Lipids extraction in animal tissues for analysis of C and N.

1. Remove the portion of skin to be tested.
2. Dry in oven at 70ºC for 24 hours.
3. Grind samples using agate mortar or ball mill.
4. Prepare a solution of cloroform:metanol (2:1, v:v).
5. Cover the sample with this solution. (With the aid of a pipette add the solution to each of the samples in sufficient quantity so that they are completely immersed. This must be done in the extraction hood and complying with all safety measures (gloves, mask etc.)).
6. Shake in vortex for few minutes.
7. Cover the vials and leave them inside the extraction hood at ambient temperature for 24 hours.
8. Centrifuge at 750 rpm for 10 minutes and remove the supernatant.
9. Repeat steps 5 to 8 twice.
10. Rinse the solid residue with distilled water and shake in vortex for a few minutes.
11. Centrifuge for 10 minutes at 12000 rpm and remove supernatant.
12. Repeat the two previous steps until the solution of chloroform: methanol that was yellowish at first becomes almost transparent.
13. The solid residue is dried in the oven at least 24 hours; to grind sample using agate mortar or ball mill. Finally weighed and encapsulate.

Justification for extraction protocol.

Reduce the sources of ^{13}C variation and compare tissue samples from different taxonomic groups, which may be different in their biochemical composition (eg, different fat content).
The extraction process allows comparisons between organisms without the confusing effect of differences in lipid content.