Processing PolyCYPs® extracts for purification of metabolites & derivatives – advice and tips

Suggested procedure:

- Stop the reaction by adding an equal volume of acetonitrile (ACN) to precipitate the proteins.
- Collect the extract and further wash reaction wells with additional solvent, e.g. 50% aq ACN, or 75% ACN.
- Pool extract with washings into a suitable container e.g., spark-proof seal centrifuge tubes.
- Separate precipitated proteins by centrifugation. A 10 minute spin at 3,000 g should be sufficient but if the supernatant is still cloudy, then repeat at a higher speed /for longer time.
- Collect the supernatant and wash the pellet again with more 50% aq ACN, or 75% ACN if compound is a little more apolar. Re-centrifuge as above.
- Pool the supernatants from the original extract and the pellet wash.

From here there are two options:

Option 1: Freeze out the organic

- Place the extract in a spark-free freezer and the layers will separate – collect the organic fraction and check localisation of your target – if it is not preferentially migrated, try option 2.
- If the target compound is localised in the organic layer, collect this and repeat with an equivalent volume of fresh ACN to recover the target compound further from the aqueous layer.
- If the target compound is localised in the aqueous layer, you'll need to recover using hydrophobic resin – remove the ACN by rotary evaporation, centrifuge to separate insoluble materials (check these in case they contain the target compound!) before column loading., e.g. on to an SPE cartridge or open column of a DVB resin e.g. HP20 or XAD16). The column can then be eluted with solvent in the usual manner to collect the concentrated extract ready for preparative LC.

Option 2: Salt out the organic layer

Before applying to the bulk of the material test out the method on a small portion using ammonium sulphate in excess (*ca.* 10% w/v) until the phases separate; check this is compatible with the product and no degradation occurs. Cooling to 4°C or centrifugation may also aid phase separation.

Once you are satisfied that the method is appropriate for the target compound, process the bulk of the extract in the same way. We have only seen stability issues using this approach when purifying *N*-glucuronides; hydroxylated products should be OK.

Purification of metabolites / derivatives

- Once you have the concentrated extract, use preparative HPLC to purify the target compound(s).
- We typically directly dilute the ACN extract with water and load via the pumps using one of the solvent lines. Make sure that dilution with water does not cause the solute to precipitate, or the flow path and/or column will become blocked. Alternatively, if you prefer to inject via a concentrate, redissolve the extract in water (if the compound is soluble) or e.g., 50-75% aq ACN for loading (centrifuge to clarify).
- The sample for preparative chromatography can also be buffered as for the LC mobile phase to achieve the best chromatography. Avoid the sample sitting in the buffer for too long as this could risk degradation – we sometime add the buffer in portions or just before injection as a precaution to avoid prolonged exposure.

Other processing tips:

To minimise the chances of compound degradation, undertake pH/solvent stability tests on small portions first. Common ways in which loss of compound is observed is from addition of DMSO, or through changing the pH.

If you have any questions or need specific advice for your application, email us at **support@hyphadiscovery.com**