

US EPA ARCHIVE DOCUMENT

Determination of Phosphamidon Residues

in Hops

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PRINCIPLE:

Phosphamidon (cis and trans isomers) and desethylphosphamidon are extracted from green or dry hops, following rehydration, through multiple blendings with methylene chloride and saturated sodium sulfate solution. The methylene chloride extracts are combined, cleaned up in an alumina column and subjected to gas chromatographic analysis.

REAGENTS:

Alumina - neutral, activity grade V, ICN Biochemicals
Phosphamidon (cis/trans isomer mixture) and desethylphosphamidon -
standard reference materials available from Chevron Chemical Co.,
Richmond, California
Sodium sulfate - anhydrous
Sodium sulfate solution - saturated
Methylene chloride - nanograde
Chloroform - nanograde
Ethyl Acetate - nanograde
Hexane - nanograde

APPARATUS:

Besides the usual laboratory equipment, the following items are required:

Micro-Tek Model 220 gas chromatograph equipped with a flame
photometric detector in the phosphorous mode.
Gas chromatographic column - 4' x $\frac{1}{4}$ " od glass column packed with
5% EGSS-X on 100/120 mesh Gas Chrom Q.
Chromatographic column - glass, 22 mm x 40 cm, with course fritted disc.
Omni mixer
Vacuum evaporation apparatus - steam bath/vacuum or rotary vacuum
Stainless steel screen - 16 meshes/inch

PROCEDURES:

Rehydration:

Hops are rehydrated by placing 20 g of ground sample in a 1 qt. Mason jar. Add proper amount of water (75 ml for green hops, 150 ml for dry hops). Seal lid tightly on jar and let stand 4-6 hr. shaking occasionally to distribute moisture.

Extraction: (1)

To Mason jar containing rehydrated sample, add 25 g anhydrous sodium sulfate and stir, then add 38 ml saturated sodium sulfate solution and stir again. Add methylene chloride (200 ml for green hops, 400 ml for dry hops) and blend for 10 min. on Omni-mixer. Cover Mason jar with stainless steel screen and decant liquid layer into 500 ml separatory funnel. Allow layers to separate. Drain lower methylene chloride layer through sodium sulfate into 1 L erlenmeyer flask. Return aqueous layer to Mason jar containing the substrate and add methylene chloride (100 ml for green hops, 200 ml for dry hops). Blend for 5 min. on Omni-mixer, decant as before and combine methylene chloride extract with the first in 1 L erlenmeyer flask. Repeat the previous methylene chloride extraction, combining this extract with the previous two. Using a graduated cylinder (500 ml for green hops, 1000 ml for dry hops) make volume to 400 ml for green hops or 800 ml for dry hops with methylene chloride.

For green hops, transfer an 80 ml aliquot to a 150 ml beaker and evaporate to dryness at 40°C. For dry hops transfer a 160 ml aliquot to a 250 ml beaker and evaporate to dryness at 40°C. In either case redissolve residue in 10 ml hexane. Proceed with column cleanup.

Column clean-up: (2)

Prepare an alumina column by placing in a 22 mm x 40 cm chromatographic column a plug of glass wool, 3 cm alumina and 1 cm sodium sulfate. Wash with 25 ml hexane.

Add sample hexane extract to column. Wash the column with 50 ml hexane and discard. Elute phosphamidon and desethylphosphamidon with 100 ml 50% chloroform in hexane, collecting eluate in 150 ml beaker. Evaporate eluate to 5 ml. Transfer concentrated solution to test tube and evaporate to dryness. Redissolve residue in 2 ml ethyl acetate. Proceed with gas chromatographic analysis.

Gas Chromatographic analysis:

Equilibrate the gas chromatograph as follows: injector temperature, 240°C; detector temperature, 200°C; column temperature, 210°C; nitrogen carrier gas flow, 100 ml/min. Condition column overnight at 220°C with 30 ml/min. carrier gas flow. Prior to analysis, make several injections (2-4 µl) of a mixed standard solution containing 10 µg/ml of phosphamidon and desethylphosphamidon in ethyl acetate to sensitize the column to these compounds.

Adjust attenuator settings on electrometer so that injection of 2.0 ng each phosphamidon and desethylphosphamidon from a mixed standard solution produces a desethylphosphamidon response which is 25-40% full scale. Under these conditions, the cis isomer of phosphamidon will produce a response approximately twice as large as that produced by desethylphosphamidon and approximately 5 times as large as that produced by the trans isomer of phosphamidon when the phosphamidon standard material used contains the typical 73% cis isomer and 27% trans isomer. (2)

Prepare calibration curves by chromatographing appropriate aliquots of mixed standard solutions containing 0.2, 0.5 and 1.0 µg/ml phosphamidon and desethylphosphamidon to demonstrate linearity.

Inject 4 μ l (representing 8 mg sample) aliquot of final sample extract and compare peak height of responses with those produced by injection of 4 μ l of a mixed standard solution containing 0.5 μ g/ml phosphamidon and desethylphosphamidon. Inject standard after every 3-4 samples. Under the gas chromatographic conditions described earlier, the retention times are as follows: trans-phosphamidon, 2.2 min.; cis-phosphamidon, 3.4 min.; desethylphosphamidon, 4.2 min.

Calculation is made by use of the following equation:

$$\text{ppm} = \frac{\text{peak height sample}}{\text{peak height standard}} \times \frac{\text{ng standard injected}}{\text{mg sample injected}}$$

Note: peak height of phosphamidon is the sum of the peak heights derived from the trans isomer and cis isomer components.

peak height phosphamidon = peak height trans isomer + peak height cis isomer

DISCUSSION:

The gas chromatographic method described has a detection limit of 0.03 ppm for phosphamidon and 0.05 ppm for desethylphosphamidon.

REFERENCES:

- (1) "Extraction of Phosphamidon residues", Method RM-4, Chevron Chemical Co., Oct. 1963.
- (2) J. Agr. Food Chem., Vol. 21, No. 5, Pg 846, 1973.