# Development of a Gas Chromatographic Method for Fungicide Cymoxanil Analysis in Dried Hops

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An analytical method for detecting cymoxanil {2-cyano-*N*-[(ethylamino)carbonyl]-2-(methoxyimino)-acetamide} residues in dried hops was developed utilizing liquid−liquid partitioning, automated gel permeation chromatography (GPC), solid phase extraction (SPE) cleanup, and gas chromatography (GC). Method validation recoveries from dried hops were 96 ± 12, 108 ± 11, and 136 ± 8% over three levels of fortification (0.05, 0.5, and 1.0 ppm, respectively). The hop samples from three field sites, which were treated with cymoxanil, had residue levels ranging from 0.146 to 0.646 ppm. The detection limit and the quantitation limit of the method developed in the present study were 0.022 and 0.050 ppm, respectively.

**Keywords:** *Cymoxanil; fungicide; gas chromatography; hops*

## INTRODUCTION

Cymoxanil {2-cyano-*N*-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide} was introduced in the late 1970s in response to the growing need for improved control against Phycomycete fungal pathogens (*1*). Historically, cymoxanil (Curzate) has been used to control grape downy mildew (*Plasmopora viticola*) and late blight of tomato and potato (*Phytophthora infestans*) (*2*). Cymoxanil provides both protective and curative action with foliar application (*3*). Currently, cymoxanil is being considered for registration in the United States to control downy mildew on hops.

Analysis of cymoxanil can be performed by either high-pressure liquid chromatography (HPLC) or gas chromatography (GC). These methods have been shown to be adequate for the analysis of matrixes such as grapes and tomatoes (*4*, *5*). However, hop matrixes are substantially more complex and require better cleanup processes. These processes are needed to remove unwanted resins and oils from the hop extract, which may cause chromatographic interference, complicating analysis.

The USDA IR-4 Program (U.S. Department of Agriculture Interregional Research Project 4, Minor Use Pesticide Registration Program) initiated this project to obtain residue data on the use of cymoxanil on hops for the control of downy mildew. Given the passage of the Food Quality Protection Act (FQPA), the U.S. Environmental Protection Agency (EPA) has since imposed lower pesticide tolerances. A crisis exemption has been declared under Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act authorizing use of cymoxanil on dried hops (*6*). As a result, laboratories for pesticide residue analysis have been forced to develop testing methods with greater sensitivities.

This paper reports a new and sensitive analytical method for detecting cymoxanil in dried hop samples. The new method uses an automated gel permeation chromatograph (GPC), solid phase extraction (SPE), and a GC with a nitrogen−phosphorus detector (NPD).

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## EXPERIMENTAL PROCEDURES

**Pesticide.** Cymoxanil (99.6%) was acquired from E. I. DuPont de Nemours and Co., DuPont Agricultural Products, Experimental Station (Wilmington, DE).

**Materials.** All solvents and reagents were of residue grade.

**Preparation of Stock and Fortification Solution.** A stock solution (0.2 mg/mL) was prepared by dissolving 20 mg of cymoxanil (analytical standard) to dichloromethane in a 100-mL volumetric flask. The volume of dichloromethane solution was adjusted to exactly 100 mL. A midlevel fortification standard solution (10 μg/mL) was prepared by placing a 2.5 mL aliquot of the 0.2 mg/mL stock solution in a 50-mL volumetric flask, and the volume of solution was adjusted to exactly 50 mL with ethyl acetate. A low-level fortification standard solution (1.0 μg/mL) was prepared by placing a 5-mL aliquot of the 10.0 μg/mL midlevel fortification solution in a 50-mL volumetric flask, and the volume of the solution was adjusted to exactly 50 mL with ethyl acetate. All stocks and fortification solutions were stored at −20 °C until use.

**Preparation of Solutions for GC Calibration Curve.** GC calibration solutions were prepared by adding 800 and 400 μL of the 10.0 μg/mL to ethyl acetate in 25-mL volumetric flasks to make 320 and 160 pg/μL solutions, respectively. Other GC calibration solutions were prepared by adding 200 μL of the 10.0 μg/mL solution to ethyl acetate in 25-, 50-, 100-, and 200-mL volumetric flasks to make 80, 40, 20, and 10 pg/μL solutions, respectively. All calibration solutions were stored at 5 °C until use.

**Collection of Field Samples.** A total of 12 hop samples (6 treated and 6 untreated controls) were collected from IR-4 (Interregional Research Project 4) field trial sites in Oregon, Idaho, and Washington. Cymoxanil was applied four times to the field at a rate of 0.156 lb (active ingredient)/acre. The final application was 7 ± 1 days prior to harvest. Final application dates for the fields of Hubbard, OR (Nugget variety); Parma, ID (Galena variety); and Prosser, WA (Nugget variety) were September 2, 1998; August 25, 1998; and September 2, 1998, respectively. The hop samples were dried in a manner consistent with commercial drying methods and transferred, in a frozen state, to our laboratory.

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**Sample Preparation for GC Analysis.** Hop samples (~300-g each) were chopped with equal amounts of dry ice using a Hobart food chopper (Hobart Corp., Troy, OH). Each chopped sample was stored in a 1-qt jar, and a lined lid was loosely closed on top to allow the dry ice to dissipate during storage at −20 °C.

The following extraction, liquid partition, and silica SPE steps are modifications of the 1994 method by Cicotti and Zenide (*7*) for the determination of cymoxanil in fresh and dried hops. A 10-g aliquot of dried hops was weighed into a 500-mL Erlenmeyer flask. One hundred milliliters of 30% hydrochloric acid solution was added to the flask, which was subsequently shaken with a gyrotory shaker (New Brunswick Scientific Co., New Brunswick, NJ) for 30 min. After shaking, 200 mL of acetone was added and the sample was homogenized using a Ultra-Turrax T-25 (Janke & Kunkel) for 3 min at 13500 rpm. The homogenized sample was then filtered under mild vacuum through a Büchner funnel fitted with Whatman 934-AH filter paper, backed by Whatman No. 1 filter paper, and covered with a teaspoon of Celite 545 (Fisher Scientific, Fair Lawn, NJ). The homogenizing flask was rinsed with 30 mL of acetone and added to the filter cake.

After the sample was filtered, the entire filtrate was transferred to a 1000-mL separatory funnel. The filtration flask was rinsed with 50 mL of hexane and added to the separatory funnel. Sodium chloride (3 g) was added to the sample, and then the separatory funnel was gently shaken for 3 min. After 3 min, 100 mL of dichloromethane was added and shaking continued for an additional 2 min. The lower aqueous phase was drained off, and the upper organic layer was decanted through a funnel plugged with glass wool and anhydrous sodium sulfate into a 500-mL graduated cylinder. A 4-g aliquot was placed into a 250-mL round-bottom flask. The remaining extract was stored at 5 °C. Glacial acetic acid (0.1 mL) was added to the aliquot, and then the sample was concentrated to near dryness using a rotary evaporator under reduced pressure (water bath at 35 °C). The concentrated sample was redissolved into 10 mL of dichloromethane/cyclohexane (1:1, v/v) solution for cleanup via GPC.

**GPC.** The GPC system consisted of a Kontes Chromaflex gel permeation column (Kontes, Vineland, NJ), a Foxy 200 X-Y fraction collector (Isco, Inc., Lincoln, NE), and a Benchmate II Workstation (Zymark Corp., Hopkinton, MA). The Benchmate was programmed to automatically weigh, vortex, and filter (PTFE, 0.45 μm Millipore filter disk, Millipore Corp., Bedford, MA) each sample prior to injection (5-mL sample loop is equal to 2-g sample on column) onto the GPC column. The column was 62 cm × 2.5 cm i.d. packed with 200/400 mesh S-X3 (Bio-Rad, Richmond, CA). The column bed length was 50 cm. The sample was developed with a dichloromethane/cyclohexane (1:1, v/v) solution at 5 mL/min. Once the sample was loaded onto the column, the fraction collector was programmed to discard the first 120 mL (24 min) of eluate and then collect the next 60 mL (12 min) of eluate into a 250-mL TurboVap tube (Zymark Corp.). The GPC column was regenerated with 250 mL of mobile phase prior to the next sample injection.

After GPC cleanup, the samples were concentrated to dryness using a TurboVap II Concentration Workstation with dry nitrogen (water bath at 35 °C). The concentrated samples were then redissolved in 1 mL of ethyl acetate.

**SPE.** Hexane (9 mL) was added to the above 1-mL ethyl acetate solution, and then the sample was sonicated briefly. Prior to the loading of the sample, the silica (1 g/6 mL) SPE was conditioned with 1 column volume (CV) of an ethyl acetate/hexane (1:9, v/v) solution. As the solvent reached the top of the packing, a 20-mL reservoir was attached, and the sample was then loaded to the SPE. Once the sample was loaded onto the SPE, the TurboVap tube was rinsed with 15 mL of an ethyl acetate/hexane (1:9, v/v) solution and the rinsate was added to the SPE. Following the 15-mL rinsate, the SPE was washed with 20 mL of an ethyl acetate/hexane (1:9, v/v) solution and the eluate was discarded. Cymoxanil was eluted with 10 mL of an ethyl acetate/hexane (4:6, v/v) solution into a 50-mL round-bottom flask. The sample was then rotary evaporated to dryness and redissolved in a appropriate amount of ethyl acetates for GC analysis.

**Instrumental Analysis.** Sample analysis was conducted with a Hewlett-Packard (HP) 5890 Series II GC (HP, Avondale, PA) equipped with a 15 m × 0.53 mm i.d. ($d_f$ = 1.5 μm) Restek Xti-5 megabore column (Restek Corp., Bellefonte, PA) and an NPD. The injector and detector were operated at 250 and 280 °C, respectively. An HP model 7673 autoinjector was used to inject 3 μL of sample in splitless mode. The oven temperature was initially held at 110 °C for 1 min and then programmed at 20 °C/min to 280 °C and held for 1 min. The NPD gases consisted of air at 110 mL/min, hydrogen at 3.5 mL/min, and helium (makeup gas) at 20 mL/min. The carrier gas was helium at a flow rate of 10 mL/min, which resulted in a retention time of 4.88 min for cymoxanil.

**Storage Stability Study.** The six untreated samples of dried hops were fortified at 1 ppm and stored at −20 °C until analysis. Three stability samples were analyzed after all of the field samples were analyzed. The three remaining samples were kept for long-term storage test.

## RESULTS AND DISCUSSION

There are very few references on the determination of cymoxanil in complex matrixes such as food crops. One such method utilized multidimensional multicolumn high-pressure liquid chromatography (MC-HPLC) with UV detection to determine cymoxanil residues, as low as 0.050 ppm, in grapes (*4*). Although the MC-HPLC method provided good sensitivity and relatively short sample analysis times, the HPLC column and UV detector would not provide the needed chromatographic resolution and selectivity required for the analysis of a considerably more complex matrix such as dried hops.

Another method, developed by Holt at DuPont, utilized gas−liquid chromatography with NPD detection and provided method sensitivity down to 0.04 ppm on potato, tomato, grape, and wine matrixes (*5*). Later, Cicotti and Zenide (*7*), also from DuPont, modified the aforementioned method for cymoxanil on fresh and dried hops by the addition of a Florisil column cleanup and achieved a method sensitivity of 0.1 ppm.

Figures 1 and 2 show typical gas chromatograms of cymoxamil isolated from dried hop samples in the present study. The method sensitivity and limit of detection in the analysis of cymoxanil in dried hop samples were 0.050 and 0.022 ppm, respectively. These results are comparable to the method reported previously (*7*). Although the acetone extraction proved effective in the current and previous pesticide residue methods on crops, it was necessary to utilize gel permeation cleanup to achieve lower sensitivity (*7*, *8*). The use of GPC provided substantial sample cleanup by separating cymoxanil from resins, waxes, and oils present in the hop extracts. In addition, the automation of the GPC increased efficiency by allowing several samples to be extracted during normal working hours and subsequently processed by the GPC during the night. Also, the use of capillary columns for separations provided superior resolution of peaks and sensitivity compared to the packed glass column used previously (*7*).

The results of recovery tests on cymoxanil from dried hops were 96 ± 12% ($n$ = 6) for 0.050 ppm, 108 ± 11% ($n$ = 6) for 0.500 ppm, and 136 ± 8% ($n$ = 6) for 1.000 ppm. The values are mean ± standard deviation. The recovery efficiency of cymoxanil from a dried hop sample at the level of 1 ppm after 217 days of storage was 133

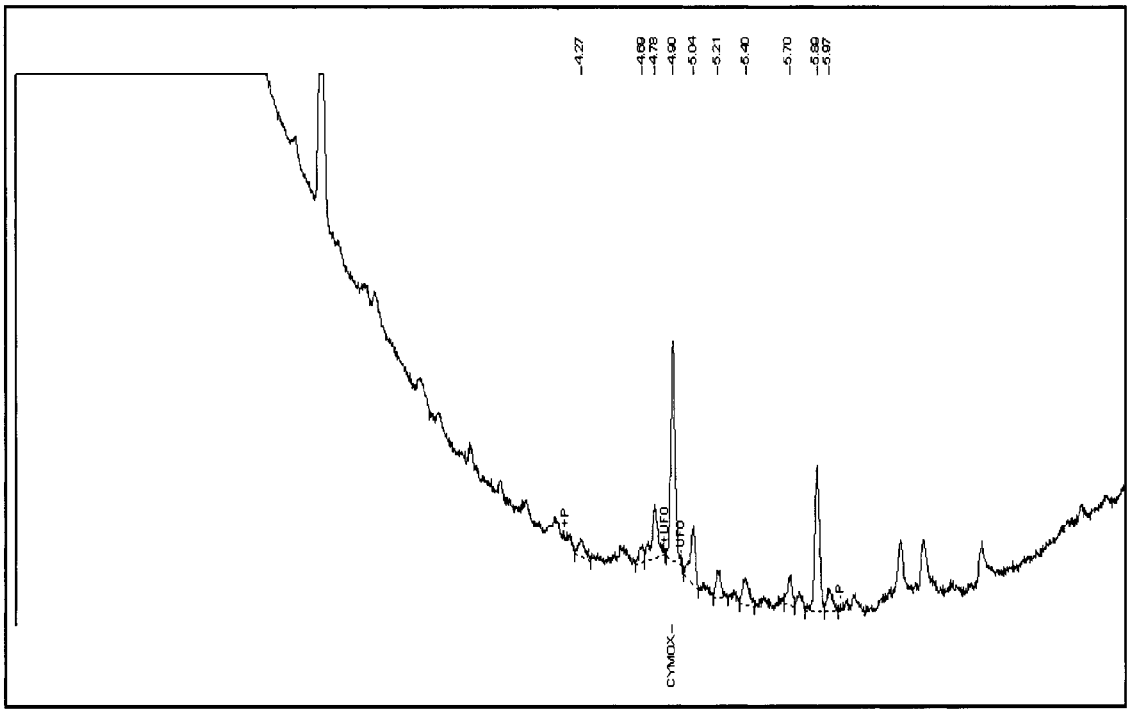


**Figure 1.** Sample chromatogram of treated Idaho field sample (0.570 ppm, 0.24 mg injected, Galena variety), cymoxanil $t_R$ = 4.90 min.

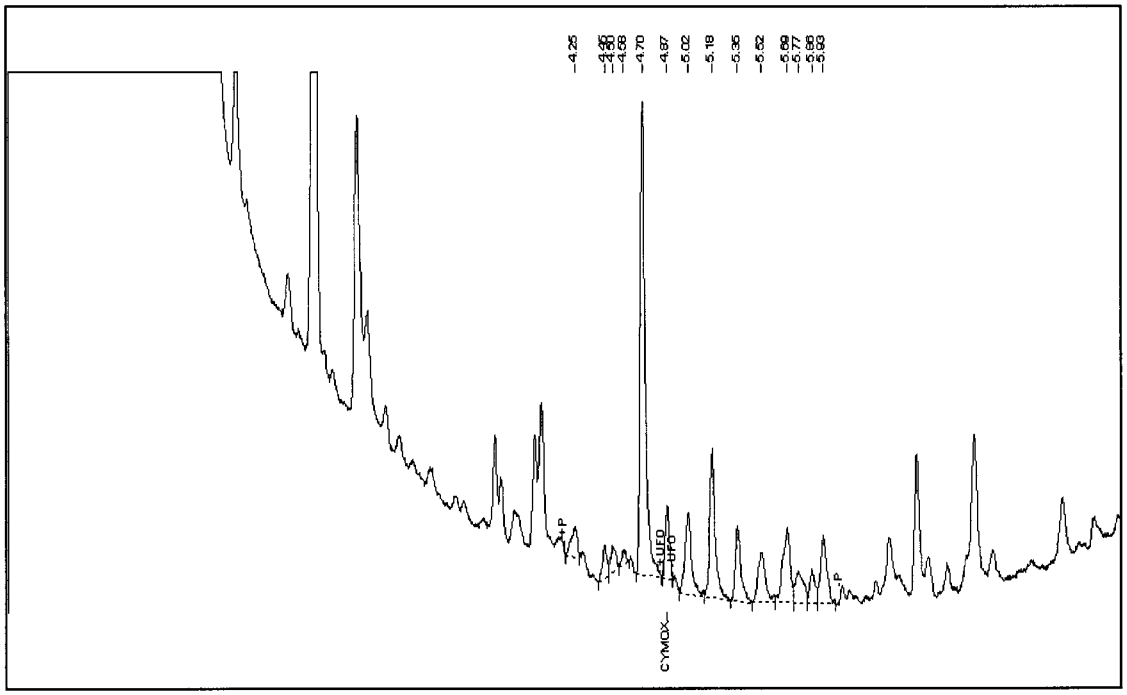


**Figure 2.** Sample chromatogram (NPD) of 12255V0.05R3 (0.050 ppm recovery, 1.2 mg injected, Nugget variety), cymoxanil $t_R$ = 4.87 min.

**Table 1. Amounts of Cymoxanil Residues (Parts per Million) Found in Field Samples**[a]

| Idaho | | Oregon | | Washington | |
|---|---|---|---|---|---|
| control | treated | control | treated | control | treated |
| <0.050 | 0.570 | <0.050 | 0.165 | <0.050 | 0.549 |
| <0.050 | 0.646 | <0.050 | 0.146 | <0.050 | 0.414 |

[a] Each value is an average of duplicate sample analyses.

± 3% ($n$ = 3). The value is mean ± standard deviation. The recovery data from the storage study suggests that cymoxanil exhibit no breakdown during extended storage in −20 °C conditions.

Table 1 shows the results of cymoxanil analysis of field samples. Hop samples treated with cymoxanil in the field showed residues ranging from 0.146 to 0.646 ppm. No control sample had residues above the method sensitivity (0.050 ppm).

The new method developed in the present study provides for the determination of cymoxanil at the current tolerance level of 1 ppm, as well as for expected future lower tolerance levels.

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