



Analysis of Malachite Green and Metabolite Leucomalachite Green by HPLC

Product Number: EUBCX256

Malachite Green CAS 569-64-2

1. Sample Preparation

- a) Add 1 g of sample to a 50 mL centrifuge tube
- b) Add 50 μ L TMPD* solution at 1mg/mL
- c) Spike using malachite green and leucomalachite green at 0.1 μ g/mL
- d) Add internal standard
- e) Allow to sit for 10 minutes
- f) Add 10 mL of McIlvaine's** Buffer/Methanol 1:1
- g) Shake for 1 minute
- h) Centrifuge at 5000 rpm for 10 minutes
- i) Collect the supernatant
- j) Repeat steps f) through i) until a volume of 20 mL of supernatant is obtained

2. Condition Cartridge

- a) Add 5 mL of methanol to cartridge EUBCX256 and soak for 1 minute
- b) Add 5 mL of water and draw through
- c) Add 5 mL of McIlvaine's buffer

Note: Do not let the cartridge go dry otherwise repeat steps a) through c)

3. Extraction

- a) Load supernatant from step 1j
- b) Adjust vacuum for flow of 1–3 mL per minute

4. Wash Cartridge

- a) Add 5 mL 0.1N HCl and slowly draw through
- b) Add 10 mL water and draw through
- c) Add 5 mL 1:1 MeOH:water and draw through
- d) Add 10 mL hexane and draw through
- e) Dry under vacuum for 10 minutes

5. Elute cartridge

- a) Elute at 1–3 mL per minute using a solution of 50% ethyl acetate, 45% methanol, 5% ammonium hydroxide
- b) Carefully evaporate to dryness at 50°C using N₂
 - a) Reconstitute with LC mobile phase (50% acetonitrile and water containing 0.05 M p-toluene sulfonic acid (TSA) as a counter ion

6. Analyze by LC/MS

a) Use a reverse-phase C18 analytical column

Solutions:

* TMPD – N,N,N',N'-tetramethyl-1,4-phenylenediamine dihydrochloride, CAS 637-01-4

**McIlvaine's buffer pH 2.6 – mix equal parts of 0.1M citric acid monohydrate with 0.2M disodium hydrogen phosphate dihydrate (Na_2HPO_4)• H_2O