

Vimala College (Autonomous)
Thrissur



PG Department of Zoology

**Standard Operating Procedure
For
BSc Zoology Practical**

Funded by

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Purchased item	Practical Core course title and code	Practical performed	SOP
Column chromatography	Core course practical III: VZO6BPL3-Biochemistry, Physiology and endocrinology	Study Chromatography techniques	<p>This method used to purify individual chemical compounds from mixtures of compounds. The technique uses an organic solvent as a mobile phase.</p> <p>SOP</p> <p>Application of the sample absorbed to stationary phase:</p> <ol style="list-style-type: none"> 1.1. Dissolve the sample in a suitable solvent in a suitably sized round bottom flask 1.2. Add a small amount of dry silica 1.3. Evaporate the solvent. This process requires special attention and should be supervised by an experienced user. 1.4. Scrape the silica mixture away from the sides of the flask with a bent spatula and apply the dry residue on top of the packed column. <p>Eluting the column:</p> <ol style="list-style-type: none"> 1. Ensuring the tap is closed, fill the column with the eluent once more, allowing the solvent to run gently down the walls of the column so as not to disturb the top of the silica bed. 2. Put a suitably sized beaker under the column, open the tap and apply N₂ pressure so that the liquid volume decreases with a rate of roughly 1 mm/s. It is recommended to use a t-adaptor with stopper on one end, which can be shot out to release excess gas if present, preventing the breakage of the column. 3. Collect fractions with the recommended volume. 4. Elute the column until the liquid is a few cm above the gel surface. Release the pressure and close the stopcock. Check the fractions. If all the wanted products have departed the column, the elution can be stopped. If not, the column can be refilled with more eluent, and the elution continued. <p>After the procedure:</p> <ol style="list-style-type: none"> 1. Disassemble the column and dispose silica and TLC as silica waste. 2. Wash glassware