

Working Method for Mocap (Ethoprop) and metabolite (M-1) in/on Hops

Reference: Adaption by Carol Weisskopf (Formerly of WSU) of “*Method for the Analysis of Mocap®-Related Residues Utilizing Methanol Extraction, Methylene Chloride Partitioning, GPC, and Silica Gel 60 Column Chromatography (version 2.0, for Lima Bean Pods, Vines, and Hay)*” by Rhône-Poulenc Ag Company.

Modifications:

1. Reduced subsample to 1 g. Instrument sensitivity allows for smaller sample size and allows for better cleanup of complex hop matrixes.
2. Substituted Varian SCX SPE (2 g/12 mL) for Dowex cation exchange resin. The SCX SPE was in house and provided sufficient cleanup and eliminated the 20 min. extraction step for Dowex cation exchange resin.
3. Substituted Varian Nexus SPE (0.5 g/12 mL) for nuchar/attaclay cleanup. Nexus SPE provides better cleanup and can be run in tandem with SCX SPE.
4. Added ethyl acetate partition. With the addition of water to improve SPE efficiency, a partition was necessary remove any water present in the sample extract. Water will affect the efficiency of the diazomethane.
5. Eliminated ethylene glycol addition. Not needed for this analysis since sample will not be going to dryness prior to methylation by diazomethane.
6. Produced large batch of diazomethane for methylation instead of making up smaller set sized batches.
7. Omitted methylene chloride partition step. Not required for this analysis since sample will be cleaned up by NH₂ SPE instead of GPC and silica gel.
8. Omitted use of GPC and changed silica gel cleanup to NH₂ SPE for improved sample cleanup.
9. See Section VIII for instrumental parameters.

Method Overview:

Hop samples will be extracted with methanol using homogenization. Following extraction an aliquot will be taken for cleanup via SCX and Nexus SPE. The methanol will be removed to aqueous remainder and partitioned with ethyl acetate. The resulting extract will be concentrated and derivatized with diazomethane (for M-1 metabolite). The reacted sample will be concentrated and further purified using a NH₂ SPE prior sample analysis by GC-FPD (phosphorus mode).

I. Preparation of Standard Solutions

Stock Solution: 0.0510g of Ethoprop (Chem Service, 98%, Lot#328-92A, expiration date 9/30/2007, stored in a freezer [$< 0^{\circ}\text{C}$]) was dissolved into 50 mL of methanol, resulting in a 1.00 mg/mL solution (324-1). The stock solution was stored in brown bottles in the laboratory freezer ($< 0^{\circ}\text{C}$). 0.0553g of M-1 (Bayer, 98%, Lot#0924200403, expiration date 1/23/2006, stored in a freezer [$< 0^{\circ}\text{C}$]) was dissolved into 50 mL of methanol, resulting in a 1.00 mg/mL solution (325-1). The stock solution was stored in brown bottles in the laboratory freezer ($< 0^{\circ}\text{C}$).

Fortification Solutions: 0.5 mL of stock solution 324-1 (ethoprop) and 0.5 mL of stock solution 325-1 (M-1) was diluted up to 50 mL with methanol, resulting in a 10 µg/mL solution (324-1M1). A low level fortification solution was prepared by taking 5 mL of the 10 µg/mL solution and diluted up to 50 mL with methanol, resulting in a 1.0 µg/mL solution (324-1M2). The fortification solutions were stored in brown bottles in the laboratory freezer (< 0 °C).

Calibration Stock Solution: 0.5 mL of stock solution 325-1 (M-1) was reacted with ~ 2mL of diazomethane solution and allowed to react for 1 hour. Following the reaction, 0.5 mL of stock solution 324-1 (ethoprop) was added and diluted up to 50 mL with acetone, resulting in a 10 µg/mL solution (324-1M3).

Calibration Solutions: 1000, 500, 250, 100 and 50 µL aliquots of the 10 µg/mL solution (324-1M3) were diluted up to 50 mL with hexane, resulting in 200, 100, 50, 20 and 10 pg/µL calibration solutions (324-1M4 to 324-1M8, respectively). Fifty microliters of the 10 µg/mL solution (324-1M3) was diluted up to 100 mL with hexane, resulting in a 5 pg/µL solution (324-1M9). The calibration solutions were stored in brown bottles in the laboratory freezer (< 0 °C).

II. Extraction

- 2.1 Weigh out a 10 g aliquot of sample into a 250 mL Erlenmeyer flask (fortify at this point for concurrent recoveries). Add 100 mL of methanol.
- 2.2 Blend sample for 2 minutes at 16,000 rpm (Ultra Turrax).
- 2.3 Filter sample with vacuum through a Buchner funnel fitted with a Whatman 934-AH glass fiber filter backed by a Whatman #1 filter.
- 2.4 Rinse the blending flask with 40 mL of methanol and add to the filter cake.
- 2.5 Pour the sample extract into a 250 mL graduated cylinder and adjust the volume to 150 mL with methanol and mix.
- 2.6 Measure out a 15 mL aliquot (1 g) into a 25 mL graduated cylinder. Add 2 mL of water and mix.

III. SCX and NexusSPE Cleanup

- 3.1 Condition a SCX SPE (2 g/12 mL, Varian) and Nexus SPE (0.5 g/12 mL) with 1 column volume (CV) of methanol.
- 3.2 Add ~ 2 mL of methanol to Nexus SPE and attach SCX SPE on top of Nexus SPE.
- 3.3 Add the sample extract, apply mild vacuum and collect eluant in a 40 mL tube.
- 3.4 Rinse the cylinder with 5 mL of water:methanol (10:90, v/v) and add to the SCX SPE.
- 3.5 After the rinse passes through the SCX SPE, remove and rinse Nexus SPE with 5 mL of water:methanol (10:90, v/v).

- 3.6 Transfer sample to a 100 mL round-bottom flask and rotary-evaporate to aqueous remainder (waterbath @ 35 °C).

IV. Liquid/Liquid Partition

- 4.1 Add 20 mL water and 0.5 mL of concentrated hydrochloric acid to a 125 mL separatory funnel. Use caution with concentrated acid.
- 4.2 Transfer the sample to the separatory funnel and rinse the round-bottom flask with 25 mL ethyl acetate. Add rinse to separatory funnel.
- 4.3 Shake separatory funnel vigorously for 30 seconds and allow layers to separate.
- 4.4 Drain off lower aqueous phase into original round-bottom flask, and pour off ethyl acetate through a funnel plugged with glass wool and anhydrous sodium sulfate into a round-bottom flask.
- 4.5 Repartition the aqueous fraction with another 25 mL of ethyl acetate for 30 seconds.
- 4.6 Drain off lower aqueous phase into original round-bottom flask, and pour off ethyl acetate through funnel.
- 4.7 Rinse sodium sulfate with 5 mL ethyl acetate.
- 4.8 Rotary-evaporate sample to ~ 5 mL (waterbath @ 35 °C).

V. Methylation with Diazomethane

- 5.1 Place sample into fume hood and remove diazomethane from freezer. Use EXTREME caution with diazomethane (explosive, flammable, and toxic).
- 5.2 Add 6-8 mL (~ 2 mL aliquots) of diazomethane to each sample with swirling.
- 5.3 Allow sample to react in the fume hood for 1 hour.
- 5.4 Carefully rotary evaporate sample to ~0.5 mL (waterbath @ 35 °C). Do not let sample go to dryness.
- 5.5 Dissolve sample into 5 mL of ethyl acetate:hexane (20:80, v/v).

VI. NH₂ SPE Cleanup

- 6.1 Condition a NH₂ SPE (NH₂ 2 g/12 mL, Varian) with 1 column volume (CV) of ethyl acetate:hexane (20:80, v/v).
- 6.2 Load sample to SPE, apply mild vacuum and collect eluant in a 40 mL tube.
- 6.3 Rinse the round-bottom flask with 5 mL ethyl acetate:hexane (20:80, v/v) and add to SPE as the solvent sinking into the packing. Repeat twice more with 5 mL aliquots of ethyl acetate:hexane (20:80, v/v).
- 6.4 Continue sample elution with 10 mL ethyl acetate:hexane (20:80, v/v).
- 6.5 Transfer the eluant to a 100 mL round-bottom flask and concentrate the sample to ~ 0.5 mL using rotary-evaporation (waterbath @ 35 °C). Do not let sample go to dryness.
- 6.6 Dilute up the sample to an appropriate volume for analysis by GC-FPD with Hexane.

VII. Quantitation

A minimum of 4 standard solutions are prepared in the concentration range of 5 pg/ μ L to 200 pg/ μ L. Samples were injected in duplicate and residues are determined by comparison to the standard curve. Standards and samples are preferably quantitated by peak area. The limit of detection is defined as 10% below the smallest concentration within the standard curve.

Control samples are fortified with known amounts of ethoprop and M-1 prior to extraction. Percent recovery is calculated by measuring the peak area as shown below:

$$\frac{\text{pg}/\mu\text{L determined} * \mu\text{L injected}}{\text{mg crop} * 1000 \text{ (conv. Factor)}} = \text{ppm found}$$

$$\frac{\text{ppm found} * 100}{\text{ppm fortified}} = \text{Percent Recovery}$$

VIII. Instrument Parameters

Instrument: "Krusty" HP 5890 gas chromatograph with flame photometric detection (phosphorus mode).

Operating Parameters:

Column: Restek XTI-5, 30 m x 0.53 mm ID, 1.5 μ m Film
(Serial # 128163)

- Oven Program: 80 °C (1 min.) program at 25 °C/min. to 280 °C (2 min.)
- Injector Temp: 250 °C: Detector Temp: 225 °C
- Splitless Injection: 2 μ L injection vol.
- Carrier Gas: Helium at 10 mL/min.
- FPD Gases: H₂ at 75 mL/min.
Air at 100 mL/min.
Makeup (He) at 15 mL/min.
- Retention Time: Ethoprop: 5.42 min.
Methyl-M-1: 7.1 min.

Laboratory Research Director

Date

Analyst

Date