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Zooarchaeology by Mass Spectrometry (ZooMS) for bone material - Acid soluble protocol

Forked from Zooarchaeology by Mass Spectrometry (ZooMS) for bone material - Acid insoluble protocol

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.bf5bjq2n

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ABSTRACT

This collection details the different established protocols for Zooarchaeology by Mass Spectrometry (ZooMS) for use on archaeological bone. ZooMS allows for taxonomic identification by the peptide mass fingerprinting of collagen type I. These protocols can be used individually or combined depending on the preservation, sample size, and ability to do destructive analysis. All the protocols are optimized for bone as the starting material.

In the acid soluble protocol bone is pretreated with hydrocholoric acid. The acid is removed and filtered leaving the collagen which is then digested with trypsin. The peptides are purified using C18 ZipTips. This protocol can be used in conjuntion with the acid insoluble protocol which analyzes the bone shaddow left after the acid is removed.

This protocol is suitable for a wide range of preservation conditions from very good to poor preservation. It works in cases where the bone is too fragile or the preservation is too poor for a collagen shaddow to remain after demineralization. The ideal starting volume is 10-20mg of bone as a powerder or a bone chip, but in cases of very poor preservation, a larger starting volume may be required.

If you are using this protocol, please cite the DOI for the protocol and the following two papers on which it is based:

Buckley, M., Collins, M., Thomas-Oates, J., & Wilson, J. C. (2009). Species identification by analysis of bone collagen using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry: RCM*, *23*(23), 3843–3854. https://doi.org/10.1002/rcm.4316

van der Sluis, L. G., Hollund, H. I., Buckley, M., De Louw, P. G. B., Rijsdijk, K. F., & Kars, H. (2014). Combining histology, stable isotope analysis and ZooMS collagen fingerprinting to investigate the taphonomic history and dietary behaviour of extinct giant tortoises from the Mare aux Songes deposit on Mauritius. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *416*, 80–91. https://www.sciencedirect.com/science/article/pii/S0031018214003071

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Wang, N; Brown, S; Richter, K. K; Ditchfield, P; Hebsetreit, S; Kozilikin, M; Luu, S; Wedage, O; Grimaldi, S; Chazen, M; Horwitz, K. L; Spriggs, M; Summerhayes, G; Shunkov, M; Douka, K. (2020). Testing the efficacy and comparability of ZooMS protocols on archaeological bone. Under review.

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COLLECTIONS

Zooarchaeology by Mass Spectrometry (ZooMS)- Pretreatment protocols for bone material

FORK FROM

Forked from Zooarchaeology by Mass Spectrometry (ZooMS) for bone material - Acid insoluble protocol, Sandra Hebestreit

KEYWORDS

ZooMS, zooarchaeology, archaeology, mass spectrometry, MALDI, peptide mass fingerprinting, collagen, protein extraction, bone

IMAGE ATTRIBUTION

Image created by Kristine Korzow Richter. Photo by Ayushi Nayak. Bone icon by iconfield at thenounproject.com. Collagen image adapted from smart.servier.com.

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36739

PARENT PROTOCOLS

Part of collection

Zooarchaeology by Mass Spectrometry (ZooMS)- Pretreatment protocols for bone material

GUIDELINES

1. This protocol can be performed in a standard wet chemistry laboratory setting.
2. Wear your personal safety equipemt while conducting this protocol (lab coat, chemical resistant gloves and safety glasses).
3. Be aware of your specific lab guidelines regarding sample handling and storage.
4. Be aware of your country and facility specific guidelines regarding the dispose of chemical waste.

MATERIALS

NAME	CATALOG #	VENDOR
Seq Grade Modified Trypsin, 100ug (5 x 20ug)	V5111	Promega
Trifluoracetic acid for HPLC ACROS organics	Product Code. 11904951	Fisher Scientific
Acetonitrile ROTISOLV® HPLC Gradient	HN44.1	Carl Roth

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NAME	CATALOG #	VENDOR
Ammonium hydrogen carbonate 98 % pure ACROS Organics	10364072	Fisher Scientific
Hydrochloric acid fuming 37% for analysis EMSURE® ACSISOReag. Ph Eur	100317	Merck Millipore
Pierce™ C-18 Tips	87784	Thermo Fisher Scientific
Pierce™ Trypsin-Protease MS grade	90057	Thermo Fisher Scientific
Vivaspin 500 30.000 MWCO	VS0122	Sartorius

MATERIALS TEXT

- Both listed trypsin products are suitable for ZooMS applications. The resuspension buffer indicated in that protocol is used from the Kit manufactured by Promega.
- Standard glass bottles in different sizes (500 ml, 250 ml, 100 ml, 50 ml, 20 ml) e.g. Laboratory bottles, round, clear, with PP-screw cap and pouring ring, Borosilicate glass 3.3 from VWR + 20 ml brown glass bottle e.g. DURAN®, borosilicate glass 3.3, brown
- Pipette tips for different volumina (0.5 µl - 5 ml) e.g. from STARLAB or Eppendorf
- Pipettes with different volumina ranges (0.5- 10 µl, 2-20 µl, 20-200 µl, 100-1000 µl, 0.5-5 ml), e.g. Eppendorf Research® plus
- Microcentrifuge tubes e.g 1.5 ml and/or 2.0 ml, safe lock, Eppendorf
- 15 ml & 50 ml centrifuge tubes: e.g PP-screw cap 50 ml or 15 ml, SARSTEDT
- Standard tube racks for microcentrifuge, 15 ml and 50 ml tubes
- pH strips, e.g. MColorpHast (ranges from 0-6, 4-7, 0-14), Merck Millpore
- Scale for labarotory use, e.g. Fisherbrand™ analytical scale, FisherScientific
- Centrifuge with a rotor for 1.5 ml/2.0 microcentrifuge tubes, e.g. Eppendorf 5424 with rotor FA-45-24-11
- Reagent reservoirs, e.g. disposable reagent reservoirs, VWR
- Ultrapure water system, e.g. Milli-Q 7000, MerckMillipore
- Ethanol for cleaning, e.g. ROTIPURAN® ≥99,8 %, p.a., denatured, Carl Roth
- Vaccum centrifuge, e.g. Rotation Vacuum Concentrator RVC 2-18 CDplus, Christ
- Incubator, e.g. VWR, Incubator, INCU Line Prime

SAFETY WARNINGS

Be aware of your country and facility specific safety guidelines.
This protocol uses several solvents, acids and other chemicals which need special precaution. Please be aware of the international GHS hazard statements (listed below) and follow your country and institute specific precautions/guiedelines.

The **GHS hazard** (H-) **and precautionary** (P-) **statements** for the chemicals used in this protocol, are:

Ethanol (for cleaning)**:**

- H225 (Highly Flammable liquid and vapor), H319 (Causes serious eye irritation)
- P210 (Keep away from heat, hot surface, sparks, open flames and other ignition sources. - No smoking), P264 (Wash ... thoroughly after handling), P280 (Wear protective gloves/protective clothing/eye protection/face protection), P303+P361+P353 (IF ON SKIN (or hair): Take off Immediately all contaminated clothing. Rinse SKIN with water or shower), P337+P313 (IF eye irritation persists: Get medical advice/attention)

Acetonitrile:

- H225 (Highly Flammable liquid and vapor), H302+H312+H332 (Harmful if swallowed, in contact with skin or if inhaled), H319 (Causes serious eye irritation)
- P210 (Keep away from heat, hot surface, sparks, open flames and other ignition sources. - No smoking), P280 (Wear protective gloves/protective clothing/eye protection/face protection), P305+P351+P338 (IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing), P403+P235 (Store in a well-ventilated place. Keep cool.)

Trifluoracetic acid:

- H318 (Causes serious eye damage), H314 (Causes severe skin burns and eye damage), H412 (Harmful to aquatic life with long lasting effects), H332 (Harmful if inhaled), H290 (May be corrosive to metals)
- P273 (Avoid release to the environment), P280 (Wear protective gloves/protective clothing/eye protection/face

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protection), P301+P330+P331 (IF SWALLOWED: Rinse mouth. Do NOT induce vomiting), P304+P340 (IF INHALED: Remove person to fresh air and keep comfortable for breathing), P305+P351+P338 (IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing), P310 (Immediately call a POISON CENTER or doctor/physician)

Ammonium bicarbonate (AmBic):

- H302 (Harmful if swallowed)
- P264 (Wash thoroughly after handling), P270 (Do not eat, drink or smoke when using this product), P301+P312 (IF SWALLOWED: call a POISON CENTER/doctor/... IF you feel unwell), P330 (Rinse mouth)

Trypsin

- H315 (Causes skin irritation), H319 (Causes serious eye irritation), H334 (May cause allergy or asthma symptoms or breathing difficulties if inhaled), H335 (May cause respiratory irritation)
- P261 (Avoid breathing dust/fume/gas/mist/vapors/spray), P264 (Wash thoroughly after handling), P271 (Use only outdoors or in a well-ventilated area), P272 (Contaminated work clothing should not be allowed out of the workplace), P280 (Wear protective gloves/protective clothing/eye protection/face protection), P302 + P352 (IF ON SKIN: wash with plenty of water), P304 + P340 (IF INHALED: Remove person to fresh air and keep comfortable for breathing), P305+ P351+ P338 (IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing), P332+P313 (IF SKIN irritation occurs: Get medical advice/attention), P310 (Immediately call a POISON CENTER or doctor/physician), P403+P233 (Store in a well-ventilated place. Keep container tightly closed), P501 (Dispose of contents/container to an authorised landfill)

Sodium hydroxide

- H290 (May be corrosive to metals), H314 (Causes severe skin burns and eye damage)
- P280 (Wear protective gloves/protective clothing/eye protection/face protection), P301+P330+P331 (IF SWALLOWED: Rinse mouth. Do NOT induce vomiting), P305+ P351+ P338 (IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing), P308+P310 (IF exposed or concerned: Immediately call a POISON CENTER or doctor/physician)

Hydrochloric acid:

- H290 (May be corrosive to metals), H314 (Causes severe skin burns and eye damage), H335 (May cause respiratory irritation)
- P280 (Wear protective gloves/protective clothing/eye protection/face protection), P301+P330+P331 (IF SWALLOWED: Rinse mouth. Do NOT induce vomiting), P305+ P351+ P338 (IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do - continue rinsing), P308+P310 (IF exposed or concerned: Immediately call a POISON CENTER or doctor/physician)

BEFORE STARTING

1. We recommend the preperation and storage of chemicals in glass bottles only and no long-term storage of chemicals and buffers in plastic tubes/containers.
2. Preperation of buffers and chemicals at least 24 hrs prior to start (please find detailed instrutctions on solution preperation in section 1 of this protocol).
3. We recommend the following cleaning protocol for surfaces and equipment: Ultrapure water first (e.g. MilliQ water) and then desinfection with 70% (v/v) ethanol.

Reagent preparation

1

General Information:

1. *We recommend to use glassware for buffers and solutions and no long-term storage in plastic containers and/or tubes.*
2. *TFA, HCl and ACN stock solutions should be handled under a fume hood only.*
3. *Always label all chemicals with the compound name, concentraion and the date.*
4. *Chemicals are prepared with water from an ultrapure water system (e.g. Milli-Q water, see materials list)*

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2

[M]**0.6 M Hydrochloric acid (HCl)** (Shelf life: 6 months at **4 °C**)

Handling of HCl should be performed under a fume hood because of its corrosive properties. HCl can cause severe skin burns, eye and respiratory irritations. Don´t touch your skin or eyes with contaminated gloves and change them immediately.

2.1 For 1000 ml of 0.6 M HCl, add **50 mL** of [M]**37 % (v/v)** HCl to **950 mL** ultrapure water carefully. Mix gently.

Please always calculate the needed amount of HCl stock solution you need since this is dependent of the specific compound density.

3 [M]**50 mM Ammonium Bicarbonate (AmBiC) buffer**, pH**8** (Shelf life: 6 months at **4 °C**)

3.1 For 1000 ml, weigh out **3.95 g** of AmBiC powder and transfer it into a clean glass bottle.

3.2 Dissolve the AmBiC in **800 mL** of ultrapure water and mix by genty inverting.

3.3 Fill up to **1000 mL** with ultrapure water and mix by gently inverting.

4

[M]**5 % (v/v) Trifluoroacetic acid (TFA)** (Shelf life: 6 months at **Room temperature**)

Triflouroacetic acid (TFA) is very hazardous (corrosive)! Handling should only be performed under a fume hood.
Prepare this solution in a brown glass bottle since TFA is slightly light sensitive.
Always pipette water first, then add the acid slowly!
Repeated used of plastic pipettes in TFA stock solutions causes plastics to build up in the TFA stock solution and can interfere with mass spectrometry, therefore ALWAYS use a glass pipette/syringe for TFA.

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4.1 Dilute **1 mL** TFA stock solution using a glass syringe/pipette (no plastic) in **19 mL** ultrapure water and mix carefully.

5 **Wash solution: 0.1 % (v/v) TFA in ultrapure water** (Shelf life: 6 months at **Room temperature**)

5.1 

For 1000 ml, add **1 mL** of TFA to **1000 mL** of ultrapure water and mix by gently inverting.

6 **Conditioning solution: 0.1 % (v/v) TFA in 50 % (v/v) Acetonitrile (ACN)**, (Shelf life: 3 month at **Room temperature**)

ACN is a volatile compound *and if there is too much headspace in the storage bottle the concentration of ACN in solution goes down over long-term stoorage. We recommend not to store small solution amounts in large bottles over a long period of time. Either prepare new conditioning solution or store the remaining solution in smaller bottles.*

6.1 For 1000 ml, mix **500 mL** ACN with **500 mL** ultrapure water.

6.2 Add **1 mL** of TFA carefully and mix by genty inverting.

7 **Trypsin solution** (Stable once resuspended for 1 month at **-20 °C**)

7.1 Freeze dried Trypsin (**20 µg**) is resuspended in **50 µl** of Trypsin resuspension buffer (included in Trypsin Kit or per manufactures instructions). The final concentration is now **0.4 µg/µl** .

7.2 Transfer the solution in a labelled 0.5 ml tube and store at **-20 °C** .

Sample preparation and collagen extraction

8 **Weighing out the sample**

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General information

1. *All steps should take place in a dedicated wet chemistry laboratory.*
2. *Wipe down all surfaces with 1. ultrapure water, 2. 70% (v/v) ethanol).*
3. *Alliquot out the reagents you will need (HCl).*
4. *Needed after proper bone material deminaralization (see step 13): Aliquot out the reagents you will need (AmBic).*

8.1 1. **Archaeological samples:** Weigh out **10 mg** - **20 mg** of bone chips or ~ **10 mg** bone powder into a microcentrifuge tube labelled with the sample name, date and your name. Record the weight.
2. **Modern samples:** Weigh out **5 mg** - **15 mg** of bone chips or ~ **7 mg** bone powder into a microcentrifuge tube labelled with the sample name, date and your name. Record the weight.

9

Acid Demineralization (destructive)

Add **500 µl** of cold (**4 °C**) **0.6 M** HCl to the sample (or enough acid to cover the sample completely) and place the tubes in the fridge at **4 °C** . Leave until demineralization is complete (bone becomes flexible/ spongy and no bubbling is visible anymore).

This may take 1 day to 2 weeks for specific sample types. Less time will be needed for bone powders (4-10 h).

For samples with good collagen preservation, complete demineralization is not necessary. Please be aware that the more the collagen remains in acid the more it breaks down. Hence monitor samples carefully at this stage to reduce protein loss.

Don´t forget to prepare blanks at this point! This should be empty tubes with only HCl added. Perform every step with the blanks, which you do with the actual samples.

10

Centrifuge the sample for **20000 rpm, Room temperature 00:01:00** to settle the bone and (heavier) collagen to the base of the tube.

11

Remove the acid supernatant into a new tube. We recommend to label this tube with the sample number and the suffix "AC",

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Do not disturb the bone residues from your sample with the pipette.

If you want to analyse the acid insolube collagen fraction (please see the protocol "Zooarchaeology by Mass Spectrometry- Acid insoluble protocol") you should use this bone chip/bone powder fraction and store it at **-20 °C** .

12

Buffer exchange

Place 30 kDa ultrafilters into labelled tubes.

12.1 Take **250 µl** of acid supernatant and put into a 30 kDA ultrafilter.

Take care not to touch the tip to the membrane.

For well-preserved collagen samples a 30 kDA ultrafilter is sufficient. Smaller sized filters easily get clogged. For poorly preserved collagen, a 3 kDA or 10 kDA ultrafilter can be considered.

12.2

Centrifuge at **3700 rpm, Room temperature** until acid has been pushed through the filter. This can take between **00:05:00** and **00:30:00** .

Put on cap and place filters into the centrifuge with the cap strap toward the center of rotor, this ensures the special shaped ultrafilters will fit properly into the centrifuge.

You should not let the filter dry out during this stage. Small amounts of liquid above the filter are fine.

12.3 Discard the flow-through.

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12.4

Add **500 µl** of **50 mM** AmBic and centrifuge at **3700 rpm, Room temperature** until AmBic has passed through filter. This can take between **00:05:00** and **00:30:00** .

12.5 Label two new sets of microcentrifuge tubes in the meantime.

These tubes are to cellect the AmBic supernatant from your filter (see step 12.8). We recommend to label one set with the suffix "EXT" (extract) and the sample name and the second set with the suffix "SE" (second extract) and the sample name. If you are going to use 50 µl of AmBic for eluting the collagen from the filter (see step 12.7), only the EXT tube is needed.

12.6 Discard the flow-through.

12.7

Add **100 µl** of **50 mM** AmBic to the filter and mix carefully by pipetting.

If your sample amount is limited or if less sample then recommended was available, use 50 µl of AmBic.

12.8 Transfer now **50 µl** of the AmBic supernatant to the "EXT" labelled tube and the remaining **50 µl** supernatant to the "SE" labelled tube.

The "SE" labelled tube serves as back up and can be stored at **-20 °C** *. Adjust the amounts of "EXT" and "SE" if less AmBic was used (see step 12.6* and see the note).

Digestion and peptide clean up

13

Trypsin Digestion

Add **1 µl** of **0.4 µg/µl** trypsin solution to the "EXT" labelled tube.

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13.1

Incubate sample overnight at **37 °C** (approx. **12:00:00** - **18:00:00**).

13.2

Centrifuge your samples **20000 rpm, Room temperature 00:01:00** , **(or at high speed)** after incubation.

13.3

Aliquot out the TFA you need and add **1 µl** of **5 % (v/v)** TFA to stop the trypsin.

The peptides can be purified using a C18 ZipTip. If not extracting immeditely, the samples can be stored in the freezer at **-20 °C** *at this point.*

14

Preperations for C18 ZipTipping

For each sample you must prepare separate tubes of wash and conditioning solution. Prepare as follows:

1. One tube per sample containing **250 µl** conditioning solution (or you can just use a common reagent reservoir - label with "C")
2. One tube per sample containing **450 µl** wash solution (label with "W")
3. One tube per sample with **50 µl** conditioning solution- this tube is to collect your purified peptides. We recommend to label it with the prefix “COL” and the sample name.

General information:
During your extraction your peptides are bound to the C18 filter sitting in the pipette tip (ZipTip). The peptides then need to be washed and eluted. Therefore, subsequent wash and conditioning solutions will be ‘contaminated’ with your sample and so solutions after the sample has been bound to the C18 filter and cannot be used for more than one sample.

15

C18-ZipTipping

Before use, each tip needs to be conditioned. A separate tip is used for each blank and sample. The pipette

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should be set at **100 µl** *and the volume NOT changed during the time. Be careful to avoid pulling air through the filter, especially at the steps with only* **50 µl** *of solution. We recommend to use an extra* ***ZipTipping Only*** *pipette as repeated use of the filters pulls the pipettes out of calibration.*

Purify your peptides using the C18 ZipTip from the digested "EXT" sample as follows:

1. Rinse tip twice with **100 µl** of conditioning solution and discard.
2. Rinse tip twice with **100 µl** of wash solution and discard.
3. Resuspend the sample back and forth over the tip, at least 5 times.
4. Rinse tip twice with **100 µl** of wash solution and discard.
5. Elute sample into the prepared "COL" labelled tube with the prepared **50 µl** of conditioning solution and pass through tip at least 5 times.

Be careful not to push air through the C18 filter (never go past the first stop point of the pipette)!

The sample(s) are now ready to be spotted on the MALDI plate. If not spotting immediately, store sample(s) in freezer at **-20 °C** *.*

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