ACCOMPANYING MATERIAL 4 GC-MS PROCEDURE

Introduction

The following method for the extraction and preparation of microscopic lithic residues was modified from the standard operating procedure titled ‘Solvent extraction of organic residues from food crusts and burnt residues preserved on archaeological ceramics’, last edited 22/06/2015, developed by Oliver Craig and Matthew von Tersch for use in the BioArCh laboratories at the University of York, UK. Stone tools and their associated soils from Star Carr, as well as authentic resinous materials from the reference collection were obtained and prepared for gas chromatography-mass spectrometry (GC-MS). First, the steps taken to prepare samples will be detailed in the standard operating procedure. This is followed by a description of solvent extraction, derivatisation, instrumentation, and gas chromatograph analysis.

GC-MS Standard Operating Procedure (SOP)

SOP Name/Title: SOLVENT EXTRACTION OF ORGANIC ‘RESINOUS’ RESIDUES FROM ARCHAEOLOGICAL STONE TOOLS

Modified from SOP: SOLVENT EXTRACTION OF ORGANIC RESIDUES FROM FOOD CRUSTS AND BURNT RESIDUES PRESERVED ON ARCHAEOLOGICAL CERAMICS

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LOCATION:
Environment Building

COSHH REF:
Refer to the COSHH risk assessment for the GC process.

PRINCIPAL:
Solvent extraction of lipid residues preserved on archaeological stone tools.

SAMPLE TYPE:
Archaeological resins, stone tools.

CAUTION:
Dichloromethane (DCM) and Methanol are toxic, use fume extraction at all times. Wear eye protection, laboratory coat and gloves at all times when using DCM. DCM will degrade gloves over time, always monitor the condition of your gloves if splashing occurs. All users of the Nitrogen blow down system must be trained to use the gas cylinder and blow down equipment before use.

MATERIALS REQUIRED:
Aluminium foil, beakers or scintillation vials of sizes appropriate to the stone tools, C34 alkane standard, C36 alkane standard, Dichloromethane (HPLC grade), Hexane (HPLC grade), Methanol (HPLC grade), Pasteur pipettes, sterile glass wear, fine permanent marking pen. Glass wool (if needed to filter soil samples or dirty. Glass wool should be cleaned with DCM in sonic bath for 15 min prior to use).

1.0 PREPARATION PROCEDURES:

1.1 Make sure all glassware is sterile (scrubbed clean with Decon and water, dried, then baked at high temperature wrapped in aluminium foil). Then rinse all glassware being used to hold artefacts in solvent (3x rinsing in DCM).

1.2 No more than twelve samples to be processed in one batch (11 samples + 1 method blank or 10 samples + 1 method blank + 1 stone tool blank) (5 samples + 5 associated soil samples + 1 method blank)

2.0 LABELLING

2.1 Label both vials and lids with unique artefact identifier

3.0 SAMPLE RETRIEVAL (if removing residue from a particular area of the stone tool by scalpel):

3.1 Collect the sample on aluminium foil, weigh it, and transfer to labelled scintillation vial.

4.0 SOLVENT EXTRACTION

4.1 Place stone tools in hatch tubes or beakers if needed. (If processing soil samples at the same time, take pinch samples of the soil sample associated with each lithic and add to scintillation vial).

4.2 Clean needle 5 x with DCM before and after adding C34 alkane standard. Add 100 µl of the C34 alkane standard to the beaker.

4.3 Add about 30 ml DCM:MEOH 2:1 v/v per stone tool and soil sample. Add enough to immerse the tool or area of interest. The volume added does not need to be identical for all samples because the standard was added in a known volume.

4.4 Cover all samples with aluminium foil.

4.5 Place samples in sonic bath, arranging beakers so that none can fall over during sonication- use empty spacer beakers if necessary. Sonicate for 15 minutes at 25°C.

4.6 Carefully pipette off the liquid extract into a clean, labelled hatch tube.

4.7 Repeat steps 4.3 to 4.6 twice more, combining the extracts. If there is too much extract to fit it all into the hatch tube, dry down the extract under a stream of N_2 before adding more extract to the tube.

4.8 Prepare to dry down samples in hatch tubes with N_2. Clean needles with DCM in a beaker and sonicate for 5 min before locking into position. Line up and insert needles into samples for drying. Close off all white valves except the banks being used. Block any empty spaces not being used in the bank with a spacer that has no needle. Write down the order in which samples are placed in the nitrogen blow down banks as a grid. Remove and place sample caps on this grid (outside the fume hood since they will blow away). Carefully lower needles into sample vials, close to the top of the liquid, but not immersed in it. Slowly turn on the nitrogen flow to the point where the liquid is shimmering but not spitting any sample up or out of the vial. Reduce volume of extracts to about 2 ml under a stream of nitrogen gas with gentle heat, about 30°C. If any samples are completely evaporated, add 1 pasteur pipette shot (10 ml) of hexane before transfer to hydrolysis vials.

4.9 Using sterile pasteur pipettes, transfer to a clean, labelled hydrolysis small vial and continue to dry down with N_2. When finished with N2 blower, make sure all white valves on the banks are open so no pressure builds up.

4.10 Store in refrigerator at 4°C (short-term) or in a freezer at -20°C (long-term).

5.0 BLANKS

5.1 For every run a method blank should be included.

5.2 GC/GC-MS analysis of blanks will provide a measure of contamination introduced during the above procedure.

6.0 DERIVITISATION

NB: Only derivitise if you are going to process samples in GC right away (within 2 days or less).

NB: Do not use BSTFA that is older than 1-2 months old.

6.1 With a glass pasteur pipette, add 3-6 drops of N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trichloromethylsilane (TCMS), cap vials and vortex. If pipette touches rim of vial, throw away and use a new one. Dry down vials individually with N_2 and cap immediately (this is easier with two people).

6.2 Heat samples for 1 hour at 70°C with caps on. After 10 minutes on heater, tighten caps as they may have loosened.

6.3 Remove samples from heater and dry down completely with N_2 blower (remember to use N_2 needles cleaned in DCM for 5 min). This should take about 4 minutes.

6.4 Label and prepare blue cap autosampling vials with sterilised inserts. Open vials, placing their caps in the same order on the workbench as the vials are laid out in their holder.

6.5 Clean syringe 10 x before starting with Hexane or 1:1 Hex:DCM, labelled for cleaning purposes. Continue to clean needle 10x between samples. NB: If the syringe needle become blocked with particulate matter during sample transfer, clean it with methanol.

6.6 Re-dissolve sample for analysis by adding 100 µl of 1:1 Hexane:DCM to sample hydrolysis vial. Vortex the samples to mix.

6.7 Take C36 alkane internal standard (for quantification) out of the freezer, vortex and warm up in your hand until no crystals are seen. Add 10 µl of C36 to all empty autosampling vials.

6.8 Transfer 90 µl of each dissolved sample in the hydrolysis vial to autosampling vials with inserts. Make sure only the liquid part is aspirated with the syringe, as particulates can block the needle. Clean the syringe 10 x with Hexane or 1:1 Hex:DCM between samples.

6.9 Store samples prior to GC in freezer at -20°C.

Or further process lipid extracts through saponification (use of methanolic sodium hydroxide) and methylation (BF3 methanol) for IRMS.

Solvent extraction: stone tools and authentic resin samples

Star Carr lithics and lithics containing reference resins were placed in sterile hatch tubes and/or small beakers and 100 µl of C34 long-chain alkane standard was added to each hatch tube. Lipids were solvent extracted from lithics and reference resins by immersion in dichloromethane/methanol (DCM:MeOH, 2:1 v/v) and ultra-sonicating for 15 min. In one case (tool 91234), the suspected residue was removed with a scalpel for analysis, and then was placed in solvent and sonicated. The supernatant was removed to a hatch tube. Additional solvent was added to the samples and then sonication step was repeated twice more, and the supernatant extracts combined.

Solvent extraction: soils

Soil samples were laid out on aluminium foil-lined trays, loosely covered with foil, and air dried at ambient temperature (~20 °C) to remove moisture (Figure AM4.1). Each soil sample was then crushed with an agate mortar and pestle to break up organics and expose more surface area for the solvent to act upon. The mortar and pestle was cleaned 3 x with DCM between each soil sample. Crushed soil samples were weighed to 1 g and placed in hatch tubes. 100 µl of C34 alkane standard was added to each hatch tube. Lipids were solvent extracted from soils using DCM:MeOH (2:1 v/v) and ultra-sonicating for 15 min and centrifuging at 3,000 RPM for 10 min. The supernatant was removed to a hatch tube. Additional solvent was added to the samples and then sonication and centrifugation steps were repeated twice more, and the supernatant extracts combined.

Figure AM4.1. Air drying soil samples.

Solvent extraction: all extracts

All samples in hatch tubes were then dried down under a stream of nitrogen (N_2) to a volume of 2 ml, and then transferred to hydrolysis vials. All soils and some tool extracts were filtered through sterile glass pasteur pipettes stuffed with sterile glass wool to remove particulate matter (Figure AM4.2). All samples in hydrolysis vials were evaporated to dryness under a stream of N_2 on a heating block at 70 °C (Figure AM4.3) and stored in the freezer at -20 °C prior to derivatisation and analysis by GC-MS.

Figure AM4.2. Filtering soil samples through sterile glass wool.

Figure AM4.3. Samples being evaporated under a stream of nitrogen while being heated at 70 °C.

Derivatisation

Samples were silylated with N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trichloromethylsilane (TCMS), vortexed, and heated for 1 h at 70 °C.

Then, samples were evaporated to dryness under a stream of N_2. Derivatised samples were redissolved in Hexane:DCM (1:1 v/v), and 10 µl of C36 long-chain alkane internal standard was added to all autosampling transferred to sterile autosampling vials (Figure AM4.4), and analysed directly by GC-MS.

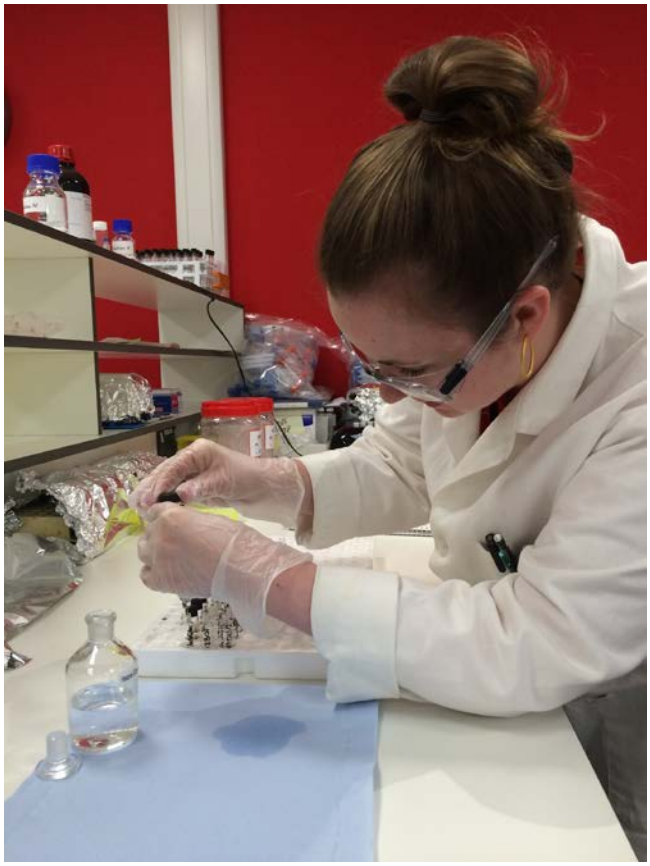

Figure AM4.4. Transferring aliquots of extracted residue samples to autosampling vials.

Instrumentation

An 7890A Series chromatograph attached to a 5975 C Inert XL mass-selective detector with a quadrupole mass analyser (Agilent Technologies, Cheadle, UK) was used. The type of ionisation used was electron impact ionisation with an ionisation energy of 70 eV. The GC column used was a DB-5ms (5%-phenyl)-methylpolysiloxane column (30 m × 0.250 mm × 0.25 µm; J&W Scientific, Folsom, CA, USA) and helium was the carrier gas. The temperature for this column was set at 50°C for 2 min, then raised by 10°C min^{-1} to 325°C, where it was held for 15 min. Data acquisition time was 44.5 min. The samples were injected with a splitless injector and the injector temperature was 300°C. The instrumental parameters used are listed below.

Mass spectrometer: 5975 C Inert XL mass-selective detector with a quadrupole mass analyser (Agilent Technologies, Cheadle, UK)

Sign of ions/type of ionization: positive electron ionisation. Electron impact ionisation, aka electron ionisation (EI)

Ionization energy: 70 eV

Gasses used: Helium carrier gas

m/z scale calibration: spectra were obtained by scanning between *m/z* 50 and 800?

Data acquisition time: 44.5 min

Gas chromatograph manufacturer, model and type: 7890A Series chromatograph (Agilent Technologies, Cheadle, UK)

Injector type: splitless injector

Injector temperature: 300°C

GC column: DB-5ms (5%-phenyl)-methylpolysiloxane column, 30 m × 0.250 mm × 0.25 µm (J&W Scientific, Folsom, CA, USA).

Carrier gas, linear velocity, flow rate: He

Column oven temperature details: The temperature for this column was set at 50°C for 2 min, then raised by 10°C min^{-1} to 325°C, where it was held for 15 min.

Other detectors: N/A

Data analysis: Agilent MSD Chemstation software, version G1701EA E.02.02.1431, where acquired

Gas chromatograph analysis

For analysis of spectra, Agilent MSD Chemstation software, version G1701EA E.02.02.1431, was used. Compounds in spectra were identified by comparison with the National Institute of Standards and Technology (NIST) Mass Spectral Database, published literature, and the gas chromatograms of authentic resins. Individual peaks

in chromatograms were searched for matches with compounds in the library using NIST MS Search Program 2.0.