SOP: Extraction of active ingredient (AI) from a treated filter paper

August 2023
1. Purpose

2. Background

3. Materials and equipment
   - Hole punch
   - Gloves
   - Lab coat
   - 10ml glass tubes
   - Internal standard dicyclohexyl phthalate (DCP)
   - Acetone
   - Sonicator (Ultrawave u500h Sonicator)
   - Pipette
   - Compressed air
   - Acetonitrile
   - Vortex
   - Eppendorf tubes
   - Centrifuge

---

1 Historical versions of SOPs can be found on the I2I website (https://innovationtoimpact.org/)
4. Procedure

Sample preparation

1. Take a representative sample of the filter paper by folding it back on itself twice and cutting in three positions using a hole punch with 0.635cm. This will give 12 circles with a total surface area of 15.201cm².

2. Place the cut-out pieces into a 10ml glass tube.

Active ingredient extraction

1. A surface extraction solution of 100µg/ml DCP in acetone is made up.

2. Add 5ml of the surface extraction solution and sonicate the sample for 60minutes at ambient temperature.

3. Pipette 1ml of the sonicated extraction solution to a new glass tube and evaporate to dryness under compressed air at 60°C.

4. Resuspend the evaporated sample in 1ml acetonitrile, and vortex for 1minute at 2'500-3'000 rpm.

5. By hand, transfer the solution to a 1.5ml Eppendorf tube.

6. Centrifuge the sample for 15minutes at 13'000 rpm.

7. Pipette 80µl to a HPLC vial ready for injection.
5. Additional data collection

6. Deviations from standard protocol

7. Glossary of terms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C</td>
<td>Degrees centigrade</td>
</tr>
<tr>
<td>AI</td>
<td>Active ingredient</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>DCP</td>
<td>Dicyclohexyl phthalate</td>
</tr>
<tr>
<td>I2I</td>
<td>Innovation to Impact</td>
</tr>
<tr>
<td>LSTM</td>
<td>Liverpool School of Tropical Medicine</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>rpm</td>
<td>Rotations per minute</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard operating procedure</td>
</tr>
</tbody>
</table>

8. References