**Title;** Analytical Chemistry – Peptide fingerprinting (MS), Samplecontamination (GC-MS), Atomic Spectroscopy - Detecting thepresence of Zinc using ICP-OES.

**Sample analysis using selected analytical techniques;**

1. **Peptide Mass Fingerprinting**

One of the simplest and quickest ways of identifying a protein is using atechnique called peptide mass fingerprinting (PMF). The PMF of a protein isunique with information coming from a collection of peptides that occur whenthe molecule is digested. PMF is a method of identifying proteins that relies onthe use of a protease to digest the protein in question into smaller peptidefragments. A common protease is trypsin which, unmodified, cuts directlydownstream of two specific amino acids. As different proteases cleave atdifferent amino acid residues, the PMF of the protein will depend on theprotease used, but provided digestion is complete, i.e., the molecule iscleaved at all the possible sites, it will produce a set of peptides of varyingmasses. These may be observed using an appropriate mass spectrometrytechnique, with the mass (m/z, where z = 1) of each peptide beingapproximately the sum of the amino acids present including any modificationsthat those amino acids might have undergone.The use of a fingerprint to identify proteins is not always possible as it relieson the masses of the peptides being available in a database. It is importantthat the organism being analysed is genome-verified, i.e., its genome hasbeen sequenced. If no reference material is available in the database, thenPMF will not identify the protein. For the current analysis, you will digest your protein using trypsin and thenuse high performance liquid chromatography interfaced with electrosprayionisation mass spectrometry {HPLC-(ESI)MS} to obtain the m/z values of theprotonated peptides (M+H+). These values will be compared to thoseavailable in a suitable database, such as Mascot.

1. **Detecting the presence of Zinc using ICP-OES**

Your protein is a metalloenzyme containing zinc (Zn). The presence of metalsmay easily be detected by atomic spectroscopy techniques, such as atomicabsorption or atomic emission (now known as optical emission). Atomicspectroscopy requires very high temperatures (typically > 2000 K) to convertmolecules into atoms and ions. This may be achieved by a flame, furnace, orplasma. Atomic/optical emission spectroscopy (OES) may be used inconjunction with an inductively coupled plasma (ICP) system to detect thepresence of metals, such as zinc. Although samples may be analysed directly,sensitivity is usually improved when treated with nitric acid solution.

1. **Detecting a protein impurity using GC-MS,** Gas Chromatography is a separation technique suitable for relatively smallmolecular weight compounds (< 1000 Da). Thus, when attached to anelectron-ionisation mass spectrometer (EI-MS), it may be used to analysevolatile or semi-volatile compounds. Direct separation and analysis ofproteins, peptides, or amino acids by GC-MS is not possible. However,digesting proteins and peptides and then chemically modifying the resultingamino acids allows their identification by this technique.Your protein may contain an unwanted impurity that is also a protein. Theimpurity (protein X) is known to possess an unusually high level of an aminoacid. Following digestion of your protein, therefore, you will chemically alterthe amino acid using a procedure known as “extraction-derivatization” andidentify it using GC-MS.The derivatization procedure involves taking an aqueous solution of thedigested protein and reacting it with an alkyl chloroformate and an alcohol inthe presence of chloroform and pyridine. The reaction takes place at theamine and carboxylic acid groups on the amino acid to generate a derivative(see scheme 1 below) that transfers from the water layer to the chloroformlayer. A small portion of the chloroform layer is then injected into the GC-MS

**Lab protocol**

**1. Inductively- coupled plasma – optical emission spectroscopy (ICP-OES)**

**i) Preparation of sample**

Prepare a solution by adding 50.0 ml of 5% nitric acid solution to your protein sample. Ensure the solution is fully dissolved and well mixed. Aliquot 1.0 ml of this solution into a separate 100 ml volumetric flask and make up to the mark with 5% nitric acid and mix well.

**ii) Analysis by ICP-OES**

Place your protein solution in the autosampler tray. Inject the sample 3 times. Using the instrument’s calibration line determine the concentration of Zinc in parts-per-million.

**2. Detecting a protein impurity using GC-MS**

**i) Preparation of sample**

To your hydrolysed protein sample add 5.0 ml of distilled water. Make sure the sample dissolves fully and is well mixed. Aliquot 50 µl of this protein solution to a vial containing 200 µl of a mixture of ethanol and pyridine (4:1 v/v). To this mixture, add 50 µl of the derivatisation agent, Ethyl Chloroformate (ECF). Mix solution for approx. 5 seconds (you will probably see bubbles of CO2 gas released). Finally, add 300 µl of chloroform and mix well for approx. 30 seconds and leave the solution to settle into two layers.

**ii) Analysis by GC-MS**

Remove approx. 200 µl of the lower chloroform layer from the sample into a vial insert and inject 1 µl onto the GC column.

**Laboratory report;Only include introduction, presentingdata**

1. **Introduction** (10%) Write a brief introduction (no more than 100 words) to the report. The introduction should include background to the **analysis/techniques** used and the **aims** of the practical. All sources should be cited correctly and included in a reference list using to the APA 7th Harvard system.
2. **Presentation of data including answers to workshop questions (30%)**

**Describe the results of the practical using tables, graphs, chromatograms, or spectra relevant to the analysis. These should be labelled correctly with the appropriate figure legend. For the measurement of Zn2+ concentration in your sample, include a table showing the 3 values, a mean of the 3 values, the standard deviation (SD) between the 3 values, and the coefficient of variation (CV) of the 3 values. Note CV = (SD/mean) x 100**.

Answer the following questions:

1- From using the peptide mass fingerprinting technique, what is the identity of your unknown protein? - Where does trypsin cleave proteins?

- The buffer recommended for making up the tryptic solution is ammonium hydrogen carbonate

2 – what are the advantages of using this buffer?

3- The addition of too much protease can be detrimental to the results – why is this?

4- Based upon the concentration (in ppm) of Zn2+ present in the sample, work out the concentration of your protein (in moles per litre). Note that each protein molecule contains one Zn2+. Include your working out in this section.

5- From the EI-mass spectrum of your derivatised protein sample, determine the identity of the amino acid present in unusually high levels. Include the structure of the underivatized amino acid – see scheme 1 **What is the amino acid worked out?**

+ ii)results section/no experimental section/

1. Relevance, critical analysis, and depth of material used in the report, general discussion, and conclusion section (30%) Critically analyse your data with reference to the literature. Include a summary in this section./ iii) need to take each result and analyse it with reference to literature, within this section include summary of what I have done in my report
2. need to take each result and analyse it with reference to literature, within this section include summary of what I have done in my report
3. Referencing and references (10%) Include your sources in a reference list using the Harvard APA 7th system of referencing.
4. Presentation and use of figures (20%) You should use figures throughout the report where appropriate. All figures should be formatted according to the Harvard APA 7th system

**The following is a link which will take you to the MS-fit database:**

[https://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msfitstandard](https://eur01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fprospector.ucsf.edu%2Fprospector%2Fcgi-bin%2Fmsform.cgi%3Fform%3Dmsfitstandard&data=05%7C02%7CS.Kazmi4%40edu.salford.ac.uk%7C9c0d19497842465c539208dc4d80213d%7C65b52940f4b641bd833d3033ecbcf6e1%7C0%7C0%7C638470459902515375%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C0%7C%7C%7C&sdata=Sb0p6RrUN898FAwyJMfSkutPNeXtpb4m0p0hR55yLAE%3D&reserved=0)

**Group 3 results my group;**

**1/3;**

**A graph of a graph showing the amount of protein

Description automatically generated with medium confidence**

**2/3 results;ICP-OES measurement of Zn2+ concentration=** **)** Group 3 concentration measurements were 1.10 ppm, 1.51 ppm, 1.11 and 1.17 ppm

**3rd set of results;** GC-MS identification of the amino acid. (Your amino acid is one of the following: alanine, isoleucine, valine, or glycine)

**A graph of a graph showing a number of points

Description automatically generated with medium confidence**

A graph of a person with a graph

Description automatically generated with medium confidence

**Mechanism below; to help work out results;**

A screenshot of a computer

Description automatically generated

Extraction derivatization paper.pdf:

[7b. Extraction derivatization paper.pdf.pdf](file:///C:\Users\Asim1\Downloads\7b.%20Extraction%20derivatization%20paper.pdf.pdf)

A close-up of a chart

Description automatically generated

Whole point of this assignment is too work out the amino acid and analysing using resources like pdf, and mechanism as well

* m/z=523.6= to mw of protonated peptide
* workout the unknown protein using PMF each line in the spectra is a peptide +1
* 0.05g protein sample,
* Mass spectrum of the peak, look at it and what it equals to +1 unit, looking at the peaks from the chromatogram, then getting the numbers going to database, to see the sequence, a data base search needs to be done,
* **The following is a link which will take you to the MS-fit database:**
* [https://prospector.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msfitstandard](https://eur01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fprospector.ucsf.edu%2Fprospector%2Fcgi-bin%2Fmsform.cgi%3Fform%3Dmsfitstandard&data=05%7C02%7CS.Kazmi4%40edu.salford.ac.uk%7C9c0d19497842465c539208dc4d80213d%7C65b52940f4b641bd833d3033ecbcf6e1%7C0%7C0%7C638470459902515375%7CUnknown%7CTWFpbGZsb3d8eyJWIjoiMC4wLjAwMDAiLCJQIjoiV2luMzIiLCJBTiI6Ik1haWwiLCJXVCI6Mn0%3D%7C0%7C%7C%7C&sdata=Sb0p6RrUN898FAwyJMfSkutPNeXtpb4m0p0hR55yLAE%3D&reserved=0)

the link above needs to be used massively using the resultssss;;;