



# **SOP: HPLC quantification of surface and total active ingredient present on an ITN**

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<b>Title</b>	HPLC quantification of surface and total active ingredient present on an ITN
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**Timeline**

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1	17/08/23	Katherine Gleave	I2I
2			

### Version Control<sup>1</sup>

Version	Date	Updated by	Description of update(s)

### Related documents

- I2I Best Practice SOP Library, August 2023 (<https://innovationtoimpact.org/>)

## 1. Purpose

## 2. Background

## 3. Materials and equipment

- Scissors
- Gloves
- Lab coat
- 10ml glass tubes
- Internal standard dicyclohexyl phthalate (DCP)
- Methanol
- Sonicator (Ultrawave u500h Sonicator)
- Pipette
- Compressed air
- Acetonitrile
- Vortex
- Eppendorf tubes
- Centrifuge

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

- HPLC vials
- 10% 1-propanol:heptane
- Heating block (Techne Driblock DB100/3)
- Dionex Ultimate 3000 HPLC System
- Hypersil GOLD C18 column (250 × 4.6 mm)

## 4. Procedure

### Surface active ingredient

1. A representative sample of net with an area of 32.17cm<sup>2</sup> (~0.160g) is cut in triplicate and placed in a 10ml glass tube.
2. A surface extraction solution of 100µg/ml DCP in methanol is made up.
3. Add 5ml of the surface extraction solution and sonicate the sample for 15minutes at ambient temperature.
4. Following sonification, remove the net and was with cold methanol before placing into a second glass tube ready for total AI extraction (to continue with total AI extraction at this point, refer to section below).
5. Pipette 1ml of the sonicated extraction solution to a new glass tube and evaporate to dryness under compressed air at 60°C.
6. Resuspend the evaporated sample in 1ml acetonitrile, and vortex for 1minute at 2'500-3'000 rpm.
7. By hand, transfer the solution to a 1.5ml Eppendorf tube.
8. Centrifuge the sample for 15minutes at 13'000 rpm.
9. Pipette 80µl to a HPLC vial ready for injection.

### Total active ingredient

1. A total AI extraction solution of 100µg/ml DCP in 1-propanol:heptane (1:9) is made up.

2. Add 5ml of the total AI extraction solution and heat the samples at 85°C for 45minutes.
3. Remove the sample from the heat block and allow to cool.
4. Pipette 1ml of the solution to a new glass tube and evaporate to dryness under compressed air at 60°C.
5. Resuspend the evaporated sample in 1ml acetonitrile and vortex for 1 minute at 2'500-3'000 rpm.
6. By hand, transfer the solution to a 1.5ml Eppendorf tube.
7. Centrifuge the sample for 15 minutes at 13'000 rpm.
8. Pipette 80µl to a HPLC vial ready for injection.

*Note1: to confirm all AI present is extracted, steps 2-7 can be repeated a further four times to fully remove any remaining AI.*

*Note 2: Depending on the chemistries associated with the net, the composition of the surface extraction solution may vary. This is usually a mix of methanol:water. The sonification time can also vary depending on the chemistry of the net and whether it is coated or incorporated.*

### HPLC analysis

HPLC analysis is performing by injecting 20µl of the sample on a reverse-phase Hypersil GOLD C18 column (75 Å, 250 × 4.6 mm, 5-µm particle size; Thermo Scientific) at room temperature. A mobile phase of 70% acetonitrile in water is used at a flow rate of 1 mL·min<sup>-1</sup> to separate the insecticide and internal standard. Chromatographic peaks of the insecticide and DCP are detected at 226 nm with the Ultimate 3000 UV detector (Dionex) and were analysed with Dionex Chromeleon software.

The quantities of insecticide were calculated from standard curves established by known concentrations of the insecticide authenticated standards and corrected by internal standard readings in each sample relative to control. Final insecticide content in gram per kilogram (%) net material was estimated using the following equation:

$$I = (x/a) \times (0.0005/m) \times C$$

Where I is the insecticide content in %, x is the insecticide peak area at 226 nm obtained from HPLC, a is the slope of insecticide standard curve, m is the mosquito net sample mass in gram and C is the internal standard correction factor calculated from dividing the peak area of 100 µg/mL DCP by the DCP peak area obtained for the unknown.

## 5. Additional data collection

## 6. Deviations from standard protocol

## 7. Glossary of terms

<b>°C</b>	Degrees centigrade
<b>AI</b>	Active ingredient
<b>cm</b>	Centimeter
<b>DCP</b>	Dicyclohexyl phthalate
<b>g</b>	Grams
<b>HPLC</b>	High-throughput liquid chromatography
<b>I2I</b>	Innovation to Impact
<b>LSTM</b>	Liverpool School of Tropical Medicine
<b>ml</b>	Milliliter
<b>rpm</b>	Rotations per minute
<b>SOP</b>	Standard operating procedure

## 8. References