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Solid fungal extraction and C8 reversed phase chromatography

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We use this protocol and it's working

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Abstract

Protocol for freeze-drying fungal cultures and preparing extracts using C8 reversed-phase chromatography.

Materials

Plasticware

	Description	Catalogue number	Supplier
	90mm Petri Dishes	**LAB-021MR**	**Medi'Ray**

Solvents, etc

	Description	Catalogue number	Supplier
	Methanol LR	**32108-5L**	**Sigma Aldrich**
	Dichloromethanol (DCM)	21409-4L	Sigma Aldrich
	D4 Methanol	DLM-24-10	Sigma Aldrich
	C8 silica gel	LU013BM	Luknova

Equipment

- Sterile scalpel handle and blade
- Freeze dryer (Labogene)
- Rotary evaporator (Buchi)

Troubleshooting

Safety warnings

Solvent hazard classes:
Dichloromethane (DCM): 6.1(3)
Hexane: 3(2)
Ethyl acetate: 3(2)
Methanol: 6(6.1)

Crude extraction of fungal compounds

1 Subculture fungus onto ~40 Petri dishes of media of choice and seal the plates with parafilm. Grow at the appropriate growth temperature until the fungus reaches the required age or coverage.

2 Once grown, freeze plates overnight (-20 °C)

3 Freeze dry over two days (-80 °C) until plates are dry and crisp.

4 Weigh a clean 500ml beaker and break up dry fungal tissue into small pieces. Reweigh the beaker to obtain the dry weight of the fungus.

5 Submerge dry fungal tissue in methanol (MeOH) for 4 hours.

6 Filter into a 500ml round bottom flask.

7 Dry at reduced pressure on a rotary evaporator at 130 mbar.

8 Re-submerge fungal tissue in dichloromethane (CH_2Cl_2), cover with tinfoil and leave to soak overnight.

9 The following day filter into the same round bottom flask and dry at 500 mbar.

C8 reversed phase chromatograpahy

10 Remove some crude product from the flask for downstream biological testing.

11 Dissolve the remaining crude product in 10%M MeOH before loading onto C_8 reversed-phase silica gel. Elute with a gradient of H_20:MeOH (25% increments of 100ml volume) to afford five fractions (F1-F5). Apply pump pressure and collect the fractions in 250ml round bottom flasks.

Schematic of C_8 reversed-phase chromatography

12 Dry the 5 fractions at reduced pressure on the rotary evaporator at 130mbar (MeOH) and 40mbar (Water).

NMR and Samples for Biotesting

13 When flasks are dry add 0.35-0.4ml of D4 MeOH to each flask (Fraction 1 isn't necessary as it will contain sugars and salts) and mix. Using a 1ml syringe and needle draw up the methanol-fungi mixture into the syringe and add it to a clean, labelled NMR tube.

14 Cap samples gently and load them on to the AvanceIII-400mhz instrument.
Name each of the samples and use these settings:
- Proton H+
- 64 scans
- Solvent: d4 Methanol

15 Label and weigh 5 empty biotesting vials for further testing. Add the contents of the NMR tubes to each of the tubes and allow to dry. Reweigh the dry tubes and label them with the mass (in milligrams). Parafilm the lids and send back for extract testing.