Method Sheet 28

How to prepare a non-polar herb extract

Overview

This method sheet explains how to prepare a non-polar extract of a herb of interest using the solvent dichloromethane. Such preparations are often necessary in the drug discovery process, as larger quantities of specific hit extracts are required to complete additional tests in the replication phase. The method shown is equivalent to that used to prepare the stock herb extracts in the *Phytotitre* library. If any of the necessary items of equipment are not available, or if preferred for any other reason, resupplies of individual extracts are available economically and promptly from Caithness Biotechnologies.

Reagents

- 10 grammes of plant material
- 100% dichloromethane
- 100% dimethyl sulphoxide

Equipment

- 37°C oven for dessication
- Rotary evaporator
- Chemical fume hood
- Glass funnel, beaker and large diameter (30 - 70 mm) vial
- PTFE or nylon 0.2 µm syringe filters

Warning!

- ⚠ Dichloromethane is a hazardous solvent that can cause eye damage and severe respiratory distress - ensure all necessary precautions are followed when using this solvent, including full PPE (labcoat, gloves, safety glasses, respirator) and use only in a fume hood.
- Standard nitrile gloves offer very little resistance to dichloromethane, if gloves are contaminated with this solvent, replace them immediately.

Method

1) Finely chop fresh plant material of interest.
2) Dry the plant material in a dessicator at 37°C for 12 to 24 hours.
3) Add 10 g fully dried plant material to 100 ml of 100% dichloromethane solvent in a glass beaker.
4) Cover the top of the beaker tightly in foil and steep overnight in a fume hood at room temperature in the dark.

5) The next morning, filter the extract through Whatman number 1 filter paper using a glass funnel into a glass beaker.
6) Transfer the liquid to a rotary evaporator and remove most of the dichloromethane under mild vacuum with the water bath set to 30 - 35ºC.
7) Dispose of the waste dichloromethane into a chlorinated solvent waste bottle for specialist chemical disposal.
8) Weigh a clean glass vial without the lid on using a balance to an accuracy of within 0.01 grammes.
9) Pour the remaining extract into the open glass vial.
10) Allow all residual dichloromethane to evaporate at room temperature in a fume cupboard.
11) Weigh the glass vial with the lid off a second time, and subtract the previous mass of the empty vial to obtain the mass of the solids present in the extract.
12) Resuspend the dried product to 10 mg/ml in 100% dimethyl sulphoxide (DMSO) by dividing the mass of dry extract in milligrammes by 10, then adding that volume of DMSO in millilitres.
13) Vortex the suspension well to dissolve extracted compounds (can also be done after transfering to a 50 ml plastic tube if more convenient).
14) Filter sterilise the suspension into a sterile plastic tube using 0.2 µm syringe filters.
15) Pipette 1 ml aliquots of the extract into sterile 1.5 ml microtubes and store at -20ºC for up to 6 months.

Notes

- All plants present in the *Phytotitre* library are available commercially from diverse suppliers.
- If you are struggling to find a supplier of a specific herb, traditional herbal remedy outlets are good places to try to obtain the raw plant material.
- If a dessicator is not available, a dry 37ºC oven or incubator can used as an alternative.
- Plastic beakers, funnels or tips should not be used for any stage involving dichloromethane as this solvent can dissolve the plastic, but plastic products can be used after removal of the dichloromethane.