Phytotitre

Caithness BIOTECHNOLOGIES

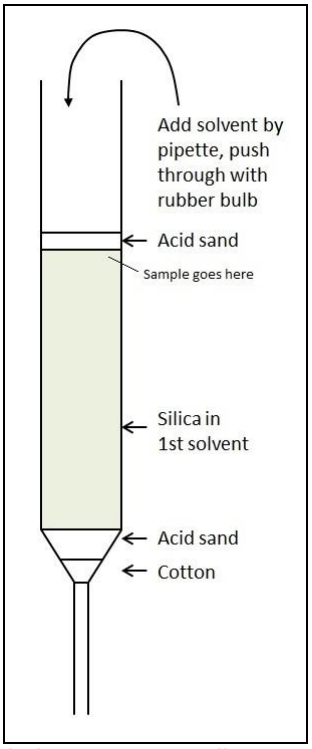
Preliminary fractionation of natural compounds from plant extracts by silica gel chromatography

Equipment and reagents required

- Vacuum concentrating evaporator
- Small clamp stand
- Clean, small bore glass pasteur pipettes (e.g. Fisher 230 mm length Pasteur pipettes, 11566963)
- Cotton buds
- 5 mg freeze-dried powder of aqueous extract, or resin of fully evaporated di-chloromethane extract of natural product of interest
- Silica 60 gel (e.g. Sigma 236799-100G)
- Acid washed sand (e.g. Sigma 18649-1KG)
- 1.5 ml microtubes or glass vials for fraction collection

Method

1) Suspend a clean glass pipette vertically in a clamp-stand (see right)
2) Insert a small piece of cotton bud from the top opening of the pipette, and tamp gently into the bottom of the column using a long piece of wire
3) Using a folded paper funnel or similar, layer ~3-5 mm of acid-treated sand on top of the cotton
4) Using a folded paper funnel or similar, layer silca 60 gel on top of the acid sand to a height reaching approximately two thirds of the way up the column
5) Wet the silica gel in the colum by pipetting into the top opening 1 ml of the first solvent to be used for separation (see below), and discard the eluate
6) Prepare a slurry of ~5 mg dried extract powder (or resin from complete evaporation of a di-chloromethane extract) by mixing with a small quantity of silica gel and the first solvent
7) Layer this slurry on top of the silica gel already in the column
8) Layer a small amount of acid-treated sand on top of the slurry
9) Prepare sufficient solvents (see below) to perform a stepwise polarity gradient elution
10) Pour 2 ml of the first solvent down the column and discard the eluate
11) Pour 1.4 ml of each solvent down the column, in order of increasing polarity, twice for each solvent
12) Allow each solvent to drip through by gravity alone and collect 1.4 ml of each eluate in numbered 1.5 ml microtubes
13) Discard the column in a glass waste bin
14) Dry the eluates in each microtube using a vacuum evaporator (continue the drying process until no trace of solvent remains)
15) Resuspend each dried extract in 0.2 ml DMSO with vortexing
16) Re-assay each fraction at a maximum final concentration of 1% DMSO



Suggested solvents,* in order of increasing polarity:

1) 10% ethyl acetate in hexane (or octane)
2) 50% ethyl acetate in hexane (or octane)
3) 100 % ethyl acetate
4) 10% methanol in ethyl acetate
5) 50% methanol in ethyl acetate
6) 100% methanol
7) 10% water in methanol
8) 50% water in methanol

* Note that the list of solvents suggested in this method is not definitive, and alternatives with similar step-changes in polarity would be equally suitable. Likewise, the solvents used for separation can be simplified if desired (e.g. to just 4 intermediate polarity solvents). If desired, larger quantities of extract can be separated using larger diameter columns (e.g. 50 mg extract can be separated on a 1-2 cm diameter glass column), with volumes scaled up accordingly.