the Bruker Ultraflex.

RESULTS

The table below provides the species identification results for all samples sent for analysis.

Lot Number (BioArCh)	Original Identifier (Client)	Species ID
11397	U.241 medium-sized mammal long bone	No ID
11398	U.241 medium-sized mammal pelvis	Fallow deer/Red deer
11399	U.2113 Ovis/Capra mandible	Goat
11400	0.3m Inv 3206	Fallow deer/Red deer
11401	1.3m Inv 3210	Fallow deer/Red deer
11402	1.7m Inv 3657	Roe deer
11403	2.2m Inv 3225	Fallow deer/Red deer
11404	2.2m Inv 3727	Fallow deer/Red deer
11405	2.6m Inv 3230	Roe deer
11406	2.9m Inv 3233	Roe deer
11407	3.0m Inv 3241	Fallow deer/Red deer
11408	3.3m Inv 3260	Roe deer
11409	3.5m Box 33	Roe deer
11410	3.6m Inv 3243	Roe deer
11411	4.1m Inv 3286.1	Fallow deer/Red deer
11412	4.1m Inv 3286.8	Roe deer
11413	4.3m Inv 3302.9	Cattle/Bison
11414	4.4m Inv 3306	Roe deer
11415	4.6m Inv 3324.3	Cattle/Bison
11416	4.7m Inv 3327-7	Roe deer
11417	4.9m Inv 3338.1	Cattle/Bison
11418	4.9m Inv 3338.6	Cattle/Bison
11419	5.3m Inv 3360.5	Cattle/Bison
11420	5.5m Inv 3371.1	Roe deer
11421	5.5m Inv 3371.4	Cattle/Bison
11422	5.7m Inv 3659	Fallow deer/Red deer
11423	6.1m Inv 3396.5	Roe deer
11424	6.2 Inv 3398	Roe deer
11425	6.5m Inv 3429.3	Cattle/Bison
11426	6.5m Inv 3429.5	Cattle/Bison
11427	6.7m Inv 3708	Fallow deer/Red deer
11428	6.9m Box 33	Fallow deer/Red deer
11429	7.1m Box 33	Fallow deer/Red deer
11430	7.3m Box 33	Roe deer
11431	7.5m Inv 3480	Fallow deer/Red deer
11432	7.5m Inv 3742	Fallow deer/Red deer
11433	7.7m Box 33	Fallow deer/Red deer

Lot Number (BioArCh)	Original Identifier (Client)	Species ID
11434	7.8m Box 33	Roe deer
11435	8.0m Inv 3518.10	Cattle/Bison
11436	8.0m Inv 3518.8	Cattle/Bison
11437	8.1m Inv 3538.1	Cattle/Bison
11438	8.3m Inv 3545.2	Cattle/Bison
11439	8.5m Inv 3572.5	Cattle/Bison
11440	8.9m Inv 3589.4	Cattle/Bison
11441	9.1m Inv 3600.1	Cattle/Bison
11442	9.2m Inv 3611.1	Cattle/Bison
11443	9.2m Inv 3608.2	Cattle/Bison
11444	9.3m Inv 3327.7	Cattle/Bison

Sample spectra for each of the identifications (as well as sheep, to emphasise the sheep/goat difference) can be seen in the ‘Figures’ section on pages 9-11. Spectra for all submitted samples can be supplied on request.

BIBLIOGRAPHY & FURTHER READING

Buckley, M., Collins, M., Thomas-Oates, J. & Wilson, J. (2009) 'Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry', *Rapid Communications in Mass Spectrometry* 23, 3843 – 3854.

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Hounslow, O., Simpson, J., Whalley, L & Collins, M. (2013) 'An Introduction to ZooMS (Zooarchaeology by Mass Spectrometry) for Taxonomic Identification of Worked and Raw Materials' in S. O'Connor and A. Choyke (eds), *From These Bare Bones: Raw materials and the study of worked osseous objects*, 201 – 207. Oxford: Oxbow Books.

FIGURES

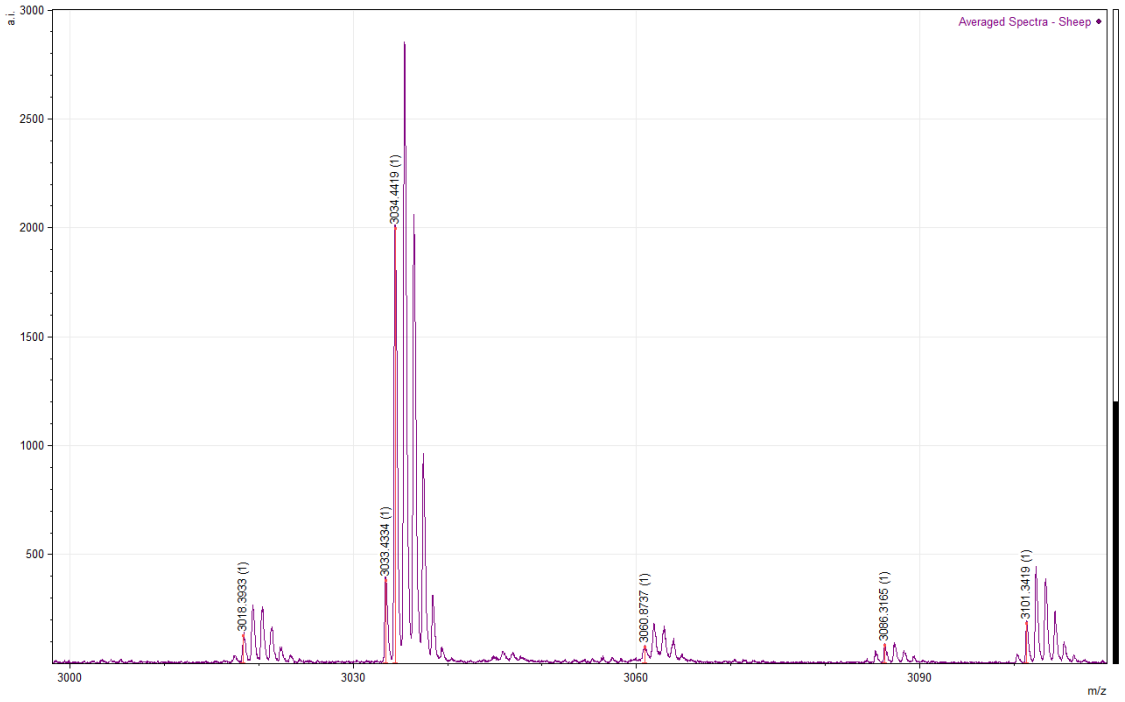

FIGURE 1: SAMPLE SHEEP SPECTRA (KEY MASS = 3033)

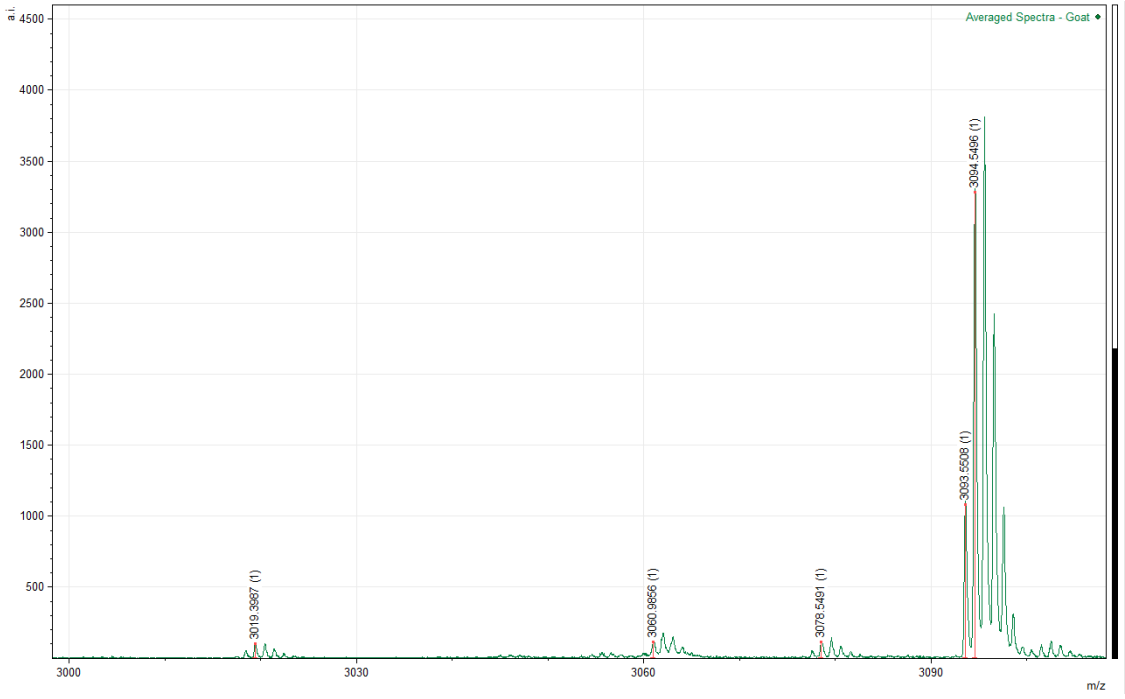

FIGURE 2: SAMPLE GOAT SPECTRA (KEY MASS = 3093)

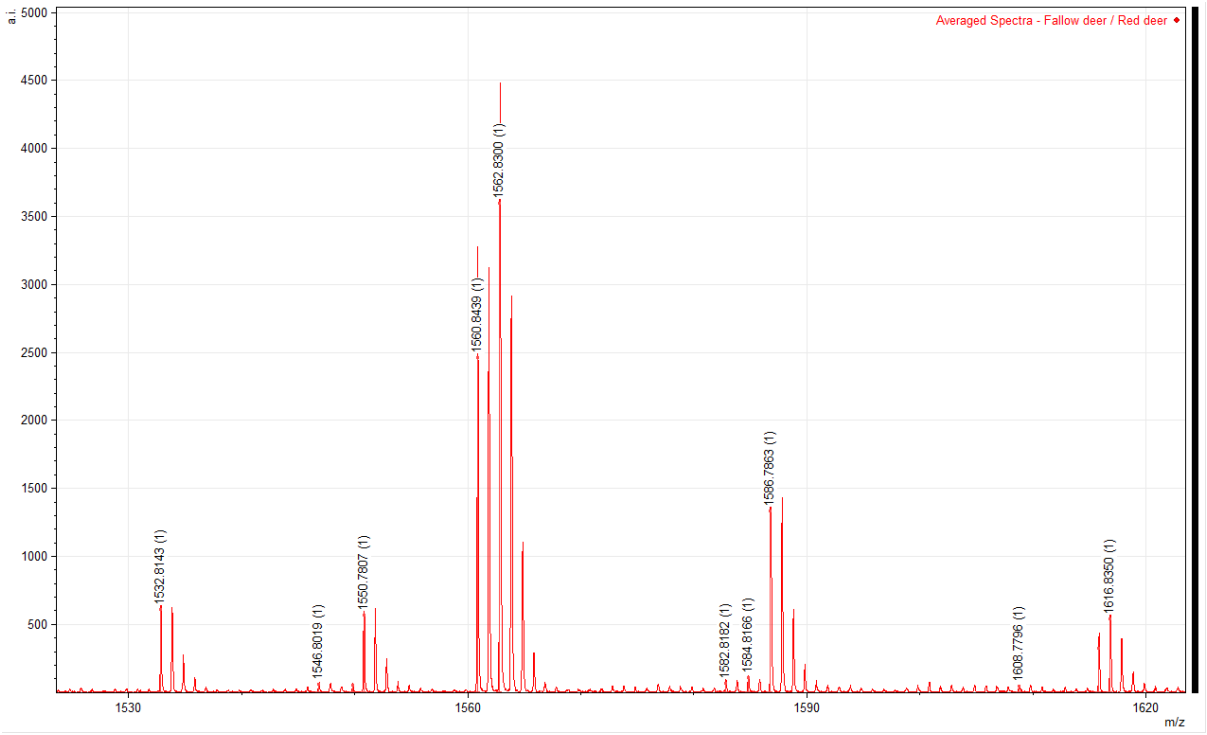

FIGURE 3: SAMPLE FALLOW DEER / RED DEER SPECTRA, 1 OF 2 (KEY MASS = 1550)

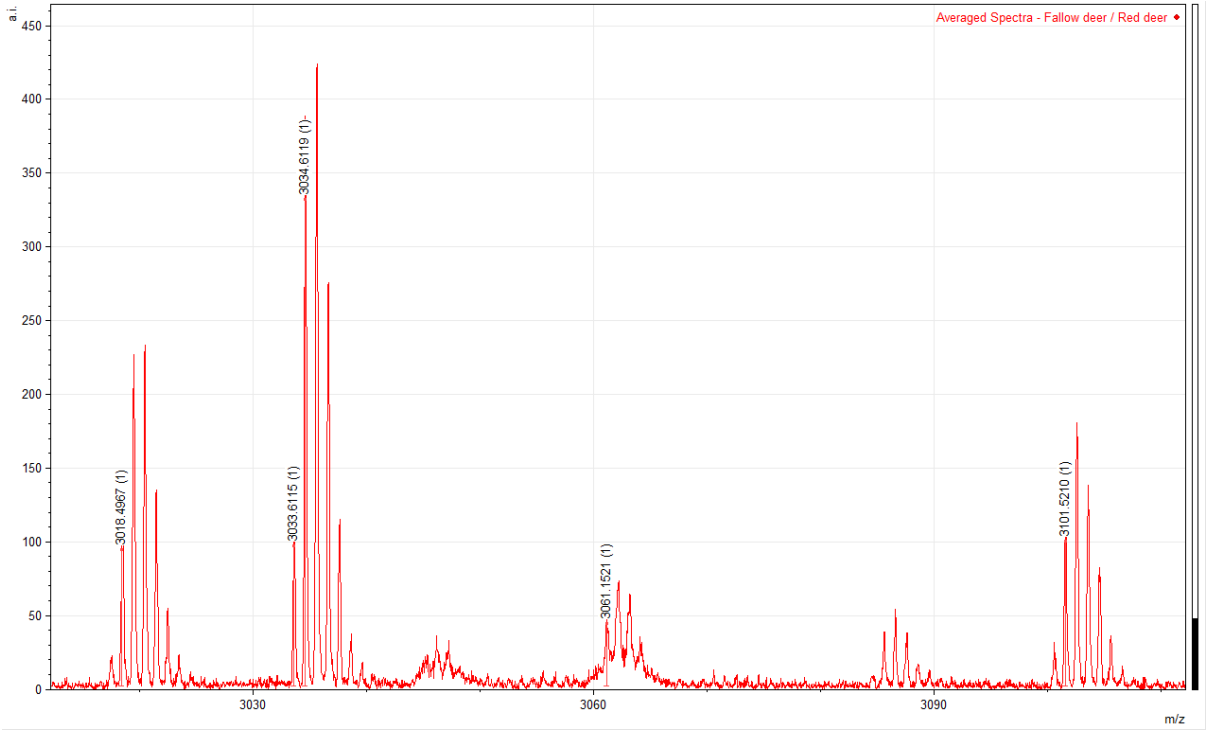

FIGURE 4: SAMPLE FALLOW DEER / RED DEER SPECTRA, 2 OF 2 (KEY MASS = 3033)

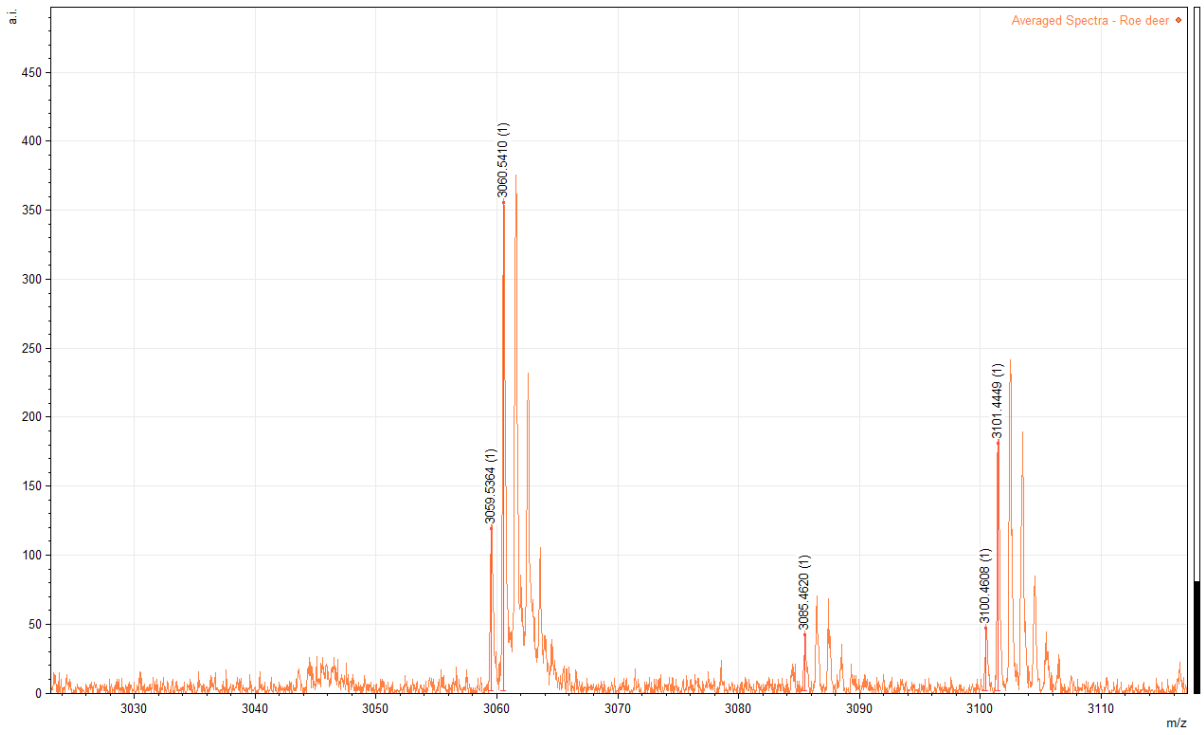

FIGURE 5: SAMPLE ROE DEER SPECTRA (KEY MASS = 3059)

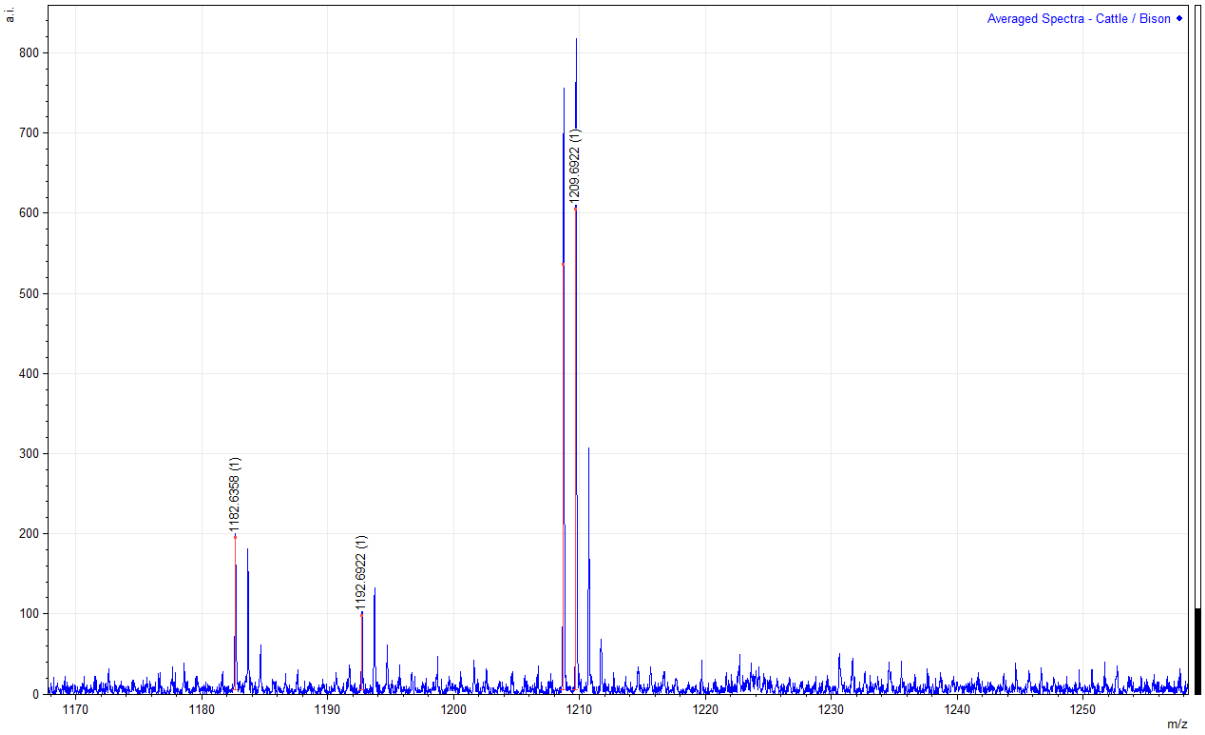

FIGURE 6: SAMPLE CATTLE / BISON SPECTRA (KEY MASS = 1208)

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