Aim: To identify the lipids in the given sample by thin layer chromatography.

Principle: This technique is similar to paper chromatography but is more convenient and less time consuming. Here instead of paper, the supporting material is either a glass plate or a plastic sheet or a piece of metal foil. A thin layer of stationary i.e., silica gel (SiO$_2$) or alumina (Al$_2$O$_3$) is laid over this inert support. The solvent system is selected according to type of biomolecule under investigation.

Requirements:
1. **Activated TLC plates:** Place thoroughly cleaned and dried glass plates (20x20 cm) and spread a uniform layer of (0.2mm thickness) Silica Gel-G slurry with the help of a spreader, dry at room temperature and then activate at 110 °C for 30 min.
2. **Thin layer chromatographic tanks:**
3. **Developing Mixture/solvent system:** petroleum ether:diethyl ether:glacial acetic acid (80:20:1 v/v)
4. **Spraying reagents for location of spots on TLC plates:** 50% sulphuric acid.
5. **Lipid standards:** cholesterol, palmitate, lecithin etc.,

Procedure:
1. Take an activated TLC plate and draw two straight lines. First one about 2 cm from the bottom and second one 1 cm from the top of the plate.
2. Subdivide the bottom line for spotting the samples with 2 cm between two samples.
3. Pipette 20 µl of all the standard and test samples, and spot in an order on the TLC plates. Air dry the plates for 5-10 min.
4. Meanwhile add solvent (mobile phase) to the TLC chamber and close it with the lid. Allow it to saturate the chamber for 10 min at room temperature.
5. Using forceps, pickup the TLC plate from the top; place the TLC plate in the TLC chamber vertically. Ensure that the solvent phase moves uniformly along the plate.
6. Leave the plate in the chamber until the solvent has moved to the top pencil line of the TLC plate. When the solvent front has moved to the top line remove the plate with the help of forceps.
7. Place the TLC plate on a clean dry surface or on tissue and allow the mobile phase to evaporate completely for about 5-10 min.
8. Spray the detection reagent carefully and heat it in an oven at 110 °C for 5-10 min. areas containing lipids get charred and appear as black spots.
9. Locate the position of the lipid spots on the plate and measure the distance travelled by the individual lipid component.

Calculate the $R_f$ value of each sample, identify and report the lipids present in the sample given.

\[
R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}
\]

<table>
<thead>
<tr>
<th>Sample</th>
<th>$R_f$ value</th>
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<tbody>
<tr>
<td>Standard (Cholesterol)</td>
<td>a</td>
</tr>
<tr>
<td>Standard (Lecithin)</td>
<td>a1</td>
</tr>
<tr>
<td>Sample No. 1</td>
<td>a</td>
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</tbody>
</table>

**Report:** The given standard No. 1 has the $R_f$ value of ---- (a) and standard No. 2 has the $R_f$ value of ----(a1).

The given sample No. 1 has the same $R_f$ value of standard No. 1. Hence the sample No. 1 might be cholesterol.