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**< Short Communication >**

# Determination of cyromazine in commercial insecticides using HPLC-DAD

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## Abstract

Each commercial cyromazine insecticide has different HPLC conditions. The aim of this study was to establish a standardized chromatographic method for the determination of cyromazine in commercial insecticides. The separation was achieved on two C18 columns - Waters® Bondapak C (4×300 nm i.d., 10 μm) and X bridge (4.6×250 nm i.d., 5 μm) using a mobile phase composed of water/methanol/ethanolamine (76:24:0.1, v/v), with UV detection at wavelengths 230 nm and 254 nm. A total of six commercial cyromazine insecticides were analyzed. In this study, the optimal high-performance liquid chromatography conditions for the analysis of cyromazine were as follows: a mobile phase of water/methanol/ethanolamine (76:24:0.1, v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 230 nm using a X bridge C18 column (4.6×250 nm i.d., 5 μm) at a column temperature of 25°C. The calibration curve was linear in the concentration range of 5～50 μg/mL, with a correlation coefficient of 0.99995. The cyromazine detection limit was 0.2 μg/mL, and the limit of quantification was 0.59 μg/mL. The percentage recovery ranged from 99.8% to 101.0% for cyromazine, and the relative standard deviation was not over 2.0%. The cyromazine concentration ranged from 92.7% to 109.4% and was within the acceptable range (90～120%) for the percent of the labeled amount. This method was found to be suitable for determining cyromazine in commercial insecticides.

**Key words :** Cyromazine, Insecticide, HPLC, Validation

## INTRODUCTION

N-cyclopropyl-1,3,5-triazine-2,4,6-triamine (cyromazine) is an insecticide used for fly control in animal feed (Yokley et al, 2000; Chou et al, 2003; Ge et al, 2014). It has been used on dogs and cats to kill dog fleas, ticks, lice, and flies. Many analytical methods such as high-performance liquid chromatography (HPLC), gas chromatography/mass spectrometry (GC-MS), and liquid chromatography/mass spectrometry (LC-MS/MS) have been developed for the analysis of cyromazine (Ge et al, 2014; Tsartsali and Samanidou, 2015). Currently, there are 20 cyromazine insecticides (19 powders and 1 granulate) from 16 companies registered with the Animal Drug Associations. Each cyromazine assay provided by cyromazine insecticide manufactures has different HPLC conditions. Most chemical formulations use a variety of excipients and raw materials, so even though they are replica drugs of the same component, content control is not performed by the same analysis method. In connection with the recent egg insecticide issue and the expansion of the companion animal industry, the improvement of standard analysis methods is required due to the increase in veterinary drugs products. For the quality control of veterinary quasi-drugs, it is necessary to establish a standard analysis conditions for other veterinary insecticides (including cyromazine), and validate the established assay.

Herein, we aimed to establish a standardized chroma-

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tographic method for the determination of cyromazine in commercial insecticides.

## MATERIALS AND METHODS

### Reagents

Cyromazine standard was purchased from the United States Pharmacopeia (USP) (Bethesda, USA). Methanol (HPLC grade) was obtained from J. T. Baker (Radnor, USA). The distilled water (HPLC grade) was obtained from Fisher Scientific (Seoul, Korea). Acetonitrile (HPLC grade) was purchased from Merck (Darmstadt, Germany). Ethanolamine (analytical grade) was purchased from Sigma-Aldrich (Steinheim, Germany).

### Instrumentation

The high-performance liquid chromatography (HPLC) system (Agilent 1260 Infinity II series, USA) was equipped with a pump (Model G7111A), an autosampler (Model G7129A), a sample thermostat (Model G4761A), a column oven (Model G7116A), and a UV-detector (Model G7117C). Chemstation Software (Version Rev C.01.10 (236)) was used for data processing and evaluation.

### Chromatographic conditions and method optimization

The separation was achieved on two C18 columns - Waters® Bondapak C and X bridge using water/methanol/ethanolamine (76:24:0.1, v/v) as a mobile phase at a flow rate of 1.0 mL/min, with UV detection at wavelengths 230 nm and 254 nm and a column temperature of 25°C.

### Sample collection

Six commercially available insecticide formulations (5 powders and 1 granulate) were selected from 20 cyromazine insecticides registered with the Animal Drug Association and purchased from their manufacturers.

### Sample preparation

Six commercial insecticide formulations contain 0.6 to 1 g cyromazine per kg of product. The formulated products were transferred into a 200 mL volumetric flask each, and 150 mL of diluent 1 (2% ethanolamine in methanol) was added and filled with diluent 2 (60% methanol). These samples were sonicated and centrifugated for 15 and 10 min, respectively. Further, after transferring 10 mL of the supernatant into a 25 mL volumetric flask, it was filled to the mark with mobile phase. Subsequently, the samples were filtered using 0.45 μm membrane.

### Preparation of standards

Cyromazine (10.0 mg) was transferred into a 10 mL volumetric flask and diluted with diluent 1. Further, 5 mL of this solution was placed in a 100 mL volumetric flask and filled with 45 mL of diluent 1 and diluent 2. The stock solution was diluted with the mobile phase into a series of standard solutions (5, 10, 12.5, 20, 25, and 50 μg/mL). Each standard was injected into the HPLC system three times.

### Method validation

We determined parameters for method validation, including linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Linearity was calculated by plotting peak areas versus different known concentrations (5, 10, 12.5, 20, 25, and 50 μg/mL), and a linear equation with correlation coefficient ($R^2$) was obtained. The accuracy of the method was determined by recovery tests. The percent recovery was calculated by the formula recovery (%)=(amount recovered×100)/(original amount+amount spiked). The precision of the method was evaluated for repeatability and reproducibility. The repeatability of the method was assessed by calculating the relative standard deviation (RSD) of the peak areas of six replicates of standard solutions with three concentrations (12.5, 25, and 50 μg/mL). Reproducibility was determined by comparing the results of two different laboratories. Biocides research Lab was the

main laboratory, and Productive and General Drugs Lab with different equipment (Agilent 1260 Infinity, USA) was the other. Precision was expressed as the RSD %.

## RESULTS AND DISCUSSION

### Optimized Chromatographic conditions

For standardization of HPLC conditions for each product, the experiments were conducted at different wavelengths (230 nm, 254 nm) under two C18 columns (Waters® Bondapak C and X bridge) using water/methanol/ethanolamine (76:24:0.1, v/v) as mobile phase for 6 commercially available cyromazine insecticides. The calibration plots were linear with the correlation coefficients ≥0.99995, and the concentrations of cyromazine were close to those of the standard and testing methods and were within the acceptable ranges (90～120%) for the percent of the labeled amount. The results show that both wavelengths (230 nm and 254 nm) can be used for the measurements, but at 254 nm, the peak area value is smaller for the same concentration; considering the peak shape, the wavelength can be standardized to 230 nm. For the columns, the retention time was lower (4.5 to 4.6 min) for the X bridge C18 column (4.6×250 nm i.d., 5 μm), than that of the Bondapak C18 column (4× 300 nm i.d., 10 μm) (5.6 to 5.8 min); thus, the analysis could be performed faster (Fig. 1). Therefore, the optimal HPLC chromatographic conditions for the analysis of cyromazine were: mobile phase of water/methanol/ethanoplamine (76:24:0.1, v/v) at a flow rate of 1.0 mL/min and a detection wavelength of 230 nm using a X bridge C 18 columns (4.6×250 nm i.d., 5 μm) at a column temperature of 25°C (Table 1).

The standardization of chromatographic conditions resulted in faster and more efficient separation of cyromazine from commercial cyromazine insecticide products with different HPLC conditions.

**Table 1.** Optimized chromatographic conditions

| Chromatographic conditions | |
|---|---|
| Column | X bridge $C_{18}$ 4.6×250 mm, 5 μm |
| Mobile phase | D.W:MEOH:Ethanolamine (76:24:0.1) |
| Wavelength | 230 nm |
| Column oven | 25°C |
| Flow rate | 1.0 mL/min |
| Injection volume | 10 μL |
| Run time | 10 min |

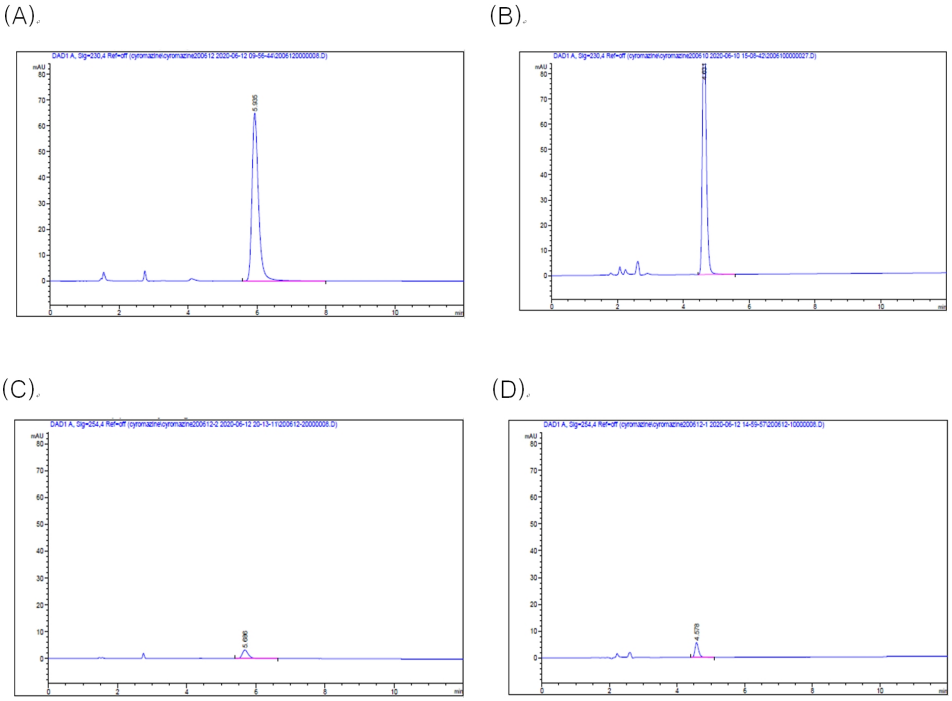


**Fig. 1.** Chromatograms of cyromazine standard solution (20 μg/mL) when using: Bondapak C 18, 230 nm (A); X bridge C 18, 230 nm (B); Bondapak C 18, 254 nm (C); X bridge C 18, 254 nm (D).

## Linearity

The calibration curve was linear in the concentration range of 5～50 μg/mL. The linear regression equation with the correlation coefficient was Y=43.17000x-2.30737 ($R^2$=0.99995) (Fig. 2). The correlation coefficient was satisfactory ($R^2$＞0.99) and indicated the reliability of this method for quantitative detection (Fig. 2).

## LOD and LOQ

The limit of detection (LOD) is the lowest concentration of analyte that can be detected, and the limit of quantification (LOQ) is the lowest concentration of analyte that can be determined quantitatively with acceptable accuracy and precision. The limit of detection of cyromazine was 0.2 μg/mL, and the limit of quantification 0.59 μg/mL (Table 2).

## Precision and accuracy

To determine the accuracy of the method, a recovery test was performed by adding known amounts of cyromazine standard (10, 20, and 40 μg/mL) to the sample and analyzing three replicates. For the precision of the method, repeatability (intra-day precision) and reproducibility (inter-laboratory comparison test) were evaluated. In this study, the mean recovery rate of cyromazine was 99.8% to 101.0% and the relative standard deviation was not over 2% (Table 3～5), indicating that the precision and accuracy of the method were satisfactory.

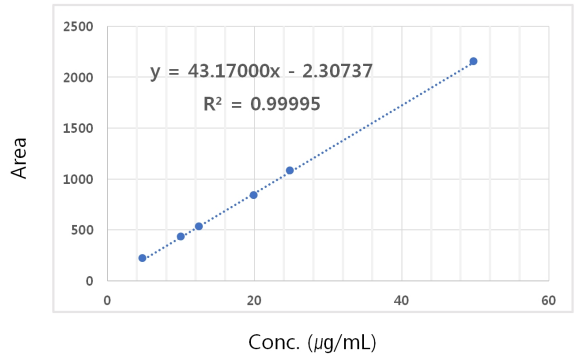


**Fig. 2.** Calibration graph for cyromazine standard.

**Table 2.** LOD and LOQ of cyromazine in this study

| Insecticide | LOD (μg/mL) | LOQ (μg/mL) |
|---|---|---|
| Cyromazine | 0.2 | 0.59 |

**Table 3.** Recovery of cyromazine

| Concentration in sample (μg/mL) | Standard added (μg/mL) | Amount recovered (μg/mL) | Recovery (%) | Mean (%) | RSD (%) |
|---|---|---|---|---|---|
| 21.13 | 10 | 31.44 | 101.0 | 100.3 | 1.24 |
| | | 30.78 | 98.9 | | |
| | | 31.45 | 101.0 | | |
| | 20 | 40.86 | 99.4 | 99.8 | 1.47 |
| | | 41.71 | 101.4 | | |
| | | 40.54 | 98.6 | | |
| | 40 | 62.60 | 102.4 | 101.0 | 1.18 |
| | | 61.43 | 100.5 | | |
| | | 61.26 | 100.2 | | |

**Table 4.** Result of the repeatability test

| Standard sample (μg/mL) | Repeatability test (n=6) | RSD (%) |
|---|---|---|
| | Average peak area | |
| 12.5 | 505.4 | 1.93 |
| 25 | 997.2 | 1.40 |
| 50 | 1193.0 | 1.10 |

**Table 5.** Result of the reproducibility test

| Standard sample (ug/mL) | Reproducibility test (n=6) | | | |
|---|---|---|---|---|
| | Laboratory A | | Laboratory B | |
| | Average peak area | RSD (%) | Average peak area | RSD (%) |
| 10 | 444.4 | 0.05 | 462.9 | 0.21 |
| 20 | 842.1 | 0.12 | 907.3 | 0.16 |
| 50 | 2196.0 | 0.29 | 2275.6 | 0.30 |

Lab A, Biocides research Lab; Lab B, Productive and General Drugs Lab.

**Table 6.** Concentration of cyromazine in samples

| Sample No | Concentration in product (g/kg) | Standard and testing method (μg/mL) | Standardized method | |
|---|---|---|---|---|
| | | | Quantity in sample (μg/mL) | Concentration (%) |
| 1 | 10 | 9.87 | 9.77 | 97.7 |
| 2 | 10 | 10.58 | 10.45 | 104.5 |
| 3 | 6 | 5.97 | 5.88 | 98.1 |
| 4 | 6 | 5.90 | 5.56 | 92.7 |
| 5 | 20 | 22.15 | 21.87 | 109.4 |
| 6 | 10 | 10.03 | 9.87 | 98.7 |

### Sample analysis

The optimized procedure was applied to the analysis of 6 commercially available products. The concentrations of cyromazine ranged from 92.7% to 109.4% and were within the acceptable ranges (90～120%) for the percent of the labeled amount (Table 6).

## CONCLUSIONS

The results of this study showed that our method is simple, rapid, accurate, precise and sensitive. Therefore, this method is suitable for determining cyromazine in the cyromazine-based insecticides. In summary, this method can be used for quantitative analysis and quality control of commercial cyromazine insecticide formulation products.

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## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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