

## **Dry Sieved Samples through Extraction** **DS→E Rev: 0.92 (1-2-10)**

**This procedure is for dry sieved samples that will be put through the ASE. “X” samples will be processed (in duplicate) in this batch.**

### **Equipment Needed:**

- Analytical balance, accurate to 0.1 mg
- Medium to large capacity oven set to  $105 \pm 5$  °C for glassware drying
- Oven set to  $40 \pm 2$  °C
- Apparatus for automatic extraction
  - Dionex Accelerated Solvent Extractor, model 200 and extraction cells, 11ml
- Evaporator, either apparatus listed or equivalent device suitable for evaporating water and ethanol
  - Rotary evaporator with trap and water bath set to  $40 \pm 5$  °C
  - Or Turbovap II

### **Chemicals and Materials Needed:**

- Deionised water.
- Ethyl alcohol, capable of making 95% ethanol from.
- Porcelain crucibles.

### **DAY BEFORE:**

1. Take 2X small empty crucibles and put them in the oven overnight at 105C (MC of dried).
2. Have 2X clean ASE cells (11ml) ready. This means that they should be assembled but missing the filters.
3. Have 2X ASE collection vials clean and in the oven at 105C.
4. Get 2X clean petridishes for air drying the extracted material. These can be put in the oven (provided that they are not plastic and do not melt) but they should be put out in the open after thirty minutes in the dessicator so they can equilibrate with the room temperature before weighing.

## DAY 1 (Extractives Removal):

1. Weighing Collection Vials:
  - a. Take the 2X collection vials from the oven (105C) and put in a dessicator.
  - b. 20 minutes later, take a collection vial from the dessicator and then close the dessicator lid again. Weigh the vial to the nearest 0.1mg. Repeat for another collection vial. These collection vials should be numbered and the numbers should match with the identity of the DS sample (and ASE cell) they will collect the extract from and the position they will be on the ASE carousel.
  - c. Repeat for the other collection vials for the other samples. Only take these vials out of the dessicator when they are ready to be weighed. Never leave unweighed glassware or samples outside of the dessicator.
2. Take a DS bottle.
3. Pour the sample from the bottle into a clean and dry “mixing box” and close the box. Shake it vigorously to mix the contents thoroughly.
4. NIR Scanning:
  - a. Take the circular NIR cell and put some of the DS material in it. Carefully position it over the detector. Scan the cell.
  - b. Save with the code NIRCODE-BATCHNUMBER in the product group “DS-E” **if moisture content determination is to be carried out** or in the product group “DS-E NO DISHES” **if moisture content determination is not to be carried out** (in the case of limited sample quantity).
5. Moisture Content Determination (if there is sufficient DS material):
  - a. Take a small crucible from the dessicator (should have been put there from the oven about 20 minutes ago). **Weigh it to the nearest 0.1mg and record the scales that were used to weigh the crucible.**
  - b. Add roughly 0.5g of the DS biomass (less if there is a small amount of DS) and **weigh to the nearest 0.1mg.**
  - c. Put in the oven and dry at 105C overnight or for at least 6 hours.
  - d. Repeat (a)-(c) for another small crucible.
6. Take a numbered (assembled) clean DIONEX ASE cell. Record the number of the cell. Place two appropriately sized glass filters in the bottom of the cell, tamping down one at a time. Put the cell on a weighing scales (record which weighing scales is being used), making sure that the lid is not on the cell, and then press TARE
  - a. Fill the cell with the sample by pushing it in with the tamping rod (not too tightly). Make sure that there is no sample on the outside, and then **weigh the cell again (to the nearest 0.1mg).**
  - b. Then screw the top of the cell on tightly. Make sure that there is no sample on the threads of the lids or cell.
  - c. REPEAT (a) to (c) FOR ANOTHER CELL for the same sample.
  - d. Put the remainder of the sample (not used for extractive removal and moisture content determination) back in the DS sample-bottle and store it.
  - e. Keep the ASE cells on one side until ready to bring them to the ASE. Make sure that the bottom (the part with the glass filters) is always at the bottom.

7. Put the two cells in the ASE (positions 1 and 2), **ensuring that the end with the filters is on the bottom**, record the position on the ASE carousel that the cell was placed.
8. Make sure that there is enough ethanol in the ASE reservoir. **NOTE THAT THE ETHANOL NEEDS TO BE 95% (v). THE ETHANOL WE HAVE IS 99.5% AND SO WILL NEED TO BE DILUTED TO 95% WITH DEIONISED WATER.**
9. Turn on the pressurized air and the Nitrogen so that gases are being supplied to the ASE.
10. Make sure that there are empty collection vials in positions 1 to 2X and in R1, R2 and R3. The numbers and positions of each collection vial should be included on the data recording sheet so that they can match with the ASE cell being processed.
11. On the ASE, check that everything is working properly by doing a "RINSE" then click "Run Schedule", select Schedule 1 and press Enter.
12. Repeat steps 2-6 for the remaining DS samples. Put the cells in positions 6,7,8,9,10,11,12,13,14,15 in that order. Make sure that the numbers, positions and dry weights of the collection vials for each ASE cell are known.
13. When the run is complete, check that all of the cells have been extracted by going to the Error Log Diagnostic screen on the ASE.
14. If some cells did not get fully processed assess why (if it was vapour threshold exceeded error then it is likely that the cell was not packed correctly and leaked). Then set up a new schedule to just process these cells.
15. Once all cells have been extracted, allow the cells to cool (approx 10 minutes) and then prepare to remove the material.
16. Take 2X numbered petri-dishes:
  - a. Record the number of each petri-dish in the appropriate column for the ASE cell.
  - b. **Weigh each large petri-dish to the nearest 0.1mg. Note that if the dish is too wide for the scales then use a support that is thinner, then Tare the scales and then weigh the petri-dish.**
  - c. Take all of the sample from an ASE cell and put in the petri-dish. Make sure not to leave any sample in the ASE cell. At this point the glass filters can be left in the petri-dish.
  - d. Clean the ASE cells. Leave the samples in the petri-dishes to dry (in the lab not in the oven) for 2 days.
17. Take the collection vials and place in the Turbovap, turn on the system and the air line and turn on the rows when the vials are present then turn on the system. Run it until there is no solvent left in the vials. Once this occurs the vials can be put in an oven set at 40C (note this whole step can be done the next day if necessary).

## DAY 2 – Petri-Dish Drying

1. After 1 day scrape off any biomass from the glass filters onto the petri-dishes, making sure not to transfer any of the filter paper to the petridish.
2. Move about the sample on the petridish with a spatula to expose wet areas so that they may dry.
3. Take the 2X small crucibles (for the moisture content of the dry sieved material) out of the oven. Find the appropriate column for each numbered crucible.
4. When it is 20 minutes after, take a small crucible (for the moisture content of the dry ground material) out of the dessicator then close the dessicator again. Weigh the crucible to the nearest 0.1mg. These crucibles can then be used for determining the ash content of the sample if necessary.
5. Repeat step 4 for the remaining crucibles.
6. **If the moisture content of the petri-dish samples is to be determined:** Put **4X** small crucibles in the oven at 105C.
7. If step 17 was not carried out yesterday do it today. If Step 17 was carried out yesterday then take the 2X collection vials and put in a dessicator. After 20 minutes take one collection vial out of the dessicator and then close the dessicator lid. Weigh this collection vial to the nearest 0.1mg. Repeat for the other collection vials. Then put the collection vials back in the oven at 40C.

## DAY 3 – MC Determination

1. If the moisture content of the petri-dish samples is to be determined: Take 4X small crucibles from the oven and put in a dessicator.
2. Weigh a petri-dish. Empty the contents into a “MIXING BOX” and close the box and shake. Fill a circular NIR cell with the sample. Carefully position it over the detector. Scan the cell. **IF YOU ARE DOING MOISTURE CONTENT DETERMINATION OF THE PETRIDISH SAMPLE:** Save with the code NIRCODE-BATCHNUMBER-PETRIDISHNUMBER in the product group “DS-E DISHES”. **IF YOU ARE NOT DOING MOISTURE CONTENT DETERMINATION OF THE PETRIDISH SAMPLE:** Save with the code NIRCODE-BATCHNUMBER-PETRIDISHNUMBER in the product group “DS-E DISHES NO CRUCIBLES”.
3. **IF YOU ARE DOING MOISTURE CONTENT DETERMINATION OF THE PETRIDISH SAMPLE:** (20 minutes after 1) weigh a small crucible then add approximately 0.2g (not much more) of the extracted biomass to it. **Weigh the exact amount that was added.** Put the small crucible on a tray to put in the oven at 105C. Repeat for another small crucible with the biomass from the MIXING BOX.
4. Put the rest of the extracted biomass in a red-test tube and label that test tube UNIVERSALCODE-“E”-BACTHNUMBER.
5. Take the other Petri-dish for that sample. Repeat steps 2-3 for that sample with a clean mixing box. Then add the remaining sample to the test tube from before.

6. Repeat 1-5 for the other samples, **each sample (2 duplicates) should have its own test-tube.**
7. **IF YOU ARE DOING MOISTURE CONTENT DETERMINATION OF THE PETRIDISH SAMPLE:** Put the small crucibles in the oven at 105C.
8. The collection vials should be weighed according to step 7 of Day 2. The data should be recorded in the appropriate cell depending on whether the vials have been in the oven for one or two days. If they have been in the oven for 2 days after weighing they can be washed until clean (it may be necessary to use NaOH) and then put in the oven at 105C.

#### **DAY 4 – Weighing Dried Extracted Samples**

1. **IF YOU ARE DOING MOISTURE CONTENT DETERMINATION OF THE PETRIDISH SAMPLE:** Take 2X of the 4X small crucibles (used to determine the moisture content of the extracted samples) out of the oven and 20 minutes later weigh these crucibles.
2. Repeat step 1 for the remaining crucibles in the oven.
3. The collection vials should be weighed according to Step 7 of Day 2. The data should be recorded in the appropriate cell depending on whether the vials have been in the oven for one or two days. If they have been in the oven for 2 days after weighing they can be washed until clean (it may be necessary to use NaOH) and then put in the oven at 105C. This step is not necessary if the collection vials have been cleaned the previous day.