Appendix II. Standard Operating Procedures for lipid residue analysis.

Derivatisation of organic residues. Silylation of organic residues for GC/GC-MS.

1.0 Purpose

This SOP provides a method for the derivatisation of organic residues of archaeological origin by silylation

2.0 Scope

This SOP is to be used for all sherds sampled for the Baltic Pottery Project

3.0 References

Evershed, R. P., Arnot, K. I., Collister, J., Eglinton, G., Charters, S. (1994). Application of isotope ratio monitoring gas chromatography - mass spectrometry to the analysis of organic residues of archaeological origin. *Analyst,* **119**, 909-914.
Regert, M., Bland, H. A., Dudd, S. N., van Bergen, P. F., Evershed, R. P. (1998). Free and bound fatty acid oxidation products in archaeological ceramic vessels. *Proceedings of the Royal Society of London B*, **265**, 2027 - 2032.
Copley, M. S., Berstan, R., Dudd, S. N., Docherty, G., Mukherjee, A., J., Straker, V., Payne, S., Evershed, R. P. (2003). Direct chemical evidence for widespread dairying in prehistoric Britain. *Proceedings of the National Academy of the United States of America,* **100**(4), 1524-1529.
Craig, O. E., Taylor, G., Mulville, J., Collins, M. J., Parker-Pearson, M. (2005). The identification of prehistoric dairying activites in the Western Isles of Scotland: an integrated biomolecular approach. *Journal of Archaeological Science,* **32**, 91-103.

4.0 Health and Safety

Health and safety issues are covered by the relevant COSHH forms available in the Lab.

5.0 Responsibilities

It is the responsibility of all members of the Baltic Pottery Project to ensure the application of this SOP to the derivatisation of organic residues.

PREPARED BY:	APPROVED BY:
NAME Val Steele	NAME Carl Heron

6.0 Procedure

6.1 Pre-preparation procedures

6.1.1 Make sure all glassware and tools are solvent rinsed (3x rinsing in DCM)

6.1.2 Wear gloves at all times

6.1.3 Samples should be processed in batches of no more than twelve (11 samples + 1 method blank/10 samples + 1 method blank + 1 pottery blank)

6.2 Labelling

6.2.1 Make sure that labels are still legible on both vials and lids before starting this procedure. Residues for silylation will be extracts A, B2 or C and should still be identified by a unique sherd identifier, a letter indicating the origin of the residue and one of the letters above.

6.3 Silylation procedure

6.3.1 Add three drops *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethyl-chlorosilane (TMCS) to each dry residue using a pasteur pipette. Alternatively add 100µl BSTFA using a micro-syringe.

6.3.2 Warm very gently on hotplate for 30 minutes.

6.3.3 Evaporate off excess BSTFA under nitrogen with gentle heat.

6.3.4 Analyse by GC/GC-MS within two days. If analysis cannot be performed within that time, repeat steps 6.3.1 to 6.3.3.

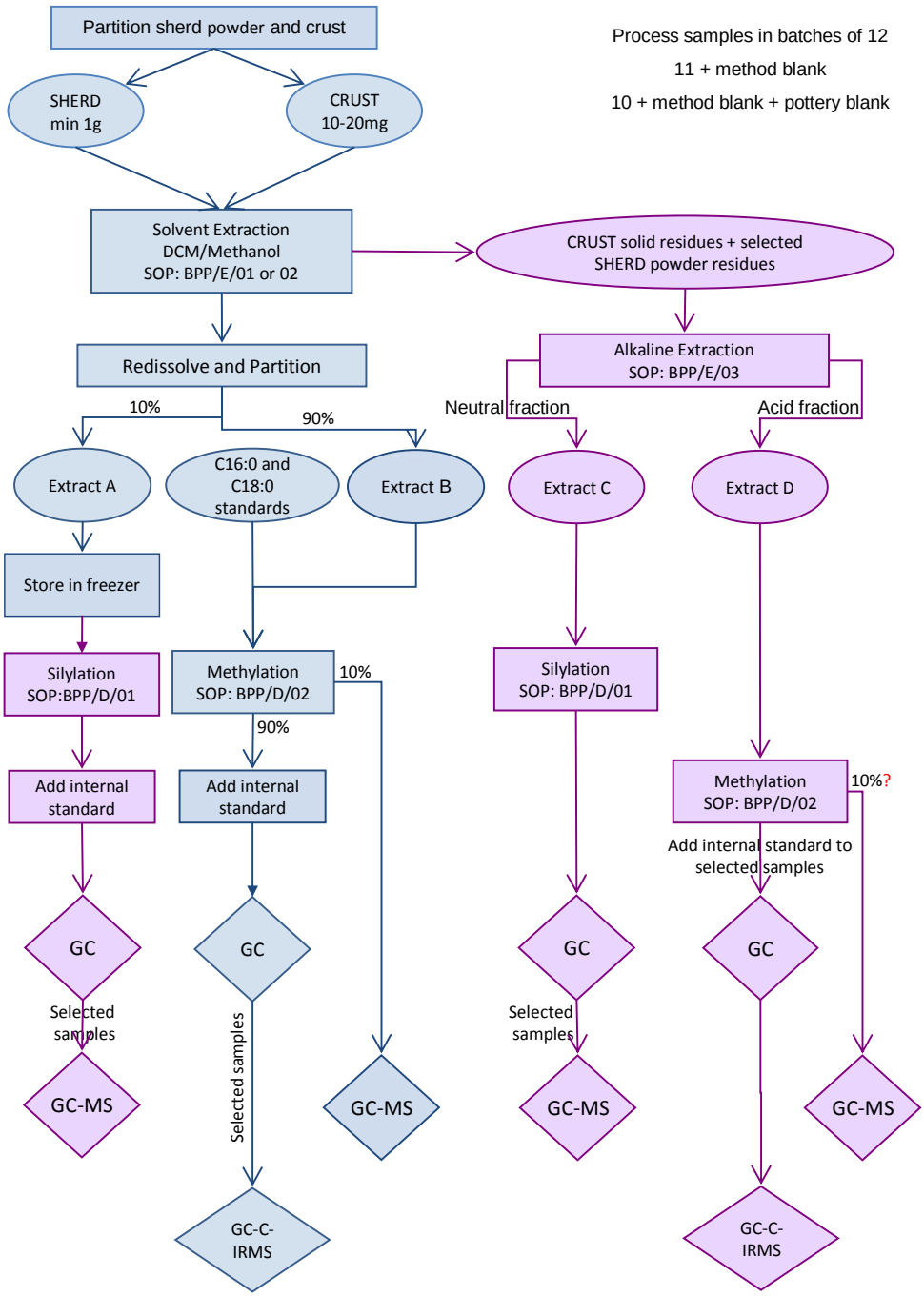
6.4 Blanks

6.4.1 Blanks from the extraction of residues should be silylated with the same batch of residues.

6.4.2 GC/GC-MS analysis of blanks will test for contamination introduced during the preparation of samples.

APPENDIX

Sample preparation and analysis flow chart



Derivatisation of organic residues. Methylation of organic residues for GC/GC-MS/GC-C-IRMS.

7.0 Purpose

This SOP provides a method for the derivatisation of organic residues of archaeological origin by methylation.

8.0 Scope

This SOP is to be used for methylation carried out on organic residues from the Baltic Pottery Project

9.0 References

Dudd, S. N., Evershed, R. P., & Gibson, A. M. (1999). Evidence for varying patterns of exploitation of animal products in different prehistoric pottery traditions based on lipids preserved in surface and absorbed residues. *Journal of Archaeological Science*, **26**, 1473-1482.

Mottram, H. R., Dudd, S. N., Lawrence, G. J. *et al*. (1999). New chromatographic, mass spectrometric and stable isotope approaches to the classification of degraded animal fats preserved in archaeological pottery. *Journal of chromatography A*, **833**, 209 - 221.

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Stott, A. (2006), pers. comm..

10.0 Health and Safety

Health and safety issues are covered by the relevant COSHH forms, which are available in the Lab.

11.0 Responsibilities

All members of the Baltic Pottery Project are responsible for following the procedure outlined in this SOP when carrying out methylation of archaeological residues.

12.0 Procedure

12.1 Pre-preparation procedures

12.1.1 Make sure all glassware and tools are solvent rinsed (3x rinsing in DCM)

12.1.2 Wear gloves at all times

12.1.3 Process no more than twelve samples in one batch (11 samples + 1 method blank/ 10 samples + 1 method blank + 1 pottery blank)

12.2 Labelling

12.2.1 Ensure that all residues are still labelled with a unique sherd identifier, a letter indicating the origin of the residue and a letter identifying which extract group the residues belong to. Methylated residues should all be extracts B or D.

12.3 Procedure

12.3.1 Add 200µl boron trifluoride methanol complex (BF_3), 14% w/v, to the dry residue in a Hach tube.

12.3.2 Heat closed tube in a heating block for 20 minutes at 70°C.

12.3.3 (Add a few drops of de-ionised water to quench the reaction)

12.3.4 When cool, add 2ml hexane.

12.3.5 Shake gently to mix.

12.3.6 Allow the hexane layer to separate out and pipette off carefully into a clean, labelled Hach tube.

12.3.7 Repeat steps 6.3.4 to 6.3.6 twice more, combining the extracts.

12.3.8 Evaporate under a very gentle stream of nitrogen with gentle warmth until about 2ml remains.

12.3.9 Pipette carefully into a clean, labelled small vial.

12.3.10 Continue to evaporate very gently to dryness.

12.3.11 Store methylated extract in a freezer at −20°C until required for analysis.

12.4 Blanks

12.4.1 Blank samples from the extraction/saponification process should be methylated with the batch of samples with which they were processed.

12.4.2 A method blank can also be included to check for contamination during the methylation procedure.

12.4.3 GC analysis of the blank samples will provide a measure of contamination introduced during preparation of samples.

Solvent extraction of organic residues.

13.0 Purpose

This SOP provides a method for the solvent extraction of lipid residues from pottery sherds.

14.0 Scope

This SOP is to be used for all sherds sampled for the Baltic Pottery Project

15.0 References

Regert, M., Bland, H. A., Dudd, S. N., van Bergen, P. F., Evershed, R. P. (1998). Free and bound fatty acid oxidation products in archaeological ceramic vessels. *Proceedings of the Royal Society of London B,* **265**, 2027 - 2032.

Evershed, R. P., Dudd, S. N., Charters, S., Mottram, H., Stott, A. W., Raven, A., van Bergen, P. F., Bland, H. (1999). Lipids as carriers of anthropogenic signals from prehistory. *Philosophical Transactions of the Royal Society of London, B,* **354**, 19-31.

Craig, O. E., Taylor, G., Mulville, J., Collins, M. J., Parker-Pearson, M. (2005). The identification of prehistoric dairying activites in the Western Isles of Scotland: an integrated biomolecular approach. *Journal of Archaeological Science*, **32**, 91-103.

Craig, O. E., Forster, M., Andersen, S. H., Koch, E., Crombé, P., Milner, M. J., Stern, B., Bailey G, N., Heron, C. P. (2007). Molecular and isotopic demonstration of the processing of aquatic products in Northern European prehistoric pottery. *Archaeometry,* **49**(1), 135-152.

16.0 Health and Safety

Health and safety issues are covered by the relevant COSHH forms, which are available in the Lab.

17.0 Responsibilities

All members of the Baltic Pottery Project are responsible for ensuring that the procedures detailed in the SOP are followed when carrying out solvent extraction of organic residues from pottery sherds.

PREPARED BY:	APPROVED BY:
NAME Val Steele	NAME Carl Heron

18.0 Procedure

18.1 Preparation procedures

18.1.1 Make sure all glassware and tools are solvent rinsed (3x rinsing in DCM)

18.1.2 Wear gloves at all times

18.1.3 No more than twelve samples to be processed in one batch (11 samples + 1 method blank/10 samples + 1 method blank + 1 pottery blank).

18.2 Labelling

18.2.1 Label both vial and lid with unique sherd identifier followed by I for interior surface or E for exterior surface

18.3 Sample retrieval

18.3.1 Drill if possible at least 1g of sherd from the interior surface using a modelling drill with a tungsten carbide bit.

18.3.2 Drill to a depth of 2 to 4mm.

18.3.3 Collect sherd powder on aluminium foil and transfer to labelled scintillation vial.

18.4 Solvent extraction

18.4.1 Accurately weigh about 1g sherd powder into a clean, labelled scintillation vial, leaving a portion of the sherd powder as a reserve sample if possible. Reserve sample should be stored in freezer at −20°C.

18.4.2 Add 5ml of DCM:methanol 2/1 v/v.

18.4.3 Sonicate for 5 minutes.

18.4.4 Centrifuge at 2000rpm for 5minutes.

18.4.5 Carefully pipette off the liquid extract into a clean, labelled scintillation vial.

18.4.6 Repeat steps 6.4.2 to 6.4.5 twice more, combining the extracts.

18.4.7 Reduce volume of extract to about 2ml under a stream of nitrogen with gentle heat.

18.4.8 Transfer to a clean, labelled, small vial and continue to evaporate to dryness.

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

18.5 Exterior surfaces

18.5.1 For extracting the residue from the exterior surface of the sherd repeat steps 6.1 to 6.4.

18.6 Blanks

18.6.1 For every ten samples a method blank and ideally also a pottery blank (Soxhlet extracted, unused modern flower pot) should be included. Eleven samples may be processed with a method blank.

18.6.2 GC/GC-MS analysis of blanks will provide a measure of contamination introduced during the extraction of organic residues from sherds.

Solvent extraction of organic residues from foodcrusts and burnt residues preserved on ceramics.

Purpose

This SOP provides a method for the solvent extraction of lipid residues from food crusts preserved on the interior surfaces of archaeological ceramics and burnt residues preserved on the exterior surfaces.

19.0 Scope

This SOP is to be used for all food crusts and exterior residues sampled for the Baltic Pottery Project

20.0 References

Craig, O. E., Forster, M., Andersen, S. H., Koch, E.; Crombé, P., Milner, M., Stern, B., Bailey, G. N., Heron, C. P. (2007). Molecular and isotopic demonstration of the processing of aquatic products in Northern European prehistoric pottery. *Archaeometry*, **49**(1), 135-152

21.0 Health and Safety

Health and safety issues are covered by the relevant COSHH forms, which are available in the Lab.

22.0 Responsibilities

All members of the Baltic Pottery Project are responsible for applying this SOP to the extraction of organic residues from food crusts and burnt residues.

23.0 Procedure

23.1 Pre-preparation procedures

23.1.1 Make sure all glassware and tools are solvent rinsed (3x rinsing in DCM)

23.1.2 Wear gloves at all times

23.1.3 No more than twelve samples should be processed in one batch (11 samples + 1 method blank).

23.2 Labelling

23.2.1 Label both vials and lids with unique sherd identifier followed by F for food crust or S for exterior residues

23.3 Sample retrieval

23.3.1 Remove a portion of the residue from the sherd surface by gently scraping with a clean scalpel

23.3.2 Collect the sample on aluminium foil and transfer to labelled scintillation vial.

23.4 Solvent extraction

23.4.1 Accurately weigh about 10 – 20mg of residue into a clean, labelled small vial, leaving a portion as a reserve sample if possible. Reserve sample should be stored in freezer at −20°C.

23.4.2 Remove from vial and crush sample gently in an agate pestle using an agate mortar.

23.4.3 Transfer crushed sample back into vial.

23.4.4 Add 2ml of DCM:methanol 2/1 v/v.

23.4.5 Sonicate for 5 minutes.

23.4.6 Centrifuge at 2000rpm for 5minutes.

23.4.7 Carefully pipette off the liquid extract into a clean, labelled scintillation vial.

23.4.8 Repeat steps 6.4.4 to 6.4.7 twice more, combining the extracts.

23.4.9 Reduce volume of extract to about 2ml under a stream of nitrogen with gentle heat.

23.4.10 Transfer to a clean, labelled, small vial and continue to evaporate to dryness.

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

23.5 Blanks

23.5.1 For every eleven samples a method blank should be included.

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

Alkaline extraction of organic residues from archaeological ceramics, food crusts and burnt residues.

24.0 Purpose

This SOP provides a method for the alkaline extraction of lipid residues from sherd powders, food crusts and burnt residues which have previously been solvent extracted.

25.0 Scope

This SOP is to be used for all alkaline extractions carried out for the Baltic Pottery Project

26.0 References

Regert, M., Bland, H. A., Dudd, S. N., van Bergen, P. F., Evershed, R. P. (1998). Free and bound fatty acid oxidation products in archaeological ceramic vessels. *Proceedings of the Royal Society of London B,* **265**, 2027 - 2032.

Regert, M., Dudd, S. N., van Bergen, P. F. et al. (2001). Investigations of solvent extractable lipids and insoluble polymeric components: organic residues in Neolithic ceramic vessels from Chalain (Jura, France). In Millard, A., *British Archaeological Reports: 939. Proceedings of Archaeological Sciences 97* (78-90). Oxford: Oxbow Press.

Craig, O. E., Love, G. D., Isaksson, S., Taylor, G., Snape, C. E. (2004). Stable carbon isotopic characterisation of free and bound lipid constituents of archaeological ceramic vessels by solvent extraction, alkaline hydrolysis and catalytic hydropyrolysis. *Journal of Analytical and Applied Pyrolysis,* **71**, 613-634.

27.0 Health and Safety

Health and safety issues are covered by the relevant COSHH forms, which are available in the Lab.

28.0 Responsibilities

All members of the Baltic Pottery Project are responsible for ensuring that the procedures detailed in the SOP are followed when carrying out alkaline extraction of organic residues from sherd powders, food crusts or burnt residues.

29.0 Procedure

29.1 Preparation procedures

29.1.1 Make sure all glassware and tools are solvent rinsed (3x rinsing in DCM)

29.1.2 Wear gloves at all times

29.1.3 No more than twelve samples to be processed in one batch (11 samples + 1 method blank/10 samples + 1 method blank + 1 pottery blank).

29.2 Labelling

29.2.1 Label both vial and lid with unique sherd identifier followed by I for interior surface, E for exterior surface, F for food crust or S for burnt residue. Also see below.

29.3 Alkaline extraction

29.3.1 Transfer sherd powder, food crust or burnt residue left after solvent extraction to a clean, labelled Hach tube.

29.3.2 Add 4ml O.5M methanolic sodium hydroxide, made up with 9:1 methanol:water v/v.

29.3.3 Heat in the closed tube for 90 minutes at 70°C.

29.3.4 Allow to cool.

29.4 Extraction of neutral fraction

29.4.1 Add 2ml hexane and agitate gently to mix.

29.4.2 Allow to separate and pipette hexane layer (upper layer) off into a clean, labelled Hach tube or scintillation vial, being careful not to extract any of the lower layer.

29.4.3 Repeat steps 6.4.1 and 6.4.2 twice more, combining the extracts.

29.4.4 Evaporate solvent off under a gentle stream of nitrogen with gentle warmth until about 2ml remains.

29.4.5 Transfer to a small vial and continue to evaporate to dryness.

29.4.6 This is EXTRACT C – the non-saponifiable or neutral fraction produced by this extraction process. Check the sample is labelled correctly and store in refrigerator until required for GC/GC-MS analysis.

29.5 Extraction of acidic fraction

29.5.1 Acidify the residue left after completing section 6.4 using *c*.2ml 0.5M hydrochloric acid – check residue is at pH 3.

29.5.2 Extract into a clean, labelled Hach tube as in steps 6.4.1 to 6.4.3

29.5.3 Evaporate to dryness under a very gentle stream of nitrogen with very gentle warmth.

29.5.4 This is EXTRACT D – the saponifiable or acidic fraction produced by the alkaline extraction. Check sample is labelled correctly and proceed to methylation procedure or store in refrigerator at −4°C until methylation can be carried out.

29.6 Blanks

29.6.1 For every ten samples a method blank and ideally also a pottery blank (Soxhlet extracted, unused modern flower pot) should be included. Eleven samples may be processed with a method blank.

29.6.2 GC/GC-MS analysis of blanks will provide a measure of contamination introduced during the extraction of organic residues from sherds.