



Cyclopiazonic acid in peanuts and corn by HPLC

Scope: This is an HPLC method for the determination of cyclopiazonic acid (CPA) in peanuts and corn. CPA is a toxic metabolite produced mainly by *Aspergillus flavus*. CPA may co-occur with aflatoxins.

1. Extraction

1. Weigh out 50 g of ground sample into a 250 mL glass blender jar or 250 mL Erlenmeyer flask. Add 100 mL of extraction solvent (methanol:2% NaHCO₃ in water; 70+30). Blend at high speed for 2 minutes (blender) or shake for one hour on gyratory shaker. If a blender is used, cool the blender jar under running water before opening it.
2. Filter through Whatman No. 4 filter paper and pipet 50 mL of the filtered extract into a 250 mL separatory funnel. Add 100 mL of *n*-hexane (to defat the extract) and mix gently to avoid the formation of an emulsion.
3. After separation of the two layers, carefully transfer the lower aqueous layer to another separation funnel and add 50 mL of 10% KCl in water. Discard the *n*-hexane. Acidify the solution with 2 mL of 6N HCl and add 50 mL of chloroform. Mix gently and collect the lower organic layer in a 250 mL Erlenmeyer.
4. Repeat the extraction process with additional 50 mL chloroform, and combine the two chloroform extracts in the same Erlenmeyer. Add 50 g of anhydrous sodium sulfate (Na₂SO₄) and let stand for 1 hour.
5. Filter the extract and collect it into a 200 mL rotary evaporator flask.
6. Take to dryness at 40°C in a rotary evaporator.

2. Purification

1. Place a solid phase Micotox® M2300 cyclopiazonic acid column onto a solid phase extraction apparatus and condition the column by passing 15 mL chloroform at a maximum flow rate of 3 mL/min. Do not let the column run dry.
2. Dissolve the dried extract from rotary evaporator flask with 10 mL chloroform and pass the solution through the column at a maximum flow rate of 2 ml/min.
3. Do not let the column run dry at any point. When top of solution reaches top of packing wash the column with 10 mL of ethyl ether followed by 10 mL of



chloroform:acetone (1+1) and 10 mL of chloroform:methanol (95+5). Discard wash solutions.

4. Elute the CPA with 10 mL of chloroform:methanol (75+25) at a maximum flow rate of 2 mL/min. Use an appropriate test tube or container to collect the eluate (e.g. a 20 mL capacity conical test tube, preferably silanized).
5. Evaporate the eluate to dryness under nitrogen or in a 40°C waterbath, using vacuum.
6. Dissolve the dry residue with 1 mL mobile phase. Mix in vortex for 30 seconds and sonicate.
7. Filter through 0.45 µm Millipore™ membrane filter and inject 20 µL into HPLC.

3. HPLC

Standard solution:

1. CPA stock solution: 100 µg/mL in methanol.
2. Working standard: Pippet 100 µL of the 100 µg/mL CPA standard solution (10 µg CPA) into an amber autosampler vial and add 0.9 mL of mobile phase. This solution corresponds to 10.0 µg/mL.
3. Inject 20 µL into HPLC (0.2 µg on column), equivalent to 0.8 µg/g CPA (ppm CPA = C/W = 0.2 µg/0.25 g = 0.8 µg/g).

Column: Merck LiChroCART® 125–4 HPLC cartridge. LiChrospher® 100 RP–18 (5 µm).
Pre-column: Merck LiChroCART® 4–4 HPLC guard column. LiChrospher® 100 RP–18 (5 µm).

Mobile phase: Methanol:water (70+30) containing 300 mg ZnSO₄·7H₂O/L.
Flow rate: 0.6 mL/min.
Detector: UV at 284 nm.
Retention time: 7.5 min (approximately).

Calculations

$W = 25 \text{ g} \times (50 \text{ mL} / 100 \text{ mL}) \times (0.02 \text{ mL} / 1.0 \text{ mL}) = 0.25 \text{ g}$
Amount of CPA standard injected on column: 0.2 µg, equivalent to 0.8 µg/g (800 µg/kg)
on sample (0.2 µg/0.25 g = 0.8 ng/g).